KADIR HAS UNIVERSITY
SCHOOL OF GRADUATE STUDIES
PROGRAM OF COMPUTATIONAL SCIENCE AND ENGINEERING

MICROALGAE-BASED BIOPLASTICS AND THEIR UTILIZATION AS FERTILIZER

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MASTER OF SCIENCE THESIS

ISTANBUL, JANUARY, 2025

MICROALGAE-BASED BIOPLASTICS AND THEIR UTILIZATION AS FERTILIZER

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A thesis submitted to
the School of Graduate Studies of Kadir Has University
in partial fulfilment of the requirements for the degree of
Master of Science

Istanbul, January, 2025

APPROVAL

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DECLARATION ON RESEARCH ETHICS AND PUBLISHING METHODS

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In addition, I acknowledge that any claim of irregularity that may arise in relation to this work will result in a disciplinary action in accordance with the university legislation.

Wiem Jebahi

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23.01.2025

ACKNOWLEDGEMENT

Family

To my dear parents, whose unwavering love and sacrifices have shaped my journey, no words can truly repay the gift of education and guidance you have given me.

To my beloved sisters, my greatest source of joy, who have always believed in me and lifted me up.

To my friends, whose encouragement and dedication have been a constant source of strength, I am deeply grateful.

To my second family in Istanbul, thank you for your kindness, support, and unwavering encouragement.

To everyone who has stood by me, may this serve as a heartfelt expression of my appreciation and profound gratitude.

Assoc. Pr. Bengü Özuğur Uysal

I would like to extend my heartfelt thanks to my exceptional professor, Bengu Usyal, for her generosity, patience, and steadfast support. Her expertise, dedication, and professionalism have been instrumental in shaping the quality and direction of my research. I am especially grateful for her insightful feedback, openness to discussions, and

encouragement to explore and develop my ideas. Thank you, Professor, for everything; I am truly grateful to have had you as my thesis advisor.

Prof. Dr. Önder Pekcan

I would like to express my gratitude to Professor Onder for his exceptional teaching in polymer science. His course opened my eyes to the field and helped in understanding the key concepts of hydrogels.

Dr. Emre Ozan Polat

I would also like to extend my heartfelt thanks to Professor Emre Ozan Polat for his invaluable support, encouragement, and guidance. His courses provided me with a deeper understanding of polymer science, a field that was previously unfamiliar to me, and greatly enriched my knowledge and appreciation of the subject.

Dr. Sercan Beytur

I would also like to extend my thanks to Sercan for his time, support, encouragement, and valuable advice and guidance.

Yağmur Poyraz

I am deeply grateful to Yağmur, whose kindness and support have been with me from my very first day at university. Her unwavering help has meant more to me than words can fully express.

ABSTRACT

This study explores the potential applications of gelatin gels enriched with Chlorella and Spirulina additives as bioplastics and biofertilizers. Comprehensive spectroscopic and structural analyses were conducted to evaluate the properties of these gels. Biodegradation tests in soil demonstrated that gelatin gels degrade within 7 days, while Spirulina-Gelatin and Chlorella-Gelatin gels take 9 and 10 days, respectively. In tests assessing their efficacy as fertilizers, all gels significantly promoted the growth of strawberry fruit seedlings and wild indigo flower seedlings within 10 days, with Chlorella-Gelatin showing significantly better results compared to other treatments. Additionally, in vivo experiments using radish plants were conducted, though the results were less promising, highlighting a potential area for further exploration and refinement. The findings confirmed that these bioplastics are environmentally friendly, degrading quickly without harm, while enhancing plant growth.

Keywords – Bioplastic, Biofertilizer, Spirulina, Chlorella

MİKROALG TEMELLİ BİYOPLASTİKLER VE GÜBRE OLARAK KULLANIMLARI

ÖZET

Bu çalışma, Chlorella ve Spirulina katkılarıyla zenginleştirilmiş jelatin jellerin biyoplastik ve biyogübre olarak potansiyel kullanımını araştırmaktadır. Bu jellerin özelliklerini değerlendirmek amacıyla kapsamlı spektroskopik ve yapısal analizler gerçekleştirilmiştir. Toprakta yapılan biyobozunma testleri, jelatin jellerin 7 gün içinde parçalandığını, Spirulina-Jelatin ve Chlorella-Jelatin jellerin ise sırasıyla 9 ve 10 gün sürdüğünü göstermiştir. Gübre olarak etkinliklerini değerlendiren testlerde, tüm jellerin çilek fidesi ve yabani indigo çiçeği fidesi büyümesini 10 gün içinde önemli ölçüde teşvik ettiği görülmüştür; özellikle Chlorella-Jelatin diğer uygulamalara kıyasla anlamlı derecede daha iyi sonuçlar vermiştir. Ayrıca, turp bitkileri kullanılarak yapılan in vivo deneyler de gerçekleştirilmiş, ancak sonuçlar daha az umut verici bulunmuş ve bu durum daha fazla araştırma ve iyileştirme alanı olarak belirlenmiştir. Bulgular, bu biyoplastiklerin çevre dostu olduğunu, hızlı şekilde zararsızca parçalandığını ve bitki büyümesini teşvik ettiğini doğrulamıştır.

Anahtar Kelimeler – Biyoplastik, Biyogübre, Spirulina, Chlorella

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LIST OF ACRONMYMS AND ABBREVIATIONS

- Min: Minutes
- Mg: Milligram
- Ml: Millilitres
- Nm: Nanometre
- UV-VIS: Ultraviolet-visible
- EPS: Exopolysaccharides
- ANOVA: Analysis of variance
- FT-IR: Fourier transform infrared

1. INTRODUCTION

Petroleum-based plastics are considered a revolutionary invention because they have profoundly elevated modern living standards by providing unparalleled convenience and versatility [2]. Their widespread adoption is due to various essential characteristics, including cost-effectiveness, the capacity for chemical manipulation to achieve diverse shapes, enhanced durability, outstanding tensile properties, and an exceptional ability to act as effective barriers against oxygen, carbon dioxide, and water vapor.[3]

However, this material causes significant harm throughout its manufacturing, use, and post-consumption phases, negatively impacting human health and the environment[4]. Plastic industries are considered one of the most industries causing environmental issues, mainly climate change, loss of biodiversity, and chemical pollution [5]. Additionally, the very features that contribute to the success of petroleum-based plastics—namely, their chemical complexity and substantial molecular weight—also present challenges regarding environmental degradation at the end-of-life stage. These attributes hinder microbial breakdown in the natural environment, leading to the persistent accumulation of these plastics over the long term [6].

Apart from the evident environmental impact, the extensive use of petroleum-based plastics has significant drawbacks. These extend to health issues like cancer, obesity, diabetes, endocrine system disorders, thyroid dysfunction, reproductive impairment, and autoimmune diseases [7]. We discuss such severe and potential health problems caused by plastics, especially when discussing those used in food packaging [8]. Unfortunately, with a predicted 50% rise in global food demand by 2050, which implies a rise in food packaging use and thus waste, it will further amplify environmental and health issues, requiring a closer look at food packaging practices.[9]

Scientists have explored recycling as a viable solution to address the environmental crisis. While recycling has gained prominence in environmental initiatives, it is crucial to recognize its inherent limitations. Existing recycling systems encounter significant

challenges, leading to a mere 9% recovery rate for the annual production of synthetic plastics, equal to 400 million tons. These limitations stem from inefficient techniques and constraints in material design[10]. Furthermore, traditional recycling primarily addresses the after-life stage, neglecting issues associated with the production phase and the health impact of plastic consumption during use.

Recognizing the limitations of recycling as a solution, the unavoidable use of petroleum-based plastics in both industries and households and acknowledging the issues they pose, there is a collective effort to explore eco-friendly alternatives. As part of this sustainable approach, researchers are investigating natural options like bioplastics derived from renewable sources such as plants or microbial species.

However, in current use, many of these bioplastics are not designed to degrade under backyard composting conditions. They must be adequately stacked, transported to commercial compost facilities, and processed. This makes it difficult for them to compete with plastics, which are already relatively low-cost. For this reason, it is essential to produce bioplastics that are easily degraded in nature, will not harm the environment when decomposed, and can even be used as fertilizer for plants. [11].

One such eco-friendly approach explores algae as a source of biodegradable materials for bioplastic production. Algae, distinct from other resources used in plastic production, have emerged as a promising candidate due to their minimal environmental footprint, no competition with food resources, and abundant presence in aquatic environments. Research has increasingly investigated the potential of microalgae and macroalgae (seaweeds) for biofilm and bioplastic production. [12] Furthermore, algae have shown promising results as biofertilizers and biostimulants, competing effectively with chemical-based alternatives.[13]

Thus, this research aims to discuss microalgae-based bioplastics' productibility and degradability and explore the possibility of their utilization as fertilizers in their afterlife phase.

1.1 Bioplastic

Bioplastics are made of two main components: biopolymers, which form the backbone of the material and can be synthesized from renewable feedstock like plant-based raw materials (Algae, vegetables waste, Banana peel, etc.), natural polymers (Carbohydrates, proteins, …) and small other molecules (fatty acids, disaccharides...) [14] or through microorganism-driven fermentation processes.[15]; and additives, which enhance its properties like reduce film brittleness and enhancing its flexibility [16].

The process to obtain bioplastic from agricultural polymers involves three main steps: breaking natural bonds to loosen the polymer chains, shaping the flexible chains into the desired form, and then stabilizing this new structure by allowing the chains to form new bonds, such as hydrogen, hydrophobic, and disulfide bonds. These interactions create a stable, three-dimensional network that holds the material's shape.[17]

Depending on the production method and chemical composition of a polymer, Bioplastics may be either non-biodegradable like Bio-based Polyethylene, Polypropylene, and Polyethylene Terephthalate (PET) or biodegradable including Polylactic Acid (PLA), Polyhydroxyalkanoates (PHA), and Polybutylene Succinate (PBS) [18] [19].

In this research, we are focusing on the last category—biodegradable Bioplastics—to achieve our goal: a safe polymer throughout all its stages, ensuring minimal impact on both the environment and human health, or even enhancing soil characteristics and promoting plant growth. Tangkoonboribun et al. proved that bioplastic when mixed with biofertilizer can reduce soil acidity and electrical conductivity [20].

The use of Bioplastics as fertilizers has also been discovered by multiple studies [21], and it has been proved that it can be degraded in soil in a shorter period than conventional fertilizer which shows not just a better environmental impact but also a better economic impact.[22]

Most of the biodegradable Bioplastics are derived from terrestrial crops like potatoes [23], corn [24] and rice [23] which creates competition with food supplies and requires significant resources (land, water, and nutrients). This makes such bioplastic production unsustainable in the long term and not suitable for mass production. In response, there is growing interest in alternative feedstock, with algae emerging as a particularly promising source for bioplastic production [25].

1.2 Algae

Algae are mostly photoautotrophic eukaryotes of ancient origin but less than 10% have been formally described They are dominant in the oceans [26].

In recent years, algae have become integral to various human activities, particularly in fields such as the healthcare sector (biotechnology, pharmacology, and cosmeceutical formulations), thanks to their diverse activities, including antioxidant and contraceptive properties [27] In the food sector, algae are utilized as food, food ingredients ,supplements and prebiotics [27]. Algae are also applied in environmental solutions for Bioremediation and Wastewater Treatment [28], production of biofuels [29], fertilizers [13] and have also been implicated in bioplastic production. In fact, their biopolymers have emerged as a promising resource for the bioplastic industry due to their economical, renewable, and abundant availability [30].

When discussing algae, we differentiate between macroalgae and microalgae. Microalgae represent an exceptionally diverse but highly specialized group of micro-organisms[31] typically grow faster than freshwater or marine macroalgae. They can also be cultivated in wastewater and even used for wastewater treatment [32]. These economic and environmental advantages have made them the primary focus of research for starch production in recent years. Furthermore, microalgae offer the advantage of not competing with food resources, either in land use or in direct food supply [33].

Chlorella sp. and *Spirulina sp*., microalgae species account for over 90% of global microalgal biomass production. Due to their abundance and beneficial properties, they are widely used across various sectors [34]. They also showed great growth rate in wastewater environment [32] and stands out for being one of the richest protein sources of microbial origin [35] knowing for better film-forming potentials [36]. In addition to proteins *Chlorella sp. and Spirulina sp*. Proceed also different other biopolymers. Their high starch content and ability to produce diverse biopolymers make them suitable for bioplastic synthesis [30].

However, extracting those polymers present economic and scalability challenges, for this reason, scientists worked on developing bioplastic out of a whole cell of microalgae. Hareesh et al successfully synthesizeda bioplastic out of whole spirulina cells in powder form having mechanical properties similar to polystyrene including a tensile strength of 34 MPa, a flexural modulus of 3 GPa, and an elongation at break of 1.6% and at the same time recyclable and biodegradable [37].

The figure 1.1 shows that spirulina-based bioplastics has a slightly better biodegradability rate than banana peels.

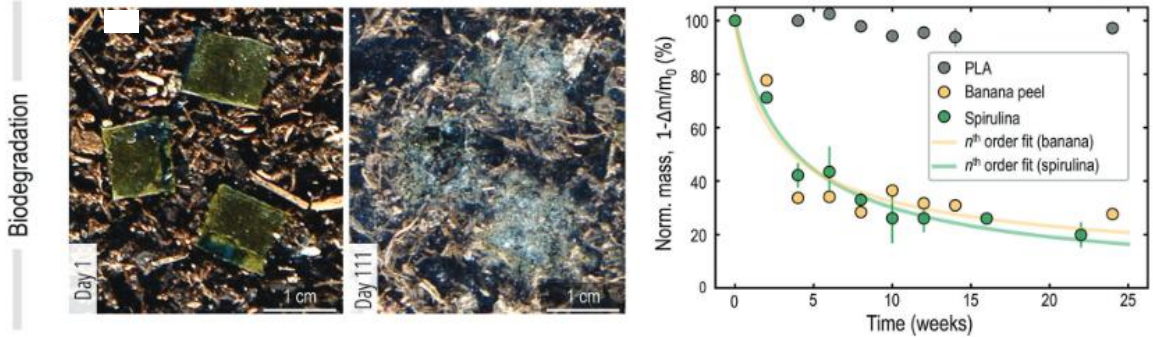

Figure 1.1 **a**) Photographs of the biodegradation of spirulina bioplastics in soil. **b)** Mass loss graph comparing the biodegradation of spirulina bioplastics in soil with banana peel as a positive control and PLA as a negative control [37].

In the other hand, microalgae role in mineralization and the mobilization of organic and inorganic, major and micronutrients, alongside the production of bioactive compounds (such as polysaccharides, growth hormones, and antimicrobial compounds), enhances plant growth, making them excellent Biofertilizer options [38]. In particular, *Chlorella* sp.

and *Spirulina* sp. are considered suitable candidates for soil conditioning and providing high nutrients (N and P) for plant growth [32]. Dineshkumar and colleagues successfully used *Chlorella vulgaris* and *Spirulina platensis* as biofertilizers through soil drench treatment, achieving a 20.9% increase in rice yield and positively impacting vegetative growth parameters such as plant height, leaf area, and fresh and dry weight [39]. Other studies have reported similar improvements in vegetative growth for different other plants like mentioned in the following Table 1.1.

Table 1.1 Impact of Microalgae Fertilizers on Crop Growth and Yield

Crop type	Microalgae specie	Key finding	Reference
Spinach	*Chlorella vulgaris*	The addition of algae fertilizer greatly increases specific leaf area compared to no fertilizer. Spinach grown with algae fertilizer have higher biomass production (Dry weight)	Rupawalla et al., 2021[40]
Tomato	*Chlorella vulgaris*	Enhance plant growth, mineral content, and shelf life of treatment method	Suchithra et al., 2022[41]
Maize	*Chlorella vulgaris* *Spirulina platensis*	Up to 51.1 % increase in plant growth, and yield	Dineshkumar et al., 2019[42]
Onion	*Chlorella vulgaris* *Spirulina platensis*	Improving photosynthesis rate, bulb length, diameter, and weight	Dineshkumar et al., 2020 [43]

1.3 Biofertilizer

1.3.1 Definition

A biofertilizer is a product that contains living microorganisms and helps improve plant growth as well as soil functions and fertility. The microorganisms colonize the plant's rhizosphere and increase the supply or availability of primary nutrients and/or growth stimulants for the target crop**[44]**. When these microorganisms are applied to seeds, roots, or soil, they help replenish the lost microflora and, consequently, enhance soil quality.

A number of soil microorganisms that primarily colonize the rhizosphere are known to exert multiple plant growth-promoting activities, mainly by acting as nitrogen fixers (such as rhizobium) or phosphate solubilizers [27].

1.3.2 Advantages

With the shift towards healthier and more environmentally friendly agriculture, and considering the harmful effects of chemical fertilizers, modern agriculture is increasingly turning to the use of biofertilizers.

The use of biofertilizers aligns with the concept of sustainable agriculture, as they are considered a renewable source of nutrients and do not deplete fossil resources, unlike chemical alternatives. Moreover, their use has no harmful effects on soil fertility and can even increase plant resistance to biotic and abiotic stress. Not to mention, they reduce environmental pollution caused by the use of chemical fertilizers.

Economically, biofertilizers are relatively inexpensive compared to chemical fertilizers, whose production costs continue to rise, and they can replace them by 25 to 30% [45] Biofertilizers can also be advantageous both ecologically and economically if the inoculation carrier used is based on waste treatment [46] and microalgae have shown significant potential in utilizing various waste streams for growth [33].

1.3.3 Types of Biofertilizers and Mode of Action

The microorganisms used as biofertilizers are either bacteria, such as Bacillus, Pseudomonas, Lactobacillus, photosynthetic bacteria, nitrogen-fixing bacteria, etc., or algae, or fungi like Trichoderma, yeast, etc or algae [47].

Microalgae have shown remarkable efficiency in nutrient cycling, absorbing and converting key nutrients like nitrogen, phosphorus, and potassium into forms that plants can easily access. Moreover, they produce bioactive compounds, including phytohormones, which directly influence plant physiological processes and stimulate growth. Microalgae also form beneficial relationships with other soil microorganisms, enhancing the growth of helpful bacteria and fungi, thereby fostering a healthy soil ecosystem. Additionally, as photosynthetic organisms, microalgae utilize sunlight to transform carbon dioxide (CO2) into organic matter, sequestering carbon and helping to mitigate greenhouse gas emissions, making them valuable contributors to sustainable agriculture.[48]

1.4 Aim of This Research Work

Building on the advantages of microalgae as both a Biofertilizer and a key player in the biodegradable bioplastic production, this work focuses on developing a microalgae-based bioplastic that is both biodegradable and functions as a Biofertilizer. As it decomposes, it enriches the soil with essential nutrients, supporting plant growth. In addition, it does not compete with human food and can be cultivated in wastewater. This, combined with the mentioned benefits and characteristics, makes this approach align with the circular economy model by reducing waste and maximizing resource use, contributing to a more sustainable and regenerative agricultural system.

In this research work, *Chlorella sp.* and *Spirulina sp.* are utilized and tested for the production of a biodegradable bioplastic that can also function as a Biofertilizer.

2. MATERIALS AND METHOD

2.1 Bioplastic Production

2.1.1 Material

Organic spirulina was purchased from …. Chlorella was purchased from Orsera.

Gelatin, to enhance the bioplastic mechanical properties [49], was purchased from Sigma–Aldrich, USA, as shown in the Figure 2.1.

Rice starch solution was produced as reported in Ref [50].

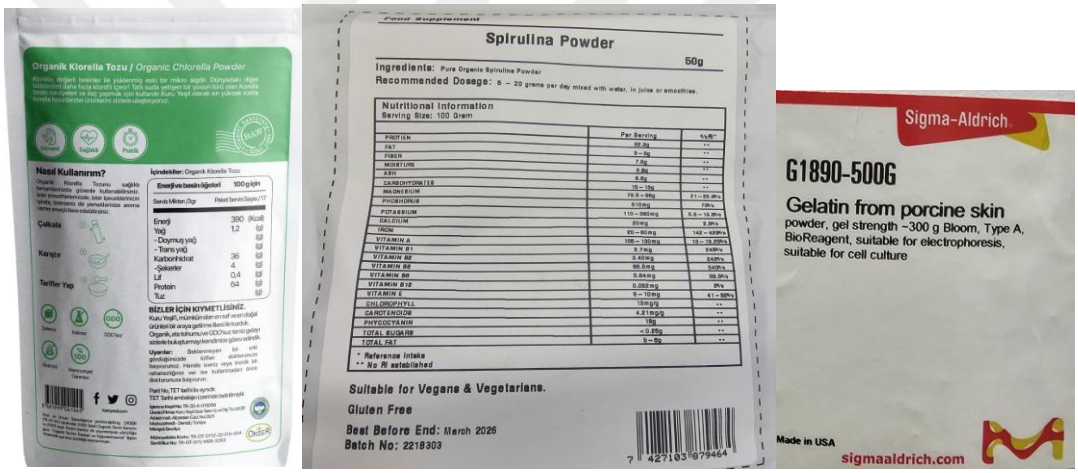

Figure 2. 1. Chlorella powder (a) Spirulina powder characteristics (b) Gelatin (c)

2.1.2 Gelation method

100 g rice was boiled in 150 mL of distilled water to provide the rice starch.

25 ml of rice water was separated and mixed with 1 g Chlorella.

1 g gelatin was added to the solution and continued to mix vigorously. In all these processes, the temperature was kept constant at 80 ° C for 45 minutes.

All procedures were repeated for Spirulina instead of Chlorella.

To see the influence of microalgae, gelatin solution was prepared using the same amount of rice water. It was observed that the solution began to condense. The final solutions were poured into leaf-shaped molds, where gel formation was observed like indicated in Figure 2.2.

The procedure was repeated using Spirulina instead of Chlorella.

To evaluate the influence of microalgae, a gelatin solution was also prepared using the same amount of rice water and all other components without adding microalgae.

This gelation method is following the solvent casting technique method.[51]

Figure 2.2. Solution poured into leaf-shaped molds for film structure

2.2 Gel Physicochemical Characterization

After the solutions began to condense (about 45 minutes), some samples were transferred into spectrometer cuvettes for FTIR and UV-VIS spectrometer analysis like it's shown in Figure 2.

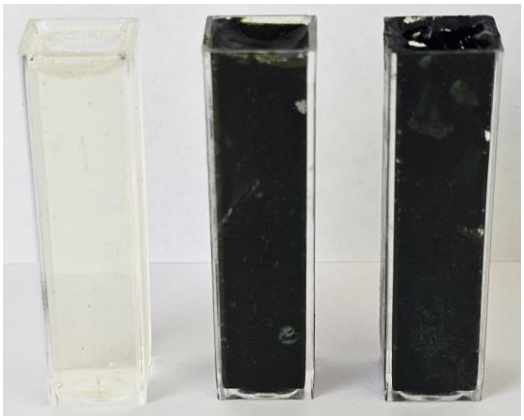

Figure 2.3. Solution poured into spectrometer cuvettes for optical measurement

2.2.1 FTIR spectra

The FTIR spectra were recorded in the 4000-400 cm^{-1} range as reported elsewhere [52] to identify the key functional groups and bonds, providing insights into the molecular structure of the bioplastic components.

2.2.2 Spectrometer UV-VIS

The gelation of the Bioplastic was measured using a Ertick Instruments 752N Plus UV-VIS Spectrophotometer. The absorption spectra were recorded in the 200-800 nm range to evaluate the optical properties and assess the transparency and absorption characteristics of the bioplastic materials.

2.3 Testing the Biodegradability and the Biofertilizer Aspect

2.3.1 Biodegradability test

Gel samples in the form of drug tablets, were prepared by pouring the prepared solution (as mentioned above) into the drug container molds (semi-spherical and tablet shaped). Gelation was observed in a short time at room temperature. Then, all samples in the molds were kept in the refrigerator for 15 minutes.

Samples in gel form removed from the molds are shown in Figure 2.4.

The sizes and masses of the samples taken from the semi-spherical mold were determined and buried in the soil. Every day they were dug up, cleaned, weighed and their sizes measured. Thus, it was determined how many days it took for them to completely decompose.

Figure2.4 Gel samples in the form of drug tablets (a) gelatin gel (b) Spirulina + gelatin gel (c) Chlorella + gelatin gel

2.3.2 Biofertilizer test

2.3.2.1 Seeds germination comparison test using gelatin gel, *Chlorella* gel and *Spirulina* gel

The strawberry and wild indigo plants have been chosen for the experiment.

The strawberry plant has been chosen due to its nutritional benefits and its vulnerability to environmental changes such as soil conditions, pollutants, or fertilizers [53]. Thus, it is an efficient choice to test the biocompatibility of our bioplastic.

Indigo plants are chosen not only to represent edible plants but also to explore the effects of the produced bioplastic on medicinal plants, which may later be utilized for pharmaceutical encapsulation.

Gel samples in the form of drug tablets were placed in the soil in the prepared multiple seedling growing assembly. All cells in the assembly were labeled with the corresponding name. Three soil cells were provided for each different sample to contribute to the statistical results and error of the measurements. One small and one big plant of each specie were planted into each cell as shown in Figure 2.5.

After 10 days, the stem length was measured and compared.

Figure 2.5 Germination of Strawberry and Wild Indigo Seeds Treated with different prepared Bioplastic Capsules

2.3.2.2 Tracking of plant growth parameters using different concentrations of Spirulina based gel

For this experiment radish plant was chosen.

Radish, an economically important root vegetable [54] and one of the horticultural crops, which are among the most widely cultivated plants globally, was used for in vivo cultivation due to their fast growth cycle, allowing us to quickly observe the effects of the treatment on root development. [55]

The exact used variety of radish is shown in figure 2.6.The seeds were sown in a cell tray filled with potting soil for a period of 10 days. Figure 2.6 shows the results after 10 days when the plants were transferred into pots and 1 ml of liquefied bioplastic (As the literature showed positive results using liquid biofertilizers based on the microalgae discussed in this study [56]), containing different concentrations of Spirulina (0%, 10%, 20%, 30%), was applied to 150g of peat substrate in each pot. Each concentration had 3 replications, along with a control group without bioplastic, which had 6 replications.

The experiment was conducted in a balcony to imitate the natural conditions. The same type of peat was used for all species and treatments, and watering was uniform for all pots, using tap water.

Figure 2.6. Cell tray of the radish plants on the day of transfer (a) Treating the plants with the liquefied bioplastic method (b)

Several parameters were measured periodically throughout the cultivation process. These parameters include the number of leaves, stem length, and the percentage of greenery, using the Canapeo [57] application and that for 1 month.

2.4 Statistical Analysis

The comparison of means for the different studied parameters was performed using analysis of variance (ANOVA) in Python Multiple comparisons of means were conducted using Tukey's test with $P < 0.05$.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1. Material characterisation

3.1.1.1 FT-IR results

The broad OH peak around 3200 cm-1, shown in figure3.2 and figure3.3, is due to the water in the gel sample [58], also if we compare it to the spectra of spirulina powder we don't have such a peak [59], Figure 3.1.

The strong peak around 1700 cm-1 for both gels is assigned to C=O stretching corresponding to the presence of esters and amino acids and the peak around 1200 corresponds to C-O stretching in the sample proving also the existence of ester groups,[60]. Hence, our material is rich in proteins and polysaccharides.

The several peaks in the fingerprint region represent the carbon-oxygen bonds of organic components in the samples and are comparable to the peaks in Figure 3.1. Microalgae samples must have these bond structures as mentioned in Ref [61], [60]. Thus, the existence of chlorella-gelatin and spirulina-gelatin biopolymers was confirmed.

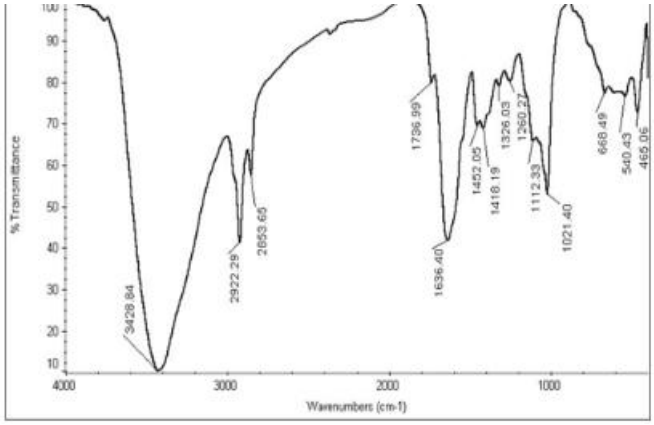

Figure 3.1. FTIR Spectrum: Identification of Functional Groups and Bonding Characteristics of *Spirulina* powder[59]

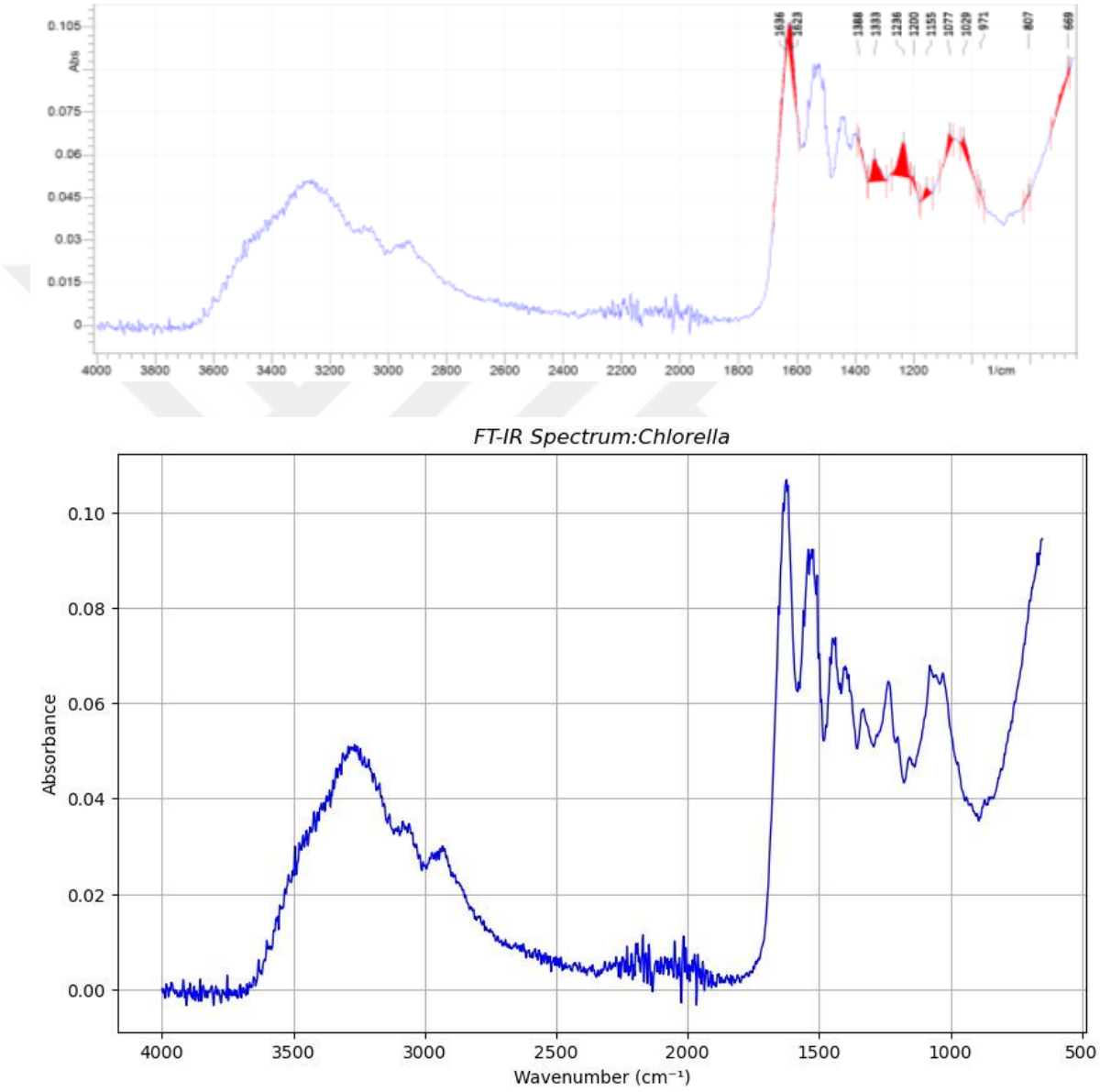

Figure 3.2. FTIR Spectrum: Identification of Functional Groups and Bonding Characteristics of *chlorella* based bioplastic

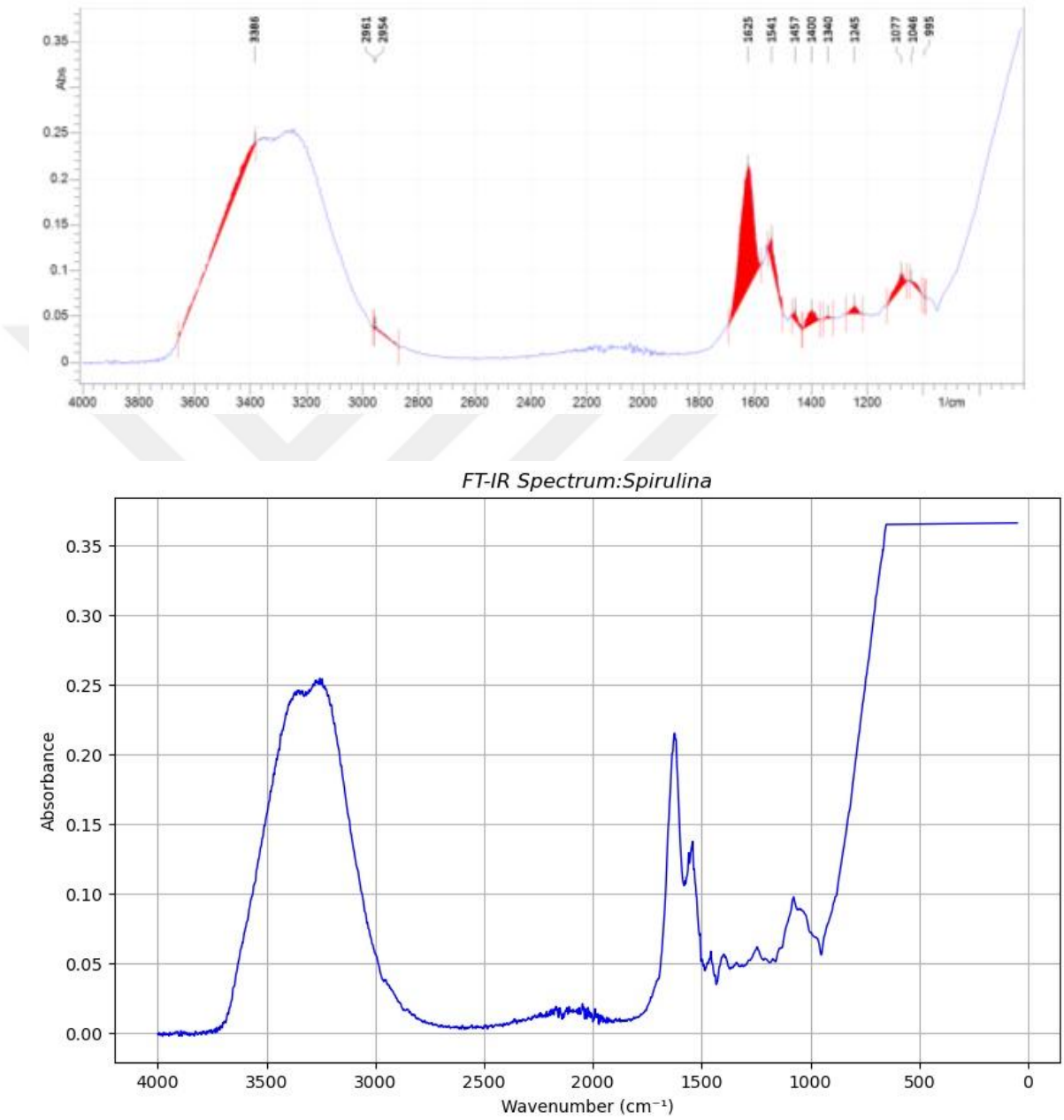

Figure 3.3. FTIR Spectrum: Identification of Functional Groups and Bonding Characteristics of *Spirulina* based bioplastic

3.1.1.2 UV-VIS spectrometer Results

UV-vis spectrometer confirms that both gels have very high absorbance. The end of the peak is also seen in the spectrometric analysis of both gels around 300 nm. The band gap energy, which is considered as the point where it intersects the axis, indicates the presence of conductive ions in the gels. We can also conclude that the Spirulina-Gelatin gel exhibits higher light absorbance compared to the Chlorella gel at the same wavelength (Figure 3.4).

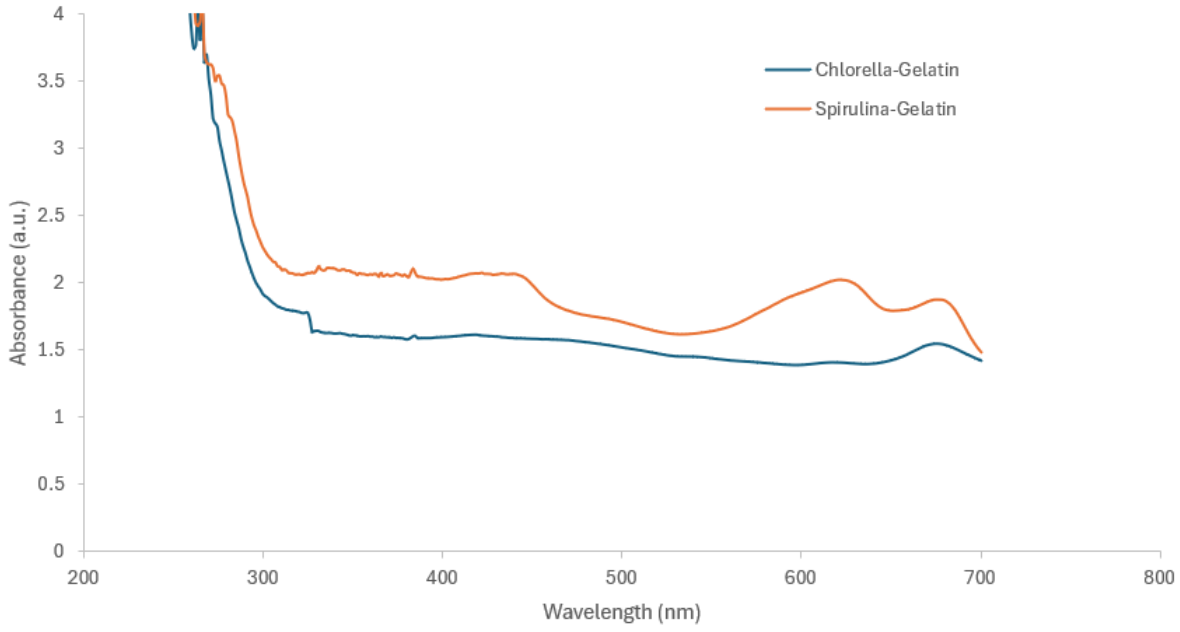

Figure 3.4. UV-VIS spectrometer Results

3.1.2. Biodegradability test results

The biodegradation test was conducted to assess the degradation rates of the obtained gel formulations in a soil environment. The results showed that the Gelatin gel fully degraded within 7 days, the Spirulina-Gelatin gel within 9 days, and the Chlorella-Gelatin gel within 10 days. The relatively similar degradation times indicate that the addition of microalgae products (Spirulina and Chlorella) does not significantly impact the biodegradation process. However, all formulations demonstrated rapid degradation, highlighting their potential as fast-biodegrading materials in soil environments.

3.1.3. Biofertilization test results

Effect of Spirulina based gel on Radish plants

Approximately 20 days after transferring the seedlings to the pots and adding the liquefied spirulina-based bioplastic, all the plants treated with the spirulina gel died. Given that we use a variety of materials in the bioplastic preparation process, it is possible that interactions between these materials may have prevented us from obtaining positive results. Therefore, the material used was simplified and the product was tested again on strawberry and wild indigo plants.

Effect of different gel formulations on the strawberry and wild indigo plants seedling

For both plant species, treated plants demonstrated significantly greater stem length compared to the control group. Seedlings of strawberry and wild indigo grew the fastest within just 10 days, with the chlorella-gelatin gel treatment showing a notably positive impact relative to other treatments ($p = 0.0 < 0.05$) (Appendix 1). This was followed by the rapid growth observed in seedlings treated with spirulina-gelatin gels. As reported in the literature, growth under the influence of pure gelatin was also noted [62].

The tracking of stem length results is presented in the tables below (Table 3.1, Table 3.2), and comparative results are illustrated in Figures 3.5 and 3.6.

Table 3.1 Effect of Gelatin gel, Chlorella-Gelatin gel and Spirulina-Gelatin gel on strawberry seedling (cm)

	Control	**Gelatin**	**Chlorella-Gelatin**	**Spirulina-Gelatin**
Strawberry seedling1	44.4	71.1	117.8	93.3
Strawberry seedling2	21.0	55.6	107.4	72.8
Strawberry seedling3	55.6	82.2	115.6	97.8
Strawberry seedling4	18.5	61.7	107.4	76.5
Strawberry seedling5	35.6	71.1	111.1	82.2
Strawberry seedling6	21.0	72.8	96.3	75.3

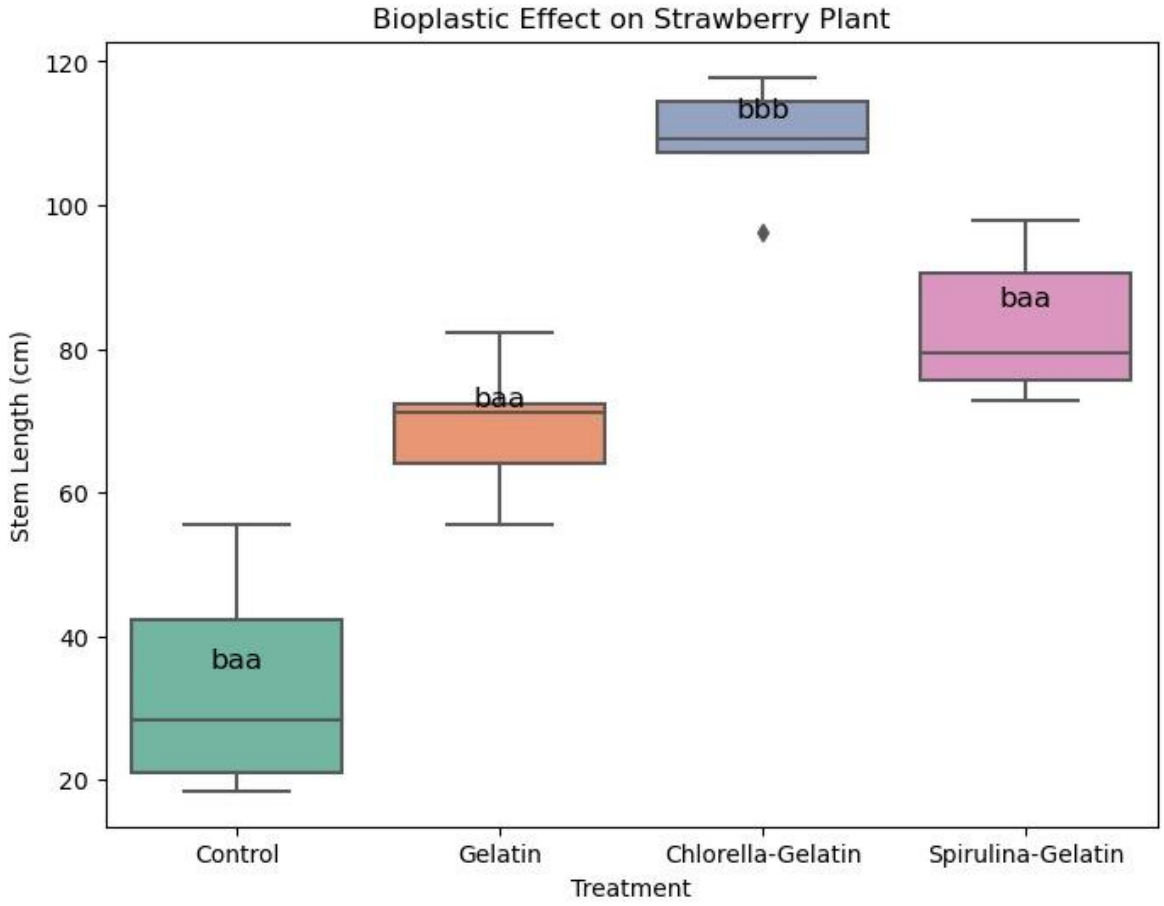

Figure 3.5 Development of Stem Length According to Treatments for Strawberry Plants.

Table 3.2 Effect of Gelatin gel, Chlorella-Gelatin gel and Spirulina-Gelatin gel on indigo strawberry seedling (cm)

	Control	**Gelatin**	**Chlorella-Gelatin**	**Spirulina-Gelatin**
Wild indigo seedling1	60.7	82.5	146.8	99.8
Wild indigo seedling2	41.2	65.9	135.1	78.6
Wild indigo seedling3	81.5	94.4	144.3	104.4
Wild indigo seedling4	38.3	72.5	135.1	82.4
Wild indigo seedling5	58.1	82.5	139.3	88.3
Wild indigo seedling6	41.2	84.4	122.5	81.2

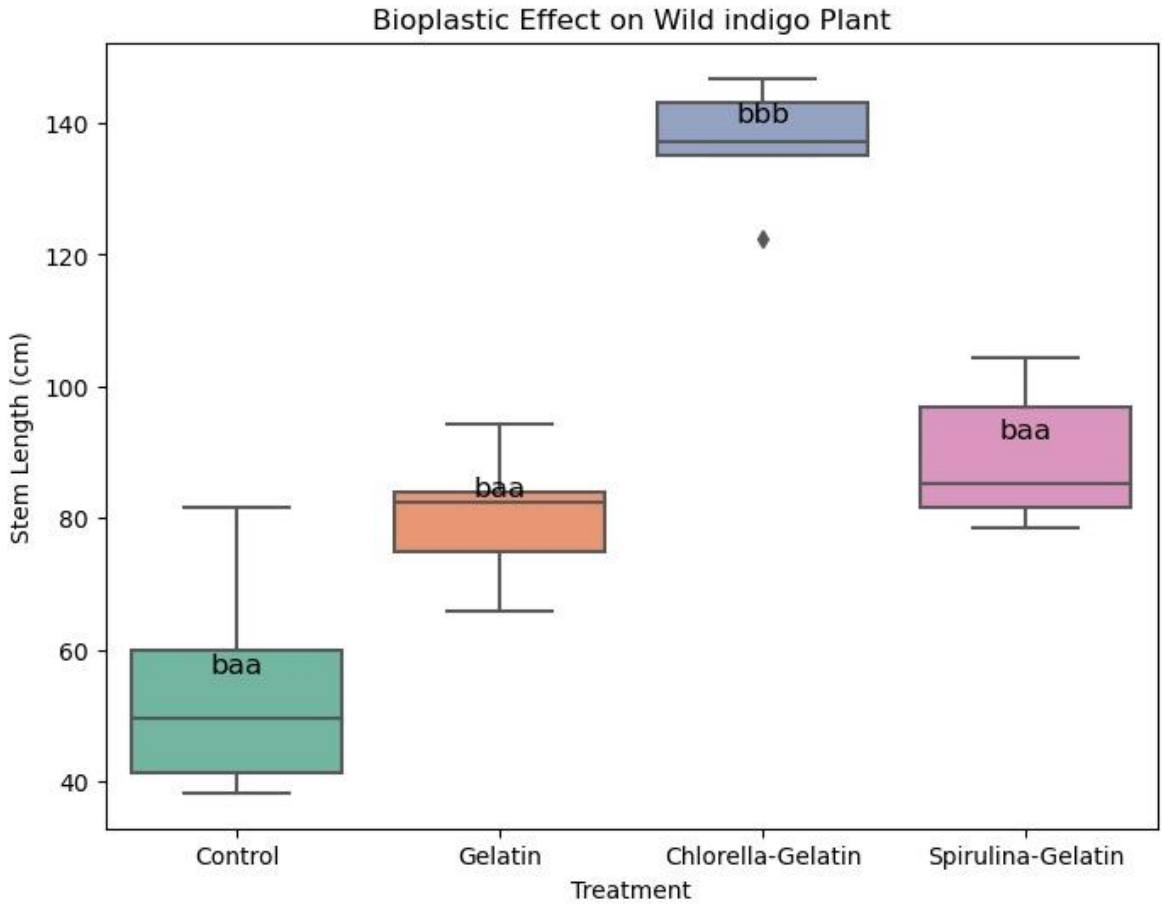

Figure 3.6 Development of Stem Length According to Treatments for Wild indigo plants.

Effect of the different concentrations of Spirulina gel on the Growth of radish plants

The parameters monitored during the in vivo trial were analysed using one-way analysis of variance (ANOVA). The model used was a mixed linear model (MLM), where the different inoculum concentrations were treated as a fixed factor and the various repetitions as a random factor. The normality of the variance was checked using the Shapiro-Wilk test.

A generalized mixed model based on a Poisson distribution was applied to the count data (number of leaves, number of flowers, etc.). When a p-value was found to be significant ($P<0.05$), mean comparisons among the different groups were performed using the Tukey test to identify the divergent group or factors.

3.2 Discussion

The exploitation of microalgae to produce safe Bioplastics proves to be a promising solution for obtaining biodegradable Bioplastics with desired functional properties suitable for various applications [63]. Microalgae have also demonstrated great potential to be used as Biofertilizers to enhance plant growth [13].

In addition, Microalgae, feedstock for bioplastic production, presents a great solution to reduce the use of fossil-based plastic while not competing with food sources [33].

In this study, For the bioplastic formulation gelatine was used to enhance mechanical properties [49] and that's due to its unique physical and functional properties making it an ideal gelling, thickening, stabilizing, and emulsifying agent ensuring Gel strength and viscosity for creating a durable bioplastic [64]. *Chlorella sp.* and *Spirulina sp*. were selected due to their significant contribution to over 90% of global microalgal biomass production [26] which means they are abundantly available for multiple use and exploitation. Both species have been tested for bioplastic production as well as for biofertilization with several documentation supporting their potential as excellent candidates for these uses [65].

The formulation included glycerol to enhance to Improve extensibility and flexibility [66]. Rice water, in the other hand, was incorporated to improve the gel fertilization activity [67].

This approach also promotes waste valorization with the aim of using wastewater from rice industries and rice irrigation, supporting a circular economy model during the scale-up phase.

The biodegradation and biofertilization properties of Chlorella and Spirulina-based bioplastics were tested through in vivo cultivation. The bioplastics were buried in soil alongside three plant species selected to represent different aspects of potential human use: a fruit, a vegetable and medicinal plant. Also the choice of strawberry, as a vulnerable plant to environmental conditions and pollutants [53], has the aim to prove the safety of the bioplastic. The experiment on the radish plants was negative suggesting the use of a better material formulation for the bioplastic.

For the strawberry plants and wild indigo plants, and after simplifying the formulation, the experiment showed an overall positive result for all treatments: gelatine, gelatine-spirulina and gelatine-chlorella.

Chlorella showed significantly better results than *Spirulina* in terms of the tested bioplastic characteristics: biodegradability and biofertilization aspects, a finding further supported by the literature [69], and that can be explained by their biochemical composition suggesting to focus further studies first to understand the difference then to better exploit *chlorella sp* in producing biodegradable bioplastics with the aim of being used as biofertilizers during their end of life stage. The literature further emphasizes that while *Chlorella sp.* exhibits better bioplastic characteristics, *Spirulina sp*. shows superior blending properties. This leads us to consider the possibility of testing bioplastic characteristics using a combination of both microalgae.

For the biofertilization and biodegradability tests, the liquefied form was used to replicate the biofertilizer formulation that previously showed positive results [70], while the gel form was used to simulate real-world conditions, considering that bioplastics used in packaging are likely to end up in the environment.

The FT-IR results, they demonstrate carbon-oxygen bonds that indicate the presence of microalgae discussed in this study. These results also emphasize that the product is rich in

carbon, which justifies its capability to enhance plant growth. It is well known that the depletion of soil organic carbon is a significant issue in croplands, leading to reduced soil quality and fertility. Microalgae, being photoautotrophs, can provide organic carbon, and some strains can even produce exopolysaccharides (EPS) which explains how microalgae can be used as biofertilizers [65]. In the present study the produced molecules weren't investigated but EPS production for both species have been discovered [71],[72]. From the FT-IR spectra the existence of ester group has been proved due to the existence of a peak around 1700 cm-1, and that may reflect the existence of EPS. [60], the peak around 1200 cm-1 enhances the presence of ester, further supporting the presence of polysaccharides. The Absorbance of Chlorella based gel in those wavenumbers is higher than Spirulina based gel one which explains the better results obtained while using chlorella-based gel.

This biopolymer is also among the biopolymers typically used in bioplastic synthesis [73] explaining the formation of films and gels in various shapes, including drug and The hemispherical forms of these bioplastics further demonstrated their potential to enhance plant growth. These samples exhibited properties that are particularly well-suited for pharmaceutical and cosmetic applications, as well as for food packaging and containers. Given the high demand for sustainable materials in the packaging industry [74], this versatility in forming various gel shapes significantly expands their potential range of applications.

This study demonstrates the potential of microalgae-based bioplastics, formulated with *Chlorella* and *Spirulina*, in producing biodegradable materials with biofertilization properties that enhance plant growth. The findings highlight *Chlorella sp.* as an optimal candidate due to its superior biodegradability and biofertilization performance, suggesting a promising path for producing bioplastics that support plant health during their end-of-life phase. Additionally, the incorporation of common food byproducts like rice water and eggshell supports sustainable waste valorization and aligns with circular economy goals.

4. CONCLUSION

Due to growing concerns about the harmful effects of chemical fertilizers and petrochemical plastics, there is increasing interest in exploring sustainable alternatives. In this study, *Chlorella sp.* and *Spirulina sp.* demonstrated excellent potential for broader applications in enhancing agricultural productivity and environmental sustainability, including the successful development of microalgae-based bioplastics. This research highlights microalgae as a dual-purpose resource, supporting both bioplastic biodegradation for environmental benefit and biofertilization to boost agricultural productivity. *Chlorella sp.* significantly enhanced plant growth and successfully produced bioplastics in both film and tablet forms, positioning it as a promising option for various applications, such as packaging.

Future studies should aim to refine these bioplastic formulations, focusing on optimizing mechanical strength and environmental durability. Specifically, investigating combined *Chlorella* and *Spirulina* formulations may improve bioplastic flexibility and stability while retaining biofertilization benefits. Additionally, while this study assessed the impact on plant growth, further research is needed to evaluate the effects of these bioplastics on animals and human health to ensure their safe and widespread application.

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Appendixes

Appendix A:

Table A1. Tukey's Test Results for Comparison of Stem Length Differences Between Treatment Groups for Indigo Plants

Group 1	Group 2	Mean Difference	p-adj	Lower	Upper	Reject
Chlorella-Gelatin	Control	-83.6833	0.0	-102.8812	-64.4854	True
Chlorella-Gelatin	Gelatin	-56.8167	0.0	-76.0146	-37.6188	True
Chlorella-Gelatin	Spirulina-Gelatin	-48.0667	0.0	-67.2646	-28.8688	True
Control	Gelatin	26.8667	0.0044	7.6688	46.0646	True
Control	Spirulina-Gelatin	35.6167	0.0002	16.4188	54.8146	True
Gelatin	Spirulina-Gelatin	8.75	0.5882	-10.4479	27.9479	False

Table A2. Tukey's Test Results for Comparison of Stem Length Differences Between Treatment Groups for Strawberry Plants

Group 1	Group 2	Mean Difference	p-adj	Lower	Upper	Reject
Chlorella-Gelatin	Control	-76.5833	0.0	-94.2786	-58.888	True
Chlorella-Gelatin	Gelatin	-40.1833	0.0	-57.8786	-22.488	True
Chlorella-Gelatin	Spirulina-Gelatin	-26.2833	0.0025	-43.9786	-8.588	True
Control	Gelatin	36.4	0.0001	18.7047	54.0953	True
Control	Spirulina-Gelatin	50.3	0.0	32.6047	67.9953	True
Gelatin	Spirulina-Gelatin	13.9	0.1578	-3.7953	31.5953	False

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