SCALE-UP AND STANDARDIZATION OF PHOTOTROPHIC MICROORGANISMS AS BIOFERTILIZER

Abstract:

A pot study was conducted at Shree Ramkrishna institute, Surat during the months of December 2022 to March 2023 to study the effect of algal biofertilizer on plants like *Solanum melongena, Solanum lycopersicum* and *Capsicum annum*. Parameters like plant height, width, number of leaves at different time intervals were measured. Carbon, Nitrogen, Phosphorous and Sulphur content from soil were measured before and after application of biofertilizers. Total 30 pots were taken, 10 for each species. Different treatment were applied to each pot, total eight different isolates were used for pot study and two pots were kept as controls. In one of the controls chemical fertilizers were added. Plant growth was significantly increased in I 1 & I 2 in *Solanum melongena*, whereas in *Solanum lycopersicum* and in *Capsicum annum* plant growth were significantly increased in all treatments as compared to control. Chlorophyll content was significantly increased in chemical fertilizer in *Solanum melongena* whereas, in *Solanum lycopersicum* and in *Capsicum annum* Chlorophyll content was significantly increased in I 1.

Keywords: Algae, Biofertilizer, Crops, Growth parameters, Scale-up, Standardization

INTRODUCTION:

Current agricultural practices depend on chemical fertilizers, pesticides, herbicides, weedicides, etc. that will have negative influence on agricultural crops, cultivated area and health of the soil. Many of the chemical fertilizers have detrimental health effects on humans. (Mushtaq *et al.,*2021).

While chemical fertilizers provide plants with some essential nutrients like Nitrogen (N), Potassium (K) and Phosphorus (P) and thereby they increase yields, they pose several undesirable health hazards. Due to these health hazards, consumers prefer using products grown using organic farming without the use of chemicals. They provide an economically attractive and environmentally friendly way to feed agricultural crops. Organic fertilizers are a cheap and renewable nutrient source that compliments chemical fertilizers with reduced hazards. To increase yields, species that stimulate plant growth are usually used. In addition to agricultural benefits, there are potential benefits for environmental applications (Shridhar, 2012).

Biofertilizers are a mixture which contains living microorganisms which, when applied to seeds, plant surfaces, or soil, colonize the rhizosphere or the interior of the plant and enhance growth by increasing availability of some primary nutrients to the host plant. Biofertilizer are essential components of organic farming which play a key role in improving crop productivity, and improving soil health The microorganisms in soil also restore natural nutrient cycle and build good soil quality.

The most commonly used biofertilizers are nitrogen-fixers, potassium solubilizers, phosphorus solubilizers, and plant growth promoting rhizobacteria (PGPR). One gram of rich soil can contain up to 10^{10} cfu, with a live weight of 2000 kg/ha. Nutrient fixation and improvements in plant growth by bacteria, on the other hand, are critical components for accomplishing future sustainable agricultural goals (Daniel *et al.,* 2022).

Microalgae are rich sources of Carbohydrates, Proteins, Lipids and Vitamins. Besides being nutraceuticals to the humans, they are useful in Cosmetics and Biofuels also (Spolaore *et al.,* 2006).

METHODOLOGY:

Collection of samples:

Algae samples were collected from the different water bodies of Valsad district like, Shanker talav, Chharvada, Chikhala and Lilapore. Collection of samples was done by clean grab method (APHA,1925). Samples of algae were cultured in BG11 medium (R.M. Atlas, 1946). Then, all the media were incubated in BOD incubator at 25°C in the presence of light for 10- 15 days.

Isolation:

Microalgae and Cyanobacteria cultures were initially cultured in BG11 broth. From the culture algae were isolated on BG 11 agar plates. All the plates were incubated in BOD incubator. Isolated organisms were differentiated based on morphological examinations and appearance of the colonies on BG 11 agar medium. This general approach of classification was used to differentiate isolates.

Microscopic identification of algae:

Algal growth was observed under wet mount preparations for the microscopic identification using compound light microscopes (Patel B. *et al.,*2021).

Quantification of Nitrogen and Phosphorus in the algal isolates :

For the estimation of Nitrogen and Phosphorous from the isolates, Nitrogen concentration was measured by nitrite-nitrogen coupling method (K. Veena and B. Narayana, 2008) and phosphorus concentration were measured by Fiske-Subbarow method (Fiske and Subbaraow, 1925; Ruth E.L.,1931).

Selection of crop plants:

Crop plants were selected on the basis of their usage in the area of study. Crops selected for the present study were *Solanum melongena, Solanum lycopersicum* and *Capsicum annum*. 8-10 days old crop were collected from the nursery and sapling of plants in pot. Size of almost all plants were same at the sapling time.

Biofertilizer application:

50% v/v algal isolates in tap water were added in different pots while one of the control pots was added with chemical fertilizer (Dineshkumar *et al.,* 2020).

Plant growth parameters:

60 days pot study was conducted. The plants from each treatment were drawn for different analyses. Plant growth parameters like plant height, plant weight and number of leaves were calculated at different time interval (Shinde, 2020, Verma *et al.,*2017).

Biochemical Analysis:

Chlorophyll content was measured at flowering and fruiting stage (Parry *et al.,*2014, Shibghatallah *et al.,*2013).

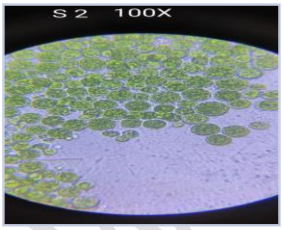
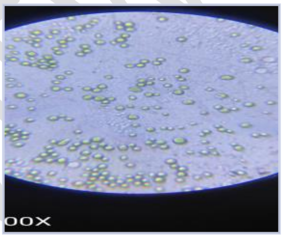
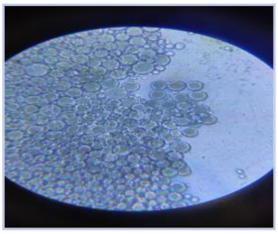
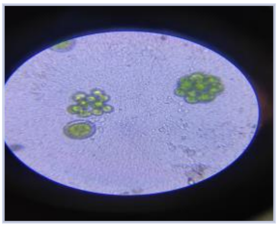
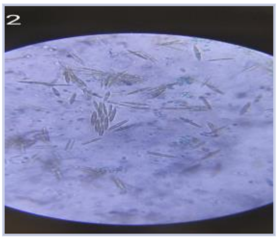
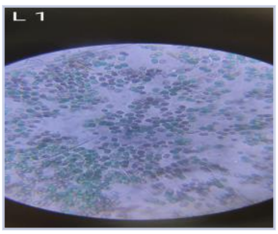
Carbon, Nitrogen, Phosphorous and Sulphur content (Alef, K., & Nannipieri, P., 1995) were measured from the soil before and after applications of biofertilizer.

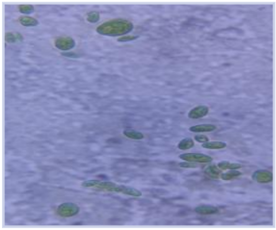
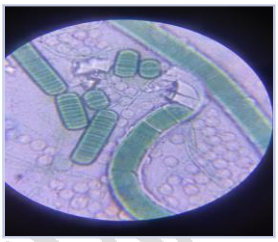
RESULTS:

Microscopic identification of algae:

Identification of isolates was based on the morphology of the individual cells following microscopic examination. Each isolate in the collection was labelled and photographed at 10x and 45x magnifications (Lee *et al.,* 2014). Microscopic identification of the obtained samples indicated presence of algal species like *Sphaerocystis, Chlorella, Chlorococcum, Gloeocapsa, Nitzchia, Nannochloropsis, Scendesmus* and *Oscillatoria*.

Table 1: Morphological characteristics of algae by microscopic examination

Isolates	**Morphological Character**	**Name of Algae**	**Figure**
I 1	Spherical colonies, with 4-32 cells embedded in mucilage	*Sphaerocystis sp.*	
I 2	Spherical cells and range 2-10µm in diameter	*Chlorella sp.*	
I 3	Cell structure is Chlorococcum like, Single young cells are ellipsoidal or ovoid	*Chlorococcum sp.*	
I 4	Unicellular or made up of small group of cells grouped within concentric mucilage envelopes.	*Gloeocapsa sp.*	
I 5	Frustules are square in girdle view	*Nitzschia sp.*	
I 6	Unicellular, Planktonic, with either 2-4 µm diameter	*Nannochloropsis sp.*	

I 7	Small, non-motile colonial green algae consisting of cells aligned in a flat plate	*Scenedesmus sp.*	
I 8	Unbranched filamentous cyanobacteria with mucilaginous sheaths	*Oscillatoria sp.*	

Observation of pot study:

(a)

(b)

(c)

Figure 1: Observation of pot study at different day (a) Day 10 (b) Day 45 (c) Day 60

Plant growth parameters of all crop:

The results presented in Tables 2 and 3 demonstrate the significant effects of different algal biofertilizer treatments on plant growth parameters in both *Solanum melongena* and *Solanum lycopersicum*. In Table 2, it was observed that the application of treatment I 7, I 8 led to taller plants, increased plant width, and a higher number of leaves at 15 days. Also, at 45 and 60 days, treatments I 1 and I 2 showed significant increases in plant growth parameters for *Solanum melongena*.

Similarly, in Table 3, treatments I 6 and I 7 showed significant results at day 15, while treatments I 5, I 6, I 7, and I 8 exhibited significant effects at day 45 for *Solanum lycopersicum*. Notably, at day 60, all treatments resulted in significant increases in plant growth parameters compared to the control.

These findings suggest that different algal biofertilizer treatments have varying effects on plant growth, with certain treatments being more effective at different stages of plant development. The observed increases in plant height, width, and number of leaves indicate the potential of algal biofertilizers to enhance the growth and productivity of *Solanum melongena* and *Solanum lycopersicum.*

Further investigation into the specific mechanisms underlying the effectiveness of each treatment, such as nutrient uptake, hormonal regulation, and soil microbial activity, could provide valuable insights into optimizing algal biofertilizer application for improved crop yields. Additionally, assessing the long-term effects of these treatments on soil health and sustainability would be beneficial for sustainable agricultural practices.

The results presented in Table 4 highlight the significant impact of algal biofertilizer treatments on plant growth parameters in *Capsicum annum*. Treatment application of I 5 and I 8 demonstrated significant effects at day 15, while treatment I 8 showed significant results at day 45. Notably, at day 60, all treatments led to significant increases in plant growth parameters compared to the control group.

These findings suggest that algal biofertilizers, particularly treatments I 5 and I 8, have the potential to enhance early-stage growth in *Capsicum annum*, as evidenced by the significant results at day 15. Treatment I 8 also showed sustained effectiveness, as it continued to produce significant results at day 45. Furthermore, the collective increase in plant growth parameters across all treatments at day 60 indicates a cumulative positive impact on overall plant development.

The consistent improvement in plant growth parameters throughout the experimental period underscores the efficacy of algal biofertilizers in promoting the growth and productivity of *Capsicum annum*. These results hold promise for the agricultural industry, offering a sustainable and potentially cost-effective means of enhancing crop yields.

Further research could delve into elucidating the specific mechanisms through which algal biofertilizers exert their effects on *Capsicum annum*, such as nutrient absorption, soil microbial interactions, and plant hormonal regulation. Additionally, exploring the long-term effects of these treatments on crop health, soil fertility, and environmental sustainability would be valuable for informing agricultural practices and maximizing the benefits of algal biofertilizers.

Table 2: Plant growth parameters of *Solanum melongena*

Treatments	Plant height (cm)			Plant width (cm)			Number of leaves/ plant		
	15 day	**45 day**	**60 day**	**15 day**	**45 day**	**60 day**	**15 day**	**45 day**	**60 day**
Control	14.7	25.3	43.5	45.5	125.6	140	5	10	12
I 1	12.4	27.2	43.2	61.5	123.1	138	4	11	15

I 2	13.6	34.6	50.5	50	107.3	136	5	13	15
I 3	11.5	23.6	36.8	45.6	105.2	127	4	11	12
I 4	10.1	24.2	38.2	51.5	103.5	125	5	10	10
I 5	12.3	15.5	22.5	36.6	87.7	95.8	4	8	8
I 6	13.8	14.3	28.4	50.4	82.5	110	4	8	8
I 7	17.1	19.4	29.2	55.1	105.2	130	4	10	11
I 8	16.3	23.5	39.2	36.2	120.4	128	5	16	16

Table 3: Plant growth parameters of *Solanum lycopersicum*

Treatments	Plant height (cm)			Plant width (cm)			Number of leaves/ plant		
	15 day	**45 day**	**60 day**	**15 day**	**45 day**	**60 day**	**15 day**	**45 day**	**60 day**
Control	18.1	32.4	50.2	44.1	86.9	81.2	5	10	22
I 1	19.2	36.2	58.1	42.6	83.6	83.2	5	11	25
I 2	19.5	39.8	49.5	46.4	96.2	98.6	6	15	25
I 3	20.5	29.1	48.2	37	74.2	78.4	5	13	27
I 4	13	35.2	56.7	33.8	100.3	108.2	5	17	30
I 5	16.2	42.5	62.2	39.2	88.6	95.3	4	15	28
I 6	20.4	50.5	70.6	35.1	95.2	102.1	5	11	27
I 7	21.5	43.1	68.2	38.4	99.3	106.6	4	12	25
I 8	18	45.3	50.2	40.6	87.5	93.1	5	15	22

Table 4: Plant growth parameters of *Capsicum annum*

Treatments	Plant height (cm)			Plant width (cm)			Number of leaves/ plant		
	15 day	**45 day**	**60 day**	**15 day**	**45 day**	**60 day**	**15 day**	**45 day**	**60 day**
Control	14	22	19.3	27.2	53	53.2	7	30	47
I 1	12.8	18	33.1	26.2	40	85.4	6	25	63
I 2	14.5	14	36.4	26.5	47	85.5	6	30	48
I 3	12.5	12.7	20.1	24.9	26.3	26.4	5	11	27
I 4	13	14	19.8	25.2	43	43.2	6	24	35
I 5	13.8	19	32.4	25.3	51.2	83.3	6	24	60
I 6	13.6	16	39	24.7	58	86.1	6	30	65
I 7	13.7	20	38.2	25.3	53.7	72.4	7	20	56
I 8	13.8	28	40.3	27.3	55	80	7	31	62

Biochemical analysis:

Carbon, Nitrogen, Phosphorous and Sulphur content were measured from the plant soil before application of biofertilizers and after application of Biofertilizer.

Solanum melongena

The study analysed the effects of biofertilizers on the Carbon, Nitrogen, Sulphur, and Phosphorous content of brinjal plants. Before the application of biofertilizers, Carbon content was higher in all pots compared to the control pot; however, after the application of biofertilizers, Carbon content was better in I 1 and I 3 labelled pots. This suggests that biofertilizers could positively enhance Carbon uptake in brinjal plants.

Before the application of biofertilizers, Nitrogen content was high in all pots as compared to the control pot. However, after the application of biofertilizers, Nitrogen content was higher in the control pot. This indicates that the biofertilizers did not significantly impact Nitrogen uptake in the brinjal plants.
Before the application of biofertilizers, Phosphorous content was high in all pots except for the one labelled I 8 compared to the control pot. However, after the application of biofertilizers, Phosphorous content was increased in the control pot. This indicates that some biofertilizers could enhance Phosphorus uptake in brinjal plants.
Before the application of biofertilizers, Sulphur content was high in the pot labelled I 1 as compared to the control pot. However, after the application of biofertilizers, Sulphur content was increased in all treatment pots compared to the control pot, suggesting that biofertilizers could enhance Sulphur nutrient uptake in brinjal plants.
Overall, the study suggests that biofertilizers can enrich the nutrient content of brinjal plants. Biofertilizers can positively affect Carbon, Phosphorous, and Sulphur uptake by the plants, but not Nitrogen uptake.

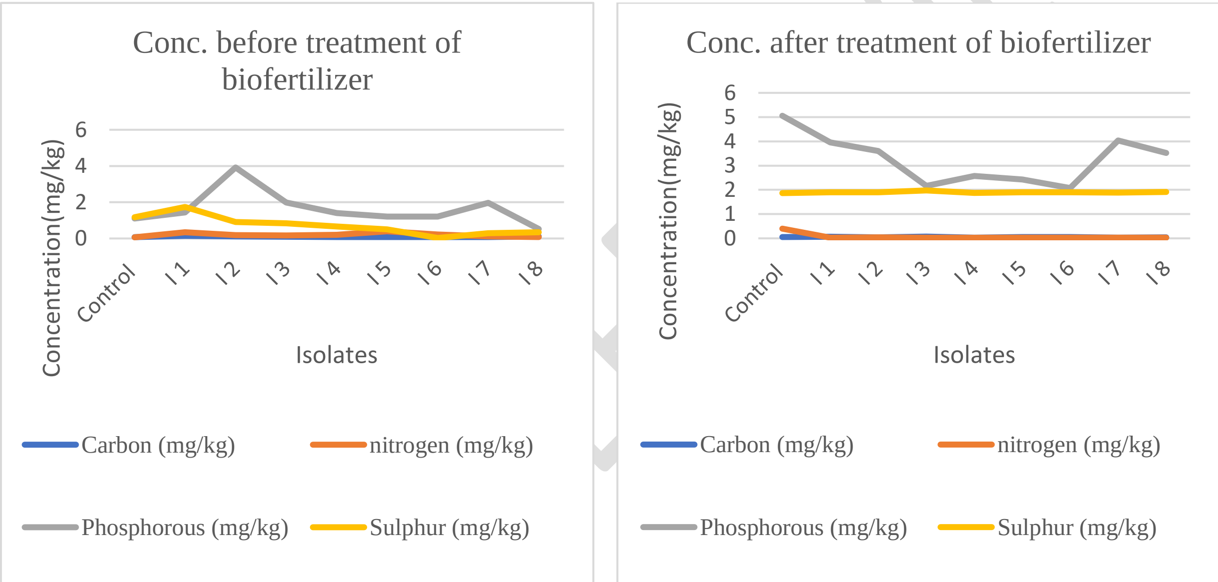


Figure 2: Carbon, Nitrogen, Phosphorus and Sulphur content from soil- before and after applications of biofertilizer from *Solanum melongena*

Solanum lycopersicum

The study analysed the effects of biofertilizers on the Carbon, Nitrogen, Sulphur, and Phosphorous content of tomato plants. The results showed that before the application of biofertilizers, Carbon content was higher in pots labelled I 4 and I 7. However, after the application of biofertilizers, Carbon content increased in all pots except for the ones labelled I 1, I 6, and I 8.
Similarly, before the application of biofertilizers, Nitrogen content was low in the control pot. However, after the application of biofertilizers, the Nitrogen content in the control pot increased. This suggests that the biofertilizers have a positive impact on the Nitrogen content of the plants.
Before the application of biofertilizers, the Phosphorous content was low in the control pot. However, after the biofertilizer application, the Phosphorous content in the control pot increased.
Before the application of biofertilizers, the Sulphur content was found to be high in all treatment pots except for I 1. After the application of biofertilizers, Sulphur content was higher

in all treatment pots in comparison to the control pot. This shows that biofertilizers can enhance the Sulphur uptake of the plants.

Overall, the results suggest that the application of biofertilizers can positively affect the nutrient content of tomato plants.

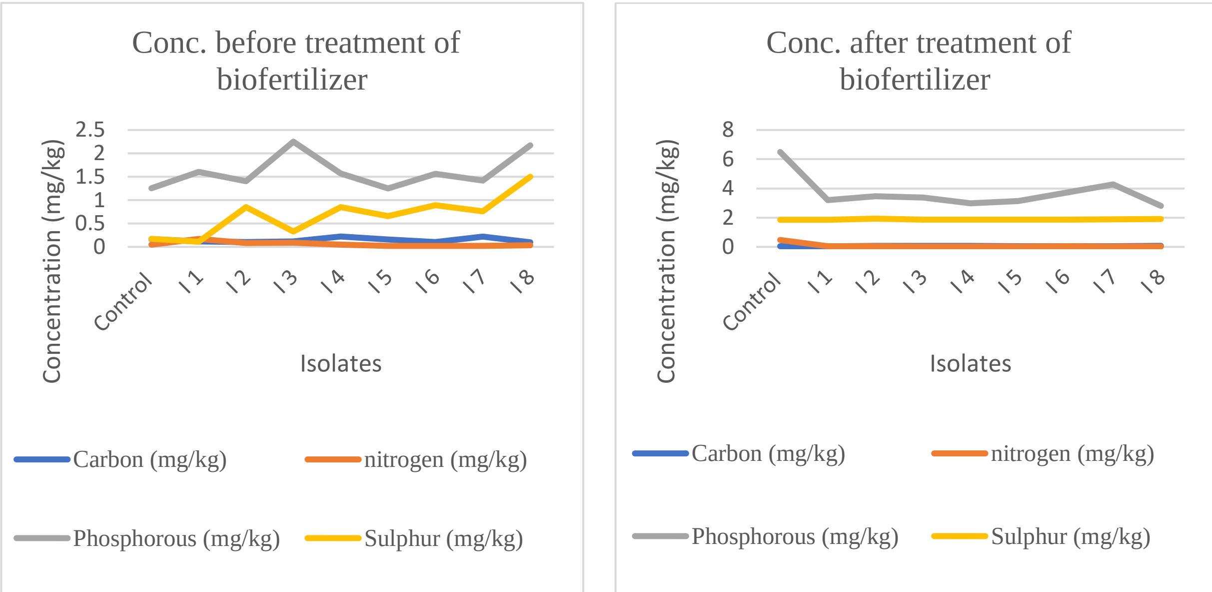


Figure 3: Carbon, Nitrogen, Phosphorus and Sulphur content from soil- before and after applications of biofertilizer from *Solanum lycopersicum*

***Capsicum annum*:**

The study analysed the effects of biofertilizers on the Carbon, Nitrogen, Sulphur, and phosphorous content of *Capsicum annum* (pepper) plants. Before the application of biofertilizers, Carbon content was found to be higher in all pots, except for I 7, as compared to the control pot. However, after the application of biofertilizers, Carbon content was higher in I 5 as compared to the control pot suggesting that biofertilizers could enhance Carbon uptake in pepper plants.

Before the application of biofertilizers, Nitrogen content was lower in the control pot whereas, after the application of biofertilizers, Nitrogen content in the control pot increased. This indicates that biofertilizers could positively impact Nitrogen uptake in pepper plants.

Before the application of biofertilizers, Sulphur content was found to be high in treatment I 5 and I 8. However, after the application of biofertilizers, Sulphur content was found to be higher in treatment I 4 as compared to the control pot. This suggests that biofertilizers can enhance the Sulphur uptake of the pepper plants.

Before the application of biofertilizers, Phosphorous content was higher in the control pot. However, after the application of biofertilizers, Phosphorous content was found to be higher in treatment I 4 as compared to the control pot, indicating that biofertilizers could enhance Phosphorus uptake in pepper plants.

Overall, the study suggests that the application of biofertilizers can positively affect the nutrient content of pepper plants by enhancing the uptake of Carbon, Nitrogen, Sulphur, and Phosphorous.

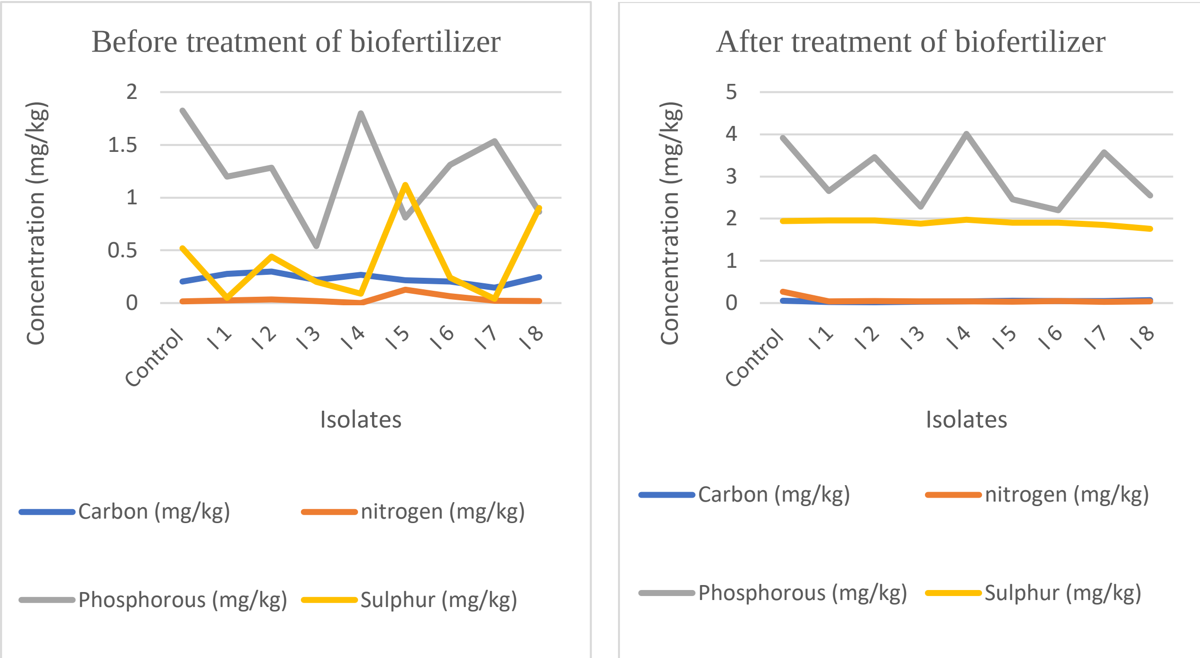
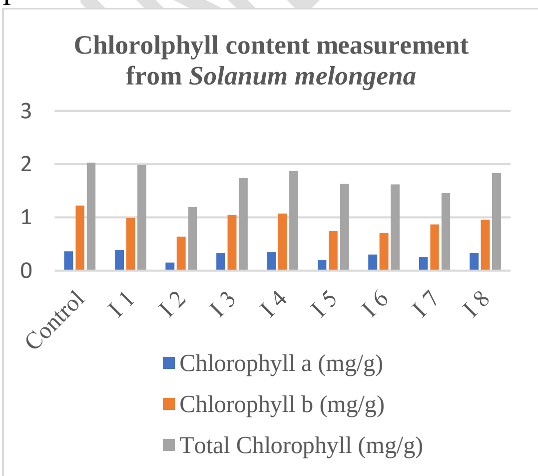


Figure 4: Carbon, Nitrogen, Phosphorus and Sulphur content from soil- before and after applications of biofertilizer from *Capsicum annum.*

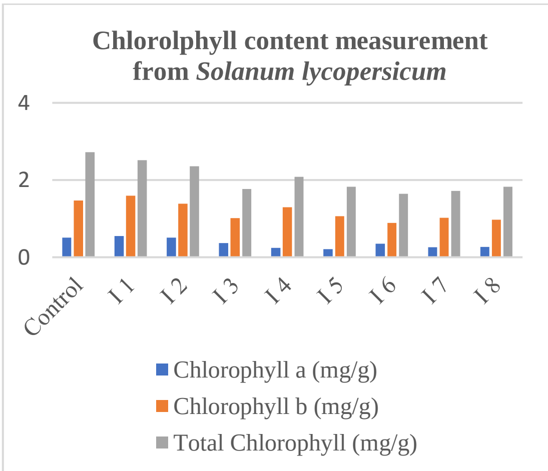
Chlorophyll content measurement:

The chlorophyll content of leaves from plants at flowering and fruiting stages were measured (Parry *et al.,* 2014). It was done to determine if the application of algal biofertilizers would have an impact on chlorophyll content. Three types of chlorophyll content were measured chlorophyll a, chlorophyll b, and total chlorophyll content in the leaves of all the plants. The results showed that in *Solanum melongena* and *Solanum lycopersicum* plant species, the chlorophyll content was higher in plants treated with the biofertilizers labelled as I 1 when compared to the control group. This means that these plants had a higher level of chlorophyll content when they were given algal biofertilizer. However, in *Capsicum annum* plant species, the highest chlorophyll content was observed in plants treated with biofertilizer labelled as I 1 and I 6 when compared to the control group. We found that these plants had significantly higher chlorophyll content when treated with these biofertilizers.

Overall, the study revealed that applying different algal biofertilizers could have a positive impact on the chlorophyll content in plants, ultimately making them healthier and more productive.



(a)



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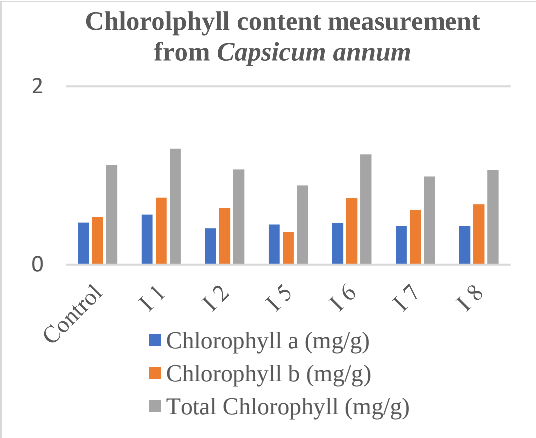

(c)

Figure 5: Chlorophyll content measurement from leaves of all plant: (a) Chlorophyll content measurement from *Solanum melongena*, (b) Chlorophyll content measurement from *Solanum lycopersicum* and (c) Chlorophyll content measurement from *Capsicum annum*

DISCUSSION:

Brinjal, Tomato and Green chilli are considered as the most important and common vegetable plant in area of study. The research aimed to explore the impact of algal biofertilizers on the growth and nutrient content of *Solanum melongena*, *Solanum lycopersicum* and *Capsicum annum* plants. The study revealed that algal biofertilizers could significantly enhance plant growth parameters, including plant height, plant width, and the number of leaves. Specifically, different treatments showed variable effectiveness at distinct stages of plant development. Additionally, the research highlighted the potential of algal biofertilizers to enrich the nutrient content of crops. Biofertilizers were shown to positively affect Carbon, Phosphorous, and Sulphur uptake in plants, but not Nitrogen uptake. Further research is needed to investigate the underlying mechanisms of these effects, improve the efficacy of treatments, and assess the long-term sustainability of algal biofertilizers for agricultural practices. The study also showed that algal biofertilizers can enhance the chlorophyll content of plants, ultimately improving their health and productivity. These findings hold significant promise for the agricultural industry, offering a sustainable and cost-effective means of enhancing crop yields.

In one of peer study checking impact of algal fertilizer on the growth of capsicum plants; results indicated that capsicum plants treated with 0.25% algae biofertilizer exhibited the highest growth and biochemical parameters, seaweed liquid fertilizer was found effective for capsicum growth (Shinde, 2020).

In another study, determining the most effective biostimulant for enhancing the growth and yield of brinjal; Humic acid at a concentration of 10 percent demonstrated the most significant improvement in growth and yield attributes compared to other treatments. Parameters including plant height, number of branches per plant, number of leaves, leaf area, stem girth, and days taken for flower initiation were notably improved by the application of humic acid at 10 percent, followed by humic acid at 5 percent (Ruban *et al.,* 2019). The similar study investigated the impact of various local seaweed extracts on the growth and yield of eggplant. Six treatments were tested, including NPK fertilizer and four types of seaweed extracts. Results showed that seaweed extracts significantly affected various growth and yield parameters of eggplant, such as plant height, leaf number, leaf area, and fruit weight. While seaweed extracts did not significantly influence certain parameters like first flowering appearance and root weight, they displayed potential to replace NPK fertilizer in enhancing eggplant growth and yield (Yusuf *et al.,*2019).

Some researchers also investigated microalgal-based biofertilizers to increase tomato yields while reducing chemical fertilizer usage, which can have harmful effects on both the environment and consumers. Three different application methods were tested, with the combined treatment of microalgae *Chlorella vulgaris* and cow dung showing the most promising results, followed by soil drenching and foliar spraying. This study highlights the potential of microalgae as eco-friendly and non-hazardous biofertilizers for sustainable agriculture (Dineshkumar *et al.,* 2021).

One of the study investigated the impact of *Chlorella vulgaris* strain on the germination and growth of tomato and cucumber seeds. Seeds were germinated in a culture medium containing the algal strain and monitored for 3, 6, 9, and 12 days. Results showed that the *Chlorella vulgaris* suspension enhanced seed growth compared to the control (sterilized culture medium), with the most effective treatments being 0.17 and 0.25 g/L of algal suspension for root and shoot lengths of tomato and cucumber seeds, respectively (Bumandalai O. and Tserennadmid R., 2019).

Another study aimed to evaluate the use of microalgae as organic slow-release fertilizers for tomato cultivation, derived from treating aquaculture wastewater and marine microalgae. Results showed comparable plant growth between microalgal and commercial organic fertilizer treatments. Microalgal fertilizers enhanced fruit quality by increasing sugar and carotenoid content, despite yielding slightly lower tomato yields. Economic evaluation suggested the feasibility of microalgae-based fertilizers, but further research is needed to optimize their composition (Grunert *et al.,* 2016).

One of the study conducted a pot experiment to assess the potential of *Sargassum wightii* seaweed liquid extracts as foliar spray for plants. The seaweed extract contains micro and macro nutrients, vitamins, growth hormones, and other beneficial constituents that can enhance crop growth. Additionally, the liquid seaweed extracts are considered eco-friendly products for sustainable agriculture (Vijayalekshmi and Renuga, 2021).

A pot culture experiments on chili was conducted, aimed to observe the influence of blue-green algae (BGA) biofertilizer on chili growth and yield. The area faces reduced soil productivity and fertility due to extensive use of chemical fertilizers. The study compared the effects of BGA alone, BGA with farmyard manure (FYM), and chemical fertilizers treatments against a control. Results showed that using BGA with FYM biofertilizer was more beneficial for chili fruit yield compared to BGA alone or chemical fertilizers (Dandwate, 2017).

CONCLUSION:

This study conducted on potted plants has revealed that algal biofertilizers are more effective at enhancing plant growth compared to chemical fertilizers. It identified various species of algae, including *Sphaerocystis*, *Chlorella*, *Chlorococcum*, *Gloeocapsa*, *Nitzchia*, *Nannochloropsis*, *Scendesmus*, and *Oscillatoria*, indicating a diverse range of potential biofertilizer sources.

The observed improvements in plant growth parameters such as plant height, width, and leaf numbers suggest that algal biofertilizer has a comprehensive impact on plant development.

Specifically, in *Solanum melongena* (eggplant), treatments labelled as I 2 and I 3 exhibited the most favourable growth parameters. For *Solanum lycopersicum* (tomato), treatments labelled as I 1, I 2, I 4, I 5, I 6, and I 8 were associated with improved growth parameters, indicating a broader spectrum of effectiveness across different treatments. Additionally, in *Capsicum annum* (bell pepper), all treatments resulted in significantly enhanced growth parameters compared to the control group, highlighting the consistent positive impact of algal biofertilizer across different plant species.

Overall, the findings suggest that the use of algal biofertilizer holds promise as an alternative to chemical fertilizers, offering a sustainable and potentially more effective approach to promoting plant growth and development. This information could be valuable for agricultural practices seeking environmentally friendly and efficient fertilization methods.

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