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Dilek YALÇIN

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PREFACE

This work presents a compilation of cutting-edge research in contemporary science, particularly in the fields of nanotechnology, plant physiology, microbiology, and environmental sustainability. The six articles assembled under different headings aim to inform and shed light on current scientific developments and discoveries for researchers, scientists, and readers from various disciplines.

The first section delves into the synthesis and biomedical applications of silver nanoparticles. Focusing on the impressive advancements of nanotechnology in the healthcare sector, this section explores the biomedical potential of silver nanoparticles.

The second section concentrates on research regarding the importance of Polygonum cognatum Meissn. Highlighting current studies in plant science and ecology, this section emphasizes significant contributions to understanding the biological diversity and ecosystem health roles of Polygonum cognatum Meissn.

The third section deals with the effects of Amaranthus retroflexus L. on seed germination and seedling growth. This research, conducted to understand the basic principles of plant physiology, sheds light on practical applications in the field of agriculture and plant breeding.

The fourth section explores microbial enzymes in the field of microbiology. Evaluating the potential of microbial enzymes in biotechnology, industry, and medical applications, this section emphasizes the crucial role of microorganisms in this field.

The fifth section introduces a revolutionary approach to sustainability. It addresses the economic aspects of ecologically friendly biodiesel production from waste vegetable oil and examines its positive contributions to the global economy.

The final section focuses on a study investigating the length-weight relationship and condition factor of Chub Squalius sp. (Teleostei: Leuciscidae) in the Kızılırmak Basin, Turkey. This research provides valuable insights into understanding biological diversity in aquatic ecosystems.

Editor

Assoc. Prof. Dr. Dilek YALÇIN

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CHAPTER I

Synthesis And Biomedical Applications of Silver Nanoparticles

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Introduction

Nanotechnological developments are a rapidly growing new field of science because they are beneficial to humans, animals and the environment. Therefore, nanotechnology is of great importance as one of the fundamental areas in the application of nanoparticles in industry and biomedicine innovations (Malik & et al., 2023). Nanotechnology is a promising field of study that has a wide range of uses, from fuel catalysts to cancer treatment to tissue engineering

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to cosmetics, food, clothes, and home appliancesAs research advances, nanotechnology will play an increasingly important role in shaping our future (Ahmed & et al., 2022).

Nanotechnology has revolutionized medicine, energy, and environmental sustainability. It has been used to create innovative treatments for diseases, green technology to improve environmental sustainability, and renewable energy to fabricate carbon nanotubes for energy storage, automobile parts, thin-film electronics, coatings, and other applications (Thapa & et al., 2017). Nanomedicines can also be developed to release drugs in a regulated manner, resulting in constant therapeutic effects and reducing the requirement for repeated dosing. Nanotechnology has the potential to enhance traditional drug delivery by improving solubility and stability, thereby increasing bioavailability. This offers great potential for the development of targeted medicines and the treatment of chronic diseases (Afzal & et al., 2022).

Nanoparticles (NPs) have a diameter of smaller than 100 nm and are categorised into various classes, mainly based on their biochemical properties, size and morphology (Altammar & et al., 2023). The fundamental pathways of individual atoms and molecules alter in this size range, changing all attributes (chemical, physical, and biological). Chemical reduction, electrochemical, photochemical and physical vapor condensation (Khan & et al., 2020) are some of the techniques used to create NPs. Nanoparticles are shaped by the chemicals used to reduce and stabilize them (Mukherji & et al., 2019). Researchers have recently regarded the production of nanoparticles by plants, fungi, and bacteria as a biocompatible and environmentally friendly process (Ashokkumar & et al., 2014). Plant leaf and fruit extracts may be a better option than prior nanoparticle production methods due to their low cost, safety, non-toxicity, and environmental friendliness. Nanoparticles (NP) or ultrafine particles are used in medicine, computing, textiles, energy, and defense (Vijayaram & et al., 2023). Physicochemical techniques have been used to create AgNPs, but these have drawbacks such as high energy consumption, hazardous waste, and

environmental health risks (Sriramulu & et al., 2017). The green synthesis (GS) technique utilizes the synthesis of microbes and plants, which is environmentally friendly and does not contaminate the environment (Akintelu & et al., 2021). The suitability of silver nanoparticles (AgNPs) for biomedical applications was confirmed through examination of their physical and chemical characteristics using various techniques such as AFM, SEM, TEM, XRD, DLS, FTIR, and UV-Vis spectroscopy (Borase & et al., 2014). New studies and technologies to understand disease mechanisms for the design of new drugs and silver nanoparticles (AgNPs) are used for the prevention and control of different infections with their antibacterial effects (Franci & et al., 2015). Silver nanoparticles (AgNPs) are used as bactericidal agents in wound dressings, optical sensors, medical device coatings, pharmaceutical and food industries, diagnostics, orthopedics, drug delivery, and surgical instruments. They are also used as disinfectants in biomedical applications, cosmetics, electronics, and textile engineering (Ferdous & Nemmar, 2020). Silver nanoparticles (AgNPs) are used in many industries, such as wound healing, catalysis, and sensing.

In this review, AgNPs have been studied for their potential applications in various fields. They have antibacterial, antifungal, antiviral, anti-inflammatory and anti-cancer, properties, making them a promising candidate for biomedical applications. This chapter discusses the synthesis method and their applications in antimicrobial, antimalarial, antibiofilm, antioxidant, and anticancer activities.

Silver Nanoparticle Characteristics

Scanning electron microscopy (SEM), transmission electron microscopy (TEM), atomic force microscopy (AFM), Fourier transform infrared (FTIR), dynamic light scattering (DLS), X-ray photoelectron spectroscopy (XPS), and X-ray diffraction (XRD) are powerful characterization techniques used to study nanomaterials like silver nanoparticles (AgNPs). These techniques provide insights into the morphology, size, composition, and structure of AgNPs,

aiding in the development of new materials and devices for applications in catalysis, sensing, imaging, and biomedicine. (Figure 1). The appearance of brown color in the prepared AgNPs was initially confirmed using the naked eye, but their preparation was further confirmed using several sophisticated studies (Sathishkumar & et al., 2012). AgNP SPR is measured by UV-vis spectroscopy, which shows an absorption peak at 420–450 nm. While FTIR analysis reveals compounds influencing AgNP formation and validates enzyme-substrate interactions and functional molecules covalently binding to AgNP surfaces, DLS analysis uses light interactions to assess the nanoparticle size distribution (Mourdikoudis & et al., 2018). The disadvantage of SEM inspection is that while it can reveal information about particle purity and degree of aggregation, it cannot view the internal structure of the particles (Ling & et al., 2018). AgNPs can be examined using TEM and high-resolution TEM (HR-TEM) to gain a better understanding of their morphology, lattice fringes, and material size (Wulandari & et al., 2022). The preparation of the materials on the grid determines the quality of the images, but the SEM cannot produce images with high spatial resolution or extra analytical data (Haase & et al., 2011). Three main modes of AFM are commonly employed to examine the size, shape, dispersion, and aggregation of nanomaterials (Naghdi & et al. 2017). Still, there are certain drawbacks, such as overestimating dimension and choosing the operating mode as a crucial part of sample analysis (Ruggeri & et al., 2019).

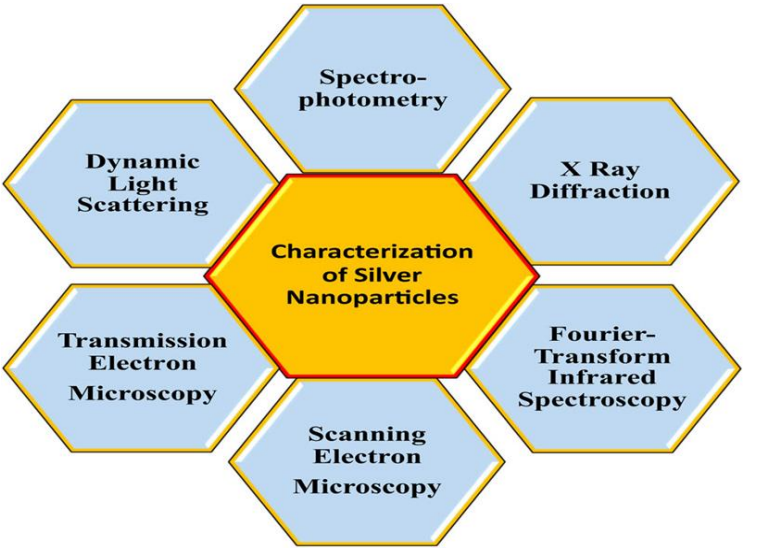

Figure 1. Numerous approaches for characterizing silver nanoparticles (Almatroudi, 2020).

Synthesis of Silver Nanoparticles

The manufacture of silver nanoparticles (AgNPs) is a significant topic in nanotechnology. The manufacturing process determines nanoparticles' size, shape, structure, physical, chemical, and biological properties. Several synthetic techniques were documented by the researchers. Three of the most important techniques have mainly been covered.

1. Chemical techniques

a. Chemical reduction

b. Electrochemical reduction

c. Pyrolysis

d. Irradiation-assisted chemical reduction

2. Physical techniques

3. Biological techniques

Chemical reduction is the most widely used technique for synthesizing AgNPs, reducing Ag+ species to Ag° using reducing agents like $NaBH_4$, $LiAlH_4$, etc. Physical methods require energy, while biological approaches use fungi, plants, and bacteria without harmful reducing agents (Arif & Uddin, 2021) (Figure 2). However, harmful chemicals are more common than physical methods (Roy & et al., 2019). Living organisms produce proteins, carbohydrates, and antioxidants to replace toxic agents in biological synthesis of AgNPs (Siddiqi, Husen & Rao, 2018).

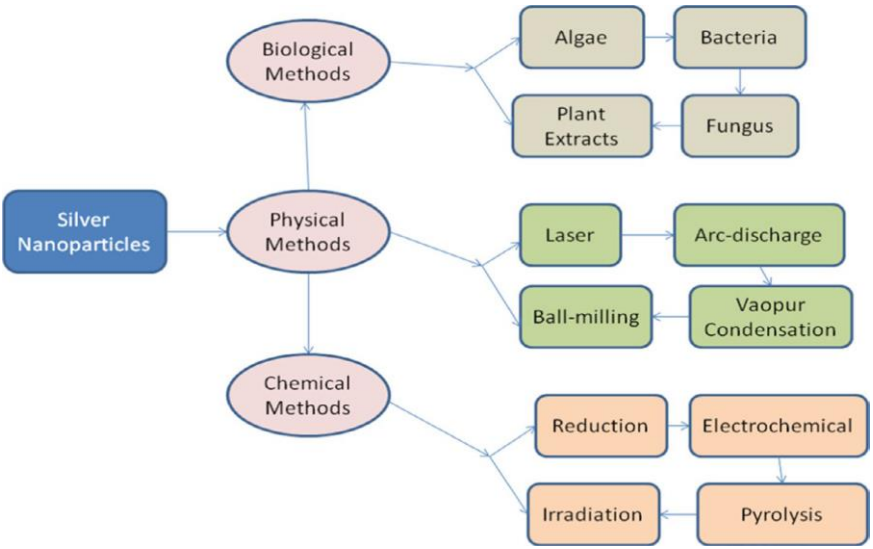

Figure 2. The silver nanoparticle synthesis schematic diagram (Arif & Uddin, 2021)

Producing Silver Nanoparticles

A multitude of methods, broadly categorized as physical (1), chemical (2), and biological (3) procedures, can be employed to create AgNPs. AgNPs are produced by physical processes such as arc discharge, energy ball milling, vapor condensation, and direct current (Zhang & et al., 2017). Silver nanoparticles contribute to various industries, but high energy consumption is a disadvantage.

Magnetic sputtering approaches are also used for 360 nanoparticle therapies (Asanithi, Chaiyakun & Limsuwan, 2012). Despite the narrow size and shape distribution benefit of physical-mediated synthesis, the high energy consumption is a significant disadvantage (Zhang & et al., 2018).

Bioapplications of Silver Nanoparticles

Nanotechnology is a field that focuses on the preparation, scheme, and modification of nanoparticles with various properties. Nanotechnology has gained attention in various fields, including physics, chemistry, biology, and medicine (Rahman & et al., 2020). These nanoparticles have various applications in medicine, biological sciences, and various industries. Scientists have concentrated on using metals such as the metal copper, zinc, iron, titanium, palladium, gold, and silver to manufacture nanoscale particles. AgNPs, or silver nanoparticles, are special because of their optical, catalytic, and electromagnetic characteristics. Because they produce reactive oxygen species and control signaling pathways, they have applications in biomedicine. Because of their special chemical and physical characteristics, silver nanoparticles (AgNPs) have several promising biomedical uses. Nanotechnology's expanding credo emphasizes AgNP preparation and Ag-tethered nanocomposite material development for improved biomedical treatment approaches (Almatroudi, 2020) (Figure 3).

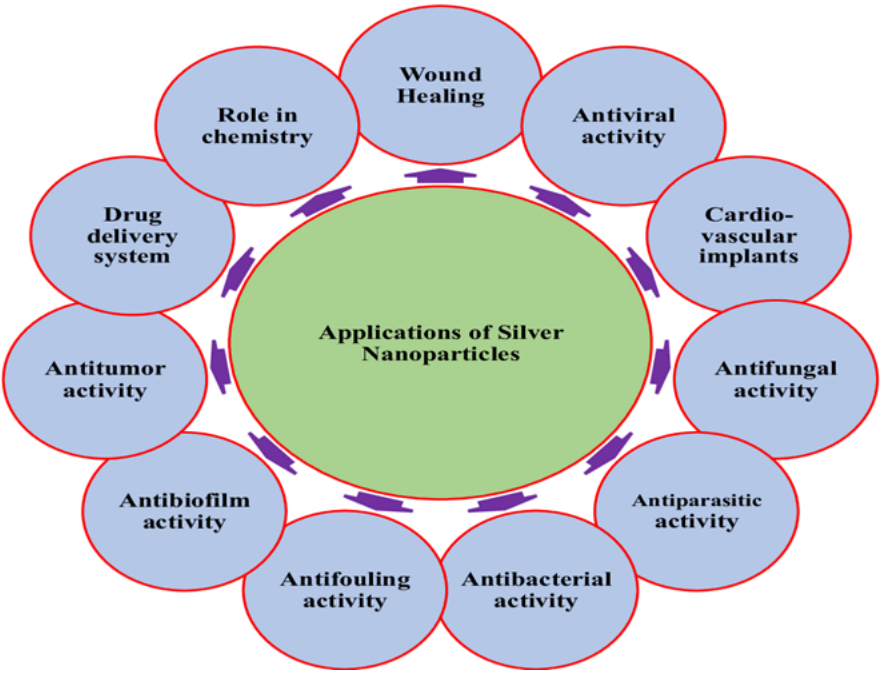

Figure 3. Biomedical applications of silver nanoparticles (Almatroudi, 2020).

Silver Nanoparticle Applications in Medicine

Silver nanoparticles have numerous biological applications, including bioimaging, biosensing, anticancer, antibacterial, and anti-diabetic drugs. Biosynthesis has led to the development of additional therapeutic silver nanoparticles due to their therapeutic potential. Environmentally friendly synthesis methods are becoming more and more important due to the affordability, bioavailability, and environmental friendliness of nanoparticles. According to new studies, synthesized nanoparticles of silver exhibit biological sensing, antibacterial, antiangiogenesis, healing of wounds, neuroregenerative, biological imaging, anticoagulant, cancer prevention, and biological sensing qualities (Kotcherlakota, Das & Patra, 2019) (Figure 4).

Figue 4. Biosynthesized silver nanoparticles for disease treatment and diagnostics (Kotcherlakota, Das & Patra, 2019)

Antimicrobial Properties of Silver Nanoparticles

Because of its appealing and special nano-properties, such as its non-toxic nature and antibacterial capabilities, nanoparticles of silver have garnered a lot of interest in the biomedical area. AgNPs have shown promise in recent times for use in tissue scaffolding, wound dressing, and protective wear (Gudikandula & et al., 20017). The AgNPs have a wide range of bactericidal activity and may affect all Gram-positive and Gram-negative bacteria's physical characteristics, which renders them one of the nanoparticles of metal with the greatest utility in modern antimicrobial activities. Multiple investigations have shown that AgNPs bind to the bacteria surface and enter inside of cells, causing both death of cells and functionality (Yan & et al., 2018).

Commercial antibiotics were once effective in preventing infectious diseases (Aminov, 2010), but the rise of multidrug-resistant (MDR) infections has raised questions about their future use (Singh & et. Al., 2017). AgNPs are a well-known antimicrobial nanomaterial used in the biomedical industry for antimicrobial purposes (Azharuddin & et al., 2019).

Antibacterial Activity

According to Singh & et al. (2017), the antimicrobial characteristics of nanoparticles of silver have led to notable progress in nanotechnology. In order to avoid diseases and encourage rehabilitation, they have been included in a number of medical equipment and products, which have enhanced the health of patients and decreased healthcare expenditures (Tang & et al., 2018).

Silver nanoparticles have also been explored for their potential environmental applications, such as disinfecting water and wastewater to address waterborne diseases and pollution. However, their widespread use has raised concerns about the development of antibiotic-resistant bacteria and the accumulation of silver in ecosystems (Palani & et al., 2023). As the field of nanotechnology continues to advance, there is potential for further developments related to silver nanoparticles' antibacterial activity. Research is focused on improving the efficacy and safety of silver nanoparticles, developing new delivery systems, and exploring their potential applications in food packaging and agricultural practices (Saha & et al., 2022). Silver nanoparticles have the potential to revolutionize the treatment and prevention of bacterial infections in healthcare. However, they also present risks such as resistance and environmental impacts (Xu & et al., 2020).

Antiviral Activity

The scientific community is working hard to develop new antiviral medications and prevention techniques. Because of their unique features, nanomaterials are vital in this sector, and silver

nanoparticles in particular have demonstrated efficacy against a variety of viruses, as well as a potent antibacterial effect. Silver nanoparticles have demonstrated the ability to limit viral reproduction, including that of influenza, HIV, and herpes simplex virus. AgNPs have broad-spectrum antiviral activity, according to our computerized search. AgNPs have been shown to lower the infectivity of 17 different families of viruses, containing 31 individual viruses. According to recent study, AgNPs have antiviral activities against SARS-CoV-2 (Jeremiah & et al., 2020), highlighting their utility as a supplemental method in resolving the present pandemic. A number of studies have shown that AgNPs can be effective against a wide range of viruses, including respiratory viruses, such as influenza and SARS-CoV-2, and blood and sexually transmitted viruses, such as HIV and hepatitis B and C. AgNPs have been shown to inhibit viral replication, prevent viral entry into cells, and induce apoptosis of infected cells. A number of individuals have made significant contributions to the field of AgNPs as antiviral agents. The use of AgNPs as antiviral agents has a number of potential advantages over traditional antiviral agents, including their broad spectrum of activity, their low toxicity, and their ease of production. However, there are also a number of challenges associated with the use of AgNPs, including their potential to cause cytotoxicity and their environmental impact. The use of AgNPs as antiviral agents is a promising area of research. However, further research is needed to address the challenges associated with the use of AgNPs, such as their potential to cause cytotoxicity and their environmental impact (Balagna & et al., 2021).

Antifungal Activity

Silver nanoparticles (AgNPs) have gained significant attention as antifungal agents against various fungi-related diseases. This essay explores the historical context, key figures, impact, and influential individuals in the field of AgNPs as antifungal agents. Fungi, responsible for skin, respiratory, and systemic infections, have historically posed a significant threat to human health

(Gudikandula & et al., 2017). Conventional antifungal drugs have been the primary treatment, but drug resistance in fungi has led to the need for alternative treatment options. The impact of AgNP's as antifungal agents has been significant in the field of medicine. Investigations indicate that AgNPs work to stop the development of a variety of harmful fungus, such as *A. fumigatus*, *C. albicans*, and *C. neoformans* (Mansoor & et al., 2021).

Antiparasite Activity

The putative anti-parasitic effects of silver nanoparticles (AgNPs) have drawn more interest to their application in the past few years. AgNPs have been utilized as a promising alternative approach for controlling parasites, particularly in the agricultural and healthcare sectors. Researchers are exploring alternative strategies to combat parasitic infections, such as AgNPs, due to concerns over resistance development and environmental safety. Traditional treatments, like anthelmintics and pesticides, have limitations, leading to a growing interest in AgNPs as a potential solution (Zhang & et al., 2023). AgNPs have shown promise in treating parasitic infections, particularly those caused by protozoa and helminths, in healthcare and agriculture. They have the potential to address the limitations of traditional anti-parasitic drugs, such as resistance development and adverse effects. AgNPs can also be used as an environmentally friendly alternative for controlling parasitic infections in livestock and crops, reducing reliance on chemical pesticides, and promoting sustainable practices (AlGabbani & et al., 2023).

Activity Against Biofilm

Silver nanoparticles (AgNPs) have gained significant attention in the fields of microbiology, nanotechnology, and medicine for their potential to inhibit biofilm formation and reduce harmful bacteria pathogenicity (Vaikundamoorthy & et al., 2018). The impact of AgNPs on healthcare is significant, as biofilm-associated infections are a major concern. Biofilms are highly resistant to traditional

antibiotic treatment, making it challenging to eradicate. The use of AgNPs as tools to inhibit biofilm formation has the potential to address this challenge by providing a novel approach to combating biofilm-associated infections (Dang & Lovell, 2015).

Impact of AgNPs as Antioxidants

Ag-based nanoparticles that or AgNPs, possess great promise in both the avoidance and cure of illnesses such as diabetes, cancer, and neurological diseases because of their ability to counteract oxidative damage and shield cells from harm (Bhakya & et al., 2016).The study of AgNPs as antioxidants is complex and multifaceted, with far-reaching implications for human health and environmental sustainability. While the potential benefits of AgNPs in combating oxidative stress are significant, it is essential to consider the associated risks and limitations. By advancing our understanding of the mechanisms underlying AgNPs' antioxidant activity and addressing potential risks, we can harness the full potential of these nanomaterials for the betterment of society and the environment (Kim & et al., 2009).

Activity Against Cancer

The past few years have seen a considerable surge in interest in silver nanoparticles (AgNPs) because of their special characteristics, which include enhanced surface area as well as distinct optically and electrically qualities. These properties make them an attractive candidate for use in cancer therapy, as traditional treatments often have side effects and are not always effective in treating aggressive forms of cancer (Keat & et al., 2015). AgNPs offer the potential for targeted cancer treatment, minimizing harm to healthy cells and reducing side effects associated with traditional treatments. Proponents of AgNPs argue that their ability to target cancer cells while sparing healthy cells is a key advantage over traditional treatments (Dreaden & et al., 2012). This includes developing novel synthesis methods for AgNPs, refining targeted delivery systems, and exploring combination therapies that

incorporate AgNPs with other anticancer agents (Shi & et al., 2017). As the field of nanomedicine continues to advance, there is a growing interest in the potential of AgNPs in cancer therapy (Mishra & et al., 2021).

Silver Nanoparticles for Drug Delivery Systems

Silver nanoparticles have emerged as a promising tool for drug delivery systems, with the potential to revolutionize the medical field (Cho & et al., 2008). Silver nanoparticles have strong antimicrobial and anti-inflammatory properties, making them promising candidates for treating infectious diseases and inflammatory conditions (David & et al., 2014). In wound dressings and medical implants, silver nanoparticles can prevent infections and promote tissue regeneration, improving patient outcomes and reducing healthcare costs. Recent research studies have demonstrated the potential of silver nanoparticles for targeted drug delivery in various disease models, including cancer, bacterial infections, and inflammatory disorders. Researchers have developed silver nanoparticle-based contrast agents for biomedical pictures that can enhance the detail and sensitivity of imaging modalities such as CT (computed tomography) and magnetic resonance imaging (MRI). Over the upcoming years, researchers are likely to focus on developing novel strategies to address safety and toxicity concerns, as well as optimizing their therapeutic potential (Adeyemi & et al., 2015).

Silver Nanoparticles for Catheter Modification

Catheters are essential medical devices for various procedures, but they are also prone to complications like infections and blockages. Silver nanoparticles have emerged as a promising solution to these issues, creating catheters more resistant to bacterial colonization and biofilm formation. This has led to a reduction in catheter-associated infections, a major cause of morbidity and mortality in healthcare settings (Roe & et al., 2008).

This field will likely involve the integration of silver nanoparticles into a broader range of medical devices beyond catheters, such as urinary catheters, central venous catheters, and implantable devices (Percival, Bowler & Russell, 2005). The potential for silver nanoparticles to contribute to the prevention of healthcare-associated infections and improve the performance of medical devices holds great promise for advancing patient care and public health (Randolph, Brun-Buisson & Goldmann, 2005).

Silver Nanoparticles for Dental Applications

AgNPs, or silver nanoparticles, have attracted a lot of interest in the the field of dentistry arena because of their special qualities and their uses in a range of dental treatments (Espinosa-Cristóbal & et al., 2018). Research has shown that AgNPs have antimicrobial, anti-inflammatory, and regenerative properties, making them a promising candidate for various dental applications. AgNPs may successfully disinfect root canals and encourage tissue regeneration in damaged teeth, which has the potential to completely transform the field of endodontics (Thangavelu & et al., 2021). Future developments in the field of silver nanoparticles for dental applications hold great promise for further developments that can revolutionize dental treatments and oral care. (Yin & et al., 2020).

Silver Nanoparticles for Wound Healing

Silver has been used for medicinal purposes for centuries, with antimicrobial properties known for treating wounds and infections. Silver nanoparticle-based dressings have shown promising results in treating chronic wounds, such as diabetic ulcers, pressure sores, and surgical wounds. The development of cost-effective and scalable production methods for silver nanoparticles remains a challenge, hindering their accessibility in under-resourced healthcare settings.

Multidisciplinary collaborations between scientists, clinicians, and industry stakeholders will drive the translation of research

findings into clinical applications, ensuring the safe and effective use of silver nanoparticles for wound healing (Paladini & et al., 2019).

Silver Nanoparticles for Bone Healing

Grafts of bones are frequently transplanted to replace or treat severe conditions, such as genetic abnormalities, cancers, or traumas, that irreversibly damage bone tissue. High levels of inflammation and the ensuing phenomena of implant loss and bone resorption are frequently linked to infections connected to orthopedics and bone implants. Bone may not be able to mend itself fully when bacterial activity is present in flaws in the bone. AgNPs have a more comprehensive range of antibacterial action in contrast to traditional antibiotics. In the case of antibiotic-resistant bacteria, such as methicillin-resistant *Staphylococcus aureus*, AgNPs can impede or disturb the development of mature biofilm, respectively, due to this feature (Zhang & et al., 2017).

Antiangiogenic Therapy

Antiangiogenic therapy of AgNPs is a treatment method that targets the process of angiogenesis to prevent the growth of new blood vessels that feed tumors. The field of antiangiogenic therapy of AgNPs is rooted in the history of antiangiogenic therapy and the study of silver nanoparticles for medical applications **(**Gurunathan *&* et al., 2009). The impact of antiangiogenic therapy of AgNPs has been significant, particularly in the field of cancer treatment. By targeting the process of angiogenesis, AgNPs can inhibit the growth of new blood vessels that feed tumors, limiting their ability to grow and spread. This approach has the potential to complement existing cancer treatments, such as chemotherapy and radiation therapy, and improve patient outcomes (Ghadjar *&* et al., 2008). The ability of AgNPs to selectively inhibit angiogenesis while minimizing damage to normal tissues holds great potential for the development of new therapeutic interventions (Carmeliet *&* et al., 2011).

Neuroregenerative Therapy

Neuroregenerative Therapy of AgNPs is a promising approach in the field of neuroscience, with silver nanoparticles (AgNPs) being a key player in the development of targeted therapies for neurodegenerative diseases and injuries. Their unique properties, including their ability to cross the blood-brain barrier and their potent neuroprotective and anti-inflammatory effects, make them attractive candidates for the development of targeted neuroregenerative. Besides neurodegenerative illnesses, neuroregenerative therapy of AgNPs may have effects on acute nervous system traumas including traumatic brain injury and spinal cord damage (Niu, Chen & Gao, 2019). The successful development of neuroregenerative therapies using AgNPs requires rigorous clinical evaluation to demonstrate their safety and efficacy in human subjects (Saeedi & et al., 2019). The continued advancement of Neuroregenerative Therapy of AgNPs holds great promise for addressing the unmet medical needs associated with neurodegenerative diseases and injuries.

Antidiabetic Activity

The medical field has made significant advancements in managing and treating chronic diseases like diabetes, with approximately 463 million adults living with the disease and an expected rise to 700 million by 2045. This has led to a surge of interest in silver nanoparticles (AgNPs) for their antidiabetic activity (Balan & et al., 2016). AgNPs have shown promising results in improving insulin sensitivity, enhancing glucose uptake, and reducing inflammation in preclinical studies. This field include targeted delivery systems for AgNPs, localized treatment of diabetic complications like foot ulcers and neuropathy, and the use of AgNPs in combination with other antidiabetic agents like insulin or oral hypoglycemic drugs.

The antidiabetic activity of AgNPs presents a promising avenue for diabetes management. The historical context of silver as

a medicinal agent, the impact of AgNPs on diabetes management, influential figures in the field, and various perspectives have shed light on the potential of AgNPs in treating diabetes.

Antiinflammatory Activity

In the fields of biotechnology and medicine, silver nanoparticles, or AgNPs, have drawn a lot of attention for their possible anti-inflammatory properties. These nanoparticles have the potential to significantly reduce inflammation, which is a key component of many chronic diseases such as arthritis, asthma, and inflammatory bowel diseases. Current treatments for these conditions often have limited efficacy and can cause significant side effects, making the development of new anti-inflammatory agents a top priority in medical research (Kehili & et al., 2016).

According to preclinical research, AgNPs have strong anti-inflammatory qualities that prevent the synthesis of cytokines that contribute to inflammation and lessen the amount of immune cells that infiltrate inflammatory areas. This leads to a significant reduction in inflammation severity. The field of AgNPs and their anti-inflammatory properties has been shaped by influential individuals who have made significant contributions to our understanding of this groundbreaking research (Gherasim & et al.,2020).

Anticoagulating Activity

Silver nanoparticles (AgNPs) have been a subject of significant interest and research due to their ability to prevent blood coagulation and inhibit blood clot formation. This has significant implications for various medical applications, including the development of new anticoagulant therapies and the design of advanced medical devices. The anticoagulating activity of AgNPs has the potential to revolutionize the field of hemostasis and anticoagulation, as the prevalence of cardiovascular diseases and the growing demand for safer and more effective anticoagulant therapies

are increasing. Asghar et al. (2020) suggest that the incorporation of AgNPs into medical equipment, such stents and a catheter, and implants used for surgery, may lower the incidence of thrombus and other blood coagulation-related problems (Asghar & et al., 2020).

The future of AgNPs' anticoagulating activity holds great potential for advancements in medical research and patient care. The design of AgNPs with enhanced anticoagulant properties and improved biocompatibility holds great promise for the future of hemostasis and anticoagulation.

Toxicity of Silver Nanoparticles

Many sectors have been transformed by nanotechnology, particularly consumer goods, medical care, and electricity. Due to their special qualities and possible uses, nanoparticles of silver have attracted a lot of attention. Given the ability to have harmful impacts on both individuals and the surroundings, silver nanoparticle poison has given rise to major worries. The field of toxicity of silver nanoparticles is poised for further developments that will shape the future of nanotechnology and its applications. Research efforts aim to elucidate the mechanisms of nanoparticle toxicity, identify safer alternatives, and develop methods for mitigating potential risks. The integration of nanotechnology into various industries will drive the need for robust regulatory frameworks and safety standards to ensure the responsible use of silver nanoparticles and other nanomaterials (Tortella & et al., 2020).

Conclusion

The nanoparticles of silver (AgNPs) are a viable option for enhanced biomedical uses because to their size-dependent physicochemical features and biological effects, including excellent antibacterial efficiency and non-toxic nature. AgNPs have showed potential benefits in innovative biodegradable and nanostructured substances materials and technologies for current treatment techniques. Because of their mechanical, optical, chemical, and

biological qualities, they are perfect for creating, assessing, and clinical assessing performance-enhanced biological materials and medical devices. However, extensive research is needed on their short-term and long-term toxicities and responsible toxic-related mechanisms. AgNPs also exhibit good antimalarial activity, but further research is needed to confirm their molecular and biochemical characteristics. Future research should investigate the synergistic and antagonistic effects of AgNPs on antioxidant activity and their impact on cellular and molecular processes in in vivo systems.

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CHAPTER II

Research on Importance of *Polygonum cognatum* Meissn.

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1.Introduction

Over the years, people have used various parts of the plants as natural resources for different purposes over the years. Several plants have been used as the main source of human nutrition (Pekdemir, Çiftçi & Karatepe 2020), for medicinal purposes in health care (Sofowora, Ogunbodede & Onayade, 2013), as a source of fiber in textiles (Ramawat & Ahuja, 2016), and additionally in cosmetics

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(Aburjai & Natsheh, 2003). In reports, wild edible plants can be defined as native species that grow and reproduce naturally in their natural habitat without being cultivated (Bhatia et al., 2018; Motti, 2022). The different parts of wild edible plants, such as leaves, bulbs, and roots are consumed either raw or after undergoing various cooking processes by local population in the regions where they grow. Wild edible plants are an important source of minerals, vitamins, antioxidants, carbohydrates, dietary fibers, proteins, and phenolic compounds (Khan et al., 2016). Hence, there has been an increasing attention to wild plants all over the world (Aberoumand & Deokule, 2009). Due to its climatic conditions and geographical composition, Türkiye is one of the richest countries in terms of plant diversity. It has been reported that the Black Sea region, located in the north of Türkiye, includes a great diversity of wild edible plants, which was explained by the location of the region, which is situated at the junction of the Irano-Turanian and Euro-Siberian phytogeographic regions. In this region of Türkiye, a wide variety of edible plants, such as, *Malva neglecta, Polygonum cognatum* and *Trachystemon orientalis*, are consumed. The analysis and determination of the nutritional properties wild edible plants is very important to evaluate their nutritional significance (Kibar & Kibar, 2017). Several studies reported that the important level of minerals and high nutritional potential of wild edible plants (Yıldırım et al., 2001; Kibar & Temel, 2016; Güzelsoy et al.2017; Yimer et al., 2023) could be comparable to or even sometimes higher than some of the commonly used vegetables (Turan et al., 2003; Şekeroğlu et al., 2006). Wild edible plants will become much more important as a source of human nutrition and pharmacy as sources in the future, especially with the increasing human population. Therefore, the analysis of wild edible plants has gained increasing attention in recent years.

Polygonum cognatum is one of best known and least studied edible plant. The limited studies conducted on *P. cognatum* have shown that it possesses significant herbal properties and has the potential to be used as an alternative food and pharmacy source. The

aim of this study is to provide a summary of the *P. cognatum* importance, composition and potential use in various fields.

Polygonum cognatum

Brief Information about *Polygonum*

The Polygonaceae family consists of 56 genera with 1266 species in the worldwide (Doğru 2021). There are seven genera (*Polygonum*, *Antenoron*, *Reynoutria*, *Koenigia*, *Fagopyrum*, *Pteroxygonum* and *Fallopia*) in Polygoneae (Hao, Gu & Xiao,2015). There have been reported 74 species in Türkiye (Geven et al., 2008; Doğru, 2021). *P. cognatum* is a member of the genus *Polygonum* (Doğru, 2021) which has been reported as the richest genus in the family Polygonaceae (Hao et al., 2015). This genus includes approximately more than 200 species that are distributed worldwide, mostly in temperate climate regions (Rodrigues et al., 2017) and 27 species known to exist in Türkiye (Gümüşçü et al., 2022). Many species of Polygoneae have known as popular and traditional medicinal herbs. Alkaloids, tannins, flavonoids, volatile oils, lignans and coumarins have been isolated from Polygoneae species and additionally two of them which are stilbenes and anthraquinones have been reported as characteristic medicinal compounds (Hao, Gu & Xiao,2015). It has been reported that interspecific hybridization and polyploidization are the key factors in Polygoneae diversification (Hao, Gu & Xiao,2015). In addition, phylogenetic relationship were analysed in different *Polygonum* species (Galasso et al., 2009; Guo et al., 2022;) to understand the evolutionary relationship of *Polygonum* and to develop available genetic resources (Guo et al., 2022). In phylogenetic analysis based on chloroplast genome of four *Polygonum* species suggested that the chloroplast genome of *Polygonum* could be an important marker to identify of the species. (Guo et al., 2022)

Some of the members of the *Polygonum* genus are known as important source of secondary metabolites, such as, phenolic compounds (El-Hacı et al., 2013; Gümüşçü et al., 2022), flavonoids

(El-Hacı et al., 2013) and highly volatile oil constituents (Vikram et al., 2015). Several reports have elucidated the pharmacological effects of some of *Polygonum* spp., which include antioxidant-antibacterial activity in *P. maritimum* (El-Hacı et al., 2013), anti-inflammatory compounds in *P. bistorta* (Duwiejua et al., 1999), potential of *P. maritimum* as source of compounds with anti-diabetic, anti-inflammatory and antioxidant activities (Rodrigues et al., 2017), and the good cytotoxic effect of. *P. cognatum* extracts on different types of cancer cell lines (Pekdemir, Çiftçi & Karatepe 2020). Due to the presence of dodecanal, decanal and other aldehydes in *P. minus* which has been reported as a potential application in perfume (Vikram et al., 2015).

Polygonum cognatum

Polygonum cognatum (knotweed) is a perennial herbaceous plant (Figure 1). *P. cognatum* is 15–30 cm long with a slender prostrate stem. Leaves are oblong-elliptic and often slightly mucronate. Flowers are pinkish and occur in bundles in the leaf axils. It grows between 720 and 3000 m above mean sea level (Davis, 1967; Kibar & Kibar, 2017; Gümüşçü et al., 2022). *P. cognatum* is called locally "madımak", which showed resistance to various climate conditions and may grow in different regions of Anatolia, mostly widespread in Tokat, Sivas, and Erzurum provinces in Türkiye (Demirgül et al., 2022). *P. cognatum* is located in non-agricultural regions such as over the roads, industrial areas, and field sides, and agricultural regions as well (Onen et al., 2014). It is found to have $2n = 60$ chromosome number (Sharma et. al., 2003). In madımak genotypes collected from different regions of Central Anatolia based on RAPD technique has been suggested important genetic variation (Özkurt, 2008). Taxonomic information about the species is explained below (TÜBİVES, 2023).

Regnum: *Plantae*

Division: *Magnoliophyta*

Class: *Magnoliopsida*

Familia: *Polygonaceae*

Genus: *Polygonum*

Species*:* *Polygonum cognatum*

Figure 1. *Polygonum cognatum bitkisinin genel görünüşü (Türkiye Bitkileri.com.tr)*

P. cognatum is a wild edible plant traditionally consumed as a food source particularly in rural areas in Anatolia and the Middle Black Sea Region (Onen, 2011; Kibar & Kibar, 2017; Ulusoy et al., 2018; Özbakır Özer & Aksoy, 2019). The plant is harvested from April until June in the spring season and sold in local markets (Demirgül et al., 2022). The young shoots and leaves of *P. cognatum* are commonly consumed as vegetables, particularly in meals and salads as an ingredient (Yücel, Güney & Yücel Şengün, 2010;

Gümüşçü et al., 2022). It has been reported that agricultural activities have been started to meet commercial demand and their own needs (Onen, 2011).

The Nutritional Value of the Plant P. cognatum

Wild edible plants provide protein, carbohydrates and lipids which are essential biochemicals in human diet. On the other hand, wild edible plants are the source of supplementary components such as mineral and vitamin contents which are important for maintaining the proper physiological homoeostasis of the body (Datta et al., 2019) According to the nutritional analysis, *P. cognatum* contains an important levels of nutrients, including phosphorus, protein, magnesium, potassium, and calcium (Kibar & Kibar, 2017). Knotweed (madimak) may grow at different altitudes ranging from 720 to 3000m. Interestingly, samples that were collected from two different altitiudes (1656 and 1170 meters of mountain region in Giresun, Türkiye) showed that the important parameters in the biochemistry of knotweed, such as the level of proline, total flavonoid, chlorophly, and carotenoid, may vary depending on altitude. The levels of proline and total flavonoids are higher in plants grown at higher altitudes, while the amounts of soluble sugars, carotenoids, and total chlorophyll are lower. In addition, it was suggested that the flavonoid-rich content of leaves may make *P. cognatum* potential source of natural antioxidant compounds (Macar & Kalefetoğlu Macar, 2018). *P. cognatum* includes a high level of various components such as fatty acids, α-tocopherol, flavonoids, phenolics, vitamins K and D, and proanthocyanidin, that all of which have very important effect on human health (Pekdemir, Çiftçi & Karatepe 2020). In another report, the phenolic compositions of *P. cognatum* samples collected from the Cumra and Manisa regions of Türkiye were analysed using methanol as a solvent and HPLC analysis, respectively. Both samples analysed show a particularly prominent level of isorhamnetin, rutin, and catechin phenolic components (Gümüşçü et al., 2021). Research on total phenolic compounds (TPC), total flavonoid compounds (TFC), and free

radical scavenging activity (DPPH) of *P. cognatum* extract indicated that *P. cognatum* is an important source of phenolic compounds and antioxidant activity (Bayram and Topuz, 2023). Therefore there is an increased interest to analyse and understand the potential of wild edible plants to be used as a source of human nutrition, as natural oxidant or vitamin. Since *P. cognatum* is rich source of phenolic compounds and antioxidant activity (Bayram & Topuz, 2023) which my suggest *P. cognatum* has potential as a functional dietary food or could be used as a component to enrich functional foods (Gümüşçü et al., 2022).

The Compounds and Biological Activities of *P. cognatum* Extracts

Rutin, chlorogenic acid, fumaric acid, and hyperoside are reported as the most abundant phenolic compounds in the leaf extract of *P. cognatum* (İnal et al., 2022). Chlorogenic acid is a natural phenolic antioxidant and a promising new way to prevent cardiovascular disease, diabetes, and cancer, but the specific mechanism is unclear (Miao & Xiang, 2020). Rutin is bioactive flavonoid component which widely distributed among plants (Atanassova and Bagdassaria, 2009), such as vegetables, fruit, and cereals, and was suggested as a promising candidate for cancer treatment (Satari et. al., 2021). Hyperoside, which is an active compound, may have potential in the treatment of vascular inflammatory diseases and against diabetic complications (Ku et al., 2014). In addition to these compounds, folic acid concentration of *P. cognatum* was reported using HPLC analyses. The folic acid concentration in *P. cognatum* was reported to be significiantly higher than that of other edible vegatables (Ulusoy et al., 2018).

Phenolic compounds are found in a wide variety of plant species and known vital in defense responses, such as antioxidant, anti-inflammatory and anti-aging properties (Lin et al., 2016). There are reports investigating the antiradical, antimicrobial, and *in vitro* anticancer activities of the *P. cognatum* extracts. Pekdemir, Çiftçi & Karatepe (2020) analysed total phenolic components, DDPH (1,1-

diphenyl 2-picrylhydrazyl) and ABTS (2,2'-azino bis(3-ethyl benzothiazoline-6-sulphonic acid) radical scavenging activities, and antimicrobial activities in extracts of *P. cognatum*. The plant extracts showed antimicrobial activity against all the microorganism analysed, including *Escherichia coli, Bacillus subtilis, Klebsiella pneumoniae, Staphylococcus aureus, Candida albicans*, and anti-radical activities as well. Based on the information provided, *P. cognatum* could have the potential to be used for pharmacological purposes. Ozturk et al., (2022) carried out antioxidant enzyme analyses of 12 edible plants, including *P. cognatum*, and reported high levels of catalase (CAT) and peroxidase (POX) activity, glutathione reductase (GR), and glutathione-S-transferase (GST) values. In addition, *P. cognatum* showed the highest levels of the hormones gibberellic acid (GA), indolacetic acid (IAA), jasmonic acid, salicylic acid (SA), cytokinin, and zeatin. Based on the results of the analysis of wild edible plants, which indicating their significant antioxidant properties, it is suggested that these plants could serve as important sources of natural antioxidants, and further investigations should be conducted to elucidate the potential use of these plants as functional foods.

The extracts of different *Polygonum* species, including *P. aviculare, P. cognatum, P. patulum*, and *P. setosum*, showed an inhibitory effect against cosmetics-related tyrosinase (TYR), elastase, and collagenase enzymes. The middle inhibitory effect of TYRa and the strong against to the collegenase enzyme in *P. cognatum* indicated *Polygonum* species appear promising for limiting working enzymes inhibition in cosmetics (Doğru, 2021). According to the biochemical and histopathological findings, *P. cognatum* extracts may reduce the lipid peroxidation, increase antioxidant enzyme levels, and prevent oxidative stress in diabetic rats, which may be beneficial to use in the treatment of diabetes and its complications (Onay 2019). A report verified that *P. cognatum* extract has promising antioxidant, and antiproliferative properties, moderate antimicrobial activity, and cytotoxic activity, but more

detailed studies are needed to identify which specific compound is responsible for these activities (Eruygur et al., 2020).

3.Conclusion

This review summarizes many previous reports to draw attention to the importance of *P. cognatum.* According to the studies conducted so far, *P. cognatum* has shown potential for various beneficial properties, including anticancer, antioxidant, antimicrobial activity, and high nutritional components. However, there are limited comprehensive studies reported, particularly in Türkiye, where *P. cognatum* naturally grows. Therefore, comprehensive, and more extensive studies are needed to elucidate the details of biochemical features and curative features of *P. cognatum* to use them more effectively.

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CHAPTER III

Amaranthus retroflexus L. (Redroot Pigweed): Effects Of Gibberelic Acid Treatments On Seed Germination And Seedling Growth

Betül AKIN[1]

Introduction

Amaranthus retroflexus L. is an annual herbaceous plant species native to North America. It has become a common weed in many countries in both the northern and southern hemispheres **(**Weaver & McWilliams,1980). *A. retroflexus* L. is a common weed species in agricultural areas (Assad & et al., 2017). *A. retroflexus*, also known as the redroot pigweed (Weller & et al., 2021), is a cosmopolitan and invasive plant species belonging to Amaranthus genus. This species has been used as a food source for centuries, especially in developing countries (Assad & et al., 2017). Pigweeds (Amaranthus spp.) are economically important plants worldwide and also an important grain food crop for poor people or lower-income people (Chidozie Ogwu, 2020; Weller & et al., 2021). In several field

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crops and vegetables, it is a very aggressive annual weed (Weller & et al., 2021). Previous studies have shown that C4 pathway weed species such as *A. retroflexus* positively respond to temperature increases (Guo & Al-Khatib, 2003). For this reason, temperature increase due to climate change could increase the growth and seed production of C4 weed species such as *A. retroflexus*, increasing this species' invasion potential (Hyvönen, 2011).

The number of seeds a plant produces can vary according to growth conditions. Studies report that *A. retroflexus* plants produce many long-lived and dormant seeds (10,000 to 1,000,000 seeds per plant). This means seeds can remain in the soil for many years without germination (Satrapova & et al., 2013). Wind, water, animals, and machinery are the main ways in which seeds are spread in the environment. This species can grow up to 1–2 m and compete with crops for agricultural land's water, nutrients, and light, and can reduce crop yields. It has also been the cause of significant losses in terms of product quality and quantity. Seed dormancy is an important factor influencing the emergence and timing of weeds. The newly developing seeds of redroot pigweed have a degree of primary dormancy. In amaranth species, high seed production, long seed life, and seed dormancy lead to high seed accumulation in the soil (Assad & et al., 2017).

This study aimed to investigate the effect of different concentrations of gibberellic acid on *in vitro* germination and seedling growth of red-root pigweeds. Therefore, using the tissue culture technique, we planned to study the effects of different concentrations of GA_3 on the growth of *A. retroflexus*.

Material and Methods

Field Study

Ripened seeds of *A. retroflexus* L. were randomly collected from August to September 2016 from the natural weed populations of Kütahya (Figure 1). *In vitro* studies on seed germination and

seedling growth under laboratory conditions were conducted using seeds from different populations.

Dried seeds are surface sterilized with a bleach solution (30% commercial bleach and a few drops of Tween-20) for 20 minutes. Afterward, the sterilized seeds were washed three times with sterile water. All media (pH 5.7) containing 3.0% sucrose and 0.7% agar were sterilized by autoclaving at 121°C for 20 min. Murashige and Skoog (MS) medium without plant growth regulator was used as a control for germination studies (Table 1).

The germination medium was supplemented with varying concentrations of gibberellic acid (10, 25, 50, 75, 100 mg l^{-}). Following autoclaving, GA_3 was added to the medium through a 0.22 μm Millipore filter. Seed germination was carried out in the plant growth chamber under 16/8 hours (light/dark) photoperiods, 25 °C temperature, and 70% relative humidity. Each trial was repeated at least three times; ten seeds were used in each treatment, and the trials were repeated six times.

Figure 1. General view of A. retroflexus in its habitat and seeds of redroot pigweed.

Germinated seeds were counted after a 20-day days to determine germination percentage, germination rate, seedling

lengths, and number of leaves. The criterion for germination was the emergence of the radicle through the seed coat.

Statistical Analyses

Data were analyzed using JMP 6 SAS statistical analysis software. F-test was used to reveal the differences between treatments at $p<0.05$ level. TUKEY-HSD multiple comparisons were applied to the significant treatments as a result of the F-test (JMP, 2005).

Results and Discussion

Germination is linked to the emergence of a radicle, which is the visible outcome of the germination process. The emergence of the radicle is preceded by several processes, starting with water uptake by the mature dry seed, followed by imbibition, and culminates in the elongation of the embryo axis (Sohindji & et al., 2020). In view of the fact that *A. retroflexus* seeds, which have a low germination rate under normal conditions, may be dormant, dormancy-breaking studies were carried out *in vitro* by applying different concentrations of GA in the range of 10-100 mg l^- to our species. In this investigation, 10, 25, 50, 75, and 100 mg l^- GA concentrations were used to determine the effect of gibberellic acid on the germination percentage and germination rate of *A. retroflexus* seeds. In MS medium with 50 mg l^- GA, *A. retroflexus* seeds had higher germination percent (100.00%), and germination rate (26.67) than seeds germinated in MS medium and 100 mg l^- GA concentrations. Notably, the GA concentrations had different effects on germination percentage and rate. As a matter of indeed, the optimal concentration for producing healthy germinating plants was found to be 50 mg l^- GA. The findings of this research are displayed in Figure 2 and Figure 3.

Table 1. Murashige and Skoog (MS) medium (mg l^-).

Inorganic Components		**mg l^-**
Ammonium nitrate	NH_4NO_3	1650
Calcium chloride	$CaCl_2.2H_2O$	440
Magnesium sulfate	$MgSO_4.7H_2O$	370
Potassium dihydrogen phosphate	KH_2PO_4	170
Potassium nitrate	KNO_3	1900
Boric acid	H_3BO_3	6,2
Cobalt chloride	$CoCl_2.6H_2O$	0,025
Copper sulfate	$CuSO_4.5H_2O$	0,025
Manganese sulfate	$MnSO_4.4H_2O$	22,3
Potassium iodide	KI	0,83
Sodium molybdate	$Na_2MoO_4.2H_2O$	0,25
Zinc sulfate	$ZnSO_4.4H_2O$	8,6
Sodium EDTA	Na_2EDTA	37,3
Ferrous sulfate	$FeSO_4.7H_2O$	27,8
Organic Components		
Glycine		2,0
Myo-inositol		100
Nicotinic acid		0,5
Pyridoxine • HCl		0,5
Thiamine • HCl		0,1
Sucrose		30 g/l

The present study shows that the content of the culture media significantly affects *in vitro* germination of *A. retroflexus* seeds. Our results concluded that the germination percent and germination rate of *A. retroflexus* were highly influenced by the GA concentrations of culture media (Figures 2, 3, 5). Environmental factors significantly influence seed germination stimulation or seed dormancy induction (Güneş, 2000a). Gibberellins stimulate germination in seeds and can also eliminate dormancy induced by various stresses (Gupta & Chakrabarty, 2013; Güneş, 2000b). Gibberellic acid plays a vital role in promoting germination and mobilizing stored nutrients in dormant seeds by counteracting the

effect of ABA. Upon treatment, GA triggers the acceleration and stimulation of seed germination through the hydrolysis of starch and storage proteins (Gupta & Chakrabarty, 2013; Güneş, 2000a). There have also been many reports on the effect of GA in promoting seed germination (Cerabolini & et al., 2004; Sharma & et al., 2004; Erdağ & Emek, 2005).

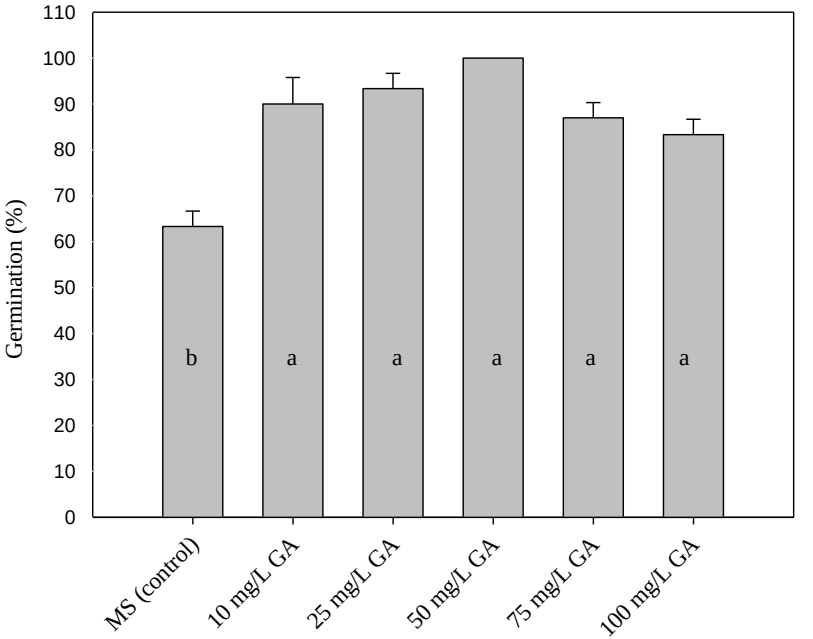

Figure 2. Effects of different concentrations of gibberellic acid on germination percent of A. retroflexus.

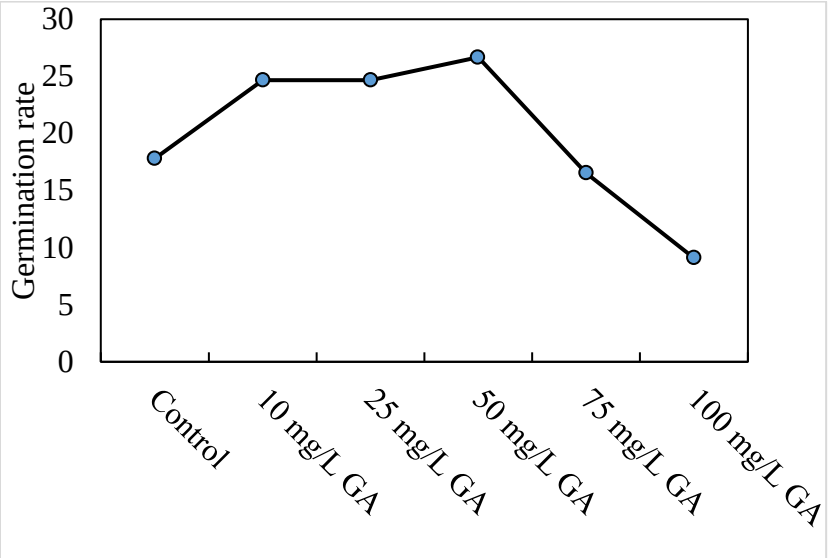

Figure 3. Effects of different concentrations of gibberellic acid on the germination rate of A. retroflexus.

We investigated the effects of different GA concentrations on the growth parameters of the *A. retroflexus* plant (Figure 4). While the most effective treatments on root length, shoot length, and number of leaves were MS and 50 mg l^{-} $GA_{3,}$ respectively, the highest shoot length (5.09 cm) was obtained in 50 mg l^{-} GA_3. Whereas the other gibberellic acid concentrations decreased the growth parameters of the plant, and the least effective concentration was 100 mg l^{-}. In this study, control and five different GA concentrations were used to identify the effect of gibberellic acid on the germination and seedling growth of *A. retroflexus* seeds. When treatments were compared, a healthy and live plant was observed in the MS and 50 mg l^{-} GA medium. 75 and 100 mg l^{-} GA_3 concentrations resulted in weak and yellowish seedlings (Figure 4).

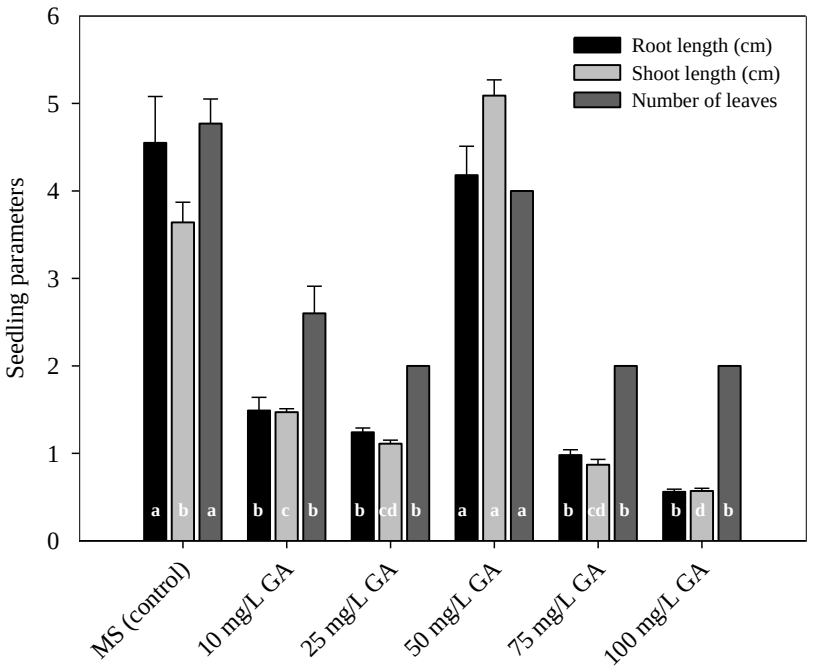

Figure 4. Effects of different concentrations of gibberellic acid on root length (cm), shoot length (cm), and number of leaves of A. retroflexus in tissue culture conditions.

It was concluded that a medium containing MS and 50 GA was more effective for seedling development of *A. retroflexus*. As Tapfumaneyi (2023) stated in their study, Amaranth and *Cleome gynandra* plant shoot length, roots length, dry weight, and vigor index I and II were significantly increased by the increase in GA_3 concentrations. Increased growth parameters of Amaranth and *C. gynandra* could be attributed to GA_3 stimulating plant growth and development. However, Palepad et al. (2017) stated that the endogenous GA_3 synthesized by the seed embryo may not be sufficient, so the external treatment of GA_3 may have improved shoot growth by increasing cell proliferation and elongation,

resulting in rapid plant growth. In this research, GA_3, at the increased concentrations especially at 50 mg l^- GA concentration, increased the percentage of germination and germination rate and, seedling growth in tissue culture conditions.

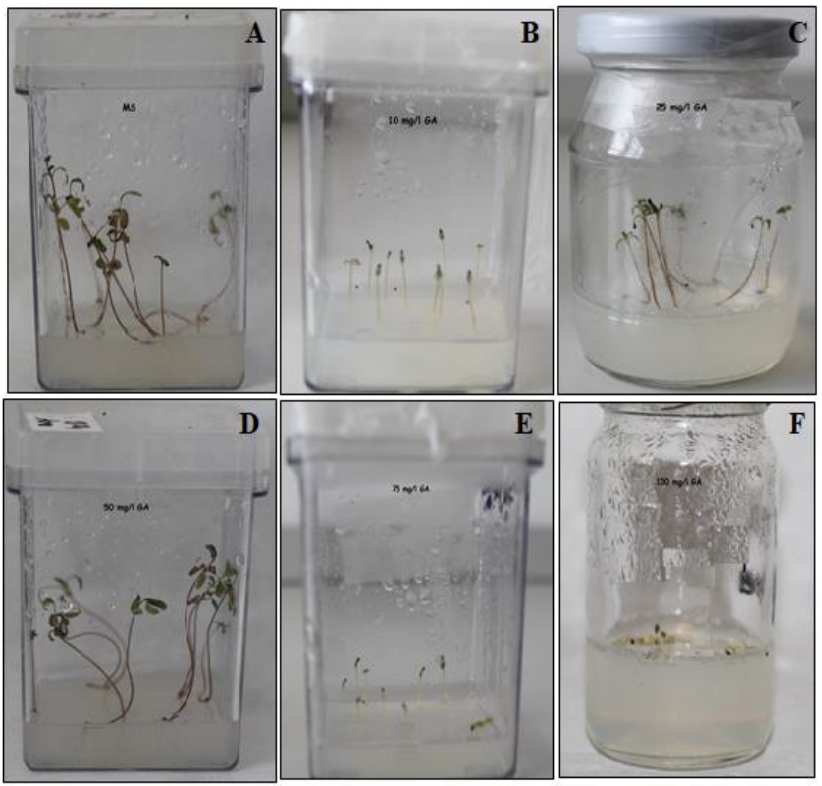

Figure 5. 30 days old seedling of A. retroflexus cultured on a. MS medium b. MS medium with 10 mg l^- GA c. MS medium with 25 mg l^- GA d. MS medium with 50 mg l^- GA e. MS medium with 75 mg l^- GA f. MS medium with 100 mg l^- GA

The study determined the optimal doses of gibberellic acid for the in vitro germination of *A. retroflexus*, a plant of medicinal importance that has problems with germination. Therefore, 50 mg l^-

GA_3 may be used as a component of MS medium in tissue culture to efficiently propagate the plant species studied. This study represents a simple and proper methodological procedure for the tissue culture and germination process of *A. retroflexus.*

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CHAPTER IV

Microbial Enzymes

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Enzymes

Enzymes are ideal metabolic catalysts that allow various biological processes to occur through a well-defined pathway (Singh et al., 2019). Enzymes expedite various biological reactions, that are very important in sustaining human life by reducing the activation energy and increasing the reaction rate without undergoing a permanent change during the reaction.

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Enzyme activities have been known for a very long time. For example one of the oldest examples of biotechnological processes is the fermentation of sugar into alcohol by yeasts (Akan, 2010).

Enzymes were first called ferments but later Berzelius (1838) used the word ‘catalyst’. The first person to use the term enzyme was Kühne (1878) (Akan, 2017).

The majority of enzymes are in protein structure and proteins are spesific and have a three-dimesional appearance, therefore the physical and binding properties of each protein are unique.

Whilen enzymes are used in many areas in industry, many of them are obtained from microorganisms.

Enzymes are found in very small amounts in plants and animals and are not suitable for industrial applications. However, enyzmes produced from microorganisms provide many advantages such as easily use, controlled rapid growth conditions, easy genetic manipulation and high production efficiency.

Microorganisms play an important role in our lives through their role in the production of valuable primary and secondary metabolites and their production of a wide variety of enzymes.

Many microbes including bacteria, fungi, yeast produce a group of versatile and attractive enzymes, it can be intracellularly or extracellularly. Many microbial enzymes including amylases, proteases, pectinases, lipases, xylanases, cellulases, citinases, pullulunases and laccases are extracellularly produced however some enzymes including catalase produced from *Saccharomyces cerevisiae* and *Aspergillus niger* are intracellular. Microbial enzymes are effective and highly spesific. In the industrial enzymes, 50% are produced from fungi and yeast, %35 are from bacteria and 15% are from plants, If we compare microbial, animal and plant enzymes, microbial enzymes have couple advantages:

1. Microbial enzymes are more active and stable

2. Microbail enzymes have high yields and easy for product modification and optimization because of the biochemical diversity and affinity to gene manipulation

Microbes have a rich source to discover to microbial enzymes by modern techniques as metagenome screening, genome mining, and exploring the diversity of extremophiles.

Microbial enzymes are of great improtance in the development of various processes on an industrial scale. Microbial enzymes have applications in various industries such as paper, leather, detergents, textiles, pharmaceuticals, food, beverages, chemicals, animal, feed, biofuels. (Singh et al. 2019).

Nowadays, thereabout 200 types of microbial enzymes from 4000 common enzymes are used commercially. Nevertheless, around 20 enzymes are produced on an industrial scale. Around 12 major producers and 400 minor suppliers are in the worldwide enzyme demand. A residual number of industrial enzymes can be offered help of the improved understanding of microbial recombination, metagenome mining, fermentation processes, and recovery methods. For instance, recombinant DNA technology can be involved to produce enzymes by microorganisms commercially. More or less 90% of industrial enzymes are recombinant version.

During recent years the industrial applications by microbial enzymes have grown intensely. Protease comprise more than 60% of all industrial enzyme sales globally, amylases represent about 30% of world's enzyme production, lipases comprise the other significant product segment. Demand for industrial enzymed in matured economies such as United States, West Europe, Japan was relatively stable however developing countries such as Asia-Pacific, East Europe, Middle East regions shown up fastest growing markets for industrial enzymes.

Depends on the application, commercial applications of enzymes can be divided into nine categories such as food and feed, detergents, etc. Food and feed comprise the largest part for industrial

enzymes, after food and feed industires, detergents followed, it is also another significant part for industrial enzymes (Liu and Kokare, 2023).

Classification

There is a spesific enzyme for each reaction. Enzymes are named according to the type of reaction they catalyze or by adding the suffix to the end of the molecule names they act on.

According to catalyzed reaction, microbial enzymes can be divided into siz types. The basis of this classification is based on the system established by the enzymes commision of the International Biochemical Union (Akan, 2010).

1. Oxidoreductases (EC 1, catalyze oxidation/reduction reactions),
2. Transferases (EC 2, transfer a functional group),
3. Hydrolases (EC 3, catalyze the hydrolysis of various bonds
4. Lyases (EC 4, cleave multiple bonds by means other than hydrolysis and oxidation
5. Isomerases (EC 5, catalyze isomerization changes within a single molecule)
6. Ligases (EC 6, join two molecules with covalent bonds)

Each enzymes has four codes (for class, subclass etc.) The majority of extracellular enzymes obtained from microbial sources are produced from varieties of *Bacillus.* Amylases and proteases from microbial source are the most widely used, and the thermostable enzymes produced by some species of this genus are of great interest. 34% of all commercial enzymes are used in detergents, 14% in dairy products, 12% in starch processing and 11% in textile application. There are 510 commercially valuable microbial enzymes in the metagenomics database (Liu and Kokare, 2023).

Amylases

Amylases are starch degrading enzymes that catalyze the hydrolysis starch into sugars as glucose and maltose. In the starch hydrolyzing enzymes, amylases play a center role in the starch processing industry. Microbial amylases have relayed the chemical hydrolysis of starch in starch processing industries, as they have a number of advantages as higher yield, specificity, greater control over amylolysis, stability of generated products. Amylases are classified into three subclasses (α, β, and γ) depend on the type of bond that they can cleave. α-Amylases (EC 3.2.1.1) catalyze the hydrolysis of internal α-1,4-O-glycosidic bonds in polysaccharides help of the retention of α-anomeric configuration in the products. Most of the α-amylases are metalloenzymes, so it need calcium ion for the activity, structural, integrity and stability (Singh et al. 2019) .They relate to family 13 (GH-13) of the glycoside hydrolase (GH) group of enzymes. β-Amylases (EC 3.2.1.2) are exo-hydrolase enzymes that treat from the nonreducing end of a polysaccharide chain by hydrolyzing α-1,4-glucan linkages to yield successive maltose units. γ-Amylases (EC 3.2.1.3) cleave α(1_6)glycosidic linkages, in addition to cleaving the last α(1_4)glycosidic linkages at the nonreducing end of amylose and amylopectin, it is not the same with the other forms of amylase (Prassana, 2005). Several bacteria, fungi, and genetically modified species of microbes can produce α-Amylase. Among the bacterial species, *Bacillus spp*., *B. amyloliquefaciens* and *B. Licheniformis* are the most widely used source. Among the Fungal sources of α-amylase, *Aspergillus* species and only a few *Penicillium* species, *P. Brunneum* are used source (Saini et al., 2017).

They offered a lot of applications in food, detergents, pharmaceuticals, and in the paper and textile industries. Amylases are useful for the saccharification of starch at the industrial scale, and saccharified starch can be used in a lot of bioprocesses. In the food industry, amylases is useful in the bread baking process, production of glucose, maltose, and in corn and chocolate syrups and also food additives. Amylases are also used in the production of biofuels, for

the brewing industry as a taste enhancers and as a desizing agent for the textile industry (Liu and Kokare, 2023)

Catalases

Catalases (EC 1.11.1.6) catalyze hydrogen peroxide to water and molecular oxygen, catalases are antioxidant enzymes. Catalases are an antioxidant enzymes expressed in living organisms such as bacteria, animals, fungi, plants under oxidative stress. Depend on the sequence and structure, catalases can be classified into three classes: monofunctional catalase or typical catalase, catalase-peroxidase, and pseudo-catalase or Mn-catalase. Catalases are key enzymes for biotechnological and pharmaceutical industries. Currently catalases gained increasing importance for bioremediation. They biodegrade the toxic environmental pollutants such as synthetic textile dyes, herbicides, pesticides, phenols, and derivatives (guaiacol, pyragallol, cresol), polyaromatic hydrocarbons, and eliminate peroxide from bleaching effluents. *Penicillium* variabile, *A. niger*, *S. cerevisiae*, *Staphylococcus*, *Micrococcus lysodeikticus*, *Thermoascus aurantiacus*, *Bacillus subtilis*, and *Rhizobium radiobacter* can produce catalases. Catalases are useful for couple of industrial applications as food, textile processing, it is necessary to remove hydrogen peroxide for sterilization or bleaching (Gan ve ark, 2022).

Cellulases

Cellulose is the most abundant and renewable biopolymer found on earth. Cellulose occurs when the 15.000 glucose unit is linked by β-1,4 glycosidic bonds. Cellulase hydrolyze β-1,4 linkages in cellulose chains. Then catalytic modules of cellulases have been divided into numerous families depend on their amino acid sequences and crystal structures. Cellulases comprise noncatalytic carbohydrate-binding modules and/or other functionally known or unknown modules located at the N- or C-terminus of a catalytic module. In nature, whole cellulose hydrolysis is mediated by a combination of three main types of cellulases. These are;

1. Endoglucanase (EC 3.2.1.4): Endoglucanases are located in the inner regions of the polysaccharide chain that forms cellulose they cut randomly and from oligosaccharides.
2. Exoglucanase (EC 3.2.1.91): Exoglucanases play a role at the reducing and non-reducing ends of the cellulose chain. They release glucose or cellobiose as the end product.
3. Glucosidases (EC 3.2.1.21): Glucosidases are enzymes that can hydrolyze cellodextrin and cellobiose to glucose.

Cellulases are synthesized by many microorganisms such as fungi and bacteria. These microorganisms can be aerobic, anaerobic, mesophilic, or thermophilic. The most extensively studied cellulose producer are the genera of *Clostridium, Cellulomonas, Thermomonospora, Trichoderma,* and *Aspergillus.* (Akan, 2017). Nowadays, the majority of laboratory and commercial cellulases are obtained from fungi: mainly *Trichoderma, Aspergillus,* and *Penicillium* according to their high enzymatic activity and hydrolysis capacity. Many bacterial genera are reported in the literature as extracellular cellulase producers, such as *Acetivibrio*, *Alteromonas*, *Bacillus*, *Clostridium*, *Cellulomonas,* and *Ruminococcus*. The most studied and the most cited genus is *Bacillus* (Balla et al., 2022).

Cellulase biotechnology was first used in animal feed and then in feed applications in 1980s. These enzymes are then used in textiles, laundry and paper industries. This enzyme accounts for approximately 20% of the world enzyme market today. Cellulases has many biotechnological applications.

Microbial cellulases find application in various application. Cellulases are useful for the production of biofuels as bioethanol, used for the clarification of fruit juices, extraction of olive oil, treatment of wines, and for improving the quality of bakery products for food industries and also used in the biostoning of denimand the

polishing of cellulosic fiber in the textile industry. Cellulases are used for the reuse of waste paper in the paper and pulp industry, and in enhancing the digestibility of animal feed.

Lipases

Lipases (triacylglycerol acyl hydrolases, EC 3.1.1.3) belong to a special class of esterases. Lipases catalyze the hyrolysis of triacylglycerols to glycerol, diacylglycerols, mono glycerol, and also free fatty acids. It is known that while lipases hydrolyze water soluble substrates, they show a higher enzyme activity against water insoluble lipid substrates. Lipases are enzymes that break down glycerol esters of long-chain fatty acids by exerting a catalytic effect against the substrate, especially on the inner surface between the water-oil phase. Bacterial lipases are divided into eight families depend on differences in their amino acid sequences and also biological properties. Lipases are produced from the plant

and animal kingdoms, also in molds and bacteria. Based on the organism that produces it, each lipase have different catalytic properties. (Girelli and Chiappini, 2022)

The family I of true lipases is the most represented one and can be classified into Pseudomonas lipase subfamily, Bacillus lipase subfamily, Staphylococcal lipase subfamily, etc. Lipases belong to the class of serine hydrolases so lipases dont need cofactor.

Among an insoluble substrate phase and the aqueous phase that the enzymes remain dissolved Lipases catalyze the hydrolysis of ester bonds at the interface. In the bulk fluid lipases do not hydrolyze dissolved substrates. In nature, lipases have important diversity in their reaction specificities. In the fatty acid side, some lipases have an affinity for short-chain fatty acids (C2, C4, C6, C8, and C10), however some have a option for unsaturated fatty acids

as oleic, linoleic, linolenic, etc.) whereas many others are nonspecific and randomly split the fatty acids from the triglycerides.

Fungi belong to the genera *Rhizopus sp., Aspergillus, Penicillium, Geotrichum, Mucor,* and Rhizomucor produce most commercially important lipase. Yeast such as Candida spp. such as *Candida rugosa, Candida tropicalis, Candida antarctica, Candida cylindracea, Candida parapsilosis, Candida deformans, Candida curvata, and Candida valida, furthermore, Yarrowia lipolytica, Rhodotorula glutinis, Rhodotorula pilimornae, Pichia spp. (Pichia bispora, Pichia mexicana, Pichia silvicola, Pichia xylose, and Pichia burtoni*i), *Saccharomycopsis crataegenesis, Torulaspora globosa*, and *Trichosporon asteroids* produce lipases.

Among bacterial lipases, Bacillus are potentail candidates for biotechnological applications. Most common lipase-producing strains are *Bacillus spp., B. subtilis, Bacillus pumilus, B. licheniformis, Bacillus coagulans, Bacillus stearothermophilus*, and *Bacillus alcalophilus* are the most common lipase-producing strains. Also, *Pseudomonas sp., Pseudomonas aeruginosa, Burkholderia multivorans, Burkholderia cepacia,* and *Staphylococcus caseolyticus* produce lipases.

Lipases have many applications in the detergent, food, leather, textile, oil and fat, cosmetic, paper, and also pharmaceutical industries.

Microbial lipases are currently used in detergent processing, flavor improvement by synthesis of esters of short chain fatty acids and alcohols, resolution of racemic mixtures and amino acid derivatives, and the construction of biosensors whixh are used as diagnostic tools for the detection of various diseases (Houde et al., 2004).

Pectinases

Pectinases catalyze pecti-containing substance degradation via depolymerization (hydrolases and lyases) and deesterification (esterases) reaction. Pectinases are mixed enzymes which hydrolyze peptic susbtances, they found in microorganims and higher plants. Based on the cleavage mode and specificity, pectic enzymes are

classified into three major gropus: pectinesterases, depolymerizing enzymes, and cleaving. pectinesterases can classified into 13 groups, these are protopectinases, pectin methyl esterases (PME), pectin acetyl esterases (PAE), polymethyl galacturonases (PMG), polygalacturonases (PG), polygalacturonate lyases (PGL), pectin lyases (PL), rhamnogalacturonan rhamnohydrolases, rhamnogalacturonan galacturonohydrolases, rhamnogalacturonan hydrolases, rhamnogalacturonan lyases, rhamnogalacturonan acetyl esterases, and xylogalacturonase. For instance pectinesterase (EC 3.1.1.11) catalyzes de-esterification of the methoxyl group of pectin to form pectic acid and methanol.). PAE (EC 3.1.1.6) hydrolyzes the acetyl ester of pectin to form pectic acid and acetate. PG catalyzes the hydrolysis of α-1,4 glycosidic linkages in polygalacturonic acid to produce D-galacturonate. PMG and PG can act in an endo- or exo-mode. Endo-PG (EC 3.2.1.15) and endo-PMG catalyze random cleavage of the substrate, however exo-PG (EC 3.2.1.67) and exo-PMG catalyze hydrolytic cleavage at substrate nonreducing end producing monogalacturonate ordigalacturonate in some cases. Microbial pectinases comprise for 25% of the global food enzyme sales and 10% of global industrial enzymes produced. (Haile and Abate, 2022).

Pectinase has a potentail applications in fruit juices preparation, papermaking, pectin containing wastewater application, degumming of plant's bast fibers, clarification of wine and oil processing and coffee and tea industry. The microbial pectinase have an important role in nature for contibuting natural recycling of carbon.

Bacteria, including actinomycetes, yeast, and fungi can produce pectinase usually almost all the commercial preparations of pectinases are produced from fungal sources A. niger is the most commonly used fungal species for the industrial application and Aspergillus niger is mostly used in the in indutrial production of pectinase (Rehman, 2022)

Protease

Proteases (it is also known as peptidase or proteinase) comprise a very large and complex group of enzymes. Protease catalyze the hydrolysis of covalent peptide bonds. Proteases can be classified depend on pH, substrate specificity and the active site amino acid. Depend on the pH optima, they are referred to as acidic, neutral, or alkaline proteases. Depend on their site of action on protein substrates, proteases are referred to as endo- or exo-enzymes. Based on their catatlytic mechanism, they are categorized as serine proteases, aspartic proteases, cysteine proteases, or metalloproteases.

Microorganisms comprise for a two-thirds share of commercial protease production in the enzyme market. Most dominant proteases are alkaline serine proteases which produced by bacteria, fungi, yeast, and actinomycetes.

Protease have an important role for the industrial sectors especially leather indutry, peptide production, recycling of waste, manufacturing of laundry detergents, treatments of diseases. In the industry, Most active protease producer is *Bacillus sp.* Currently, 29 *Bacillus* species and fungal producers have been reported for producing alkaline proteases. *Bacillus sp.* are important for protease production in a large scale, *Bacillus sp.* are used in industry such as leather, detergent, pharmaceuticals and textile. Among fungal species, *Aspergillus sp.* are studied for the production of alkaline protease (Razzaq ve ark., 2019).

Xylanases

Xylanases are a group of GH enzymes which degrade the linear polysaccharide xylan into xylose via catalyzing the hydrolysis of the glycosidic linkage (β-1,4) of xylosides. Xylan has the chemical and structural variation therefore. Xylanases have been classified based on the molecular weight and isoelectric point, the crystal structure and kinetic properties, or the substrate specificity and product profile. According to 3D structure analysis and amino

acid sequence similarity, it has been grouped as glycosyl hydrolase Family F10 and G11.

Xylanase cleaves β-1,4 glycosidic bond of xylan backbone and constituting usable products such as xylose, and xylobiose like xylooligosaccharides. Xylanases are one of the xylanolytic enzyme system that involves endoxylanase, β-xylosidase, α-glucuronidase, α-arabinofuranosidase, and acetyl xylanesterase. Endoxylanase has been known to be produced from microorganisms such as bacteria (*Streptomyces sp*., *Bacillus sp*.) and fungi (*Trichoderma sp*., *Aspergillus sp*.). α-glucuronidase has been found in several bacterial and fungal filtrates however it has been isolated to homogeneity from only few sources. α-arabinofuranosidase have been purified from bacteria (especially *Bacillus subtilis*), fungi, and plants (Alokika and Singh, 2019).

Among the all xylanases, endoxylanases and β-xylosidase are the most important beacuse of their direct involvement in cleaving the glycosidic bonds and liberating short xylooligosaccharides.

The production of xylanase from fungi, bacteria, yeast, marine algae, seeds, crustaceans, snails are reported however the main sources for these enzymes are fungi and bacteria.

Xylanases have a lot of biotechnological potential in various industrial sectors. Xylanases are mainly used in the prebleaching of kraft pulps for improving the pulp fibrillation, and restoration of bonding, for increasing the freeness in recycled fibers, and improving the biobleaching of wood pulp. Xylanases are also useful for improving the quality of bread, treatment of hemicelluloses waste, clarifying of fruit and vegetable juices, beer, bakery products softening of fruits, extraction of plant oils, bioconversion of agricultural wastes and produce biofuel, pretreatment of forage crops to improve the ruminant feed digestibility, in the production of ethanol and xylitol, and in the degumming of bast fibers such as flax, hemp, jute, and ramie (Burlacu et al., 2016).

L-Asparaginase

L-Asparaginase (L-asparagine amidohydrolase, E.C. 3.5.1.1) catalyzes the deamidation ofNL-asparagine to L-aspartic acid and ammonia which is a highly characterized and well documented enzyme. In nature, L-Asparaginase is in different forms such as dimers, tetramers and hexomers. L-Asparaginase can degrade acrylamide which is a potential human carcinogen. The substrate and product of this enzyme have an important role in various metabolic processes, from microorganisms to mammals. Human body can synthesize L-Aspargine, and its external supplementation is not required. But, cancer cells can not synthesize this amino acid, because of a lack of aspartate ligase activity. The survival of cancer cells relate to the extracellular circulatory asparagine. For cell's existence L-Asparagine is essential. L-Asparaginase supplied exogenously may hydrolyze the extracellular L-asparagine that is essential for the survival of cancer cells like lymphocytes. For the treatment of leukemia, this handling is used. (Muneer et al. 2020; Nunes et al, 2020; Wang et al.2023,).

L-Asparaginase is divided into two groups, these are plant-type and bacterial-type asparaginases. The plant type is classified into potassium-dependent and potassiumindependent enzymes. Type II bacterial asparginase play a role in tumor suppression.

L-Asparginase is produced from plants, animals, bacteria, fungi and yeast. Best source for L-Asparaginase is microbes. *Serratia marcescens* and *Enterobacter aerogenes* are bacterial sources for L-Asparinase. *E.coli* and *Erwinia carotovora* has better concentrations of the enzyme for this reason used for the treatment of acute lymphoblastic leukemia. Some Penicillim sp. are important source. Some of strains such as *Pseudomonas aeruginosa, Aspergillus tamari, Aspergillus terreus, Pseudomonas stutzeri, staphylcoccus sp. Erwinia aroideae, Proteus vulgaris, E. coli, Serratia marcescens, Vibrio succinogenes, Citrobacter freundii*, and *Klebsiella aerogenes* have ability to produce xylanase. (Muneer et al. 2020)

Chitinases

Chitinases belong to the glycosyl hydrolase family, which hydrolyzes the β-1,4 glycoside bond of N-acetyl-D-glucosamine in chitin to produce monomer and oligomer units (Rathore and Gupta, 2015).

Chitinases are classified into two categories. These are endochitinases and exochitinases (Oyeleye and Normi, 2018). Endochitinases cleave chitin at internal sites for producing chitotriose, chitotetraose, and diacetylchitobiose, exochitinases are classified into two subcategories (Le and Yang, 2019). These are;

-chitobiosidases which release the diacetylchitobiose from the nonreducing end of chitin, and -β-1,4-N-acetyl glucosaminidases which cleave the products of endochitinases and -chitobiosidases, generating N-acetyl-D-glucosamine units. According to the amino acid sequence, chitinases are grouped into three families (18, 19, and 20) of the glycosyl hydrolases superfamily. Family 18 contains chitinases from bacteria, fungi, and animals. Family 19 consists of plant chitinases and some *Streptomyces* chitinases and also family 20 consists of β-N-acetylhexosaminidase from *Vibrio harveyi, Dictyostelium discoideum*, and humans (Gomaa, 2021; Nayak et al., 2021)

Chitinases have been reported from bacteria, fungi, plants, and animals. Bacterial strains that produce chitinases belong to genera Terrabacteria, Actinobacteria, Cyanobacteria, Proteobacteria and Firmicutes. Chitinases are widely presented in Gram negative bacteria such as *Serratia marcescens, Vibrio, Chromobacterium, Klebsiella, Pseudomonas, Xanthomonas and Aeromonas,* as well as in Gram positive one *as Bacillus, Clostridium, Arthrobacter, Nocardia and Streptomyces*.

Same with those of bacteria, the fungal chitinases play an important role in degradation and also in morphogenesis, cell division, autolysis, and acquisition of chitin. It has been reported that fungal genera such as *Trichoderma, Penicillium, Aspergillus,*

Verticillium Metharhizium, Beauveria, Lecanicillium, Aphanocladium, Neurospora, Mucor, Stachybotrys, Lycoperdon, Myrothecium, Conidiobolus and *Agaricus* are chitinase producers.

Chitinases are also used for these potential applications such as bio-Control of phytopathogens, potential as bio-pesticides, production of single cell protein, fungal biomass estimation, production of oligomers, mosquito control, medical applications, waste management (Dukar and Kumariye 2020; Thakur et al., 2023).

Keratinases

Keratinases are a spesific class of proteolytic enzymes that can break down insoluble keratin subtrates. Keratinases (E.C. 3.4.21/24/99.11) are usually serine proteases or metalloproteases and they hydrolyze keratins. Keratin has highly resistant structure therefore simple proteases cannot degrade keratin only a spesific class of proteolytic enzymes called keratinases can degrade.

Keratinase has encouraging application in tanning, bio-feed, fertilizer, degradation and transformation of keratin waste, fertilizer, detergent, cosmetic, pesticide and biomedical. Many keratinases from various sources have been reported however most natural keratinase-producing bacteria have difficulties for needs of inustrial applications because of their low efficiency or poor stres resistance. Keratinase is used for tanning industry which needs keratinase with strong thermal stability and specificity, the detergent industry, the feed industry which needs heat-resistant and acid-resistant enzymes because of the animal's gastrointestinal tract's acidic environment. (Wang et al., 2023).

Keratinolytic enzymes are produced by Fungus, bacteria, Actinomycetes. They are isolated from environments and soils. Bacteria are the main players in the degradation of keratin waste especially *Bacillus spp*. The bacteria that can degrade keratin are mainly Gram-positive bacteria (Akan, 2010).

Keratinase have various industrial and biotechnological applications based on their ability to degrade keratins. These enzyme are used in detergents, pharmaceutical, biomass production, leather and textile industry, animal feed, carbon and nitrogen sources and cosmetic applications (Li, 2021).

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CHAPTER V

Revolutionizing Sustainability: Unveiling the Economic Marvels of Eco-Friendly Biodiesel Production From Waste Vegetable Oil for a Thriving Global Economy in Line with SDGs

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1. Introduction

The rise of petroleum diesel as a primary fuel for automobiles and industrial applications, owing to its widespread availability and low cost, is facing a severe issue due to the inevitable depletion of conventional resources. An urgent need to address environmental

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degradation and the depletion of scarce natural resources has consequently led to a paradigm shift towards renewable energy sources, such as solar, wind, geothermal, and biomass. This transformation is especially important on a global basis, and particularly in nations like Pakistan, where the negative environmental effects of fossil fuel emissions highlight the need for a transition to sustainable alternatives. A viable alternative is biodiesel, which comes from renewable sources and has advantages such being readily available locally, biodegradable, renewable, and significantly reducing emissions, air pollution, and global warming contributions. This study examines waste vegetable oil (WVO) and its potential for producing biodiesel, with a particular focus on WVO obtained from soybeans. The biodiesel product made from soybean WVO satisfies the requirements of the American Standard for Testing of Materials (ASTM) in terms of its physico-chemical characteristics, such as its density (0.89), sulphur content (0.0063), cloud point (-8), pour point (-12), and flash point (70). The findings indicate that locally generated WVO from soybeans has substantial potential for large-scale biodiesel manufacturing. This study contributes to ongoing efforts to revolutionise sustainability by revealing the economic miracle of eco-friendly biodiesel production from waste vegetable oil, thereby helping a healthy global economy in accordance with the Sustainable Development Goals (SDGs). There are not enough potential remains in the already available sources like in natural gas and oil because these sources are reducing with the passage of time. In this view, as a replacement of these sources a significant attention should be given on biodiesel production (Lean, 2007). Environmental benefits are also among the other benefits which make biodiesel more attractive one (Demirbas, 2003; Ali et al., 2020).

Biodiesel can be defined as an alternative fuel which is produced by a chemical process under controlled laboratory conditions. In a chemical process, the triglycerides of vegetable oil are linked with alcohol to produce biodiesel (Harun et al., 2006). It can also be defined as that the mono-alkyl esters which are produced

from different sources e.g., canola oil, soyabean oil, sesame oil and from animal fats by transestrification of the triglycerides (TGs) of fatty acids appears in these sources react with ethanol to form the methyl esters of fatty acids (FAME) (Dunn et al., 2001). In 1900, different oil sources like peanut oil was used by Redolf Diesel as an alternative fuel source, it is said as the first evidence of using vegetable oils in engines as a fuel (Shay, 1993; Khanam et al., 2021). Fats and vegetable oil which are renewable and natural are also utilized to made alternative diesel fuels (Ratledge, 1985). Biodiesel as diesel fuel is liquid in nature, higher combustion efficiency, ready availability, renewability, also include in biodiesel advantages (Ma and Hanna, 1999; Knothe et al., 2006). Environment friendly emissions, biodegradable and local resources are also the benefits of biodiesel (Zhang et al., 2003; Ahmad and Zafar, 2023).

Biodiesel produced from different sources like waste cooking oil, plant-based oil and animal oil can be used as a good substitute of high-speed diesel (HSD) and fossil fuels to use in diesel engines or in other combustion equipments without any major amendments (Meher et al., 2006; Gerpen, 2005; Canakci, 2007). A feasible and sustainable biofuel must have two important features i.e., easy availability from renewable sources and low negative impact on environment compared with fossil fuel. Presently different transesterification methods and technologies are used for production of biodiesel. Waste vegetable oil is a good renewable and alternative fuel source. Most of the waste vegetable oils have energy content very similar to the diesel fuel so it is a potentially rich source of energy for the present needs as well as future needs.

Among other alternative fuel sources, one of the most competent biodiesel sources can be waste vegetable oil. Its low price and easy availability as well as problem regarding its disposal are the major factors which promote its use as a raw material. So, by utilizing this to produce biodiesel can also help to overcome the disposal problem (Chhetri et al., 2008).

2. Materials and Methods

The current research work was conducted during 2010-2011 for the estimation of synthesis of "Biodiesel" from samples of waste vegetable oil (WVO), collected through different sources of waste oil from the local market. Most part of this study was conducted at the Biofuel Laboratories, Department of Plant Sciences, Quaid-i-Azam University, Islamabad. The study was performed under the standard protocols established in the said laboratories for the production of biodiesel from different types of oil sources. In this study the WVO obtained from the soyabean seed source was used as an alternative oil source. The study was aimed towards the successful formulation/production of the biodiesel from the waste vegetable oil, in the maximum possible percentage/s, to have economically feasible production at the lowest possible price in the current socio-economic conditions.

Experimental material: The waste oils e.g., Soyabean waste vegetable oil was collected from local whole sale market/industry (Raja Bazar, Rawalpindi).

Chemicals: Chemicals which are used in biodiesel production include Methanol, Sodium Hydroxide, Phenolphthalein, Iodine, Sodium Sulphate, Potassium Hydroxide (all are from Merck, Germany).

Experimental instruments:

Hot plate (VWR, VELP-Scientifica Berlin Germany)

Titration apparatus

Magnetic stirrer (TEFLON)

Oven (Memert, Germany)

Electrical Balance (GF-3000)

Free fatty acid estimation in WVO (soyabean oil) by aqueous acid-base titration: Free fatty acid (FFA) number of soybean oil was calculated by aqueous acid-base titration technique (Komers et al.,

1997). Two types of titrations were executed initially blank titration was done to optimize the reaction followed by sample titration.

Blank Titration: In this process of blank titration any of the two catalysts, sodium hydroxide (NaOH) or potassium hydroxide (KOH) was used. Phenolphthalein use as an indicator and isopropyl alcohol were taken in a flask. Then from burette oxalic acid was added. Change in color will be the end point of blank titration.

Sample Titration: In sample titration process phenolphthalein (indicator) and isopropyl alcohol both were in the flask. Then this mixture was titrated by using sodium hydroxide (NaOH) / potassium hydroxide (KOH). End point was the changed in the color, mostly pink color is the changed color.

Following formula was used to explain free fatty acid number of oils

$$\text{Acid Number} = \frac{(A - B) \times C}{V}$$

A= Actual Titration volume

B= Blank Titration volume

C= Mass of Catalyst in g/l

V= Volume of Oil Used

Biodiesel Production: Following steps were including in the process of biodiesel production.

Filtration: The first step was the filtration of Waste oil as it contained many impurities. Filtration was done to remove the impurities and all types of waxes. If these impurities were not removed from the oil the process, quality and yield of transesterification could be affected. Whatman filter paper # 42 was used during filtration process. Quality of oil effects the time required for filtration.

Heating: The oil after filtration was heated to a temperature around 160^{o}C on hot plate. There are two major reasons for heating the oil; first to break the fatty acid bonds present in the oil and secondly to remove any water present in it. The process usually takes 1-2 hours in the lab. conditions, but it usually extends based on the large quantity of oil in use.

Transesterification: Sodium hydroxide (NaOH) of known quantity was dissolved and mixed in distilled methanol (CH_3OH) in this step. Then magnetic stirrer was used to stir the blend during the process. Nearly 30 to 35 minutes to be taken by Sodium hydroxide for getting dissolved completely. Then sodium methoxide which acts as a catalyst was mixed with the oil at 60^{o}C at which the change of triglycerides into fatty acid methyl esters (FAME) was suppose to be at its maximum. The finishing mixture was stirred vigorously for 60-70 minutes with the help of magnetic stirrer at 600rpm for almost 1 hour. Different alkali catalysts are used for the production of methyl ester in this process (Ahmad et al., 2009).

Settling: After stirring, the solution was immediately shifted to a separating funnel and the solution was left overnight to settle down at room temperature. Ending in the formation of three different types of layers, at the top a thin layer of soap appeared, the center layer of biodiesel (FAME) appeared as the major and important portion. And at the bottom thin but dense layer of glycerine was formed.

Separation: These three layers were collected separately in different beakers with the help of separating funnel or with simple handling apparatus carefully.

Washing: Washing is done in a separate container. Double the amount of water from biodiesel was poured in a separate container along with the addition of some drops of acetic acid in order to protect it from contamination. Allow the mixture to settle down for 4-5 hours for the purpose to eradicate impurities and hanging particles and to eliminate the chance of fungal activity, leave the

mixture to completely settle down for 4-5 hours. A complete clearance of biodiesel was found after 3-4 washing.

3. Chemical Conversion

Transesterification: It is a process in which oil reacts with alcohol and esters. Function of catalyst is just to speed up the reaction time and yield. Extra alcohol may also be used to transfer the equilibrium towards the product side because the reaction is reversible. Different problems are found in plant oil mainly it contains sterols, water, impurities and free fatty acids. Because of these problems oil cannot be used as a fuel directly. So, some kinds of chemical modification are required to overcome all these problems (Canakci, 2007).

Three different kinds of catalysts and an enzyme can be used in transesterification process. The key benefit of strong alkali using as a catalyst is that it reduces reaction time as well as less amount of catalyst is required during the transesterification (Ma & Hanna, 1999; Meher et al., 2006). Different kinds of alcohols can be used in tranesterification mainly propanal, methanol, ethanol and amyl alcohol are used. Mostly ethanol and methanol are used because of their good chemical and physical properties as well as cheap in price. It can rapidly react with triglycerides and simply dissolve in it. Due to Easter the reaction moderately converted into saponification (soap production). The soap having some disadvantages as it reduces the yield of esters and renders the partition of ester and glycerol for the period of water washing. Alkali-catalyzed transesterification is suitable for triglycerides of low free fatty acids contents and acid-catalyzed transesterification is good for high free fatty acids in triglycerides (Keim, 1945). Enzymes, acids and alkalis catalysts are used in a reaction. Sodium ethoxide and methoxide and alkalis like NaOH and KOH are used. Hydrochloric acid, sulfonic acid and sulfuric acid are included in acid catalysts. Lipases may be used as biocatalysts. Alkali-catalyzed tranesterification is mostly used in commercial level and is much more useful than acid-catalyzed transesterification (Wright et al., 1944). Biodiesel synthesized from

the waste oil of soyabean was examined for various fuel properties of biodiesel including viscosity, pour point, density, acid number, cloud point, etc.

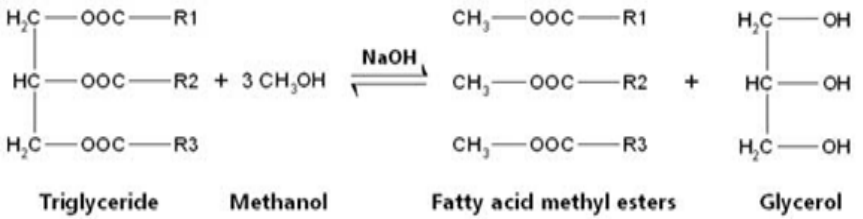


Figure 1. *General equation of Transesterification Reaction*

4. Results

The present study gives biodiesel characterization of waste vegetable oil from soyabean, which belongs to family Leguminosae. Biodiesel characterization is based on different parameters like transesterification process, free fatty acid determination, acid number, and percentage of oil to biodiesel conversion with two different kinds of catalysts used in different ratios and fuel properties in comparison with ASTM (American Standards for Testing Material) etc.

Biodiesel Finding*:* The free fatty acid (FFAs) number of WVO soyabean was also calculated before the biodiesel production. After titration the free fatty acid number of WVO of soyabean was found 2.23 mg/g against the catalyst sodium hydroxide (NaOH). In transesterification process Molar ratio of methanol: oil is 1: 6 along with temperature at 60^{o}C and used the base catalyst potassium hydroxide (KOH) and sodium hydroxide (NaOH) by changing concentration. In the process the FFAs from triglyceride breaks catalysts one by one. The methanol attached with fatty acid create bond with it and converted in fatty acid methyl ester (FAME). Hydroxyl group attached to free fatty acid and converted to soap but no soap was found in this case. The remaining result of oil settled

down in the form of glycerin. Biodiesel was synthesis from waste oil of soyabean. Various fuel properties of biodiesel were studied that included viscosity, pour point, density, acid number, cloud point, etc.

WVO Soybean Biodiesel Yield*:* The proportion of transfer from oil to biodiesel, soap and Glycerine was different with various types and concentration of base catalyst (NaOH and KOH) as shown in table 2 and 3. When 3.24g amount of NaOH catalyst is used, the percentage of biodiesel was (85%), Glycerine (14%), soap was (0%). The result of biodiesel with KOH reaction was (87%), Glycerine (10.6%) and soap (0%). By using 2.50g concentration of catalyst, the result of biodiesel with NaOH (80%) Glycerine (15%) and soap was (0%) along to it the result with KOH of biodiesel was (84%), Glycerine (11.8%) and soap (0%).If the concentration of base catalyst (NaOH & KOH) was 1.75g, the result was found in different amount. The result of biodiesel with NaOH catalyst was (77%), Glycerine (17.5%) and soap was (0%) and the result of biodiesel with KOH was (74%), Glycerine (20%) and soap was (0%).

Table 1. *Result of biodiesel, Glycerine and Soap from WVO Soybean Oil at 60^{o}C with different amount of Catalyst NaOH.*

Catalyst	**Amount of Catalyst**	**Biodiesel (%)**	**Glycerine (%)**	**Soap (%)**	**Emulsion (%)**
NaOH	3.24 g	85 %	14 %	traces	1.0%
	2.50 g	80 %	15 %	0 %	5.0%
	1.75 g	77 %	17.5 %	traces	5.5%

Table 2. *Result of biodiesel, Glycerine and Soap from WVO Soybean Oil at 60°C with different amount of Catalyst KOH.*

Catalyst	Amount of Catalyst	Biodiesel (%)	Glycerine (%)	Soap (%)	Emulsion (%)
KOH	3.24 g	87 %	10.6 %	0 %	2.4%
	2.50 g	84%	11.8%	traces	4.2%
	1.75 g	74 %	20 %	0 %	6.0%

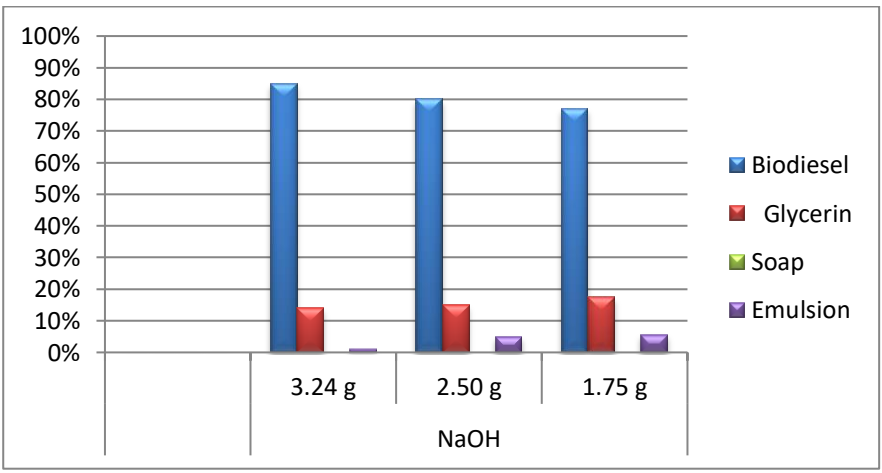


Figure 2. *Production percentages of Biodiesel, Soap and Glycerine using different amount of NaOH*

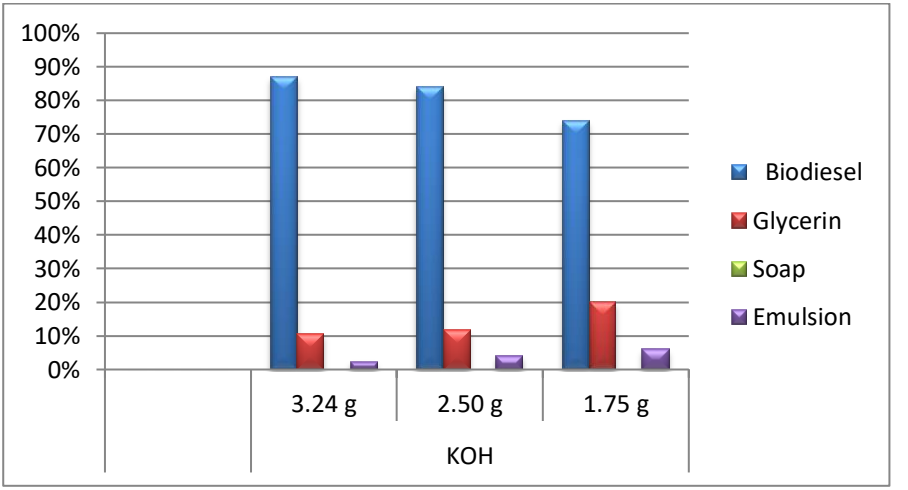
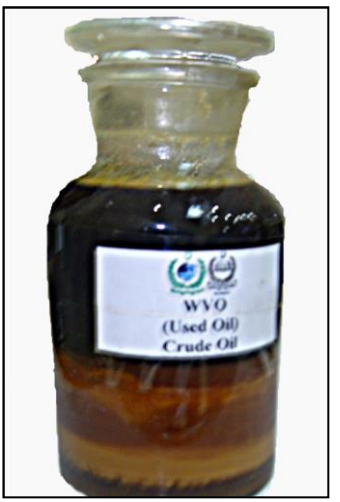


Figure 3. *Production percentages of Biodiesel, Soap and Glycerine using different amount of KOH*



WVO crude oil

WVO Biodiesel

WVO Soybean Biodiesel

Soybean Glycerine

Fuel Properties:

In the present study the waste soybean oil based biodiesel is analyzed for the physico-chemical properties. Most of the results for biodiesel properties were found in accordance with American Society for and Testing Materials (ASTM) methods and analyzed in lab. and also verified by PSO headquarters Karachi (Table 3).

Table 3. *Fuel Properties of Waste Vegetable Oil Biodiesel*

TEST	METHODS	WVO B100 %
Flash Point, oC	ASTM D-93	70
Kinematic viscosity@ 40 oC (ny)	ASTM D-445	14.52
Color Comparison	Visual	Visual
Sulfur %wt	ASTM D-4294	0.0063
Pour Point, oC	ASTM D-97	-12

Cloud Point, oC	ASTM D-2500	-8
Total Acid No.mg KOH/gm	ASTM D613	0.36
Density @ 15 oC kg/L	ASTM D-1298	0.89

WVO: Waste Vegetable Oil, B100: 100% Biodiesel, ASTM: American society for testing and materils wt: weight, C: centigrade

5. Discussion

Biodiesel potential to waste vegetable oil of soyabean

In the current research work WVO of soyabean was analyzed for the production of biodiesel. It was estimated that WVO Soyabean (*Glycine max*) is a favorable and suitable choice for biodiesel production. Prior to biodiesel production the free fatty acid number of WVO oil was also found by aqueous acid base titration against sodium hydroxide and potassium hydroxide. The free fatty acid number should be less than 3% in the oil/ WVO, so that transesterification reaction may be occurred unhindered (Dorado et al., 2002; Munir et al., 2023). The conversion percentage from WVO to biodiesel and its byproducts was changing with a choice of concentration of base catalyst (NaOH and KOH) as shown in Table 1 and 2.

It is vivid that when 3.24g of NaOH catalyst was used, the percentage of biodiesel was produced maximum i.e., (85%). While in case of KOH reaction results were again found to be maximum (87%) with minimum quantities of glycerin and soap produced (Murugesan et al., 2009). By using 2.50g concentration of catalyst, the yield decreased a little i.e., for biodiesel with NaOH (80%) and with KOH (84%).When base catalyst (NaOH and KOH) concentration was further reduced to 1.75g, the results were found in lowest yield of biodiesel with NaOH catalyst only (77%) and the result of biodiesel with KOH it was as low as (74%) (Conceicao et al., 2007) Therefore from the results it can be concluded that under

the same condition the yield of biodiesel can be disturbed considerably by reducing the catalyst percentages, NaOH and KOH gave minimum result when minimum catalyst is used and the yield increasing when we increase the catalyst amount (Refaat et al., 2008).

The influence of catalyst type on the ester yields was also observed in the study and it was found that among the two catalysts NaOH and KOH, later proved to be the best suitable catalyst when used in higher concentration (3.24). These results are found in accordance with those obtained by other studies (Meneghetti et al., 2006). In a transesterification process of used vegetable oil it is also noted that Potassium hydroxide was the most excellent catalyst (Nye et al., 1992). World over the biodiesel fuel is produced using two major sources, i.e. animal fats or vegetable oil, the biodiesel produced through this method can either be added to the petroleum or may also completely replace the conventional diesel petroleum fuel. Almost all biodiesel in the United States is created from canola or soybean oils (Borrios et al., 2007).

Biodiesel price varies from place to place and depends on the biodiesel source and its mixture percentage. Used or waste vegetable oil generally is widely available and of free from local restaurants around Europe and in North America or may cost negligible. But in developing countries like Pakistan it may cost from Rs. 20 to 65 per liter based on its properties and quality. Aside from the processing equipment initial cost, production of biodiesel costs also include chemicals utilized in the process, electricity and expenses as well as labor cost. That is estimated to be around Rs. 20 per liter. If the price of WVO is considered to be Rs. 65 the net cost per liter reach as high as Rs. 85 which is not feasible economically. Therefore, if we get WVO at the lowest possible price (or free) the process of biodiesel production become economically viable.

İn bulk Methanol (near pure) costs around Rs.300 per liter, however, you need only 1/5 a liter of methanol for each biodiesel liter that you will produce or 8 liter of methanol is required for 40

liter of biodiesel. By utilizing used vegetable oil or WVO it is calculated that biodiesel production costs may be slashed down by 50 percent, thereby making biodiesel very reasonable and can compete with pure petroleum diesel (Ayhau, 2009). Nearly all vegetable oil biodiesel shows very low or almost negligible amount of Sulphur content (Mohammad et al., 2009). The sulfur contents in biodiesel are found in B100 blend is 0.0063 whereas in petroleum diesel it is 0.05. Sulphur content is considered as significant aspect for reduction of SO_2 from exhaust emission (Kumar, 2007). Hence, many biodiesels from plant sources e.g., Brassicaceae family members, taramira and mustard biodiesel is also showing no exception in terms of low Sulphur amount in comparison to diesel fuel thus supporting it to be considered as a very environment friendly fuel. More SO_x compounds will be released in compression-ignition engine as a result if there is slightly higher Sulphur content in canola biodiesel (Chakrabarti & Ali, 2008). The difference in the viscosity between alkyl ester derivates and the parent oil is the base to monitor the biodiesel production (Fillipis et al., 1995; Ahmad et al., 2023).

Flash point of WVO soyabean (70) is very much like as compared to mineral diesel (60-80), so the high flash point is considering a positive point towards its storage and transportation (Kumar, 2007). The Kinematic viscosity was noted about 14.52 in B100 where it is 4.1 in petro-diesel. The density of biodiesel produced in this study was calculated in B100 is 0.89. This density value is very close to density of petroleum diesel 0.8343. Higher density of biodiesel outcomes in the delivery of a little greater mass of fuel similar to our results of density taramira showing 0.881 and canola showing 0.884, both are Brassicaece family member (Mohamed et al., 2009) which was close of ASTM standards as well as mineral diesel (Chakrabarti & Ali, 2008).The color comparison of biodiesel is also according to the international standards (Meneghetti et al., 2006) The temperature at which oil is cooled at a specific rate so; that cloud of wax crystal appears is known as cloud point (Lang et al., 2002; Ahmad et al., 2022). The Pour Point is that point or

temperature at which quantity of wax present out of solution is sufficient to change the fuel into gel or in other words it is the lowest temperature at fuel can easily flow. Biodiesel having high value of cloud point and pour point compared with conventional diesel. The value of cloud point in conventional diesel is -15 where it is calculated -8 in B100% blend of biodiesel and in case of pour point it is -35 to -15 and -12 respectively. Rice bran biodiesel contain cloud point of about 9 which is quite high and pour point of -2 (Sinha et al., 2008). Where pour point is calculated as -4 (Kumar, 2007).

6. Conclusion

In the current research work WVO soyabean was analyzed for the production of biodiesel. Results give a clear idea that this among the viable source for biodiesel production. The best results of biodiesel production were achieved on constant 1:6 Molar ratio at 60 °C, with different types and concentrations of Catalyst, Potassium hydroxide with 3.24gm & 2.50gm shown the best results. The Physico-chemical properties i.e., flash point, cloud point, specific gravity, kinematic viscosity and sulfur content of biodiesel present in accordance with the ASTM values. From the results it is concluded that WVO soyabean having full potential for biodiesel production and it is recommended to utilize them on large scale for biodiesel production. Used/ WVO available in local market is economically feasible source of biodiesel production specially keeping in view the recent increasing prices of diesel in Pakistan.

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CHAPTER VI

Ethnobotanical, Morphological and Ecological Characteristics of *Morina persica* L. (Morinaceae)[1]

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Büşra Gökçe Dinç[4]

Introduction

Flora studies involve the systematic study of vegetation in a given region and play an important role in our understanding of biodiversity and ecosystem Dynamics (Rees, & srk., 2001). The importance of such studies focuses on their contribution to ecological research, conservation efforts and human welfare. This importance of flora studies is emphasised by the valuable

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information they provide on plant diversity, distribution patterns and ecological interactions. At the same time, these studies include the systematic study of plant species, distribution, morphology and ecological interactions (Seppelt & ark., 2011). Plants, as essential components of terrestrial ecosystems, have an impact on the overall health and functioning of ecosystems. Through flora studies, scientists can have a comprehensive understanding of the complex relationships between plants, animals and the environment (Bernacchi & VanLoocke, 2015).

One of the main contributions of flora studies is the assessment of biodiversity. Plants form the basis of terrestrial ecosystems and provide essential ecosystem services. Through detailed field studies and data collection, researchers can identify plant species in a given area and determine the biodiversity in a given region (Bastian, 2013). Biodiversity assessments provide critical information for conservation, planning and management strategies. The identification of rare, endangered or invasive plant species allows scientists and conservationists to create targeted conservation plans (Sarkar, & ark., 2006). Understanding the ecological requirements of specific plant species assists conservationists in developing strategies to protect and restore habitats, which ultimately contributes to the overall health of ecosystems (Hamilton, 2013).

Flora studies are essential in climate change research, as plants are sensitive indicators of environmental changes. Changes in plant distribution, flowering times and abundance serve as early indicators of climate change impacts (Fitchett, Grab & Thompson, 2015). By monitoring these changes, scientists can assess the vulnerability of plant species and ecosystems to climate change impacts and facilitate adapted management strategies in the face of global climate change (Keenan, 2015).

Through flora studies, plants of medicinal and economic value can be identified. Many medicines and traditional medicines are derived from plant components. In addition, since plants are

essential resources for food, materials and ecosystem services, flora studies are of great economic importance in sustainable resource management (Shedayi & ark., 2016). As a result of the connection between humans and plants that has been going on for centuries, the science of ethnobotany, whose importance is recognised by the whole world today and where serious research is carried out, was born (Koçyiğit, 2005).

The root of the word ethnobotany, 'ethno' means the study of people and 'botany' means the study of plants or plant science. Ethnobotany, in a broad sense, refers to plant-human relations in different human communities (Tütenocaklı, 2002). The term ethnobotany was first used in 1895 by John W. Harshberger, a professor of biology, who defined it simply as "the use of plants by local people". In addition, Harshberger's "The Purposes of Ethnobotany", where the term ethnobotany was first mentioned, is the first known publication on this subject (Tütenocaklı, 2002).

Understanding the role of different plant species in ecosystem functioning is important for maintaining ecological balance. Flora studies provide information on plant interactions, nutrient cycling and ecosystem resilience. This knowledge is essential for predicting and mitigating the impacts of human activities on ecosystems (Alberti & Marzluff, 2004).

Material and Method

Collection of Plant Samples

The plant materials required for the research were collected from Çankırı province, Yapraklı district, at an altitude of 1280 m. as a result of field studies carried out in June-August 2014. For the identification of the plants, attention was paid to be collected during flowering and fruiting periods. The identification of the plants was made by using the 'Flora of Turkey and The East Aegean Islands I-X' published under the editorship of Davis (1965-1988).

Determination of the ethnobotanical use of the taxon Morina persica L.

In order to determine the ethnobotanical use of the taxon, face-to-face interviews were conducted with local people. During these interviews, it was paid attention that the people identified (3 women, 2 men) were knowledgeable about the ethnobotanical uses of the plants. These people were asked the local name of the taxon, the part of the taxon used, the way of use and the purpose of use, and the ethnobotanical use of the taxon was determined according to the information they gave.

Taking soil samples

The surface soil sample from the research area was taken in March 2015 during the vegetation period. Since the plant sample showed herbaceous taxon characteristics, soil sampling was carried out from 0-30 cm depth for surface sampling. Some physical and chemical analyses were performed on the soil samples. Some analyzes were performed below to determine the physical and chemical soil properties of the soil samples.

- pH and EC-total salt (TS) according to Mc Lean (1982) and Pansu & Gautheyrou (2006) determined in 1:5 soil and distiled water mixture

- soil organic matter (SOM)and total nitrogen (TN), according to (Jackson 1967) the modified Walkley Black method

- texture (sand, Silt and clay content) according with Gee & Bauder (1986) by hydrometer method,

- Bulk density (BD), according to Blake & Hartage (1986) with a 100 cm3 stainless cylinder and were analysed.

Lime content ($CaCO_3$), determined by Scheibler calcimetry as stated by Pansu & Gautheyrou (2006).

Determination of morphological characteristics of Morina persica L.

In order to determine the morphological characteristics of the collected specimens, it was ensured that the plant specimens were intact, leaves were complete, flowers were open and undamaged. For the morphological characteristics of the specimens, measurements and observations were made on plant height, stem and basal leaf length, leaf shape, leaf spine tooth length, flower number, calyx and coralla tube length, flower colour after the plant specimens were collected.

Results

Ethnobotanical utilisation of the taxon Morina persica L.

The ethnobotanical use of the taxon was determined as a result of interviews with local people (Table 1).

Table 1. Ethnobotanical use of the taxon *Morina persica* L.

Taxon Name	Local Name	Used Part	Usage Form	Usage Purpose
Morina persica L.	Donkey bellowing	Flowers	Food, drink and pain reliever	* The flowers of the plant are consumed by eating * Fresh flowers are consumed by adding to tea due to their pleasant odour * The flowers are crushed together with the thorns and used to relieve joint pain

Findings related to soil properties

Considering the presence of *Morina persica* L. taxon at the point where the study was carried out, sampling was carried out from 3 different points. Results of the soil chemical and physical properties state in Table 2. According to the Table 2; the study area soils were generally sandy loam in terms of texture classes, and the soil reaction was slightly acidic with 6.71-6.98. Singh & ark., (1992) stated that the Bulk density (BD) of a soil that is favourable for plant growth is 1.3 g/cm^3 while the BD that causes plant root growth to stop is 2.0. Accordingly, the average BD of the soils in the study area is 1.69 g/cm^3 and suitable for plant growth. As a result of the analyses, the soil organic matter content of the area was found to be low. According to Ergene (1993), soils with low soil organic matter content are classified as low calcareous when analysed in terms of lime content.

Table 2. Soil physical and Chemical properties of the study area

Coordinates Elevation Slope Aspect	Sample No	Physical Analyzes Bünye (%)					Chemical Analyzes									
		Sand (%)	Clay (%)	Silt (%)	Class	BD(gr/cm^3)	pH (1/5)	Class	$CaCO_3$ (%)	$CaCO_3$ Class	SOM %	SOM Class	TN %	TN Class	EC dS/m	TS (%)	Sınıfı
565872-4514878 1500m, %5, East	1	70	19	11	SL	1,72	6,71	Slightly Asidic	2,49	Slightly	0,041	Low	0,01	Low	0,834	0,03	without salt
	2	70	18	12	SL	1,65	6,90	Slightly Asidic	2.64	Slightly	0,059	Low	0,01	Low	0,917	0,04	without salt
	3	70	18	12	SL	1,70	6,98	Slightly Asidic	2.05	Slightly	0,097	Low	0,01	Low	0,590	0,04	without salt

SL: Sandy Loam; BD: Bulk Density; pH: Power of Hydrogen; $CaCO_3$: Lime content; SOM: Soil organic matter; TN: Totatl Nitrogen; EC: Electrical Condutivity; TS: Totatl Salt

Morphological findings

General characteristics of the Morinaceae family: Herbaceous perennials, Leaves in whorls, without stipules, usually spiny. Flowers arranged in rings. Calyx herbaceous and 2-lobed. Corolla long-tuberculate, 5-parted. Flowers white or pinkish. Stamens 2 or 4. Ovary subulate, 1-eyed, surrounded by involucel with unequal spines Davis (1965-1988).

General characteristics and distribution of the taxon Morina persica L.

Up to 1.5 m tall, glabrous or soft hairy, perennial plants with erect stems. Leaves are whorled, leathery, lanceolate, lanceolate, with gulf margins and long spiny teeth. Lower leaves glabrous, upper leaves glandular hairy. Flowers 4-7(-10) in a ring, fragrant. Corolla 3.5-5.5 cm, long-tubular, initially white, later pink or reddish and hairy. Involucre 6-13 mm, tubular-lanceolate, softly hairy, spiny at apex, usually 2 longer than others. Calyx 9-16 mm, 2-lobed, lobes entire or 2-lobed. Flowering occurs between May and August. Davis (1965-1988).

Figure 1. *Morina persica L. (Tübives, 2014)*

Plant height was 68 cm, basal leaves 13.0-17.0 cm, stem leaves 6.9-7.5 cm, leaf spine tooth length 4-20.0 mm, number of flowers 4-8 (-13), corolla tube 3.5-4.2 cm, involucel 6-15 mm, involucel spine (short) 3-10 mm, involucel spine (long) 17-24 mm, calyx 7-17 mm and stamens 6-8 mm (Table 3).

Table 3. *Comparative morphological character measurements of Morina persica L. taxon of Davis (1965-1988) and our study*

Morphological Character Measurements	Davis (1965-1988)	Our work
Plant height (cm)	150	68
Basal leaf (cm)	-	13.0-17.0
Stem leaves (cm)	-	6.9-7.5
Leaf spine (mm)	-	4-20.0
Number of flowers (pcs)	4-7(-10)	4-8 (-13)
Corolla tube (cm)	3.5-5.5	3.5-4.2
Involusel (mm)	6-13	6-15
Involucial spine (short) (mm)	-	3-10
Involucial spine (long) (mm)	-	17-24
Calyx (mm)	9-16	7-17
Stamen (mm)	-	6-8

Conclusion

In this study, ethnobotanical, morphological and ecological characteristics of *Morina persica* L. taxon were determined and presented for the first time. Among the morphological characters, measurements for plant height (cm) and corolla tube (cm) were in agreement with the studies of Davis (1965-1988), while differences were found between the number of flowers (number), length of involucel (mm) and calyx (mm). Other morphological characteristics of the taxon such as basal leaves (cm), stem leaves (cm), leaf spines

(mm), involucel spines (short) (mm), involucel spines (long) (mm), stamen length (mm) were revealed for the first time in this study.

The area where the study was carried out is in the mountainous land class and considering the topography of the points where plant-soil sampling was carried out, it was determined that the effects of erosion in the area and the vegetation cover were destroyed accordingly. As a result of the evaluation of the soil analyses of *Morina persica* L. plant, it was determined that the SOM content and the amount of clay in the soil were low in relation to the soil properties examined. At the same time, the volume weight values of the physical soil properties examined were determined as 1.69 g/cm^3 on average. Considering this value, the soils of the study area are suitable for plant growth.

In order to determine the ethnobotanical use of the taxon, face-to-face interviews were conducted with local people. During these interviews, it was paid attention that the people identified (3 women, 2 men) were knowledgeable about the ethnobotanical uses of the plants. As a result of the interviews with these people, it was determined that the flowers of the taxon are consumed by eating, fresh flowers are consumed by adding to tea because they give a pleasant odour, and the flowers are crushed with thorns and used to relieve joint pain.

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CHAPTER VII

Length-weight relationship and condition factor of chub *Squalius* sp. (Teleostei: Leuciscidae) (Kızılırmak Basin, Türkiye)

Özlem ABLAK GÜRBÜZ[1]

1. Introduction

The genus *Squalius* Bonaparte, 1837 includes approximately 48 species common in the Western Palearctic and is also widely distributed in Türkiye representing by 20 species (Özuluğ & Freyhof, 2011; Turan et al., 2013, Bayçelebi 2019). Chub *Squalius* are small to large fishes living in different aquatic habitats both lotic and lentic systems. Several species of this genus in Europe have quite similar appearances and, at some time, most of them were identified as wide-ranging *Squalius cephalus* since some earlier authors had contradictory views on species definitions (Kottelat &

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Freyhof, 2007). Similarly, studies on the systematics of *Squalius* have not been completed in Türkiye. According to Bayçelebi (2019), *Squalius* populations of Bolu Stream and Kızılırmak-Yeşilırmak basin differ morphologically from each other and from other species distributed in Türkiye. Therefore, it is thought that these two populations may be new species that have not yet been described. The present study was conducted in the Kızılırmak basin mentioned above. Therefore, the fish examined were evaluated as *Squalius* sp. (Bayçelebi 2019; Yoğurtçuoğlu et al., 2020) even though it was called as *Squalius cephalus* in previous studies (İlhan & Balık, 2008; Benzer & Gül, 2017).

Squalius sp. has an economic value consumed by local people in the region. Studying the biology of a fish will enable the development of more effective conservation strategies needed by the species. The length-weight relationship (LWR) is an important tool frequently used in fisheries managements, ecological studies, and biomass estimations (Froese, 2006). There are various studies on the LWR and growth characteristics of *Squalius* sp. (Çolak, 1983; Vitali & Braghieri, 1984; Altındağ, 1996; Türkmen et al., 1999; Ünver & Tanyolaç, 1999; Kırankaya & Ekmekçi, 2007; Stefanova et al., 2008; Pompei et al., 2011; Ünver & Erk'akan, 2012; Benzer & Gül, 2017; Dedeoğlu et al., 2020; Sarı & Becer, 2021). However, it is important to study fish populations periodically in order to maintain the sustainability of the ecosystem and protect biodiversity.

The aim of this study is to determine the age, length and weight distribution, sex ratio, growth parameters and condition factor of *Squalius* sp. to reveal the growth characteristics of the population in the Devrez River and compare the findings to previous studies. This study will aid in the development of sustainable management plans for the fish fauna in this basin and will provide a comprehensive database for future research on the biological parameters of the ichthyofauna.

2. Materials and Methods

The Devrez River is located in the northern part of Türkiye within the provincial borders of Çankırı, Kastamonu, and Çorum. It is a tributary of the Kızılırmak basin and approximately 200 km long, has an area of 3364 km^2 and a flow rate of 4,081 m^3/s (DSI 2014). A total of 141 individuals of *Squalius* sp. were collected by using cast nets (12-25 mm pore sizes) each month from April 2020 to March 2021 and assistance was received from local fishermen.

Fish specimens were measured for total length (TL), fork length (FL) and standard length (SL) to the nearest 1.0 mm and weighted (W) to the nearest 0.1 g. Sex was determined via macroscopic or microscopic observation of gonads. The overall ratio of males to females was evaluated using Chi-square tests. For age determination, 10–15 scales above the lateral line, below the anterior part of the dorsal fin were removed from each specimen (Lagler, 1966). The length–weight relationship was determined using the equation:

$$W = a\,L^{b}$$

where W is total weight (g), L is the FL (mm), b is the growth exponent and a is a constant. The log-transformed equation was used to establish the length-weight relationship:

$$\log W = \log a + b \log L$$

The parameters a and b were calculated using least-squares regression. The condition factor (CF) was calculated using the formula $(W/L^3)\,x\,100$ (Ricker 1975). The significance of differences in growth and mean CF values for males and females was determined using Student's t-test (Zar, 2010).

3. Results

In all, 141 *Squalius* sp. specimens were aged during the study, with the age-3 (3 years of age) group being dominant (41%) in the population followed by age-4 (33%). No male fish were captured

greater than 4 years of age and males were dominant only in age-2. There were no fish caught in age-8 and 9 and only one fish 10 years of age (Figure 1). Overall, the sex ratio of males (n= 43) to females (n= 98) was 0.44:1.00, which differed significantly from a ratio of 1.00: 1.00 (X^2= 21.452 > $X^2_{1,\ 0.05}$= 3.84).

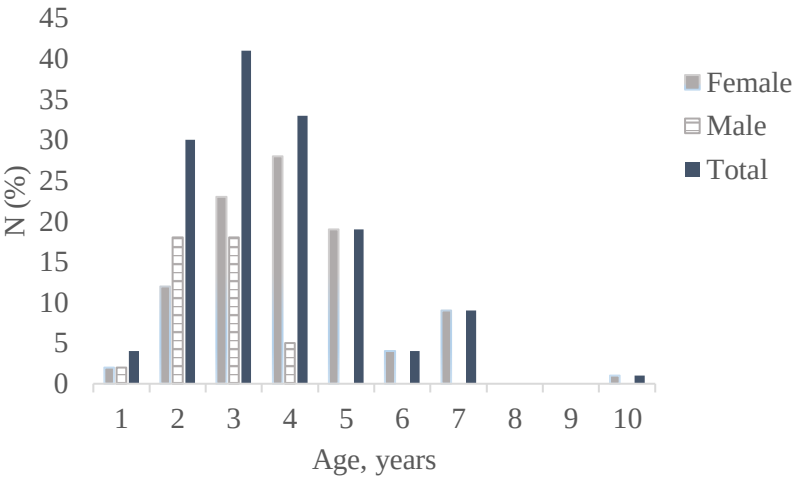


Figure 1. *Age distribution of Squalius sp. samples from the Devrez River*

The mean and minimum-maximum length (TL, FL, SL), W and CF of fish specimens were presented in Table 1. Females were longer than males in the population and the majority (28.4%) belonged to the 165- to 190-mm-FL size class. There were no significant differences in mean FL between males and females in same age groups (Student's t-test, t = 0.50, P > 0.05).

Mean CF values ranged from 1.03 to 1.69 for both sexes (Table 1) with a mean (±SD) of 1.35± 0.1. The CF values were calculated as 1.36 and 1.35 for females and males respectively (Figure 2). There were no significant differences in mean CF values between males and females in all age groups (Student's t test, t = 0.44, P>0.05).

Table 1. *Length, weight, and condition factor values in Squalius sp. population living in the Devrez River (min: minimum, max: maksimum, SD: Standard Deviation, TL: Total Length, FL: Fork Length, SL: Standard Length: W: Weight: CF: Condition Factor)*

	Female			Male			Total		
	min	max	mean±SD	min	max	mean±SD	min	max	mean±SD
TL (mm)	102	410	195.4±47.9	106	193	153.1±24.8	102	410	182.5±46.5
FL (mm)	95	391	183.9±44.3	100	182	144.4±23.8	95	391	171.9±43.2
SL (mm)	85	353	165.8±39.9	89	165	129.6±21.3	85	353	154.8±39.1
W (g)	11	1011	104.2±118.1	14.4	81	43.9±20.3	11	1011	85.8±102.8
CF	1.14	1.69	1.36±0.1	1.03	1.61	1.35±0.1	1.03	1.69	1.35±0.1

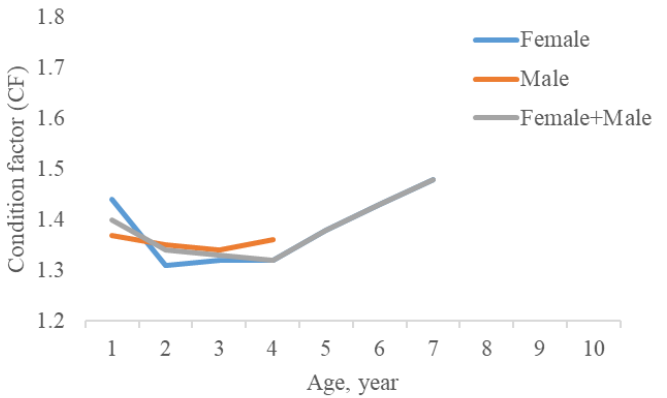


Figure 2. *Condition factor of Squalius sp. living in the Devrez River*

The estimated relationships between length and weight of chub *Squalius* sp. were given as follows:

Female $W = 0.000007 \times L^{3.11}$

$\log W = -5.154901 + 3.11 \log L$

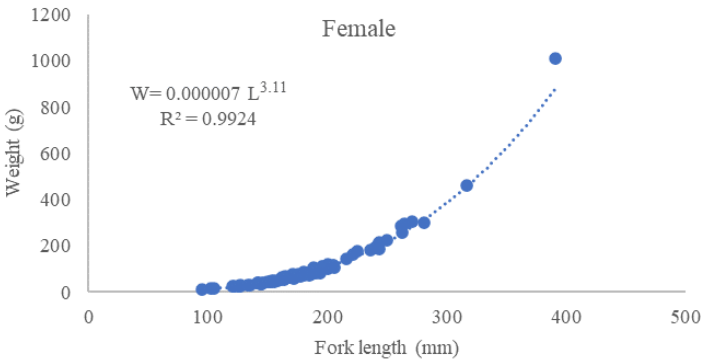
Male $W = 0{,}00001 \times L^{2{,}99}$

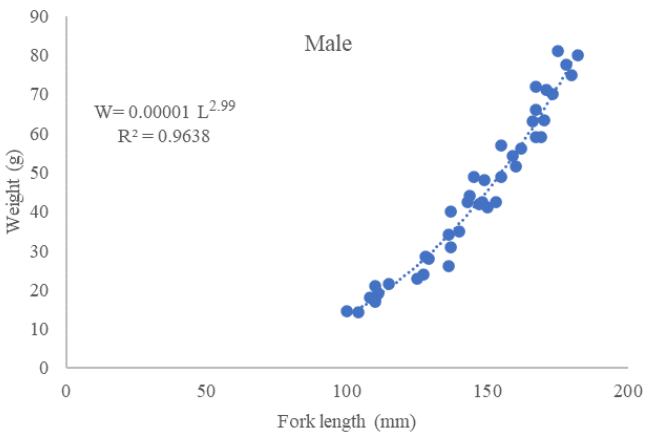
$\log W = -5.00 + 2{,}99 \log L$

Female+Male $W = 0{,}000009 \times L^{3.07}$

$$\log W = -5{,}045757 + 3.07 \log L$$

The growth exponent *b* generally lies between 2.5 and 3.5, but the relationship is reported to be isometric when *b*= 3 and allometric when *b* is a value other than 3 (Ricker 1975). According to *b* value, females showed positive allometric growth while males negative (Figure 3).





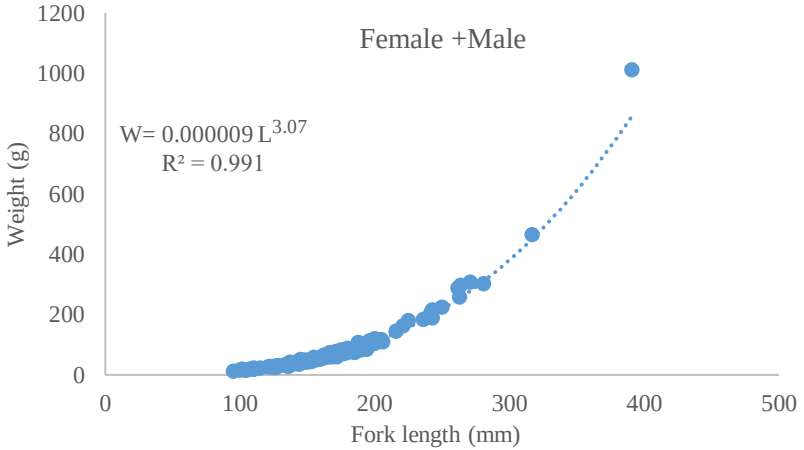


Figure 3. *Length–weight relationships for Squalius sp. inhabiting in the Devrez River*

4. Discussion and Conclusion

Since the species definition of the examined *Squalius* sp. in the Kızılırmak basin is not yet definitive, a comparison was made with other species from the genus. *S. cephalus* species has been examined in many studies in terms of growth characteristics (Table 2). The sex ratio of males to females of chub *Squalius* sp. was 0.44: 1.00 in this study. Similarly, some studies have found that the number of females in the population is higher than males. (Ünver & Tanyolaç, 1999; Ünver & Erk'akan 2012). Although the sex ratio for the majority of freshwater fish species is close to 1, but it differs considerably from species to species, varies from one population to another of the same species and may vary from year to year in the same population (Nikolsky, 1963).

In this study, the maximum age was the same or close to the ages reported by Ünver & Tanyolaç (1999) and Ünver & Erk'akan (2012) in Tödürge Lake, Pompei et al. (2011) in the Assino Creek,

Italy, and Sarı & Becer (2021) in Oymapınar Dam Lake, Türkiye. Length and weight distribution were found to be higher than in the previous study (Benzer & Gül, 2017) conducted in the same habitat.

The CF of *Squalius* sp. in the Devrez River was greater than that of *S. anatolicus* in Oymapınar Dam Lake (Sarı & Becer, 2021), but similar to that reported by Benzer & Gül (2017) in their study of the same habitat in the Devrez River. Analysis of population dynamics has shown that high condition factor values are correlate with favorable environmental conditions, including factors such as abundant habitats and food availability, while lower values indicate less favorable environmental conditions (Blackwell et al., 2000). The CF varies due to the interactions among feeding conditions, parasitic infections, and physiological variables (Le Cren, 1951).

The slope (*b*) values of the length–weight relationship in was found as a 3.07. Similar *b* values for *S. cephalus* were reported earlier (Ünver & Tanyolaç, 1999; Ünver & Erk'akan, 2012; Stefanova et al., 2008; Benzer & Gül, 2017). Higher values of *b* were reported by Dedeoğlu et al. (2020) and Sarı & Becer (2021) in Borçka Dam Lake and Oymapınar Dam Lake respectively than the values estimated in the present study. The variation in the exponents might be linked to differences in gonad maturity, sex, age, and species. Additionally, environmental factors such as season, time of day (due to changes in stomach fullness), and diseases may have also affected the value of *b* (Bagenal, 1978).

In conclusion, the findings of the present study indicate that the chub population of the Devrez River appears to exhibit many similar growth characteristics to species of this genus in various habitats, with a few exceptions. The length–weight regression results indicated a positive allometric growth characteristic for these specimens. Yet, the ongoing construction of the Devrez Kızlaryolu Dam is believed to pose the a significant threat to fish within the Devrez River (Ablak Gürbüz, 2023). This is primarily due to expected changes in the flow regime and the inadequate volume of reserved water allocated for sustaining aquatic organisms. To ensure

the conservation and sustainability of fish stocks, fish should be given the opportunity to ensure the continuity of their generations, while periodic assessments are conducted to mitigate any adverse factors.

Table 2. *Comparison of some growth characteristics of chub Squalius sp. in different habitats*

Location	Species	N	FL (mm)	W (g)	CF	a	b	R^2	Age	References
Devrez River	*Squalius* sp.	141	95-391	11-1011	1.35	0.000007[a]	3.11[a]	0.99[a]	I-X	Present study
						0.00001[b]	2.99[b]	0.96[b]		
Tödürge Lake (Sivas)	*S. cephalus*	674	53-287	1.5-347.1		0.0101 [a]	3.09[a]	-	I-VII	Ünver&Tanyolaç, 1999
						0.0121 [b]	3.03[b]	-		
Tödürge Lake (Sivas)	*S. cephalus*	373[a] 93[b]	53-279 [a] 66-201 [b]	1.5-320.8[a] 3.8-120.5[b]		0.010 [a] 0.012 [b]	3.10[a] 3.03[b]	- -	I-VII	Ünver&Erk'akan, 2012
Gelingüllü Dam Lake	*S. cephalus*	460	119-312	32-600	0.68-1.98	0.0248	2.87	0.91	I-V	Kırankaya& Ekmekçi, 2007
Maritza River (Bulgaria)	*S. cephalus*	161	121-150		2.52[a] 2.01[b]	0.0148	3.05	0.99	I-IV	Stefanova et al., 2008
Assino Creek (Italy)	*S. squalus*	1311	41-488*	0.50-1233			3.19[a] 3.12[b]	0.99[a] 0.98[b]	0-XI	Pompei et al., 2011
Devrez River	*S. cephalus*	170	87.9-196.4	9.7-126.6	1.432[a] 1.378[b]	0.0108[a] 0.0146[b]	3.11[a] 2.97[b]	0.90[a] 0.91[b]	I-VI	Benzer&Gül, 2017
Borçka Dam Lake (Artvin)	*S. orientalis*	392	141-532	28.1-2267		0.0035[a] 0.0058[b]	3.36[a] 3.21[b]	0.99[a] 0.99[b]	III-XVI	Dedeoğlu et al., 2020
Oymapınar Dam Lake (Antalya)	*S. anatolicus*	422	200-551	93.4-2307.9	1.278	0.0064[a] 0.0068[b]	3.20[a] 3.18[b]	0.98[a] 0.98[b]	II-VIII	Sarı&Becer, 2021

[a] Female, [b] Male, * Total Length

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CHAPTER VIII

Hydrothermal Liquefaction Of Hazeulnut Shell With - Solvent Glycerol And Alkaline Catalyst Hazelnut

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Levent BALLİCE[2]
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Giriş

Concerns about the threat of fossil fuel depletion have grown in recent years due to the rise in demand for fossil fuels and the increase in environmental pollution brought on by their widespread use. As a result, the search for alternative renewable and sustainable energy sources for fuels has started. The resources to be found

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should be used as an alternative source for traditional fossil fuels, which should be sustainable, provide energy security and reduce global warming.

This leads to a focus on the search for alternative resources such as biomass. Biomass sources include wood waste, energy crops, aquatic crops, agricultural products and their waste products, and municipal and animal waste; and are considered potential sources of fuel and chemical raw materials. The production of fuel from lignocellulosic biomass and the using renewable energy sources for this purpose are very important in terms of sustainability. In this context, it is possible to obtain valuable chemical and structural transformations by applying various processes. In addition, the catalysts to be used in biochemical and thermochemical processes directly affect the yield and composition of the product to be obtained at the end of the process. While the substances that catalyze the structural degradation in biochemical transformation processes can be enzymes or microorganisms, thermochemical transformation processes provide the necessary decomposition in the lignocellulosic structure with the help of temperature and acidic/base catalysts. Since thermochemical conversion processes can be applied directly or indirectly, the processes can be grouped into two. In the direct conversion process, if the purpose is only to generate energy, the combustion process is applied. However, if it is aimed to produce valuable chemicals from the structure, pyrolysis and gasification processes can be applied in different process conditions to obtain solid, liquid, and gaseous products.

In recent years, hydrothermal methods have been studied intensively, and besides the clean solvent and cheapness of water, the presence of water in the wet biomass structure provides significant advantages in use. The supercritical hydrothermal treatment in water, depending upon changes and it occurred in the physical properties apolar showing solvents behavior, high-resolution effect, such as high diffusion effects, as well as fluids such as gas, water is an ideal solvent to a large extent makes it advantageous to use in production.

Lignocellulosic biomass can be dissolved more efficiently with the aid of solvents and the formation of valuable chemicals after structural degradation makes its use more efficient. It is important to investigate the use of the solvent as a by-product and a waste of another reaction rather than being a pure compound in such studies. Thus, many benefits can be obtained both in terms of waste disposal and use, and in the production of valuable chemicals, as well as contributing to the structural breakdown of renewable biomass and contributing to the production of valuable chemicals.

There is no such comprehensive study in the literature. It is also important for the originality of the study. Glycerin penetrates the structure during the process and shows swelling behavior in the structure, the positive effects it will cause to dissolve the solid matrix structure by the hydrogen bonds it will form with the structure, and the thrust behavior of the glucose units forming the cellulosic structure by penetrating into the gaps that will form in the cellulosic structure, effects, such as showing, can be cited as the reasons for using glycerin as solvent in this study. The studies to be performed and the data obtained will show how much this hypothesis is compatible with reality. Within the scope of the study, it is also aimed to increase the solubility efficiency of the lignin, especially in the structure, by using some alkali catalysts. It is expected that the alkali catalyst to be used will allow the structure to penetrate into the structure and thereby cause the decomposition in the structure of the lignin, due to the weakening of the lignin-carbohydrate interaction by using glycerin in the thermal degradation processes to be carried out in the low and moderate temperature range, and subsequently by the effect of the glycerin, especially at high temperatures, by reducing the solution surface tension. Thus, it is predicted that high conversion rates can be achieved.

In this study, it is aimed to analyze the effects of the glycerin structure to penetrate the lignocellulosic structure by using glycerin, which is a by-product of biodiesel and a polyalcohol, as a solvent for this purpose. Especially in high temperature processes that require high activation energy, the solvent effect was investigated. In the

liquefaction process, the high boiling temperature of the glycerin and its thermal stability, as well as its contributions to the dissolution of the biomass and the formation of valuable chemicals by creating a direct solvent effect at lower temperatures was evaluated comprehensively. In addition, by adding alkali catalysts to the medium, the catalyst effect on biomass conversion was examined.

1. LITERATURE REVIEW

One of the most effective ways of converting lignocellulosic biomass to biofuel is the hydrothermal liquefaction method. The reason why heterogeneous catalysts use the HTL process is more efficient than homogenous is because it can be recovered at the end of the reaction. This means that it is used several times more in heterogeneous catalysts than homogeneous catalysts. In this regard, Scarsella et al., investigated the effects of heterogeneous catalysts consisting of four groups (alkaline earth metals, transition metals, lanthanides oxides and zeolites) on the efficiency and high heating value (HHV) of bio raw materials. Catalysts based on heterogeneous alkaline earth metals are widely used; especially in carbohydrate dissociation reactions. These catalysts are calcium oxide, colemanite ($Ca_2B_6O_{11}$~$5H_2O$), magnesium-based oxides (MgO and $MgMO_x$, where M can be Mn, Ni, Fe, Cr, Zn and Al) and hydrotalcite. ($Mg6Al_2(CO_3)$ (OH)16~$4H_2O$). In all studies except $MgMnO_2$, it was found that the quality of the bio-crude product increased when the yield increased; Constructed without catalysts has a lower HHV compared to subjects. High liquid product yield is obtained due to the catalytic activities in the reactions of Transition metals, (Nickel, Copper, Cobalt, Iron, Manganese, Zinc, Vanadium, Palladium and Ruthenium.). The catalytic activity of lanthanide oxides in the HTL of lignocellulosic biomass has also been investigated in recent years. During the experiments with La_2O_3, Dy_3O_3 and CeO_2, it was observed that the biomass obtained more bio-oil yield and its quality increased in the presence of CeO_2 catalyst. Results from subjects with zeolites showed a slight improvement in the yield and quality of the biological raw material of zeolites. As a result, transition

metals and lanthanides oxides resulted in a 38% increase in bio-crude yield and a 19% increase in HHV, alkaline earth metals catalyst led to the highest yield of biological raw materials. But in experiments without a catalyst, it was revealed that 10% lower HHV was obtained from crude oil. (Scarsella et al., 2020)

The researchers found the effect of solvent type to be different when liquefying the lignocellulosic biomass. But acetone, phenol and 1,4-dioxane turned out to be more effective in liquefying. Solvents with high polarity and hydrogenation properties must be used to obtain higher efficiency. Huang and Yuan, compared some of the studies they compiled: The liquefaction of Phragmites australis in methanol and ethanol, and the Verbascum stalk in methanol, ethanol and acetone, the time of the process and the results of the experiment compared the bio-oil yields. Although the bio-oil yield of P. australis was higher in the highest ethanol experiment, the bio-oil yield of Verbascum stalk showed the lowest yield in the experiment with ethanol and the highest yield in the experiment with acetone. (Huang and Yuan, 2015)

During the research made with Typha latifolia, giant fennel (Ferula orientalis L.) and Onopordum heteracanthum, 5 different organic solvents were used. Which of the methanol, ethanol, 2-propanol, acetone and 2-butanol is the more effective liquefaction solvent was examined and the highest efficiency was achieved in the experiments with acetone. This was because the lignin and acetone contained the same functional groups. (Huang and Yuan, 2015)

It was observed that polar protic solvents were more suitable than non-polar solvents for the liquefaction time of oil palm empty fruit bunch fiber and sawdust. Ethylene glycol (EG), water and ethanol as polar protic solvent, acetone as polar aprotic solvent and toluene as non-polar solvent were used during liquefaction time. The highest bio-oil and gas yields were achieved when polar protic solvents were used. When the solvent-water mixture was used, higher conversion of the experiments, increased lignocellulosic degradation and higher biooil yield were obtained. When the effect

of methanol-water, ethanol-water and phenol-water mixtures on corn stalk was examined, methanol-water was more effective than the others. The conversion rate of liquid products reached 88.1% and the yield of 52.4% in 30 minutes and 300 °C. The highest liquid product yield (70%) was obtained with 65% vol. at 250 °C for 90 minutes. (Huang and Yuan, 2015)

Hydrothermal co-liquefaction has attracted the attention of researchers as it increases the yield and quality of biomass. Yang et al. examined how the hydrothermal co-liquefaction process of several biomasses affected the biochar yield during the time. The observed synergistic effects should be critically evaluated because of the inconsistent reaction/separation conditions, the uncertain statistical significance of the experimental data, and the various indicators of co-liquefaction effect in the reported studies. The amounts of cellulose, hemicellulose, lignin, lipid and protein in the biomass were also meticulously investigated to find the source of the effect of co-liquefaction (Xu et al., 2016). Progress made is reviewed, including models used to predict the yield relative to biomass chemical composition or reaction parameters, and kinetic models of the network of reactions between components of biomass. Optimizing the raw material mix and liquefaction time of biomass are useful models for investigating yield. The statistical significance of the co-liquefaction impact, evaluation of the co-liquefaction effect under identical liquefaction and separation circumstances, and molecular comprehension of the co-liquefaction effect are all cited as key issues in the study of co-liquefaction. Biomass is often bulky, has a low energy density, and has variable properties/compositions throughout the year. It's difficult to collect enough of one form of biomass in a given area to make overall production economically viable. As a result, co-liquefaction can drastically lower the costs of collecting and transportation. The improved processability of slurry feedstock for continuous operations is another advantage of co-liquefaction. More crucially, by altering the biological content of feedstock mixes, co-liquefaction has the ability to boost yield and vary the physicochemical qualities of generated biocrude. Finally,

hydrothermal co-liquefaction of multiple biomasses is thought to be preferable to hydrothermal liquefaction of individual feedstocks because of the possible synergistic influence on biological raw product yield/quality and low logistics costs for raw material collection and delivery. (Yang et al., 2021)

Hydrothermal liquefaction is a promising method for the future as it is attractive in terms of energy consumption. The use of homogeneous and heterogeneous catalysts has also become widespread in order to achieve greater efficiency. Toor et al. conducted an experimental investigation with both groups of catalysts. In the experiment with corn straw, it was observed that the bio-crude product yield increased from 33.4% to 47.2% when 1% Na_2CO_3 was added to the biomass. Additionally, adding an alkali catalyst to wood biomass at a temperature of 280oC for 15 minutes raises the yield from 17.8% to 33.7%. Other than that, potassium salts outperformed sodium salts in terms of catalytic activity, with K_2CO_3 coming in first followed by KOH, Na_2CO_3, and then NaOH. The catalysts increased liquid yields while lowering solid residue levels. Homogeneous catalysts also have other advantageous effects, such as improved fatty acid decarboxylation. In conclusion, introducing catalysts has been found to improve the liquefaction process and can boost the yield as well as quality of liquid products. (Toor et al., 2011)

In general, the optimum HTL conditions for various feedstocks are the temperature range of 250– 375 °C and pressure of 4–25 MPa. The presence of water in HTL facilitates the easy hydrolysis, degradation and repolymerization of cellulose, hemicellulose and lignin present in biomass and converts them to biofuels such as bio-oil, char and gases. There are some major factors that affect the reaction and products such as; reaction temperature, heating rate, pressure, retention time, feed ratio, solvents, type of catalyst and the environment used. Hydrothermal liquefaction of biomass feedstocks has been studied in the temperature range of 180–400 °C with mid temperature range (50– 350 °C) and higher temperature range (300–400 °C). There have been reports of heating speeds of 3 °C/min, 4

°C/min, and 10 °C/min (Mathanker et al., 2020). In this situation, Akhtar et al. found that once supercritical conditions were reached during liquefaction, pressure had little to no impact. In a series of studies, Wang et al. and Cheng et al. used several solvents to create alcohol (methanol, ethanol), as well as discovering other solvents to be highly efficient. As a result, we can conclude that catalysts are crucial in determining the course of a reaction, the formation of products, and their quality. Additionally, Minowa et al. noted that the addition of alkali increased oil output while reducing char formation. Additionally, the alkaline pretreatment of biomass improves cellulose's surface accessibility. In order to understand the effects of various alkali hydroxides and carbonates, Karagoz et al. and Akhtar et al. carried out a series of experiments and ordered the results as follows: K_2CO_3 > KOH > Na_2CO_3 > NaOH > H_2O. A parametric research on HTL of corn stover was conducted as part of the work's conclusion and found it to be a potential method for producing biofuels. They also noticed that the yield of HO (heavy oil) dropped as temperature increased above 300 °C and retention duration increased above 0 min. The maximum yield of HC (Hydrochar) is achieved at 350 °C when using HTL primarily for hydrochar. While retention time has no impact on HC yield, pressure increase has a negative impact on it. The total gas phase increased with higher pressure, longer retention time and above 350 °C. (Akhtar et al., 2010, 2011; Wang et al., 2013; Cheng et al., 2010; Minowa et al., 1998; Karagoz et al., 2005)

In the process of liquefying biomass, the use of glycerol as a solvent encourages the diffusion of lignin degradation products from the wood to the solvent and the penetration of alkali into the particle structure, ensuring that the reagents are distributed uniformly throughout the biomass. In this context, Demirbaş conducted an experiment in which sawdust, glycerol and water were loaded into the autoclave with or without Na_2CO_3 or NaOH catalyst. Initially, direct glycerol liquefaction of poplar sawdust was investigated and it was found that the overall liquefaction efficiency increased with increasing reaction temperature. In addition, alkali (5% Na_2CO_3) and

(5% NaOH) glycerol liquefaction studies were performed and it was found that the total liquefaction efficiency increased with increasing reaction temperature. (Demirbaş., 2008).

Parameters such as reaction temperature, heating rate, pressure, holding time, feed rate, solvents, catalyst type and medium were used to affect the reaction and products. Hydrothermal liquefaction of biomass was investigated in medium temperature (50-350 °C), high temperature (300-400 °C) and 180-400 °C temperature ranges. Three different heating rates (3 °C/min, 4 °C/min and 10 °C/min) were used during the experiment. (Ankit et al., 2020).

In the hydrothermal liquefaction process, the glycerin/water ratio and the addition of catalyst have important effects. Cao et al. used glycerin as a solvent during hydrothermal liquefaction time of rice straw and the change in oil yield was investigated. Experiments with different glycerin/water ratios were carried out in a high pressure batch reactor. The elemental composition, heating value, water content, ash content, and acid number of the resulting bio-oil were all examined to determine the quality of the product. The most efficient result was obtained during the experiment with 1:1 volume glycerol/water ratio containing 5% Na_2CO_3 by weight, which lasted for 1 hour at 260 °C. Under these conditions, 50.31% by weight of bio-oil and 26.65% by weight of solid residue were produced. According to the results obtained, glycerin HTL can use time as a co-solvent and help to obtain higher efficiency. It has been investigated that the presence of Na_2CO_3 further increases the effect of glycerol. Since lignin is the most difficult to decompose among the components of biomass, an increase in lignin content during liquefaction time causes an increase in solid residue. (Cao et al., 2016)

The hydrothermal liquefaction process is a very efficient chemical way of generating energy and generating resources from lignocellulosic biomass. Glycerol, a by-product of biodiesel, is a commodity chemical used in a wide variety of commercial

applications. Crude glycerol, the unrefined by-product from the transesterification step in biodiesel production, is mostly a bulk emulsion of glycerol, transesterification residues such as salts (FFAs) free fatty acids (FFAS) and trapped fatty acid methyl esters (fames). It caused the increased production of glycerol and the formation of glycerol excess. This helps to lower the market value and provide opportunities for alternative energy generation. In several investigations on the subject, it was looked into whether wood could entirely liquefy in glycerol in the presence of an alkaline catalyst. The main aim is to experimentally investigate the synergistic effects of co-liquefaction of crude glycerol and poplar wood under supercritical water conditions and to try to establish a reaction framework for the conversion process. Pedersen et al. aimed to examine the effects of raw material composition (pure/crude glycerol/poplar wood mass ratio) and process conditions on product quality. For this, 14 experiments were performed to evaluate the liquefaction time processing aspects and synergies. It was observed that the mixing of poplar wood with the formation of glycerol charcoal could be significantly reduced. In the case of poplar wood alone, a charcoal yield of about 18% drops almost exponentially to 2.7% when glycerol alone is processed. Additionally, the crude glycerol to aspen wood ratio had a big impact on the biocrude quality in terms of the effective hydrogen-to-carbon ratio (H/C_{eff}). The H/C_{eff} was 1.6 at crude glycerol to aspen wood ratio of 3:1 as opposed to 0.96 with aspen wood alone. The two primary feedstock components were shown to have a direct impact on the chemical composition of the biocrude, making it easy to forecast the biocrude's composition and, consequently, manage the biocrude's attributes through feedstock mixing. (Pedersen et al., 2015)

2. MATERIAL AND METHODS

2.1 Materials

In the study, the biodiesel by-product glycerin was prepared from pure glycerin and was used as a model solution. The shells of hazelnut were used as the biomass sample, which is widely produced

in our country and which has an important place in the production maintenance in the world. Potassium hydroxide (KOH) and potassium carbonate (K_2CO_3) was used as the alkaline catalyst. The hazelnut shells used in the working time were grown in Giresun, located in the Black Sea region, and 600,000 tons of hazelnut shells were produced in the last 5 years on an area of approximately 350,000 hectares. This production meets 70-75% of the total crop in the world. (Erdogan and Strossman, 2018).

The equipment used in this study for the characterization of biomass consists of a Thermobalance SDT 2960 from TA Instruments coupled to a NEXUS FTIR spectrometer from Thermo Nicolet via a short stainless steel heated line (at 150 °C). A flowing inert gas was used as the carrier during the TGA testing, and the flow rate was 100 mL/min. To avoid secondary reactions in the oven and achieve the right component concentrations in the pyrolysis gas, the carrier gas flow was modified.

The biomass was ground into particles smaller than 100 μm. Biomass samples were divided into lignin and cellulose using the standard test method of Van Soest detergent. (Van Soest et al. 1991). NDF (neutral detergent fiber) to determine the amount of cellulose, hemicellulose and lignin in the compound, ADF (acid detergent fiber) to determine the total amount of cellulose, and ADL (acid detergent lignin) to find the total amount of lignin were performed. The procedures performed with the Van Soest method were repeated three times and no deviation was found. Analysis results are given in Table 1 and 2 (Ballice et al., 2020).

Table 1. Elemental analysis results of Hazelnut shell. (Ballice et al., 2020).

Elements (% wt)	Hazelnut Shell
C	52,20
H	5,90
N	0,25
S	0,03
K	0,59
Ca	0,42
Mg	0,08
Al	0,03
Fe	0,02

Table 2. The composition of Hazelnut shell (Ballice et al., 2020).

Properties	Hazelnut Shell
Moisture (%wt)	6,80
Ash (%wt)	1,67
Crude Protein (% wt)	3,97
Total Extractives (% wt)	8,60
Cellulose (% wt)	38,20
Hemicellulose (% wt)	12,10
Lignin (% wt)	40,00

2.2 Methods

2.2.1 Experimental System

Liquefaction experiments were carried out in a 100 mL batch autoclave system that can be operated under reaction conditions up to 500 atm pressure and 600°C. It includes gas distribution pipes, furnace, heat and pressure control units, autoclave mixing system, gas collection and metering unit and high pressure valves. Solvent glycerin as a model solution of pure glycerin in three different ratios as the water content (6 mL: 12: mL), (9 mL: 9 mL) and (12 mL: 6 mL) solution was used. After 2 g of hazelnut shell and 18mL of

glycerin+water in the ratio specified in the experimental condition, the material was placed in the reactor, the air in the reactor was removed with N_2 gas and the valves were closed. It is heated up to the reaction temperature at heating rates of 5-10K/minute and studies are carried out at this temperature for an hour. At the end of the reaction period, the reactor is cooled, then the gas valve in the reactor is opened and water saturated with CO_2 at room temperature is collected in a gasometer and its volume is measured and samples are taken for analysis. The aqueous portion of the reactor is separated from the coke and unreacted biomass particles that remained at the end of the operation were separated by filtration process and then weighted.

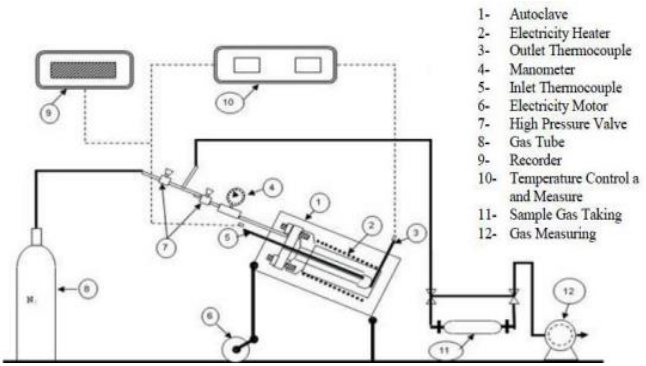


Figure 1. Batch reactor system.

2.2.2 Experimental procedure

In experimental studies within the scope of the thesis, independent variables (factors) and levels defined in the experimental program created by Box-Behnken experimental design were as follows:

- Glycerin concentration: 0.333, 0.500, 0.666 mL glycerin / mL solvent mixture
- The recommended temperature for the reaction: 200, 300 and 400°C
- Reaction time: 30, 60, and 90 minutes.

All experiments in the program will be repeated at least three times. The same program will be repeated without catalyst and in the presence of two type catalysts (K_2CO_3, KOH).

Table 3. Experiment design table of the study with hazelnut shell and glycerine+water mixture in the presence of an alkaline catalyst (KOH and K_2CO_3)

Run number	**Temperature, °C**	**Time, min**	**Glycerin concentration, mL glycerin/ mL solution**
1	300	30	0,666
2	200	30	0,500
3	400	60	0,333
4	400	90	0,500
5	300	30	0,333
6	200	90	0,500
7	200	60	0,333
8	400	30	0,500
9	400	60	0,666
10	300	90	0,666
11	300	60	0,500
12	300	90	0,333
13	300	60	0,500
14	300	60	0,500
15	200	60	0,666

After the mixture of biomass and solvent was added to the reactor, the air was removed from the reactor with nitrogen stream. According to the experimental design table, it was waited until it reached the appropriate temperature for each test condition. During this time, temperature and pressure values are noted every ten minutes. After reaching the determined temperature, it continued to monitor the pressure and temperature. After the reaction time is over, the reactor is cooled. It is then rapidly cooled to room temperature again using an ice water bath.

During the gas analysis experiments, the cooling of the reactor to 25°C is followed. Under atmospheric conditions, the output gas volume was calculated with the help of gas-tight syringes and some amount was obtained. The accuracy of the gas volume is measured to be ±10%.

The products remaining in the reactor are washed and filtered through filter paper. Liquid product was stored in ISOLAB Bottle - P.E - Transparent - Wide Neck - Screw Cap - 100 ml containers for analysis. The solid residue was oven dried. During some experiments, sticky solid products formed in the reactor appeared. To analyze these products, they were dissolved in acetone and kept in a flask for analysis in GC-MS. After all treatments, the reactor was washed. After washing, tetrahydrofuran (THF) was removed again. The quantities of liquid, solid and gaseous products were measured.

The products to be obtained after the experiments was analyzed in GC, GC-MS, HPLC and TOC-SSM devices, and the amount and composition of the solid, liquid and gas product to be obtained was determined.

Table 4. Devices for product analysis.

Products	**Components**	**Equipment**
Gas	Methane, Ethane, Propane, Butane H_2 and CO, CO_2	GC
Liquid	Organic acids and sugars	HPLC
	Total Organic Carbon Content	Total Organic Carbon
	Qulitative Analysis of possible compunds	GC-MS
Biomass and solid residue	Total Organic Carbon	TOC-SSM

2.3 Analytical methods

2.3.1 Gas phase analysis

Gas products was analyzed using the HP7890A gas chromatographer equipped with two thermal conductivity detectors (TCD) and a flame ionization detector (FID). At the time of analysis, the sample goes through 3 stages.

Hat Gaz Company in Kocaeli, Turkey, provided a standard gas mixture for GC calibration. Gas chromatographic methods were used to determine the composition of the gaseous products (H_2, CO_2, CO, and C_1–C_4 hydrocarbons). The standard deviation of the gas composition results was determined to be less than 2%. Table 4.3.1.1 and Figure 4.3.1.1 provide some technical characteristics and operating conditions of gas chromatography, as well as a picture of it.

Table 5. Analytical condition of gas chromatography measurements.

Gas Chromotography Model	**HP7890A**
Columns	Hayesep Q 80/100 mesh (0.5 m x 1/8"), Column 1. Hayesep Q 80/100 mesh (6 feet by 1/8"), Column 2. Molsieve 5A 80/100 mesh (8 feet by 1/8"), Column 3. Hayesep Q 80/100 mesh (3 feet by 1/8"), Column 4. Molsieve 5A 80/100 mesh (8 feet by 1/8"), Column 5. DB-1 (pre-column), Column 6. HP-Plot Al2O3 S (25 m x 0.32 mm x 8), Column 7.
Temperture Program	60˚C / min, 20˚C/ min speed to 80˚C 30˚C/ min speed to 120˚C 2.66 min.

Carier Gas	He (Helium)
Operating Mode	Split (Flow: 60 mL/min, Range: 80:1)
Injection Temperature	250˚C
Detector Types and Operating Temperatures	Front signal – FID- 250˚C Back signal – TCD- 250˚C Auxiliary signal – TCD- 250˚C

2.3.2 High Performance Liquid Chromatography (HPLC)

High Performance Liquid Chromatography (HPLC-Agilent) is currently one of the most widely used instruments for the analysis of organic acids and carbohydrates. Its usefulness enables it to be used in many areas of the industry. The device consists of four parts called pump, degasser, column and detector.

The degasser removes gas bubbles that are due to injection or formed in the mobile phase. The pump pulls the mobile phase from the needle injection port and moves it along the column. The column sends the sample to the units by keeping the flow rate and pressure under control. Detectors detect components of the solute. Analysis was performed using 0.5 mM H_2SO_4 solution as mobile phase. The method used, features and working conditions are shown in Table 6.

Tablo 6. HPLC specifications and operating conditions for method.

Device	**HPLC-RI (Agilent 1200A)**
Column	HPX-87H column
Mobile Phase	5 mM H2SO4
Flowrate	0.6 mL/min
Detector	Refractive Index
Colon Temperature	30°C
Detector Temperature	30°C
Injected Volume	20 µL
Analysis Time	50 min

2.3.3 Total organic carbon. (TOC)

It is easier to perform high precision TOC analysis using the new model TOC-VCPH. Since the TOC-VCPH is equipped with a multi-functional 8-port valve and a 5mL syringe, functions such as automatic acid addition and non-pure organic carbon (NPOC) analysis, mixing and spraying in the syringe for dilution can be performed. This instrument is also for measuring the total nitrogen value. is also used.

The carbon value in the liquid samples was determined using a total organic carbon device (Shimadzu TOC-VCPH). Total carbon value and organic and inorganic carbon values were also calculated separately. As indicated in the experimental procedure phase, solid residues were recovered after gasification by filtering the reaction byproduct. After that, the solids are dried in an oven that is kept at 100°C all night. Many solid samples, including soil, sludge, and sediments, can be analyzed using the SSM in conjunction with a TOC analyzer in addition to aqueous samples. Figure 4.3.3.1 displays a TOC-SSM analyzer.

3. EXPERIMENTAL RESULTS

The effects of temperature, experiment duration, and catalyst on the production of gaseous, liquid, and solid products during the hydrothermal liquefaction of hazelnut shells were examined in this experimental investigation. Three different ratios of water and glycerin were utilized as the solvent (0.333, 0.500 and 0.666). According to the Box-Behnken design, the presence and absence of catalyst was conducted at 200, 300, and 400 °C temperatures, and 30, 60, and 90 min experiment times. As catalysts, KOH and K_2CO_3 were employed. As a result of the experiment, various products appeared and the expected modifications were observed in these products with solid, liquid and, gas analyzers (GC, GC-MS, HPLC, TOC, TOC-SSM). Tables 7 present the experimental findings.

3.1 Gas Chromatography analysis

When we look at the gas analysis of the three runs of 2 g hazelnut shell at 400 °C with a 0.333 solution of glycerin and uncatalyzed water mixture, with KOH, and K_2CO_3 catalysts, it is seen that the highest hydrogen yield (37.8%) reached in the experiment with KOH but methane (13.7%) and ethane (5.2%) conversion were obtained highest result in the experiment without catalyst as seen in Fig.2.

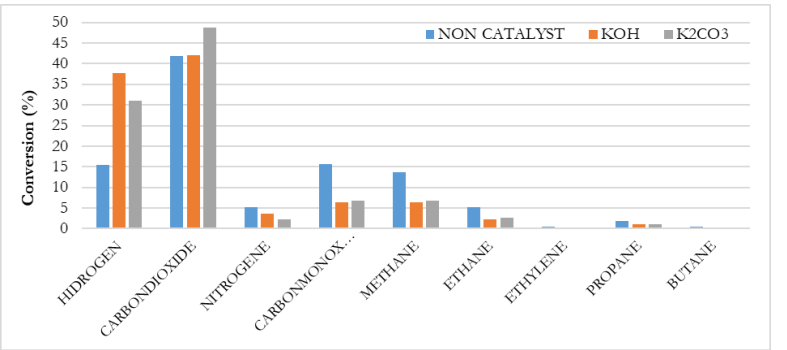


Figure 2. GC analysis of the conversion chart of results under 3rd experimental conditions (400°C, 60 min and 0.333 glycerine concentration)

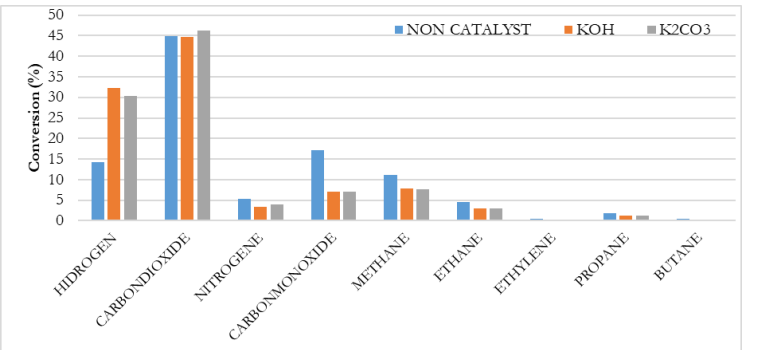


Figure 3. GC analysis of the conversion chart of results under 4th experimental conditions (400°C, 90 min and 0.500 glycerine concentration)

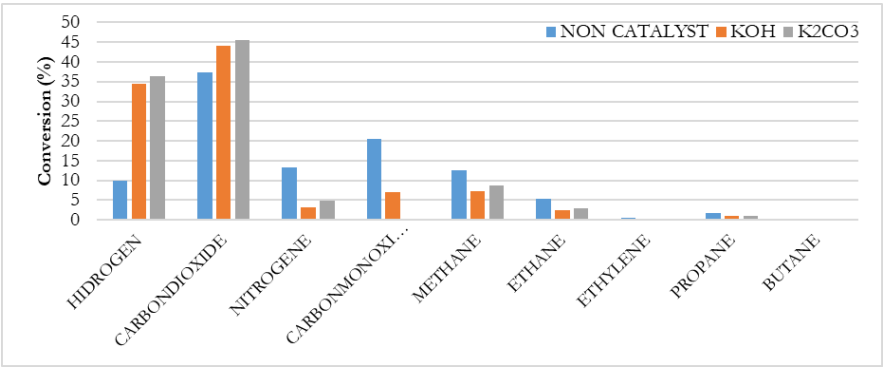


Figure 4. GC analysis of the conversion chart of results under 8th experimental conditions (400°C, 30 min and 0.500 glycerine concentration)

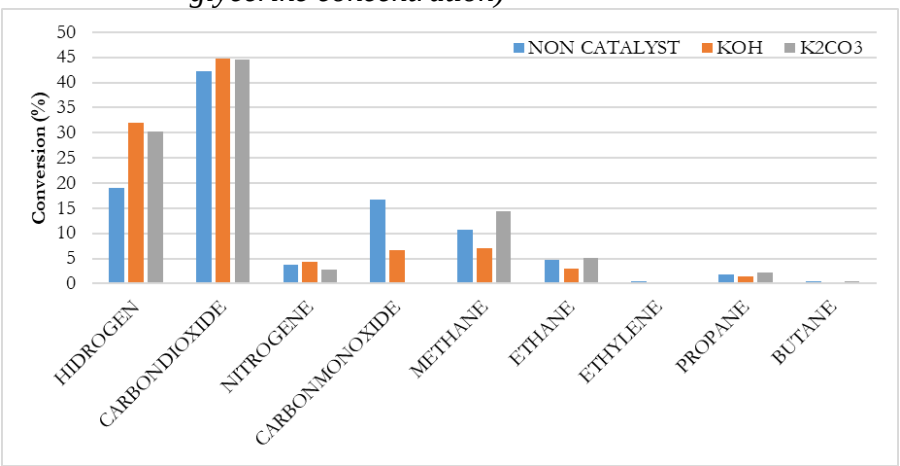


Figure 5. GC analysis of the conversion chart of results under 9th experimental conditions (400°C, 60 min and 0.666 glycerine concentration)

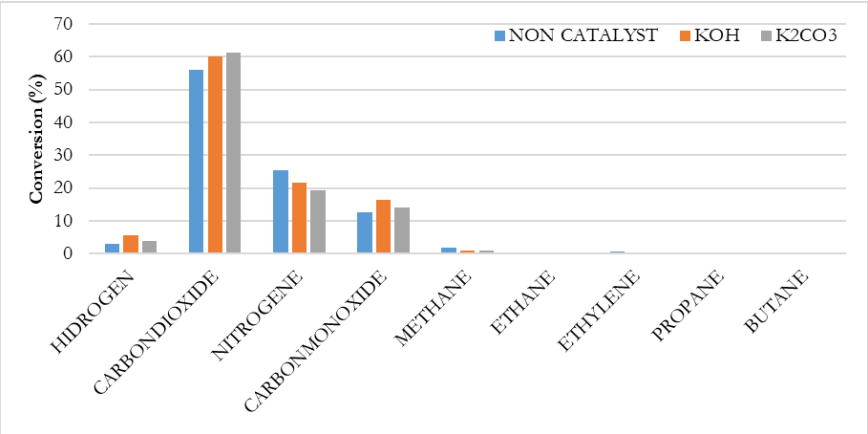


Figure 6. GC analysis of the conversion chart of results under 12th experimental conditions (300°C, 90 min and 0.333 glycerine concentration)

Gas analyses of the experiment conducted at 400 °C were made, but not much gas was obtained under the experimental conditions at 300 °C. To determine how much gas was formed at 300 °C only gas analyses of the experiment in only the 12th experiment condition were made. Table 7 displays conversions for both gases and liquids. The total of the solid residue and tar fraction that makes up the remaining conversion percentage was determined.

As a result of the experiments lasting 90 minutes at 400°C temperature and 0.5 glycerin/water ratio, the highest hydrogen efficiency was obtained with KOH catalyst as displayed in Figure 3. Methane (11.2%), and ethane (4.5%) obtained as the highest ratio in the result of the experiment without catalyst. As a result of the experiments, which lasted for 30 minutes at 400°C temperature and 0.5 glycerin/water ratio, the highest hydrogen (36.4%) ratio was obtained with the K_2CO_3 catalyst as shown in Fig. 4. The highest methane (12.7%), and ethane conversion (5.5%) were the results of the experiment without catalyst. As a result of the experiments lasting for 60 minutes at 400 °C temperature and 0.666

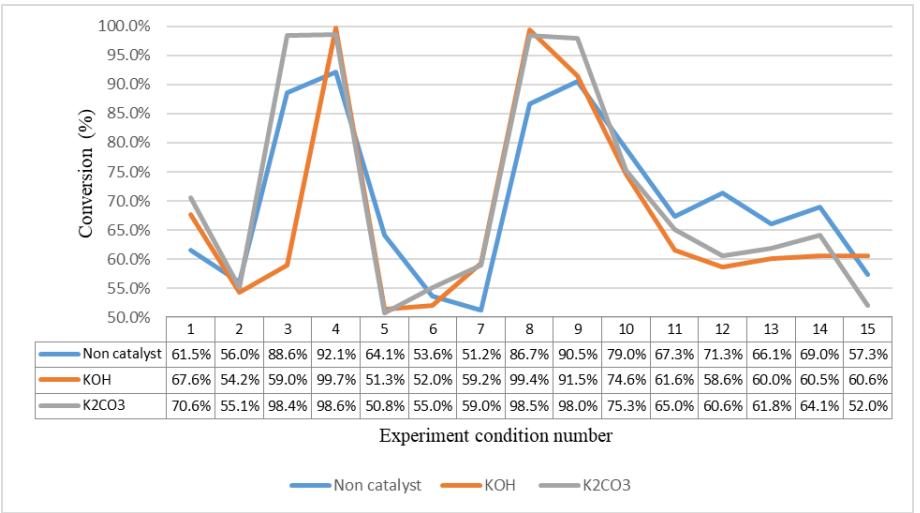
glycerin/water ratio, the highest hydrogen yield (32%) was obtained with the KOH catalyst as seen in Fig. 5. The highest Methane (11.5%) and Ethane (5.2%) conversion were obtained in experiment with K_2CO_3.

As a result of the experiments lasting 90 minutes at 300°C temperature and 0.333 glycerin/water ratio, the highest hydrogen (5.6%) yield was obtained with the KOH catalyst, and he results are given in Fig. 6. It was found that methane, ethane, ethylene, propane, and butane ratios very little.

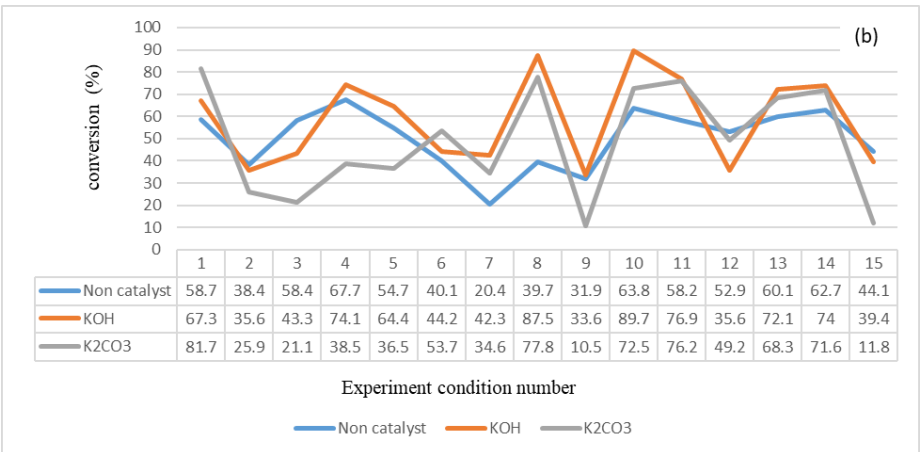
Table 7. Total conversions of experiments at high temperature.

Temperature= 400 °C, time= 60 min. and glycerin/solvent con= 0.333			
Conversion % (based on C)	Gas	Liquid	Total
Non Catalyst	45.4	36.8	82.2
KOH	22.6	58.1	80.7
K_2CO_3	44.2	27.2	71.4
Temperature= 400 °C, time= 90 min. and glycerin/solvent con= 0.500			
Conversion % (based on C)	Gas	Liquid	Total
Non Catalyst	51.7	24.5	76.2
KOH	40.3	14.4	54.7
K_2CO_3	52.3	15.6	67.9
Temperature= 400 °C, time= 30 min. and glycerin/solvent con= 0.500			
Conversion % (based on C)	Gas	Liquid	Total
Non Catalyst	37.7	23	60.7
KOH	51.5	15	66.5
K_2CO_3	54.9	24.2	79.1
Temperature= 400 °C, time= 60 min. and glycerin/solvent con= 0.666			
Conversion % (based on C)	Gas	Liquid	Total
Non Catalyst	55.6	21	76.6
KOH	53.4	17.2	70.6
K_2CO_3	62.2	17.1	79.3

Temperature= 300 °C, time= 90 min. and glycerin/solvent con= 0.333			
Conversion % (based on C)	Gas	Liquid	Total
Non Catalyst	5.3	56.9	62.2
KOH	7.5	66	73.5
K_2CO_3	9.3	64.3	73.6



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Non catalyst	61.5%	56.0%	88.6%	92.1%	64.1%	53.6%	51.2%	86.7%	90.5%	79.0%	67.3%	71.3%	66.1%	69.0%	57.3%
KOH	67.6%	54.2%	59.0%	99.7%	51.3%	52.0%	59.2%	99.4%	91.5%	74.6%	61.6%	58.6%	60.0%	60.5%	60.6%
K2CO3	70.6%	55.1%	98.4%	98.6%	50.8%	55.0%	59.0%	98.5%	98.0%	75.3%	65.0%	60.6%	61.8%	64.1%	52.0%



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Non catalyst	58.7	38.4	58.4	67.7	54.7	40.1	20.4	39.7	31.9	63.8	58.2	52.9	60.1	62.7	44.1
KOH	67.3	35.6	43.3	74.1	64.4	44.2	42.3	87.5	33.6	89.7	76.9	35.6	72.1	74	39.4
K2CO3	81.7	25.9	21.1	38.5	36.5	53.7	34.6	77.8	10.5	72.5	76.2	49.2	68.3	71.6	11.8

Figure 7(a) Gycerine conversion and (b) Hazelnut shells conversion

Glycerin is not a product, however a product was obtained from it. Therefore, the amount of glycerin converted throughout the experiments was also investigated. We observed a maximum conversion rate of 99.7% for 90 minutes at 400 °C in the solvent with a 0.500 glycerin ratio in the presence of KOH catalyst, as shown by a comparison of the glycerin conversion rates with our experimental table. Using a 0.333 glycerin ratio and non-catalyst combination, the experiment was conducted at 300 °C for 30 minutes, yielding a minimum glycerin conversion rate of 50.8 % as shown in Fig. 7a and 7b.

Glycerin and water were employed as the experiment's solvents while hazelnut shell was used as the biomass. Both biomass and glycerin underwent transformation in the experiment's findings. Only the liquefaction of the biomass was considered for determining the total organic values of the liquid analysis. 7a and 7b illustrate how much of the biomass transforms into liquid.

In 10^{th} experimental condition using KOH catalyst, biomass conversion attained its highest value (89.7%). The experiment's 9th experimental condition, which also included the K_2CO_3 catalyst, produced the least conversion results.

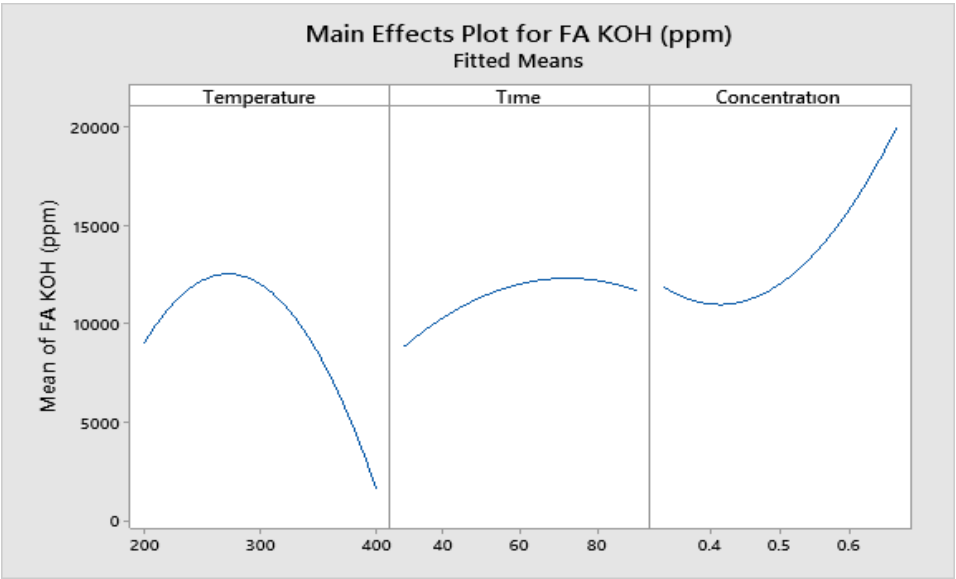


Figure 8. The variation in formic acid amount with parameters and catalyst KOH.

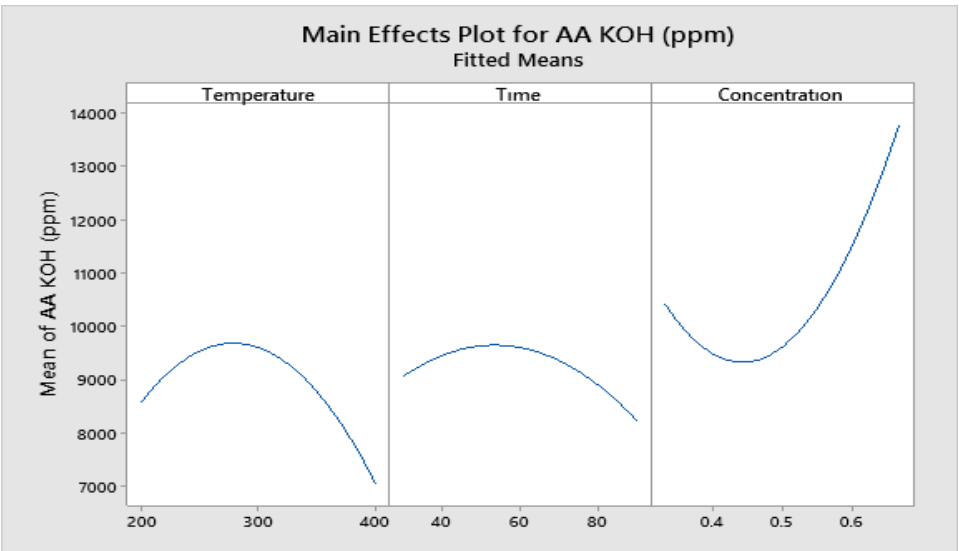


Figure 9. The variation in acetic acid amount with parameters and catalyst KOH.

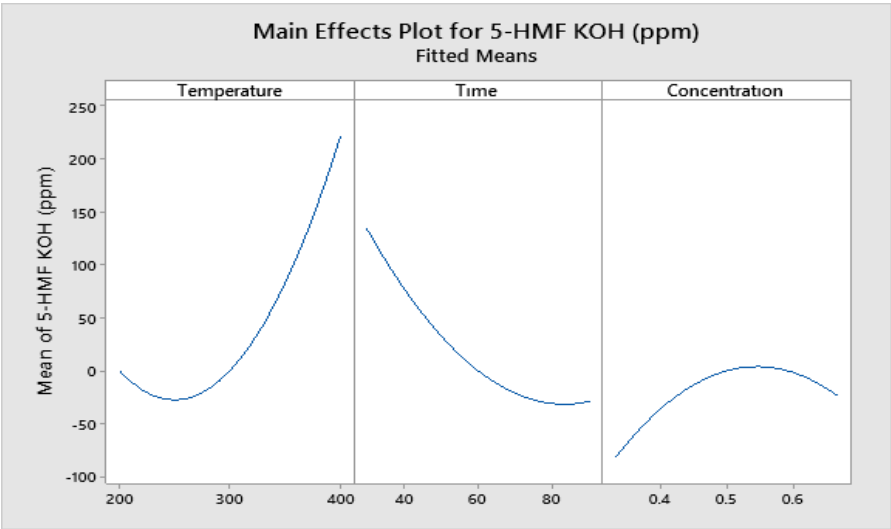


Figure 10. The variation in 5-HMF amount with with parameters and catalyst KOH.

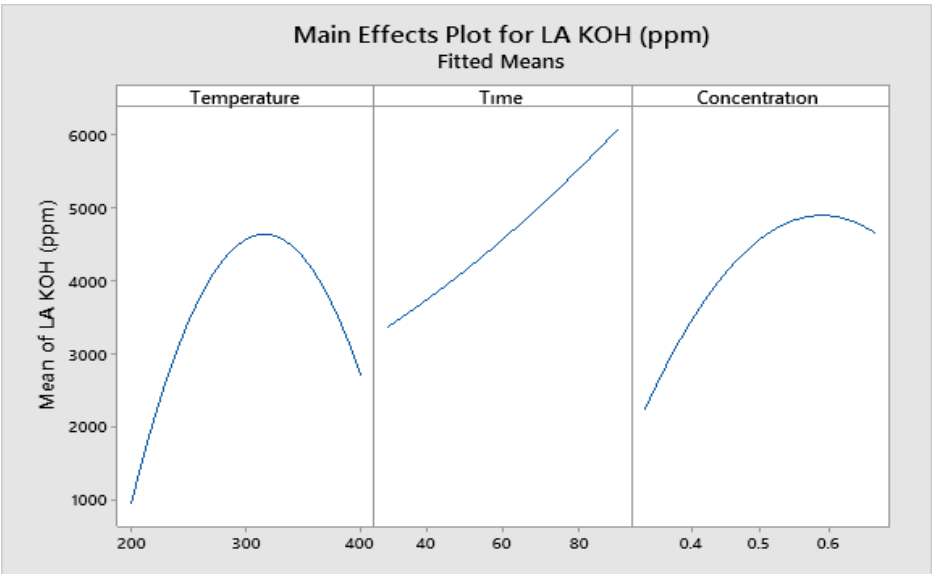


Figure 11. The variation in levulinic acid with parameters and catalyst KOH.

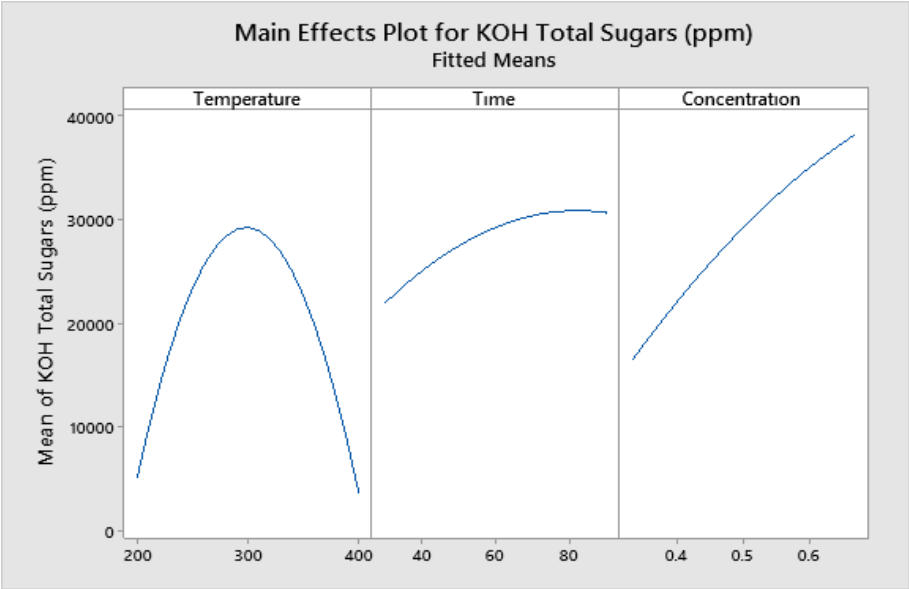


Figure 12. The variation in total sugars amount with parameters and catalyst KOH.

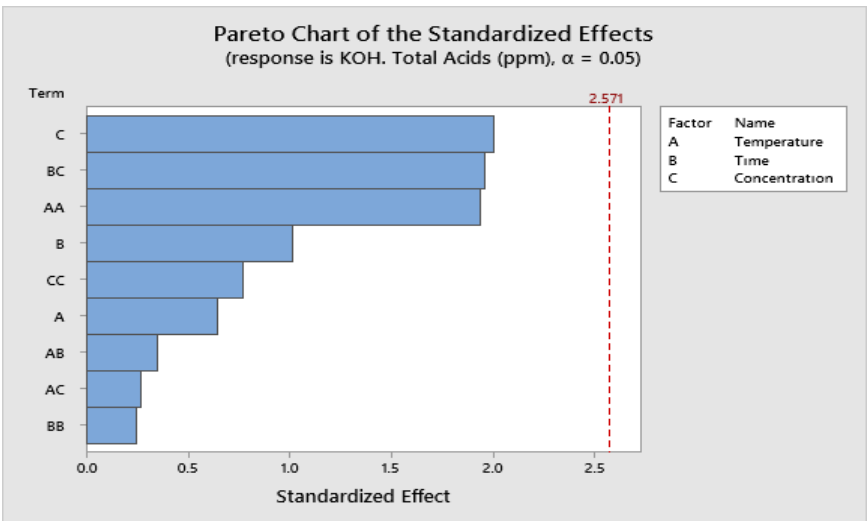


Figure 13. Pareto chart of the experiments with KOH on total acid products.

The model successfully fulfilled the observed response, as evidenced by high R^2 values of 0.73 (73%) in the presence of KOH catalyst, and 0.76 (76%) in the presence of K_2CO_3 catalyst

Total Acids yield (KOH) = 2675 + 576 Temperature - 847 Time - 192483 Concentration
- 0.989 Temperature*Temperature - 1.41 Time*Time
+ 142708 Concentration*Concentration
+ 0.57 Temperature*Time
- 79 Temperature*Concentration + 1925 Time*Concentration

Total Acids yield(K_2CO_3) = -6843 + 975 Temperature - 1405 Time - 366031 Concentration
- 1.503 Temperature*Temperature + 5.17 Time*Time
+ 319980 Concentration*Concentration
- 1.14 Temperature*Time
- 67 Temperature*Concentration + 2514 Time*Concentration

Table 8. Analysis of variance for total acid yield obtained from experiments with KOH.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	9	1360909866	151212207	1.57	0.323
Linear	3	525867977	175289326	1.82	0.261
Temperature	1	40004276	40004276	0.41	0.548
Time	1	98887500	98887500	1.03	0.358
Concentration	1	386976200	386976200	4.01	0.101
Square	3	446248789	148749596	1.54	0.313
Temperature*Temperature	1	360928738	360928738	3.74	0.111
Time*Time	1	5973508	5973508	0.06	0.813
Concentration*Concentration	1	57789857	57789857	0.60	0.474
2-Way Interaction	3	388793101	129597700	1.34	0.359
Temperature*Time	1	11828441	11828441	0.12	0.740
Temperature*Concentration	1	6998670	6998670	0.07	0.798
Time*Concentration	1	369965990	369965990	3.84	0.107
Error	5	481983059	96396612		
Lack-of-Fit	3	476502717	158834239	57.97	0.017
Pure Error	2	5480342	2740171		
Total	14	1842892925			

In the study conducted according to Box-Behnken design evaluations by using Minitab 19 package program to make statistical

evaluations, it was examined how acids and sugars changed with the effect of temperature, experiment time and glycerin/solvent ratio. As a result of the experiments carried out with KOH, the effect of temperature, time and glycerine ratio on the experiment results in the acid analysis was investigated. As the temperature increased, FA, AA and LA acid yields increased up to a certain value of 300 °C and decreased at elevated temperatures as given in Figs 8, 9 and 11 while the variation in the amount of HMF is different. HMF formation enhanced with temperature after reaching a minimum at around 250°C. Time affects the amounts of AA and FA positively at earlier reaction periods while they decrease after reaching an optimum. Amount of LA increases with time during the whole time but HMF amount decrease with proceeding reaction time as expected. HMF converted to LA during the reaction time and its amount decreased while LA amount increased. As seen in main effects plots, glycerine concentration causes a minimum point for AA and FA while a maximum point was reached for LA and HMF.

The effect of temperature, time, and glycerine ratio on the experiment results in total sugar analysis was investigated as seen in Fig. 12. As the temperature increased, the total acid yield increased and then decreased, when the experiment time was 60 minutes, the least sugar yield was reached and then increased again. When the glycerin concentration increased, the total sugar yield increased continuously.

The results of the experiment were performed at different glycerin+water ratios and different experimental times, the total sugar yield increased as the temperature increased, but a decrease was observed afterward. When we evaluate the glycerine ratios according to the experimental time, the total sugar content decreased at first and then started to increase. If we look at the 3 graphs in general, when the glycerin ratio is 66%, the total sugar yield is high in the experiments conducted at 300 °C for 90 minutes.

Fig. 13 indicates the effect of parameters on the amount of total acids. The most significant parameters were determined as

concentration of glycerine, and square terms of time*concentration and temperature*temperature, respectively. This figure shows that the other terms of the prediction equation are not effective because a sharp decrease is seen in their standardized effect values. Table 8 includes detailed information about the F and P values of each term in the model equation. It verifies the Pareto chart indications.

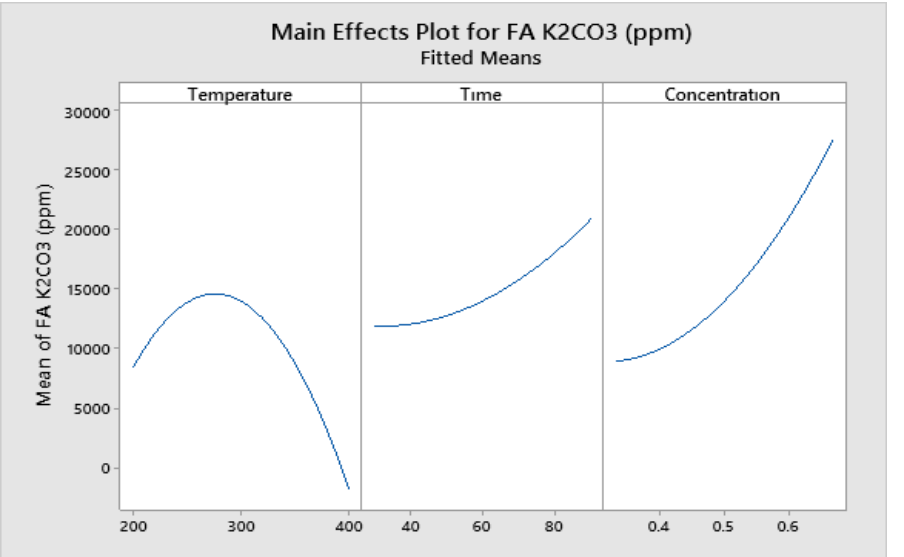


Figure 14. The variation in formic acid amount with parameters and K_2CO_3

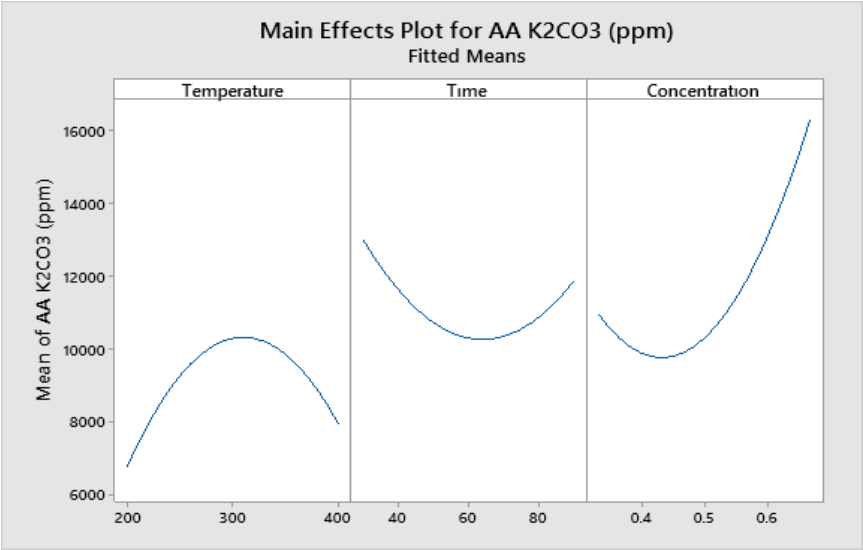


Figure 15. The variation in acetic acid amount with parameters and K_2CO_3

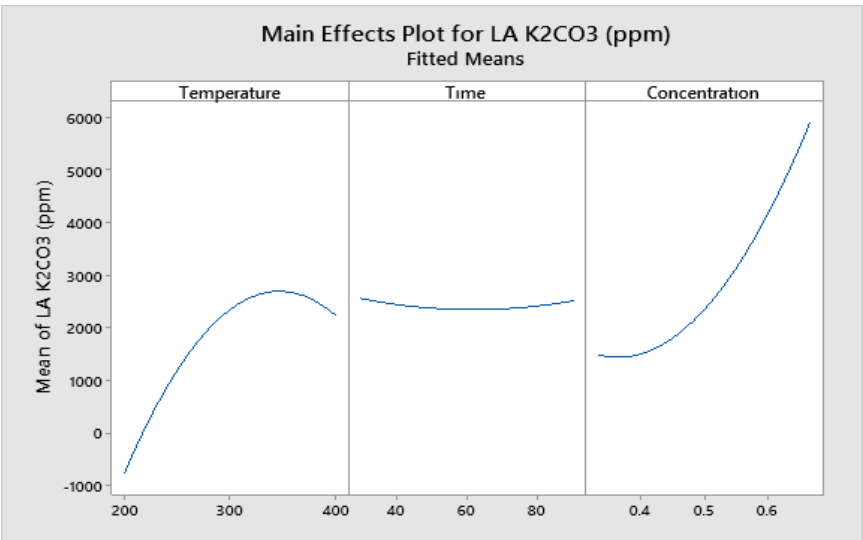


Figure 16. The variation in levulinik acid amount with parameters and K_2CO_3

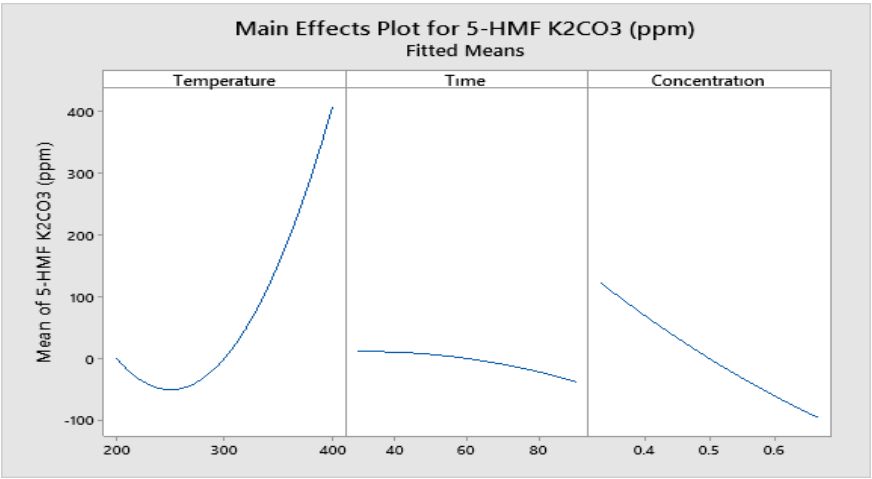


Figure 17. The variation in 5-HMF amount with parameters and K_2CO_3

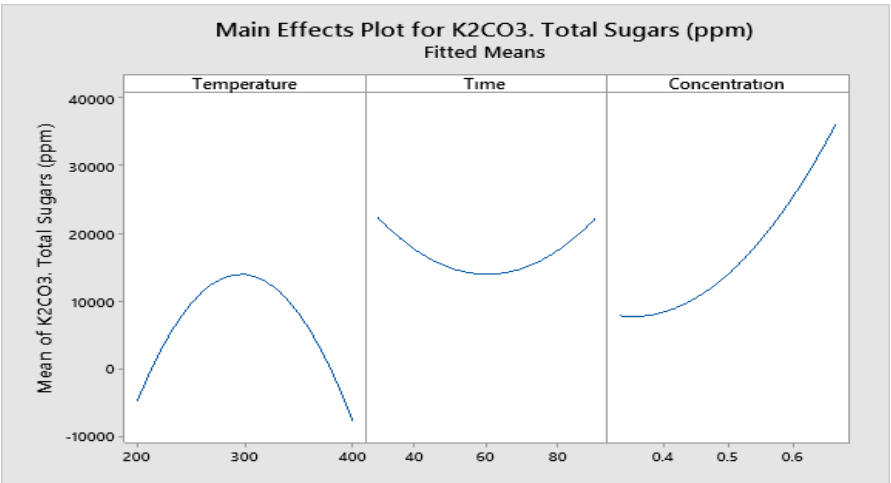


Figure 18. The variation in total sugars amount with parameters and K_2CO_3

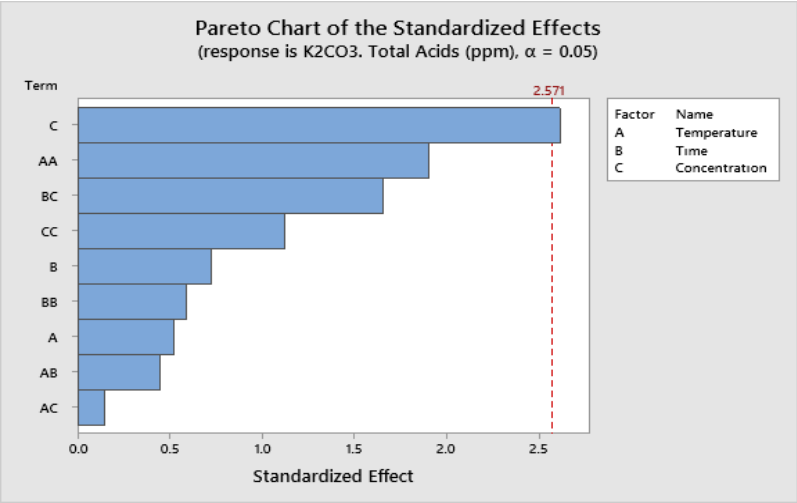


Figure 19. Pareto chart of the experiments with K_2CO_3 on total acid products.

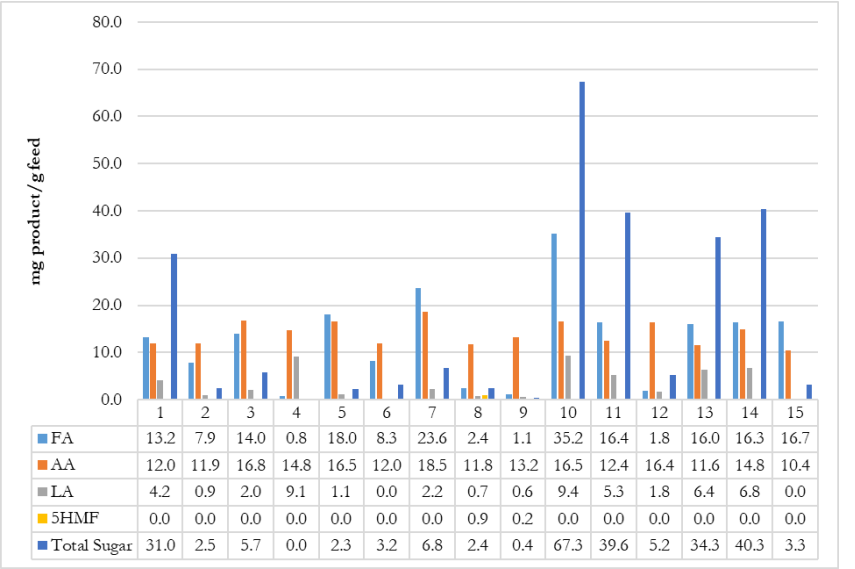
Table 9. Analysis of variance for total acid yield obtained from experiments with K_2CO_3.

Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	9	3744855026	416095003	1.81	0.267
Linear	3	1758641047	586213682	2.55	0.170
Temperature	1	63199714	63199714	0.27	0.623
Time	1	120924576	120924576	0.53	0.501
Concentration	1	1574516757	1574516757	6.84	0.047
Square	3	1303915639	434638546	1.89	0.249
Temperature*Temperature	1	833811241	833811241	3.62	0.115
Time*Time	1	79826193	79826193	0.35	0.582
Concentration*Concentration	1	290536169	290536169	1.26	0.312
2-Way Interaction	3	682298340	227432780	0.99	0.469
Temperature*Time	1	46635241	46635241	0.20	0.671
Temperature*Concentration	1	5025443	5025443	0.02	0.888
Time*Concentration	1	630637656	630637656	2.74	0.159
Error	5	1151064821	230212964		
Lack-of-Fit	3	1148764365	382921455	332.91	0.003
Pure Error	2	2300456	1150228		
Total	14	4895919847			

In studies with K_2CO_3, as the temperature increased in three different glycerin concentrations and three different experimental periods, the total acid yield first increased and then decreased. As seen in Figures 14-16, AA, FA, and LA amounts are increasing towards a maximum and then begin to decrease while for HMF it is observed as inversely. The amount of HMF decreased towards a certain temperature increase and then started to increase as shown in Fig. 17. When we look at all the graphics of the experiment results with the K_2CO_3 catalyst, it is concluded that the most optimal conditions are 66% glycerin ratio and a temperature of 300 °C for 90 minutes.

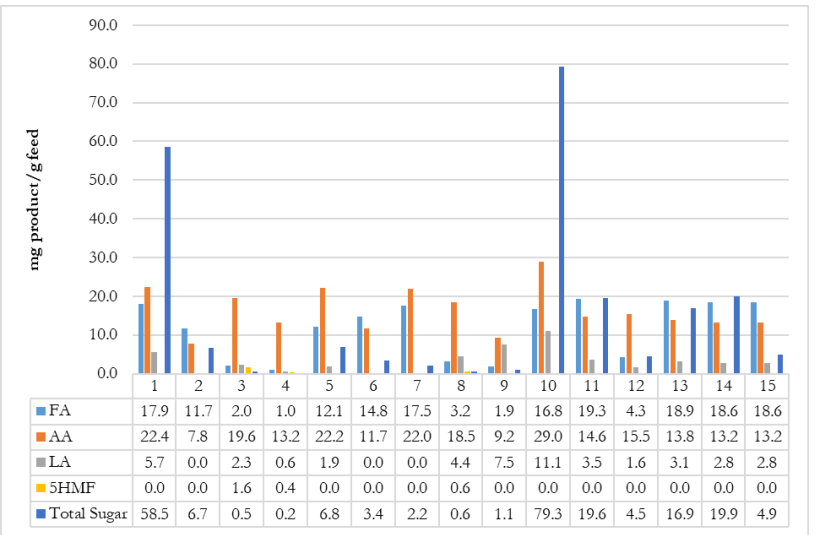
The interaction of glycerine gave the highest value to the total sugar yield. The lowest interaction was observed as the effect of time. In Fig. 18, as the temperature increased, the total sugar value first increased and then decreased. It was observed that the total sugar yield decreased and then increased as the experiment time increased. When the glycerine ratio increased, the total sugar value also increased.

A factorial plot analysis with a 95% confidence level was used to assess the significance of the parameters on total acid yields (Minitab-19 software). This analysis led to the development of a model equation, from which the "goodness fit" of these models was assessed. The equations above can be used to calculate the yields for acid, either alone or collectively. Fig. 19 indicates the effect of parameters on the number of total acids with K_2CO_3. The most significant parameters were determined as the concentration of glycerine, and square terms of temperature*temperature, and time*concentration respectively. These results are very similar to the findings with KOH as a catalyst. Table 9 gives detailed information about the F and P values of each term., the highest F values are found for the most significant terms as expected.



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
FA	13.2	7.9	14.0	0.8	18.0	8.3	23.6	2.4	1.1	35.2	16.4	1.8	16.0	16.3	16.7
AA	12.0	11.9	16.8	14.8	16.5	12.0	18.5	11.8	13.2	16.5	12.4	16.4	11.6	14.8	10.4
LA	4.2	0.9	2.0	9.1	1.1	0.0	2.2	0.7	0.6	9.4	5.3	1.8	6.4	6.8	0.0
5HMF	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.9	0.2	0.0	0.0	0.0	0.0	0.0	0.0
Total Sugar	31.0	2.5	5.7	0.0	2.3	3.2	6.8	2.4	0.4	67.3	39.6	5.2	34.3	40.3	3.3

Figure 20. Acid and total sugar values of glycerin and hazelnut shells under different experimental conditions with KOH



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
FA	17.9	11.7	2.0	1.0	12.1	14.8	17.5	3.2	1.9	16.8	19.3	4.3	18.9	18.6	18.6
AA	22.4	7.8	19.6	13.2	22.2	11.7	22.0	18.5	9.2	29.0	14.6	15.5	13.8	13.2	13.2
LA	5.7	0.0	2.3	0.6	1.9	0.0	0.0	4.4	7.5	11.1	3.5	1.6	3.1	2.8	2.8
5HMF	0.0	0.0	1.6	0.4	0.0	0.0	0.0	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total Sugar	58.5	6.7	0.5	0.2	6.8	3.4	2.2	0.6	1.1	79.3	19.6	4.5	16.9	19.9	4.9

Figure 21. Acid and total sugar values of glycerin and hazelnut shells under different experimental conditions with K_2CO_3.

It was observed that the FA and AA values were higher in the 3rd, 4th, 8th, and 9th experimental conditions without a catalyst. On the other hand, LA achieved high results only in the presence of K_2CO_3 catalyst under 9th experimental conditions. It can be concluded from this that the catalyst-free environment provided a more suitable environment for acids during the experiments at high temperatures (Figs. 20 and 21).

When the FA and AA values were examined, it was observed that the test time in the presence of K_2CO_3 catalyst reached a high value. LA, on the other hand, gave high results in experiments with more KOH catalysts. It was observed that 5-HMF was formed in general without a catalyst. Examining the experiment findings generated by the experiment table. The acquired acid values were compared, and under different experimental settings, high yields of acids were obtained. Levulinic acid obtained the maximum value in the absence of a catalyst in the 10th experimental condition (Conditions: 200 °C, 30 minutes, 2 g Hazelnut Shell and 9mL Glycerin + 9mL H_2O, without catalyst and with alkaline catalyst (K_2CO_3 and KOH)), while Formic acid reached the highest value in the presence of a K_2CO_3 catalyst. Under the 4th experimental condition (Conditions: 400 °C, 90 minutes, 2 g Hazelnut Shell and 9mL Glycerin + 9mL H2O, without catalyst and with alkaline catalyst (K_2CO_3 and KOH) without a catalyst 5-HMF and acetic acid reached the highest value.

On the other hand, total sugar reaches highest result in the experiment performed in the presence of KOH catalyst in the 10th experimental condition (Conditions: 200 oC, 30 minutes, 2 g Hazelnut Shell and 9mL Glycerin + 9mL H_2O, without catalyst and with alkaline catalyst (K_2CO_3 and KOH).

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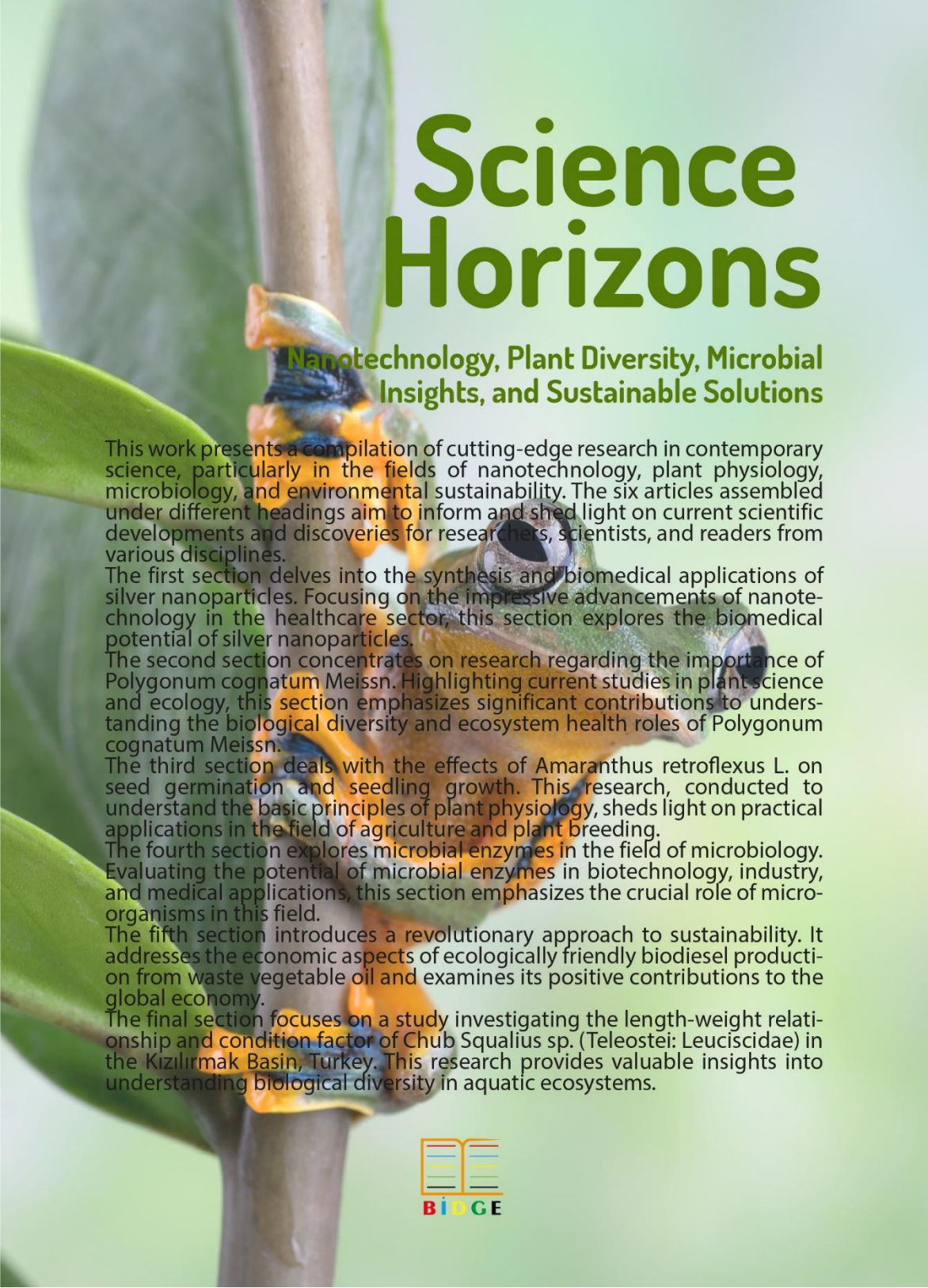

Science Horizons

Nanotechnology, Plant Diversity, Microbial Insights, and Sustainable Solutions

This work presents a compilation of cutting-edge research in contemporary science, particularly in the fields of nanotechnology, plant physiology, microbiology, and environmental sustainability. The six articles assembled under different headings aim to inform and shed light on current scientific developments and discoveries for researchers, scientists, and readers from various disciplines.

The first section delves into the synthesis and biomedical applications of silver nanoparticles. Focusing on the impressive advancements of nanotechnology in the healthcare sector, this section explores the biomedical potential of silver nanoparticles.

The second section concentrates on research regarding the importance of Polygonum cognatum Meissn. Highlighting current studies in plant science and ecology, this section emphasizes significant contributions to understanding the biological diversity and ecosystem health roles of Polygonum cognatum Meissn.

The third section deals with the effects of Amaranthus retroflexus L. on seed germination and seedling growth. This research, conducted to understand the basic principles of plant physiology, sheds light on practical applications in the field of agriculture and plant breeding.

The fourth section explores microbial enzymes in the field of microbiology. Evaluating the potential of microbial enzymes in biotechnology, industry, and medical applications, this section emphasizes the crucial role of microorganisms in this field.

The fifth section introduces a revolutionary approach to sustainability. It addresses the economic aspects of ecologically friendly biodiesel production from waste vegetable oil and examines its positive contributions to the global economy.

The final section focuses on a study investigating the length-weight relationship and condition factor of Chub Squalius sp. (Teleostei: Leuciscidae) in the Kızılırmak Basin, Turkey. This research provides valuable insights into understanding biological diversity in aquatic ecosystems.

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