

Original Article

## Microalgae-Dark Green (DG), Light Green (LG), and *Bmt cya mi* (Baramati cyano-microalgae) Collected from Krishi Vigyan Kendra (KVK), Baramati, Pune, Maharashtra, India, can enhance the Growth of *Trigonella foenum-graecum* L.

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### Abstract

Microalgae are becoming promising biostimulants with an extensive variety of phytohormones, pigments, vitamins, amino acids, and bioactive metabolites. This work studies the biostimulant properties of three microalgal cultures: Dark Green (DG), Light Green (LG), and *Bmt cya mi* in relation with the growth and yield of fenugreek (*Trigonella foenum-graecum*). Microalgae were grown collected from Baramati, from freshwater in BG-11 medium. The culture were then tested for the fenugreek growth in a pots. Monitoring was conducted on the basis of growth parameters (shoot length, root length, and leaf number) as well as biochemical characteristics (chlorophyll and carotenoid content). The most improvements in growth were reported in DG microalgae. The shoot length and the root length improved by 50.94% and 20% respectively as compared to NPK controls. LG and BMT *cya mi* also promoted growth albeit in a smaller magnitude. Pigment examination indicated that chlorophyll and carotenoid concentrations were much greater in plants treated with microalgae. The statistical analysis was able to prove the significant variations in the root length ( $p < 0.05$ ).

### 1. Introduction:

Rising global concern with the need to practice sustainable agriculture has heightened demand for biologically derived plant-growth regulators, especially the biostimulants made with microalgae. Recently, agriculture sector has problems of soil degradation, excessive chemical fertilizer use, and the accumulating

impacts of abiotic stresses such as climate change, salinity, drought, and temperature variations [1]. Over fertilization not only disrupts the regular presence of microbes in the soil but also causes the build-up of heavy metals and water pollution which eventually increases environmental susceptibility [2]. These growing concerns have driven a shift towards more

nature-based solutions that can boost plant productivity while also restoring ecological resilience.

Microalgae have become the new potential candidate in this regard as a result of their biochemical richness and their extraordinary physiological plasticity. Their cell wall includes phytohormones, amino acids, vitamins, polysaccharides, antioxidants, and mineral nutrients that have been known to promote root growth, chlorophyll production, nutrient uptake, and abiotic-stress resistance [3,4].

Various reports indicate that microalgae like *Chlorella vulgaris* not only improve nutrient use responses but also positively impact anti-oxidative responses and metabolic activities of plants in drought or salinity conditions [5,6]. These have hormone-like functions that are similar to auxins, gibberellins and cytokinins and they interact with plant signaling pathways even at low concentrations in application [7]. In addition to being biochemically rich, microalgae have ecological significance as fast-growing microorganisms with carbon-fixing potential (and soil stabilization), better microbial community structuring [8].

Microalgae are a promising substitute for synthetic fertilizer because of their role in sustainable nutrient cycling, specifically the mobilization of nitrogen and phosphorus. Microalgal biostimulants unlike traditional agro-inputs cause agronomic advantages of physiological actions without being dependent on their nutrient composition and thereby cause a decreased chemical load on the soil ecology [9,10].

The nutritionally and medically attractive leguminous crop with extensively promiscuous growth in India, for example, *Trigonella foenum-graecum* L., can serve as an excellent model system in assessing the potential of microalgal biostimulants. Moreover, the plant species under study, in spite of its great adaptability is vulnerable to soil nutrient restrictions and other environmental stressors that have adverse effects on growth, pigment production, and yield [11].

It is noted by the prior studies that the algae-based formulations have a significant positive impact on morphological characteristics, biomass growth, and metabolic pigment rates in *Trigonella foenum-graecum* L., which indicate their potential to improve physiological and biochemical performances [12]. Since the problem of minimizing the usage of synthetic fertilizers and switching to environment friendly cultivation system has been growing in importance,

exploring microalgae as biostimulants becomes of utmost importance.

Nevertheless, there are still some gaps in terms of the species-specific impacts, the most effective application methods, and the competitive performance of chemical fertilizers. This research study assesses the effectiveness of isolated microalgal cultures as biostimulants in *Trigonella foenum-graecum* L. in the context of the dynamics of growth, root-shoot development, and chlorophyll and carotenoid profiles in comparison with standard NPK treatment. The study can help further develop microalgal-based agricultural innovations and implement them in a sustainable crop production model.

## 2. Materials and Methods

### 2.1 Sample Collection and Isolation of Microalgae

Microalgal samples were collected from agricultural fields near the Krishi Vigyan Kendra (KVK), Baramati, Pune, Maharashtra, India (figure 1a, figure 1b and 1c). Three morphologically distinct microalgal types—Dark Green (DG), Light Green (LG), and *Bmt cya mi* (Baramati cyano-microalgae) were isolated from surface water by using aseptic collection procedures.

Water samples were transferred into Erlenmeyer flasks containing Blue-Green 11 medium and filtered through standard filter paper before inoculation [13]. Blue-Green 11 medium was prepared according to the standard composition containing sodium nitrate (0.1g/L), dipotassium phosphate (0.25 g/L), magnesium sulphate (0.051 g/L), ammonium chloride (0.0051g/L), calcium chloride (0.005g/L), and ferric chloride (0.0005 g/L). Cultures were incubated at 25 -30°C for 15-20 days to promote initial growth.



Figure1a: Dark green microalgal culture was isolated from surface water by using aseptic collection procedures.

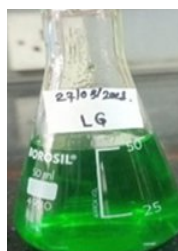


Figure 1b: Light green microalgal culture was isolated from surface water using aseptic collection procedures.



Figure 1c: *Bmt cya mi*-microalgal culture was isolated from surface water using aseptic collection procedures.

## 2.2 Culture Maintenance and Sub-Culturing

Following initial proliferation, microalgal cultures were transferred into 150 mL Erlenmeyer flasks containing 50 mL Blue-Green 11 medium. The media was sterilized by autoclaving at 121°C for 20 minutes. Cultures were incubated at ambient temperature and sub-cultured every 21 days to expand biomass. Each subculture was transferred sequentially into fresh Blue-Green 11 medium, with progressively increasing culture volumes to maintain exponential growth.

## 2.3 Microscopic Identification of Microalgae

Morphological identification was performed using a compound light microscope at 40X magnification. Wet mounts were prepared by placing a drop of actively growing culture on a clean slide, covering it with a cover slip and examining the sample for colony morphology, pigmentation, and cell structure. Identification was based on morphological descriptors reported in previous studies on microalgae.

## 2.4 Soil Collection and Test Crop Selection

Soil used for cultivation experiments was collected from farm land adjacent to the college campus. Fenugreek (*Trigonella foenum-graecum* L.) seeds (SK Double Dipak variety) were procured from a local agricultural supplier. Seeds of uniform size and appearance were selected to ensure experimental

consistency, as *Trigonella foenum-graecum* L. is a well-established medicinal crop with significant agronomic relevance [14].

## 2.5 Experimental Design and Pot Assay

*Trigonella foenum-graecum* L. plants were cultivated under three treatment conditions. 1. Soil with *Trigonella foenum-graecum* L. 2. Soil with *Trigonella foenum-graecum* L. supplemented with microalgae. 3. Soil with *Trigonella foenum-graecum* L., supplemented with microalgae and an unknown biofertilizer (Figure 2).



Figure 2: *Trigonella foenum-graecum* L. plants were cultivated under three treatment conditions. Top two rows show Soil+ fenugreek, middle two rows show Soil+ fenugreek + Algae and bottom two rows show Soil+ fenugreek + Algae + unknown Biofertilizer treatment conditions.

Microalgal cultures were quantified using a Neubauer counting chamber. Optical density was measured at 664 nm followed by a ten-fold dilution to standardize cell concentration. Approximately  $1 \times 10^6$  cells were applied to plants every alternate day. All plants were grown under identical conditions with temperature and humidity monitored (average temperature: 28 °C). Biostimulants were added to the plants (table 1).

Table1: Addition of biostimulants to the plants

Microalgae	Average optical density	Average cells count $10^6$
Dark Green	0.482576923	1.120092308
Bmtcyami	0.326469231	1.198115385
Light Green	0.410915385	1.0517

## 2.6 Growth Parameter Measurements

Growth responses were assessed at weekly intervals and after 15 days. Plant height was measured from the base of the shoot to the apical tip using a centimeter scale. Root length was recorded by gently washing and

measuring roots. Leaf number was counted manually at each time interval. These parameters were selected based on established plant growth indicators reported in microalgal biostimulant studies [15].

### 2.7 Pigment Extraction and Quantification

To determine chlorophyll and carotenoid content, 0.1 g of *Trigonella foenum-graecum* L. leaves were homogenized in 1.5 mL of 90% acetone. The extract was sonicated in the dark and centrifuged at 3000 rpm for 10 minutes. Supernatants were collected, and absorbance was measured at 630, 645, 663, and 480 nm using a spectrophotometer, following the protocol of Oo et al. (2017) [16]. Chlorophyll a, chlorophyll b and carotenoid concentrations were calculated using standard formulae based on absorbance values.

### 2.8 Statistical Analysis

All measurements were performed in triplicate. Data were analyzed using Microsoft Excel. Means and standard errors were computed, and a two-way ANOVA was performed to determine statistical significance at  $p < 0.05$ . Root length was identified as significantly influenced by treatments based on ANOVA results.

## 3. Results:

### 3.1. Isolation and Morphology of Microalgae

All microalgae cultures exhibited visible growth after 15–20 days of incubation. They showed characteristic algal pigmentation and cellular arrangement. Dark Green (DG) cultures displayed dense green pigmentation, Light Green (LG) cultures exhibited lighter chlorophyll accumulation, while *Bmt cya mi* showed cyanobacterial features consistent with field observations.

### 3.2. Growth Performance of *Trigonella foenum-graecum* L. Under Different Treatments

#### 3.2.1 Plant Height

*Trigonella foenum-graecum* L. plants treated with microalgae demonstrated enhanced shoot elongation compared to the NPK control. Dark Green (DG) treatment produced the greatest improvement, showing a 50.94% increase in shoot length relative to NPK[23]. Light Green (LG) treatment showed an 18.76% increase [17] while *Bmt cya mi* maintained shoot lengths comparable to the control. Untreated soil-grown plants showed minimal growth, confirming the stimulatory effect of microalgal supplementation.

#### 3.2.2 Root Length

Significant differences ( $p < 0.05$ ) were observed in root length across treatments. Dark Green(DG)-treated

plants exhibited a 20% increase, while *Bmt cya mi* treatment resulted in a 12% increase compared to NPK [17]. Light Green (LG)-treated plants showed similar root lengths to controls.

#### 3.2.3 Number of Leaves

Leaf count increased notably under microalgal treatments. Light Green (LG) and *Bmt cya mi* both recorded a 15% increase in leaf number compared to NPK. The Dark Green (DG) treatment showed leaf counts similar to those of the control despite superior shoot and root performance.

#### 3.2.4 Pigment Analysis

##### 3.2.4.1 Chlorophyll Content

Chlorophyll a and b levels were highest in Dark Green (DG)-treated plants, measuring 5.44  $\mu\text{g/mL}$  (Chlorophyll a) and 6.30  $\mu\text{g/mL}$  (Chlorophyllb) [24]. Light Green (LG) and *Bmt cya mi* treatments showed intermediate chlorophyll levels, while NPK plants had the lowest pigment content (Chlorophyll a: 2.05  $\mu\text{g/mL}$ ; Chlorophyll b: 2.47  $\mu\text{g/mL}$ [18]. Dark Green (DG) treatment nearly doubled pigment concentration relative to NPK, indicating enhanced photosynthetic potential.

##### 3.2.4.2 Carotenoid Content

Carotenoid concentration followed a similar trend, with Dark Green (DG)-treated plants exhibiting the highest value (2.32  $\mu\text{g/mL}$ ), followed by *Bmt cya mi* (1.48  $\mu\text{g/mL}$ ) and LG (1.40  $\mu\text{g/mL}$ ) [18]. NPK-treated plants presented the lowest carotenoid levels (0.85  $\mu\text{g/mL}$ ).

## 4. Discussion

Shoot length, roots, and pigment accumulation indicate that microalgae provide a well-balanced combination of bioactive molecules, which can control plant physiology at various regulatory levels [19]. Similarly, microalgae are documented to produce amino acids, vitamins, phytohormones, polysaccharides, antioxidants as well as minerals, most of which have been observed to resemble and mimic plant hormone signal transduction crucial to growth and resilience to environmental challenges [20].

The much higher shoot height with Dark Green (DG) treatment that is over 50.94 cm greater than with NPK, indicates that there is a strong anabolic effect, which could be due to the presence of microalgal auxin-like or cytokinin-like substances, which have been known to promote cell growth in length and division [20, 21]. These were observed previously with the growth of

vegetation in different crops improved using microalgal extracts, such as *Vigna radiata* L., *Solanum lycopersicum* L., and *Cucumis sativus*[1].

Dark Green (DG) treatment may have a superior composition of growth-modulating compounds than Light Green (LG) and *Bmt cya mi*, contributing to the enhanced apical growth stimulation activity. Dark Green (DG) and *Bmt cya mi* had significant positive effects on root development, with increases of 20% and 12%, respectively over NPK.

This is consistent with established effects of microalgae on root initiation, rhizosphere microbial activity, and nutrient uptake efficacy [15]. Cyanobacteria and green algae can synthesize extracellular polysaccharides and organic acids, and can especially induce root hair formation and alter soil microstructure [8]. The increase in the power of root length in this case is probably associated with the better nutrient mobilization and the interaction of the rhizosphere, which results in the health and resilience of the plant in general.

Species-specific phenotypic variations between the two organisms reveal distinct responses: microalgal treatments like LG, *Bmt*, and *cya mi* strongly boosted leaf production but had milder impacts on shoot height. These patterns highlight tailored regulation of branching and leaf development pathways across species.

Such outcomes align with findings that different microalgal genera possess unique metabolic routes influencing specific morphological characteristics [22]. LG and *Bmt cya mi* might harbor secondary metabolites that induce patterns of axial growth more aggressively, Dark Green (DG) suggests axial growth. The high stimulatory value of microalgal application is further confirmed by pigment analysis. The Dark Green algae-treated plants showed almost twice as much chlorophyll a and b as compare to NPK-treated plants, and considerably more carotenoid content. These results are complemented by previous studies showing that microalgal extracts enhance chlorophyll biosynthesis and photosynthetic efficiency by increasing precursor and cofactor levels involved in pigment biosynthesis [16].

High carotenoids also indicate an increase in the antioxidant capacity, which is essential in protecting against abiotic stress to retain chloroplast integrity and optimal photosynthetic activity [6]. The measured

changes in chlorophyll and carotenoid levels are consistent with the earlier reports on the fact that horticultural biostimulants, such as microalgae have been effectively used to enhance pigment synthesis and photosynthetic qualities in various plants [19].

Notably, this paper points out that microalgal applications can deliver equal or better capabilities that match those of conventional NPK fertilizers, and this is in line with the move to lessen the reliance on chemical fertilizers around the world. Excessive chemical fertilizer use has long been linked to soil erosion, excessive deposition of heavy metals, and environmental negative consequences [2]. The promising alternative provided by microalgal biostimulants is a biologically active, environment friendly input that can improve plant performance alongside promoting soil health [23].

## 5. Conclusion

This research shows that microalgae-based biostimulants are far better than the traditional NPK fertilizer in terms of the growth and physiological functions of the *Trigonella foenum-graecum* L. plants.

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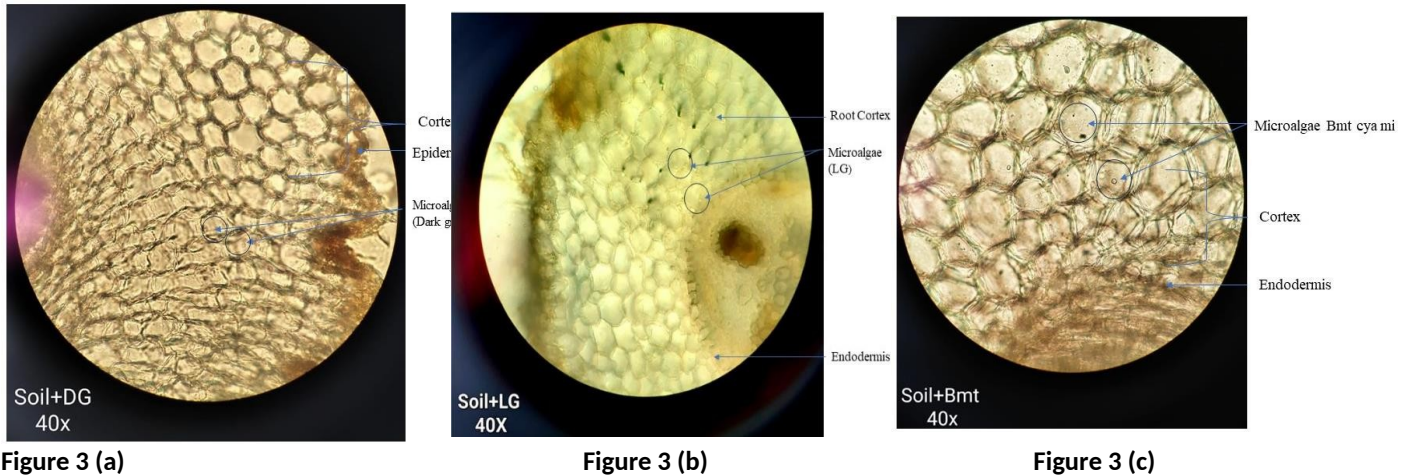
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Maharashtra, India, can enhance the Growth of *Trigonella foenum-graecum* L. *Int. J. Micro. Sci.* 2025; 6(2):18-24.

### Appendices



**Figure 3:** Microscopic view of root association of microalgae (Set 1 of Soil). a-Dark Green Microalgae observed in association with *Trigonell afoenum-graecum* L. root under 40X objective. b-Light Green Microalgae observed in association with *Trigonella foenum-graecum* L. root under 40X objective. c-*Bmt cya mi* Microalgae observed in association with *Trigonella foenum-graecum* L. root under 40X objective.



**Figure 4 (a)**

**Figure 4 (b)**

**Figure 4 (c)**

**Figure 4:** Comparative assessment of *Trigonella foenum-graecum* L. plants treated with -a. Soil with *Trigonella foenum-graecum* L. supplemented with Dark Green microalgae. b. Soil with *Trigonella foenum-graecum* L. supplemented with Light Green microalgae. c. Soil with *Trigonella foenum-graecum* L. supplemented with *Bmt cya mi* microalgae

**Table 2: Optical density at different wavelengths of microalgal cultures.**

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Microalgae	480	630	645	663
Dark Green	0.5819	0.5698	0.5556	0.5634
Light green	0.3522	0.3468	0.3451	0.3436
Bmt cya mi	0.3724	0.3618	0.3572	0.3595
NPK	0.2134	0.2096	0.2128	0.2131

**Table 3: Chlorophyll content analysis of leaves of microalgal cultures**

Microalgae	Chlorophyll content of leaves		
	Chl a (µg/ml)	Chl b(µg/ml)	Carotenoid(µg/ml)
Dark Green	5.44335	6.302934	2.3276
Light green	3.306108	3.979031	1.4088
Bmt cya mi	3.467298	4.087772	1.4896
NPK	2.052276	2.472098	0.8536