

CHITOSAN COATED IMMOBILIZED ALGAL CELLS AS PLANT GROWTH PROMOTER ISOLATED FROM SOUTH GUJARAT

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. Algal biofertilizer are major source of Phosphorous and Nitrogen. 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. Measured percentage of seed germination and also measured shoot length and root length. Carbon, Nitrogen, Phosphorous, Sulphur concentration was measured from the mung bean. Chlorophyll content was measured from the leaves of mung bean. The result revealed that seeds treated with Chitosan treatment of algal beads (T1 and T3) it gives significant growth as compared to Control (T0). Carbon, Nitrogen and Phosphorous concentration 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*)

Introduction:

Fertilizers provide plants with essential nutrients such as Nitrogen (N), Potassium (K) and Phosphorus (P). While these fertilizers increase yields, they pose several environmental risks. 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 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 production of various biofertilizers. The production of such biofertilizers from various microorganisms (Boraste *et al.*,2009). The product acts on plants both vegetation and reproduction growth stages. Promotes efficient tillation of available applied biofertilizers

resulting in improved crop quality and higher yield. It is also effective in producing the plant during critical stress periods such as drought, temperature variation etc.

In the soil and root nodules of legume crops, they fix nitrogen and make it available to the plant. They also solubilize the insoluble form of phosphate, such as tricalcium, iron, and aluminium phosphate into an available form, and produce hormones and antimetabolites that promote root growth. They also decompose the organic matter. When biofertilizers are applied to the seed and the soil they increase the availability of the nutrient to the plant (Bhattacharjee and Dey,2014). Chemotrophic microorganisms are using available chemicals in ecosystem for their constitutive or induced metabolic pathways (Ratna Trivedi, Jay Bergi; 2022).

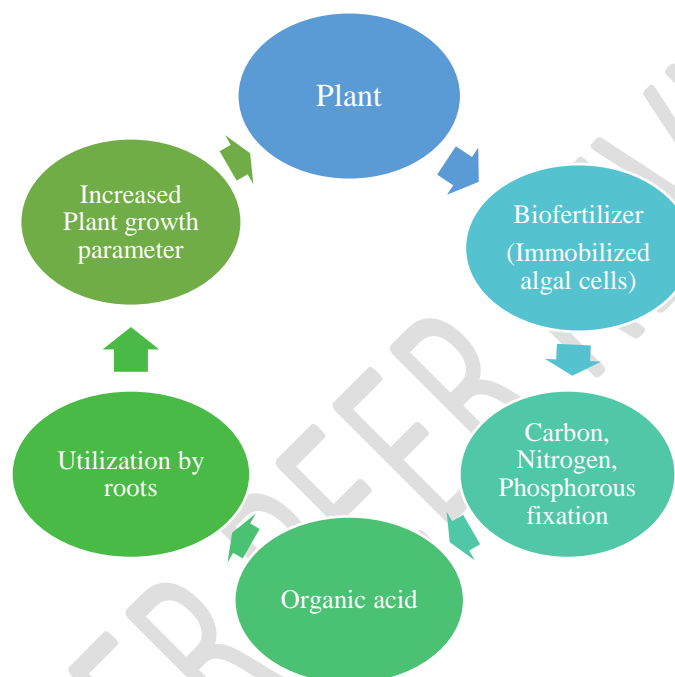


Figure 1: Effect of algal biofertilizer on plant.

Common storage product for cyanobacteria are polyphosphate as a phosphorous storage compound whereas, cyanophycin and phycobilin protein pigment as nitrogen storage product (Kromkamp, 1987).

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 (APHA,1995).

Algal isolation: Samples of algae were cultured in BG11 medium (R. M. Atlas, 1946). Media were incubated in BOD incubator at 25°C in the presence of light for 10- 15 days. From these Culture, algae have been isolated on BG 11 agar plates and stored on BG 11 agar slant.

Microscopic identification of algae: Algal growth was observed for microscopic identification by using wet mount preparation techniques (Patel B. *et al.*, 2021). Identification of these isolates was based on the morphology of the individual cells following microscopic examination.

To quantify Nitrogen and Phosphorous content of isolates: Phosphorous content was measured by Fiske and Subbaraow method (Fiske and Subbaraow, 1925) and Nitrogen concentration was measured by Nitrite-Nitrogen coupling method (K. Veena and B. Narayana, 2008).

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. Also, use photobioreactor for the mass cultivation. 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 °C- 25 °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 were mixed with 50 ml 5% Sodium alginate. Above mixture was filled in syringe and added drop-wise into ice cold 0.7% CaCl₂. Intact beads were transferred in 0.7% CaCl₂ solution and stored for 2 hours at 4°C. After that, beads were washed with 0.2M phosphate buffer (pH-7). (Da Costa *et al.*, 1991; Soares *et al.*, 2022) Beads dried on filter paper. From the prepared beads half of the beads were coated with chitosan and other half remained uncoated. Sun dried of all algal beads (with chitosan and without chitosan treatment) 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, 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.

Measured growth parameter: 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.

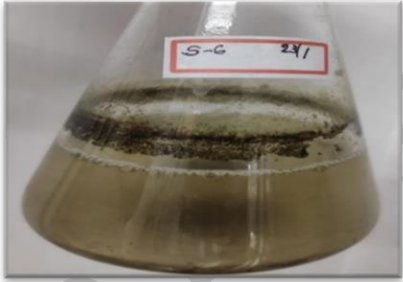
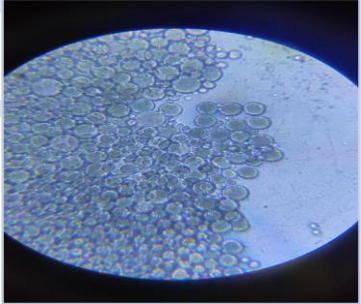

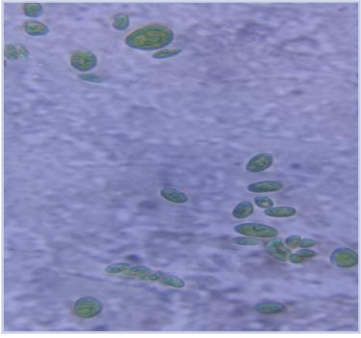
Biochemical analysis: Study of Carbon, Nitrogen, Sulphur and Phosphorous content of cocopeat from *Vigna radiata* (Alef, K., & Nannipieri, P., 1995). Chlorophyll content was measured from the leaves of the mung bean at day 7 (Parry *et al.*, 2014).

Result and Discussion:

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 I1 was belonging to *Chloococcum 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		

Immobilized Algal beads

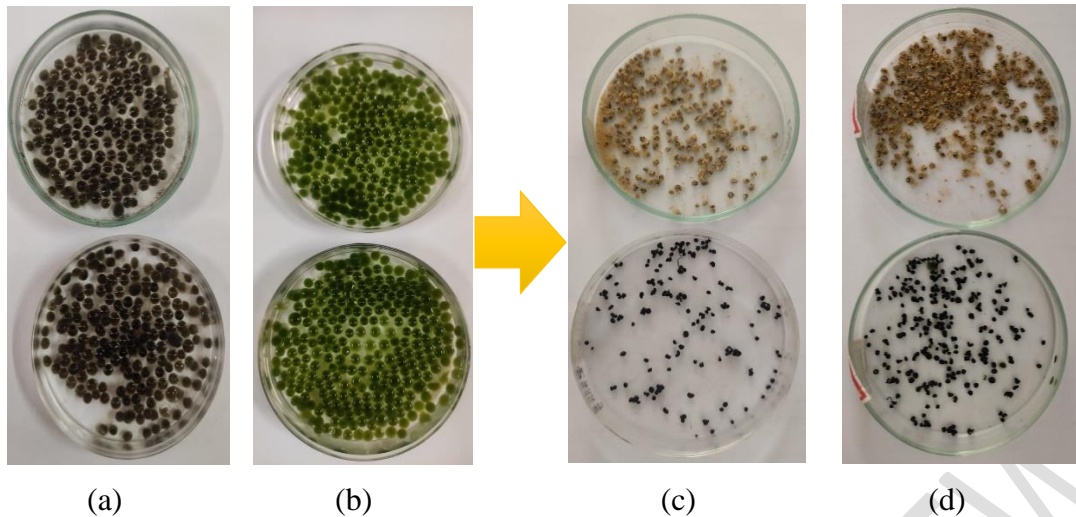


Figure 2: Observation of (a) *Chlorococcum* bead before sun drying; (b) *Scenedesmus* bead before sun drying; (c) *Chlorococcum* bead with and without Chitosan (after sun drying) and (d) *Scenedesmus* bead with and without chitosan (after sun drying).

In this study created immobilized algal beads that they further divided into two groups. Half of the beads were coated with Chitosan, 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 dry 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, stored the algal beads 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.

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).



Figure 3: (a) Observation at day 1; (b) Observation at day 4 and (c) Observation at day 7

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.

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.

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 (Dineshkumar *et al.*, 2020).

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.

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

Table 2: Germination percentage of mung bean

Sample	T0	T1	T2	T3	T4	T5
Germination (%)	100%	100%	100%	100%	100%	100%

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.

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.

Table 3: Measurement of shoot length and root length

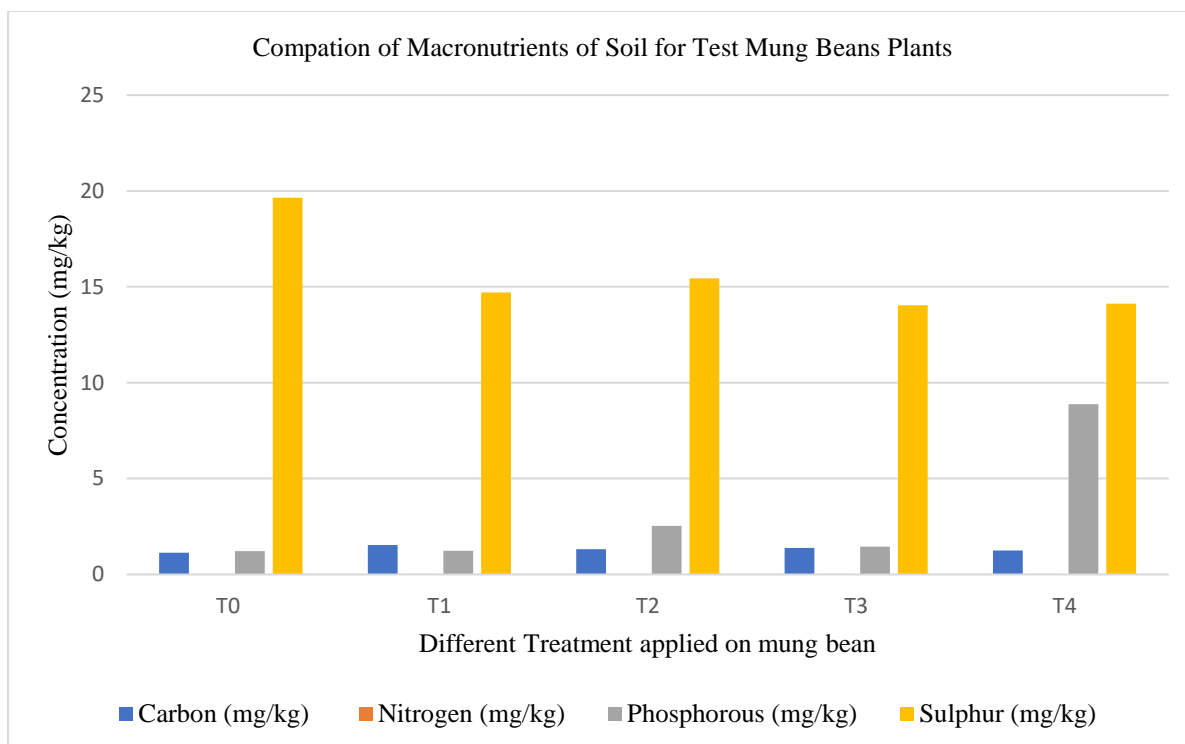
Shoot length (cm)		Root length	
Treatment	Day - 5	Day - 7	Day 7
T0	4.22 ± 0.61	6.7 ± 0.76	7.2 ± 2.29
T1	5.84 ± 0.63	8.12 ± 0.78	8.2 ± 2.63
T2	3.9 ± 1.31	5.86 ± 0.5	5.9 ± 2.24
T3	5.42 ± 0.73	7.2 ± 1.18	10.2 ± 0.55
T4	5.04 ± 0.87	6.5 ± 0.26	7.4 ± 0.7

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 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.



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

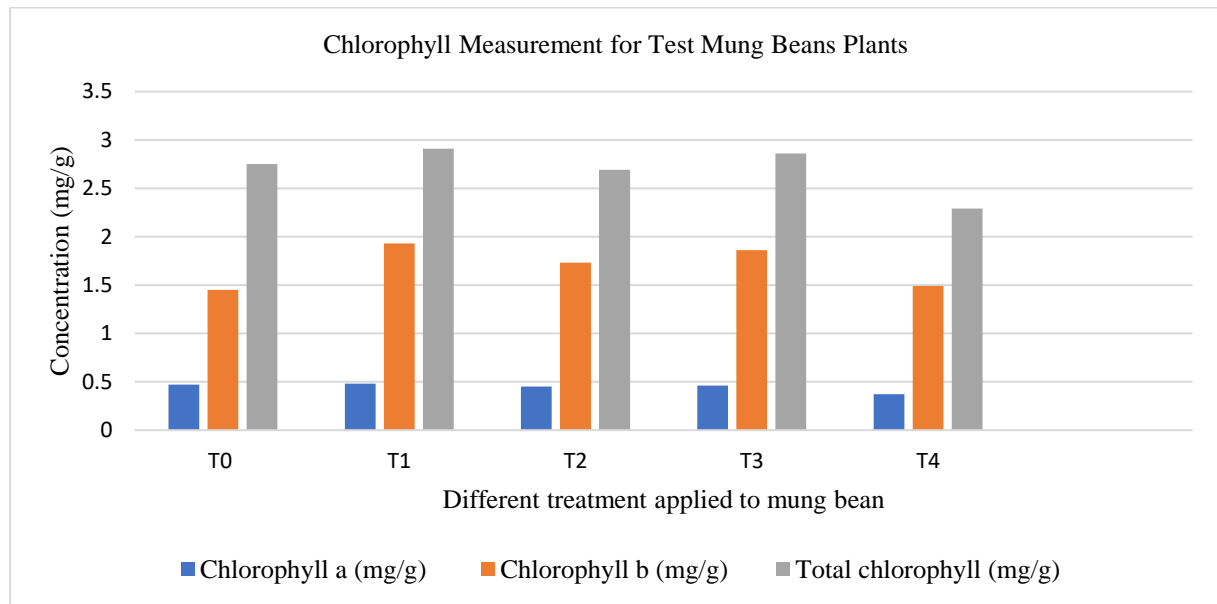
Chlorophyll content measurement from *Vigna radiata*

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.



Graph-2: Concentration of Chlorophyll in treated Test Mung Bean Plants

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. Chitosan, a natural biopolymer, has several useful properties that can improve the bioavailability of nutrients in the soil, aid in plant growth promotion, and enhance resistance to biotic and abiotic stress.

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.*, 2020 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. Refaay D. A. *et al.*, 2021 investigated that assess the impact of foliar spraying with *Chlorella vulgaris*, *Arthrospira platensis*, and *Tetrademus dimorphus* suspensions as biostimulants on common bean growth

and yield characteristics. Foliar spraying with *C. vulgaris* and chemical fertilizer exhibited the most significant increase in various growth parameters and yield attributes compared to the control group.

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.*, 2024 *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.

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.

References:

1. Alef, K., & Nannipieri, P. (Eds.). (1995). *Methods in applied soil microbiology and biochemistry* (pp. xix+-576).
2. APHA. Standard methods for examination of water and wastewater. 19th ed. Washington DC: American Public Health Association; 1995 AWWA WEF.
3. Bhattacharjee, R., & Dey, U. (2014). Biofertilizer, a way towards organic agriculture: A review. *African Journal of Microbiology Research*, 8(24), 2332-2343.
4. Boraste, A., Vamsi, K. K., Jhadav, A., Khairnar, Y., Gupta, N., Trivedi, S., & Joshi, B. (2009). Biofertilizers: A novel tool for agriculture. *International Journal of Microbiology Research*, 1(2), 23.
5. Da Costa, A. C. A., & Leite, S. G. F. (1991). Metals biosorption by sodium alginate immobilized *Chlorella homosphaera* cells. *Biotechnology Letters*, 13, 559-562.
6. Dineshkumar, R., Duraimurugan, M., Sharmiladevi, N., Lakshmi, L. P., Rasheeq, A. A., Arumugam, A., & Sampathkumar, P. (2020). Microalgal liquid biofertilizer and biostimulant effect on green gram (*Vigna radiata* L) an experimental cultivation. *Biomass Conversion and Biorefinery*, 1-21.
7. Fiske, C. H., & Subbarow, Y. (1925). The colorimetric determination of phosphorus. *J. biol. Chem*, 66(2), 375-400.
8. Gharib, F. A. E. L., Osama, K., Sattar, A. M. A. E., & Ahmed, E. Z. (2024). Impact of *Chlorella vulgaris*, *Nannochloropsis salina*, and *Arthrospira platensis* as bio-stimulants on common bean plant growth, yield and antioxidant capacity. *Scientific Reports*, 14(1), 1398.

9. K Veena and B Narayana (2008). Spectrophotometric determination of nitrite using new coupling agents. *Indian Journal of Chemical Technology*.
10. Kromkamp, J. (1987). Formation and functional significance of storage products in cyanobacteria. *New Zealand Journal of Marine and Freshwater Research*, 21(3), 457-465.
11. Parry, C., Blonquist Jr, J. M., & Bugbee, B. (2014). In situ measurement of leaf chlorophyll concentration: analysis of the optical/absolute relationship. *Plant, cell & environment*, 37(11), 2508-2520.
12. Patel B., Shah K.B., Shah B. (2021). Scope of a Algae as a Third Generation Biodiesel Production. *Journal of Emerging Technologies and Innovative Research*, Volume 8, issue 7.
13. R.M. Atlas (1946). *Handbook of microbiological media*.
14. Ratna Trivedi, Jay Bergi (2022). Role of chemotrophs in Agricultural Rich Soils. *ARER*, Vol:10 (1), 1-11.
15. Refaay, D. A., El-Marzoki, E. M., Abdel-Hamid, M. I., & Haroun, S. A. (2021). Effect of foliar application with *Chlorella vulgaris*, *Tetradesmus dimorphus*, and *Arthrospira platensis* as biostimulants for common bean. *Journal of Applied Phycology*, 33, 3807-3815.
16. Soares, R. C., Zangirolami, T. C., Giordano, R. L., Demeke, M. M., Thevelein, J. M., & Milessi, T. S. (2022). Cell immobilization using alginate-based beads as a protective technique against stressful conditions of hydrolysates for 2G ethanol production. *Polymers*, 14(12), 2400.