Asian Journal of Soil Science and Plant Nutrition

Volume 10, Issue 3, Page 26-36, 2024; Article no.AJSSPN.117104
ISSN: 2456-9682

Effect of Chitosan Coated Immobilized Algal Cells as Plant Growth Promoter in *Vigna radiata L.*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/ajsspn/2024/v10i3317

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/117104

Original Research Article

Received: 28/03/2024
Accepted: 03/06/2024
Published: 06/06/2024

ABSTRACT

The present study is design to investigate effect of immobilized algal cells as a growth promoter for improving quality and quantity of Leguminosae plant such as mung bean (*Vigna radiata*). The experiment was conducted in plant growth chamber during month of January 2024. The experiment was performed in plastic glass using cocopeat. Immobilized beads were coated with Chitosan and followed by sun drying of that bead. The experiment consists of five treatments which was T0: Control, T1: With chitosan *Chlorococcum* beads, T2: Without chitosan *Chlorococcum* beads, T3: With chitosan *Scenedesmus* beads and T4: Without chitosan *Scenedesmus* beads. The result revealed that seeds treated with Chitosan treatment of algal beads (T1 and T3) it gives significant growth as compared to Control (T0). Shoot length and root length were significantly increased in treatments (T1 and T3) as compared to control. Carbon, Nitrogen and Phosphorous concentration

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Cite as: *H. Patel, Bijal, Ratna Trivedi, and Jay Bergi. 2024. "Effect of Chitosan Coated Immobilized Algal Cells As Plant Growth Promoter in Vigna Radiata L". Asian Journal of Soil Science and Plant Nutrition 10 (3):26-36. https://doi.org/10.9734/ajsspn/2024/v10i3317.*

were significantly increased in all treatment as compared to control whereas, Sulphur concentration was increased in control. Chlorophyll content was also, significantly increased in all treatment compared to control.

Keywords: Immobilized algal beads; biofertilizer; chitosan; mung bean (Vigna radiata).

1. INTRODUCTION

Fertilizers provide plants with primary nutrients such as Nitrogen, Potassium, Phosphorus and other plant nutrients. While these fertilizers increase yields, they pose several environmental risks like, reduce soil fertility and cause a lot of degradation of soil and land. The use of chemical fertilizers reduces soil microorganisms and causes groundwater pollution. Due to numerous environmental risks, consumer preferences are changing in favor of using products grown in organic farming without the use of chemicals.

It has become important for us to increase productivity in agriculture by using various chemical fertilizers due to the increasing demand. But with the tremendous use of these products the soil has been affected badly because of the depletion in the essential minerals of the soil. So, to overcome this problem it has become important for all of us to use a different remedy for the use of various biofertilizers. The use of such biofertilizers from various microorganisms, algae and cyanobacteria increased plant growth [1]. Algae can be used as biofertilizers, providing nutrients to the soil and increasing crop productivity [2].

To enhance the stability of encapsulated bacteria and adhesion of flagellated bacteria in alginate microbeads, the microbeads can be coated with chitosan. Bacteria-encapsulated alginate microbeads with 1% chitosan coating remained structurally stable up to 72 hours, while control groups without chitosan showed degradation after 24 hours. Chitosan-coated alginate microbeads also enhance the adhesion of flagellated bacteria, resulting in microrobots with 4.2 times higher average velocities. Therefore, chitosan coating enhances the structural stability and movement of bacteria-based alginate microrobots [3]. Chemotrophic microorganisms are using available chemicals in ecosystem for their constitutive or induced metabolic pathways [4].

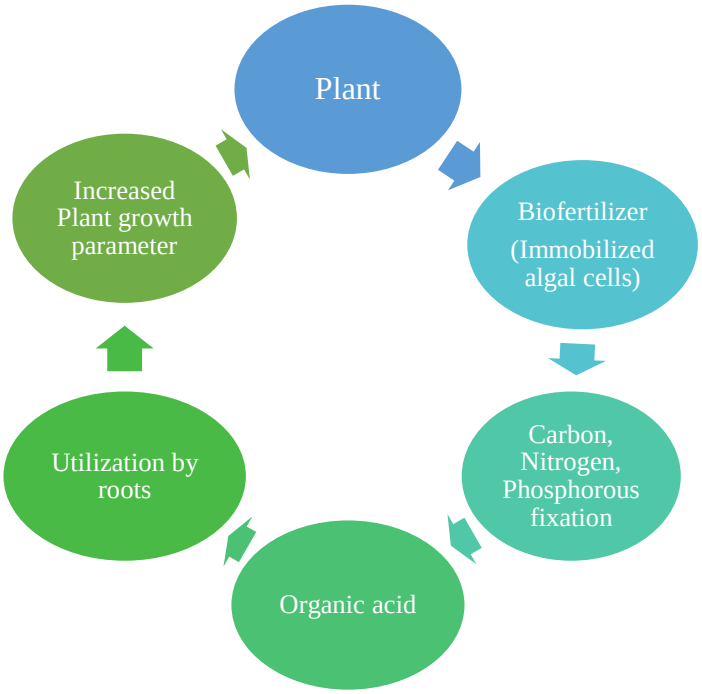


Fig. 1. Effect of algal biofertilizer on plant

Common storage product for cyanobacteria are polyphosphate as a phosphorous storage compound whereas, cyanophycin and phycobilin protein pigment as nitrogen storage product [5].

2. MATERIALS AND METHODOLOGY

Collection of samples: Algal samples were collected from the ponds of two different regions of Valsad district, Chharvada and Lilapore. Collection of samples was done in sterile screw capped glass bottles by clean grab method [6].

Algal isolation: Samples were collected from water bodies and diluted. Different dilutions were plated and allowed to grow in BG 11 medium [7]. After growth, cultures were streaked on sterile plates containing various growth media and incubated. Media were incubated in BOD incubator at 25°C in the presence of light for 10-15 days. This procedure was repeated until a single colony of algae was obtained [8].

Microscopic identification of algae: Algal biomass was observed for microscopic identification by using wet mount preparation techniques [9]. Identification of these isolates to the genus level was based on the morphology of the individual cells following microscopic examination [10].

Quantification of Nitrogen and Phosphorous content of isolates: Phosphorous content was measured by Fiske and Subbaraow method [11] and Nitrogen concentration was measured by Nitrite-Nitrogen coupling method [12].

Mass cultivation of algae and harvesting: Algal cells were pre cultured in a sterile BG11 medium, every two weeks half of the culture volume was replaced by freshly prepared growth medium. Then, all the media were incubated in BOD incubator at 25°C in the presence of light for 10-15 days. Additionally, a photobioreactor can be used for mass cultivation of the isolated algae. In photobioreactor constant mixing of the algal culture in the tank was provide by the aeration. The temperature of the mass culture in tank remained between 20 ^{0}C- 25 ^{0}C. Large volume harvesting of the microalgae was achieved by the centrifugation. Algal biomass was prepared by washing it with demineralization water. At first it was centrifuged and the growth medium supernatant was replaced by demineralized water. The biomass was vortexed and centrifuged again afterwards. After the centrifugation, the supernatant was discarded and biomass were collected.

Immobilization of Algae: For preparation of immobilized cell, 2.5 g Algal biomass was mixed with 50 ml 5% Sodium alginate. Above mixture was filled in syringe and added drop-wise into ice cold 0.7% $CaCl_2$. Intact beads were transferred in 0.7% $CaCl_2$ solution and stored for 2 hours at 4^0C. After that, beads were washed with 0.2M phosphate buffer (pH-7) [13,14]. Beads dried on filter paper. From the prepared beads half of the beads were coated with chitosan and other half remained uncoated. The algal beads, both those treated with chitosan and those without chitosan treatment, were dried in the sun and checked its activity on mung bean (*Vigna radiata*) growth.

Application of immobilized algal beads on mung bean: In plastic container added 50g cocopeat and 5 seeds per container. Following treatments were applied to mung bean - T0: Control (without any treatment), T1: With chitosan *Chlorococcum* beads, T2: Without chitosan *Chlorococcum* beads, T3: With chitosan *Scenedesmus* beads, T4: Without chitosan *Scenedesmus* beads. Kept all the container in plant growth chamber. Checked the moisture content visually every day and added water if needed. At third day observed for the seed germination and measured germination percentage.

Measurement of growth parameter: Statistical analysis, ANOVA test was performed by using Microsoft excel. Growth parameters like shoot length and root length were measured using centimeter scale. Shoot length was measured at day 5 and day 7 and root length was measured at day 7.

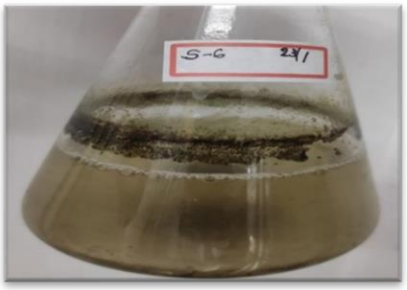
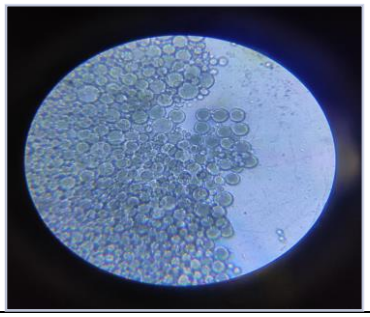
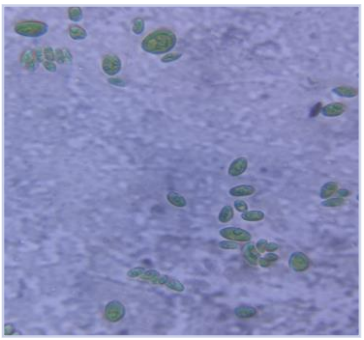
Biochemical analysis: Statistical analysis, ANOVA test was performed by using Microsoft excel. Study of carbon, nitrogen, sulphur and phosphorous content of cocopeat from *Vigna radiata* [15]. Chlorophyll content was measured from the leaves of the mung bean at day 7 [16].

3. RESULTS AND DISCUSSION

3.1 Isolation and Microscopic Observation

Followings were the result of algal growth of the isolates labelled I1 and I2 in BG 11 medium was observed and microscopy at 45x magnification. From the microscopic observation it was observed that the isolates labelled I1was belonging to *Chlorococcum sp.* and isolates labelled I2 was belonging to *Scenedesmus sp.*

Table 1. Observation of algal isolates and BG11 medium and Microscopy

Isolates	BG 11 Medium	Microscopy (45x)
I 1		
I 2		

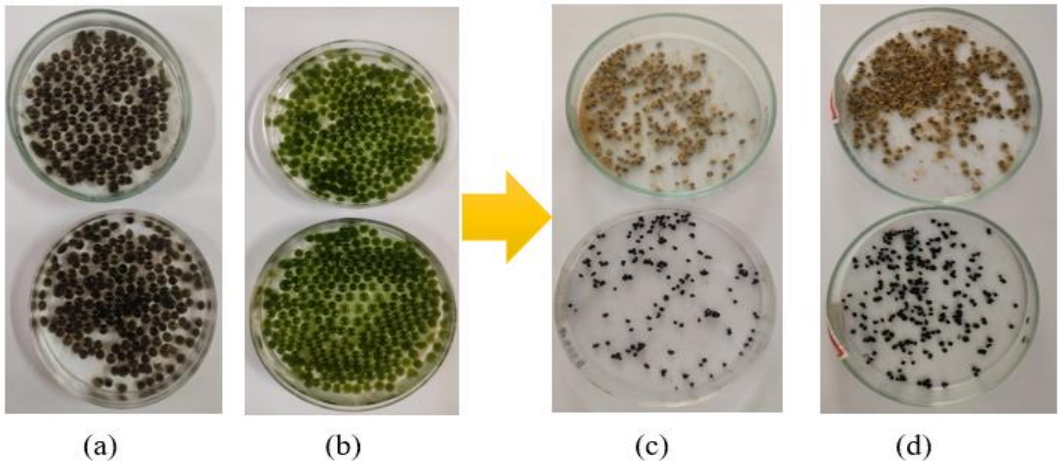


Fig. 2. Observation of (a) *Chlorococcum* bead before sun drying; (b) *Scenedesmus* bead before sun drying; (c) *Chlorococcum* bead with and without Chitosan (after sun drying) and (d) *Scenedesmus* bead with and without chitosan (after sun drying)

3.2 Immobilized Algal Beads

In this study created immobilized algal beads that they further divided into two groups. Half of the beads were coated with Chitosan powder, which is a biopolymer known to improve the stability of the beads, whereas the other half were left without Chitosan coating. To apply the coating, covered the beads surfaces with a layer of Chitosan and then allowed them to sun dried completely. This drying process was critical to prevent contamination and ensure that the Chitosan coating effectively adhered to the surface of the algal beads.

After drying, the algal beads were stored for further investigation. Proper storage is essential to maintain the structure of the beads and prevent contamination. Hence, took the necessary precautions to store the beads under suitable temperature and lighting conditions.

Overall, the Chitosan coating was an essential step in the study as it provided the immobilized algal beads with additional structural and mechanical support. The beads can now be used for future experimentation and investigation to determine their effectiveness.

3.3 Observation of Mung Bean Growth

In the figure presented, the study focused on the observation of mung bean growth and the effect of different treatments on mung bean growth (Fig. 3).

It was observed that the seeds began to germinate at day 2. At this point, the beads that were coated with chitosan treatment were observed to have germinated. This indicated that the chitosan treatment might have promoted earlier germination of the mung bean seeds.

By day 3, all the seeds in all containers had germinated. This indicated that the seeds had been successfully planted and were growing. At day 4, observed visible growth in the length of the shoots. The shoots seemed to have grown longer, indicating that growth was taking place. This growth continued to be observed on day 5 and day 7, indicating that the seeds continued to grow and develop at a steady pace. The study noted that these observations of growth were made despite the different treatments applied to the mung bean plants.

(a)

(b)

(c)

Fig. 3. (a) Observation at day 1; (b) Observation at day 4 and (c) Observation at day 7

Overall, this study's observations showed that the mung bean seeds grew and developed at a steady pace. The chitosan coating treatment appeared to promote earlier germination of the seeds, indicating that it may have a positive effect on mung bean growth. However, more investigation is needed to determine the full impact of chitosan coating treatment on mung bean growth.

3.4 Germination Percentage of Mung Bean

In the study, measured the germination percentage of mung bean seeds on the third day of the study. The germination percentage was calculated on the basis of the number of seeds that had germinated per container. The results showed that in all the treatments, the germination percentage was 100%. This meant that all the seeds that had been planted successfully germinated within three days. The high germination percentage indicated that the planting methods used and the conditions provided were suitable for the mung bean seeds to germinate successfully [17].

Overall, the high germination percentage observed in all the treatments indicated that the seed germination was successful, and the seeds were growing as expected. The observed growth over subsequent days confirmed that the germination percentage obtained at day three correlated with the healthy growth of the mung bean plants. The study's results suggest that the planting methods and conditions used are suitable for the cultivation of mung bean plants.

$$\text{Germination (\%)} = \frac{\text{No. of seed germinated} \times 100}{\text{No. of seed in glass}}$$

3.5 Seedling Vigour index

Vigour index [18] of seedling was also calculated by following formula,

Vigour index = [Root length + Shoot length] × Seed germination %

3.6 Measurement of Shoot and Root Length of Mung Bean

In this study measured the length of the shoots on day 5 and day 7 following the planting of the mung bean seeds. Additionally, root length was measured on day 7. The data presented in the table revealed that the mung bean seeds treated with algal beads coated with chitosan produced better results compared to algal beads without chitosan and the control. Specifically, on day 5, the length of the shoots measured in the algal beads coated with chitosan treatment was 5.84 cm and 5.42 cm, higher than the uncoated algal beads (3.9 cm and 5.04 cm), and the control (4.22 cm). The difference in shoot length between chitosan-coated algal beads and uncoated ones becomes more significant on day 7, with the algal beads coated with chitosan resulting in a length of 8.12 cm and 7.2 cm, and uncoated algal beads and control resulting in 5.86 cm and 6.5 cm and 6.7 cm, respectively.

Table 2. Vigour index of mung bean

Treatments	Germination percentage (%)	Vigour index
T0	100	1390
T1	100	1630
T2	100	1176
T3	100	1740
T4	100	1390

On day 7, the root lengths of the mung beans were also measured. The chitosan-coated algal beads had the longest roots, with a length of around 8.2 cm and 10.2 cm, while the uncoated algal beads and control only produced root lengths of approximately 5.9 cm and 7.4 cm and 7.2 cm, respectively. The results revealed that the application of chitosan coating had a positive effect on mung bean growth, increasing both the length of the shoots and roots. From these results, it can be concluded that the use of chitosan coating might have been an effective method to promote better growth and development of mung bean seeds.

The ANOVA results (Table 3) showed that there is a statistically significant difference between the three groups at a 95% confidence level (P-value = 0.005). The F-value is 8.4763, and the f-critical value is 3.8853. Based on these results, we reject the null hypothesis that there is no significant difference between the three groups. We conclude that the sample means of at least two groups are different and that some treatments were more effective than others in promoting shoot and root growth.

3.7 Study of C, N, S, P Content of Cocopeat from *Vigna radiata*

In this study, analysed the carbon, nitrogen, sulphur, and phosphorus content of mung bean

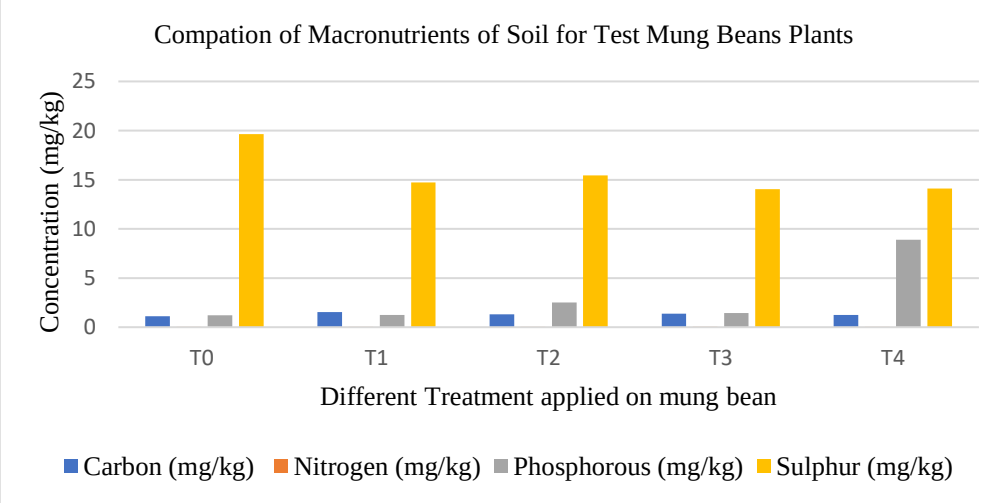
coated seeds treated with different methods. The data showed that the Chitosan treated algal beads had higher carbon, nitrogen, and phosphorus content than the control group. However, the sulphur content was found to be higher in the control group compared to the treated ones.

Chitosan is a natural biopolymer derived from chitin that has been found to have a positive impact on plant growth. In this study, the chitosan treated algal beads had shown higher concentrations of carbon, nitrogen, and phosphorus compared to the control group. Carbon is an essential component of plants, providing structure and aiding in energy production. Nitrogen is critical in plant development, as it is a key component of chlorophyll, which aids in photosynthesis. Phosphorus plays a significant role in plant growth, including cell division and energy transfer. In contrast, the sulphur content was found to be higher in the control group compared to the treated ones, indicating that the chitosan treatment may have negatively affected the sulphur content of the mung bean seeds. Overall, the data showed that the chitosan treatment of algal beads had a positive impact on the Carbon, nitrogen, and phosphorus content of the mung bean seeds, which could have contributed to their growth and development. However, it is important to note that the impact of the treatment on sulphur content warrants further investigation.

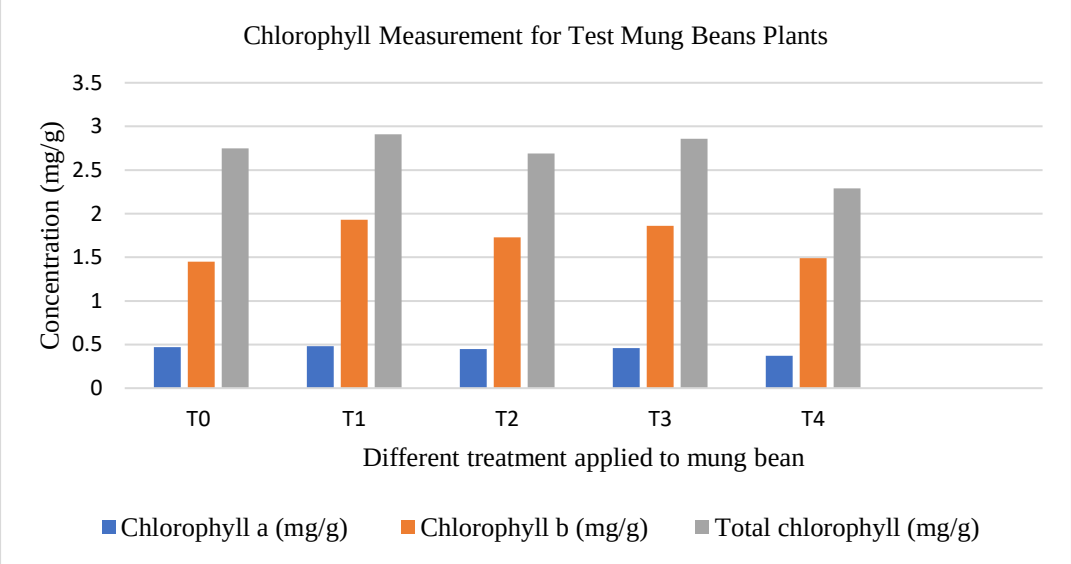
The ANOVA test results showed that there is a statistically significant difference between the four groups at a 95% confidence level (P-value = 4.53891E-09). The F-value is highly significant at 62.80087627, and the f-critical value is 3.238871517. Based on these results, we reject the null hypothesis that there is no significant difference between carbon, nitrogen, phosphorus, and sulphur groups. We conclude that there is a statistically significant difference between the four groups. Sulphur had the highest mean followed by phosphorus, carbon and nitrogen.

Table 3. Measurement of shoot length and root length

Treatments	Shoot length (cm)		Root length
	Day - 5	Day - 7	Day 7
T0	4.22 ± 0.61	6.7 ± 0.76	7.2 ± 2.29
T1	5.84 ± 0.63	8.12 ± 0.78	8.2 ±2.63
T2	3.9 ± 1.31	5.86 ± 0.5	5.9 ± 2.24
T3	5.42 ± 0.73	7.2 ± 1.18	10.2 ± 0.55
T4	5.04 ± 0.87	6.5 ± 0.26	7.4 ± 0.7
p-value	<0.05		
F-value	8.47634		
F critical	3.885294		



Graph 1. Comparison of Macro-nutrients presented in Soil samples after addition of immobilized cells



Graph 2. Concentration of Chlorophyll in treated Test Mung Bean Plants

3.8 Chlorophyll Content Measurement from *Vigna radiata*

The Chlorophyll contents were measured in the leaves of mung bean on Day 7. Chlorophyll has an essential role in the photosynthesis process of plants, allowing them to absorb energy from sunlight to produce food. Chlorophyll content was measured using the spectrophotometric method. The measurements were taken for Chlorophyll a, Chlorophyll b, and Total Chlorophyll content. The Chlorophyll a content was measured by taking absorbance readings of the mung bean leaves at a wavelength of 645nm, and Chlorophyll b content was measured at a wavelength of 663nm. The Total Chlorophyll content was measured at a wavelength of 652nm, which includes both Chlorophyll a and Chlorophyll b.

The data obtained showed the Chlorophyll content of the mung bean leaves. The Chlorophyll a and Chlorophyll b contents were then combined to obtain the Total Chlorophyll content. The results of the study revealed that the mung bean plants treated with Chitosan-coated algal beads had higher Chlorophyll contents, including Chlorophyll a, Chlorophyll b, and Total Chlorophyll content, than the control plants. The data obtained indicates that the Chitosan-coated algal beads are beneficial for the growth and development of the mung bean plants. Specifically, the increased Chlorophyll content could mean that the plants were producing more energy through photosynthesis.

In conclusion, the spectrophotometric method was used to measure the Chlorophyll content in the leaves of mung bean on Day 7. The Chlorophyll a, Chlorophyll b, and Total Chlorophyll content were measured, and the data obtained showed that the Chitosan-coated algal beads had a positive impact on the Chlorophyll content of the mung bean plants.

The ANOVA test results showed that there is a statistically significant difference between the two groups at a 95% confidence level (P-value = 1.26996E-09). The F-value is highly significant at 176.3277, and the f-critical value is 3.8853. Based on these results, we reject the null hypothesis that there is no significant difference between chlorophyll a and chlorophyll b groups. We conclude that there is a statistically significant difference between chlorophyll a and chlorophyll b groups. The Chlorophyll b group had a higher average than the chlorophyll a group.

4. DISCUSSION

The present study aimed to investigate the effect of immobilized algal cells on the growth and nutritional quality of mung bean plants. The findings suggest that the use of algal beads coated with Chitosan can be a potential source of plant growth promoter, leading to improved quality and quantity of leguminous crops such as mung bean. The use of Chitosan as a coating material for the algal beads has also shown a

positive impact on the growth of mung bean plants. Biopolymer ‘‘Chitosan’’ has received much interest for potential wide application in agriculture due to its excellent biocompatibility, biodegradability and bioactivity. This naturally occurring molecule with interesting physiological potential has been getting more attention in recent years [19].

The study showed that the highest percentage of seed germination was observed in all treatment groups compared to the control. The length of the shoot and root was also significantly higher in treatments T1 and T3 compared to the control group. This indicates that the immobilized algal cells had a positive impact on the growth of the mung bean plants. Dineshkumar et al. [17] studied that, use of microalgae-based biofertilizers, *Chlorella vulgaris* and *Spirulina platensis*, in enhancing the growth of green gram (*Vigna radiata*). Treatment with these extracts resulted in improved growth parameters such as shoot and root length, weight, number of root nodules, plant height, branches, leaves, and leaf area index. De-Bashan et al. [20] studied that the use a simple experimental model for studying plant-bacterium interaction. This model involves using a unicellular microalga called *Chlorella* species as the plant and a plant growth-promoting bacterium called *Azospirillum* species. These two will be immobilized together in small alginate beads. This method allows for close interaction between the pant and bacterium while avoiding interference from external bacterial contaminants.

The analysis of biochemical parameters showed that carbon, nitrogen, and phosphorus concentrations were significantly higher in all treatment groups compared to the control group. This result indicates that the use of immobilized algal beads can be a potential source of nitrogen and phosphorus for the mung bean plants, leading to an improved nutrient content. However, this study showed that the Sulphur concentration was decreased in all treatments compared to control group.

Moreover, the Chlorophyll content was found to be significantly higher in all treatment groups compared to the control group. This indicates that the use of immobilized algal cells can potentially improve the photosynthesis process of mung bean plants, leading to a higher yield of crops. In one experiment Gharib F. A. E. L et al. [21] *Chlorella vulgaris*, *Nannochloropsis salina,* and *Arthrospira platensis* (*Spirulina platensis*) extracts was evaluated on common bean plants. Among the extracts, *N. salina* showed the highest content of growth hormones and auxins. Application of these extracts significantly improved various growth parameters, yield attributes, chlorophyll content index, antioxidant capacity, and reduced oxidative stress markers in common bean plants.

Overall, the results of this study demonstrate that the use of immobilized algal cells as a plant growth promoter could be a promising technology for the agriculture industry. However, further research is required to understand the mechanism of action behind the positive impact on plant growth and nutritional quality. Additionally, future studies should investigate the long-term effects and potential negative consequences of using immobilized algal cells as a plant growth promoter.

5. CONCLUSION

The findings of the study suggest that the use of algal beads coated with Chitosan has the potential to act as a plant growth promoter, leading to the improvement of both the quality and quantity of leguminous crops, specifically mung bean. Algal biofertilizers have been found to be a major source of Nitrogen and Phosphorous, which are two of the most important macronutrients needed for plant growth. The immobilized algal beads contain significant amounts of these nutrients, which are made available to the plant once they interact with the soil. The experimental results suggest that seeds treated with Chitosan treatment of algal beads (T1 and T3) exhibited significant growth as compared to the control group (TO). This was also reflected in the significant increase of Carbon, Nitrogen, and Phosphorus concentrations in all treatment groups compared to the control group, indicating the positive effect of the algal beads on the nutrient content of the mung bean plants. This result indicates that the algal beads treatment had a positive effect on the nutrient content of the mung bean plants. Additionally, the study measured the Chlorophyll content in the mung bean plants. The Chlorophyll content was found to be the highest in T1, followed by T3, compared to the control group. This result indicates that the algal beads coated with chitosan treatment had a positive effect on the Chlorophyll content of the mung bean plants, which is essential for the photosynthesis process. In conclusion, the experiment findings suggest that the use of coated immobilized algal

cells are significant for plant growth promoter. They could have a positive impact on shoot length, root length, nutrient content, and Chlorophyll content of mung bean plants. However, the negative impact on Sulphur content needs further investigation.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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