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# Effect of foliar application with *Chlorella vulgaris*, *Tetradesmus dimorphus*, and *Arthrospira platensis* as biostimulants for common bean

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**Abstract**
The aim of the present investigation was to study the impact of foliar spraying with *Chlorella vulgaris*, *Arthrospira platensis*, and *Tetradesmus dimorphus* suspensions as biostimulants on growth and yield characteristics of common beans such as *Phaseolus vulgaris*. Seven treatments were tested during the study: T1 (soil amended with N-urea but no microalgae foliar application), T2 (foliar spraying with *C. vulgaris* but no N-urea added to the soil), T3 (foliar spraying with *T. dimorphus* but no N-urea added to the soil), T4 (foliar spraying with *A. platensis* but no N-urea added to the soil), T5 (foliar spraying with *C. vulgaris* and soil amended with N-urea), T6 (foliar spraying with *T. dimorphus* and soil amended with N-urea), T7 (foliar spraying with *A. platensis* and soil amended with N-urea) and control (untreated). Foliar spraying was applied after 7, 25, and 77 days from sowing using the test microalgae suspensions in concentration of 10 g 100 $mL^{-1}$. Plant growth and biochemical parameters were measured at the end of both vegetative and fruiting growth stages. Compared with control, the treatments from T1 to T7 showed noticeable increase in all growth parameters and yield attribute. The foliar application with *C. vulgaris* and chemical fertilizer treated plants (T5) exhibited the maximum increase in total plant height (26.9%), dry weight (37.28%), protein content ($48.06 \pm 2.403$ mg $g^{-1}$ fresh wt.), and total carbohydrate ($394 \pm 19.7$ mg $g^{-1}$ dry wt.) during vegetative stage as well as number of pods per plant ($5.2 \pm 0.26$), number of seed/pod ($3.5 \pm 0.18$), pods dry weight ($0.95 \pm 0.05$ g $plant^{-1}$) during fruiting stage. Thus, it is advisable to use *C. vulgaris* as a biostimulant for enhancing *P. vulgaris* growth and crop production.

**Keywords** Foliar spraying · Microalgae biostimulants · Common beans · Vegetative growth · Yield attribute

## Introduction

Common beans are among the world's largest cultivated crops used for direct human consumption. About 20 leguminous species are consumed as dry grains in substantial amounts, considered poor man's meat in third world countries where protein energy malnutrition considered major nutrition problem (Lin et al. 2008; Priya and Manickavasagan 2020). Among the most consumed legumes in the world, *Phaseolus vulgaris* occupies an important position in human nutrition, with a commercial value exceeding that of all other bean crops (Porch et al. 2013). Despite being low in methionine and cysteine, *P. vulgaris* dried seeds, or "pulses." are a major source of nutritional protein which is 2–3 times that of cereal grains (Siddiq et al., 2010). It also contains high amounts of starch, dietary fiber, minerals, vitamins, and variety of phytochemicals such as anthocyanins, flavonoids, proanthocyanidins, phenolic acids, and isoflavones that play important roles in the prevention of cardiovascular disease, obesity, diabetes mellitus, and cancer (Díaz-Batalla et al. 2006; Lin et al. 2008).

Despite their economic and nutritional importance, common beans are low yield crops that cannot meet food demands of growing populations. In recent years, numerous studies have been carried out to develop substances of biological origin as alternatives to chemical inputs and able to enhance plant growth, crop yields, and quality with less environmental damage (Mourice and Tryphone 2012; Dias et al. 2016). Biofertilizers and biostimulants are alternatives to chemical fertilizers that, when applied at low

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concentrations to soil, seeds, or crops, regulate the physiology of plants through different pathways such as enhancing crop growth, increasing nutrient uptake, resistance to abiotic stresses, and longer the shelf life of harvested products (Kawalekar 2013). Moreover, they are eco-friendly products that contain living microorganisms such as bacteria, actinomycetes, fungi, or algae alone or in combination.

In this regard, microalgal biomass is rich in different biomolecules that are essential for an optimal crop growth and development such as free amino acids, organic acids, and phytohormones. It is widely known (Shaaban 2001a, b; Tarakhovskaya et al. 2007; Khan et al. 2009; Battacharyya et al. 2015; Mógor et al. 2018) that different microalgae species are rich in growth-promoting substances such as auxins, cytokinins, gibberellins that are essential for plant growth and sustainably.

Several studies have investigated the effect of microalgae fertilizers on different crops. For example, foliar spraying with *Arthrospira platensis* and *Scenedesmus* sp. hydrolysates increased the flowers number, the root dry weight, number of leaves, and shoots of *Petunia* x *hybrida* (Plaza et al. 2018). Similarly, Mógor et al. (2018) observed cytokine-like effects of *A. platensis* hydrolysate on lettuce seedlings and Bumandalai and Tserennadmid (2019) found a stimulatory effect of foliar spraying with *Chlorella vulgaris* suspension on tomato and cucumber seeds germination. Other studies have investigated the impact of *C. vulgaris* and *A. platensis* fertilizers on rice and maize plants and they found an increase in seed quality, seed germination, yield production, and plant growth parameters (Dineshkumar et al. 2018, 2019).

Based on the above information, the present study aimed primarily at investigating the effect of foliar application with *C. vulgaris*, *Tetradesmus dimorphus*, and *A. platensis* suspensions on different vegetative growth and yield aspects of *P. vulgaris* plants.

## Material and methods

### Algal culture

Freshwater samples were collected from the River Nile system at Delta region in the front of Mansoura University (31° 2′ 45.7620″ N, 31° 21′ 18.6444″ E) (Raschke and Schultz 1987) and centrifuged at 2688 × *g* for 10 min then supernatant was discarded and the pellets were picked up by sterile needle and streaked on Bold Basal Medium (BBM) (Bischoff 1963) solidified with 1.5% (w/v) of bacteriological agar for purification. The plates were incubated for 2 weeks at 25 ± 2 °C under continuous light of 45 µmol photons $m^{-2}$ $s^{-1}$ and then examined. Unialgal colonies were transferred to liquid BBM and left to grow to obtain enough biomass for identification (Stein et al. 1973).

Three species were isolated and identified according to Komárek et al. (1983) and Anagnostidis and Komárek (1988): *Chlorella vulgaris* Beijerinck, *Tetradesmus dimorphus* (Turpin) M.J.Wynne, and *Arthrospira platensis* Gomont.

Modified *Navicula* medium (Starr 1978) was used to grow *C. vulgaris* and *T. dimorphus* and Zarrouk's medium (Zarrouk 1966) was used for *A. platensis* growth. Cultures were incubated under continuous illumination of 45 µmol photons $m^{-2}$ $s^{-1}$ at 25 ± 2 °C, pH = 7 for *C. vulgaris* and *T. dimorphus*, and at 35 ± 2 °C, pH = 9 for *A. platensis*.

### Preparation of algal suspension

Samples of each of the test microalgae were harvested at the end of growth stationary phase by centrifugation then pellets were collected, spread as a thin layer on surface of glass plates, and left to dry under mild air current. The air-dried biomass was then collected, put in a porcelain crucible, and left to dry at 50 °C until constant weight. A weight of 10 g dry weight biomass dried at 50 °C was suspended in 100 mL distilled water for foliar spray of the tested potted plants.

### *Phaseolus vulgaris* experimental cultivation

The experiment was carried out in the green house of Botany Department Faculty of Science Mansoura University. The experiment was conducted for 85 days. Seeds of *Phaseolus vulgaris* were selected then surface sterilized by soaking in 0.01% $HgCl_2$ solution for 3 min. The seeds were washed thoroughly with tap water, divided into eight equal groups in 10 replicates; each pot contains 15 seeds. All sets of seeds were sown in similar earthenware pots (30 cm diameter and 25 cm depth), filled with clay-sand soil (2:1 w/w). Before planting some physical and chemical analysis was carried out on the used soil according to Black (1965): moisture; 1.9%, pH; 8.44, maximum water capacity (MWC); 60%, $CaCO_3$; 2%, electric conductivity; 2.15 ds $m^{-1}$, total salts; 19.5%, total nitrogen; 44.9%, sand; 22.13%, clay; 60.09%, silt; 19.79%, soil texture class; clay.

The pots were kept in the greenhouse under a normal day/night condition. According to the announcement of Ministry of Agriculture of Egypt, the following was done: (a) super phosphate fertilizer (0.5 g per pot) was added to the soil before sowing, (b) no Rhizobia were added to the soil, (c) for urea-treated plant, N-urea as nitrogen fertilizer (1 g per pot) was added to the soil during the first week of cultivation. After 7 days from sowing thinning took place with 5 uniform seedlings left to grow in each pot.

Plant foliar application was carried out three times using one-hand pressure sprayer containing different microalgae suspensions separately, in concentration of 10 g $(100\ mL)^{-1}$; the first spray was done after the first week of sowing with

50 mL, the second after 25 days with 100 mL, and the third after 77 days from sowing with 150 mL of each microalgae suspension (at vegetative, flowering, and fruiting stages, respectively). Spraying was done in the early morning when the stomata were open, allowing for better foliar penetration. Throughout the experiment, all plants were watered every 72 h, except after foliar application, when they were not watered for 24 h.

The experiment design was as follows:

**Control** (untreated): no addition of N-urea and no foliar spraying with microalgae.
**T1**: soil amended with N-urea but no foliar spraying with microalgae.
**T2**: foliar spraying with *C. vulgaris* but soil without N-urea addition.
**T3**: foliar spraying with *T. dimorphus* but soil without N-urea addition.
**T4**: foliar spraying with *A. platensis* but soil without N-urea addition.
**T5**: foliar spraying with *C. vulgaris* and soil amended with N-urea.
**T6**: foliar spraying with *T. dimorphus* and soil amended with N-urea.
**T7**: foliar spraying with *A. platensis* and soil amended with N-urea.

### Analyses of algae biomass

Total protein content was determined by the method of Bradford (1976) as modified by Stoscheck (1990), total carbohydrate content was determined according to Hedge et al. (1962), and total lipid was determined by the method of Sadasivam and Manickam (1996). Fresh frozen samples of the tested microalgal biomass, after extraction according to Shindy and Smith (1975), were sent to the Arid Land Agricultural Research and Services Center Faculty of Agriculture Ain Shams University, for phytohormone analysis (auxins; IAA, gibberellins; $GA_3$, cytokinin; CK and abscisic acid; ABA).

### *Phaseolus vulgaris* growth parameters and yield attribute

Shoot length, number of leaves per plant, shoot fresh weight, shoot dry weight per plant, root length, number of nodules, root fresh weight, and root dry weight of *P. vulgaris* were recorded at the vegetative stage (14 days from sowing). At the time of harvesting (fruiting stage), number of pods per plant, number of seeds, fresh weight of pods per plant, and dry weight of pods per plant were recorded in addition to shoot length, shoot fresh weight, shoot dry weight per plant, root length, root fresh weight, and root dry weight of *P. vulgaris* plant (84 days from sowing).

Biochemical parameters such as chlorophyll *a*, *b* were measured at 14 days from sowing according to the method described by Arnon (1949), and carotenoids were measured according to Horvath et al. (1972) as modified by Kissimon (1999). Protein content was determined according to Bradford (1976), and total soluble sugars, polysaccharides, and total carbohydrates determined according to Yemm and Willis (1954) and van Handel (1968) at 14 days and 84 days from sowing.

### Statistical analysis

Means of data obtained were analyzed by least significant difference (L.S.D) test at probability of 0.05. ANOVA analysis was done with Costat (CoHort software, USA).

## Results

### Biochemical composition of the test microalgae

Weight % of total lipid, total protein, and total carbohydrate of the microalgae are shown in Fig. 1. The highest percent of total lipid was recorded for *Chlorella vulgaris* ($10.8 \pm 0.54\%$) and the lowest was recorded for *Tetradesmus dimorphus* ($3.8 \pm 0.19\%$). *Arthrospira platensis* had relatively the highest total protein content ($57.55 \pm 2.87\%$) compared with *C. vulgaris* and *T. dimorphus*. *Tetradesmus dimorphus* had the highest percentage total carbohydrate content ($29.6 \pm 1.48\%$) compared to *C. vulgaris* and *A. platensis*.

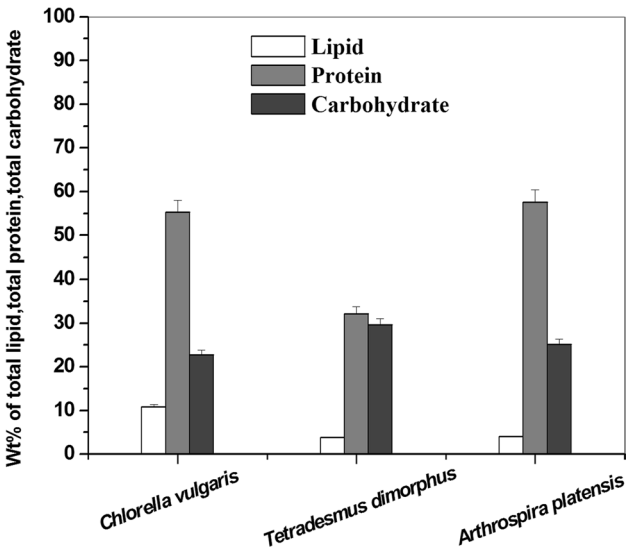


**Fig. 1** Weight percent of lipid, protein, and carbohydrate content of different tested microalgae. Data represent mean ± SD, $n = 3$

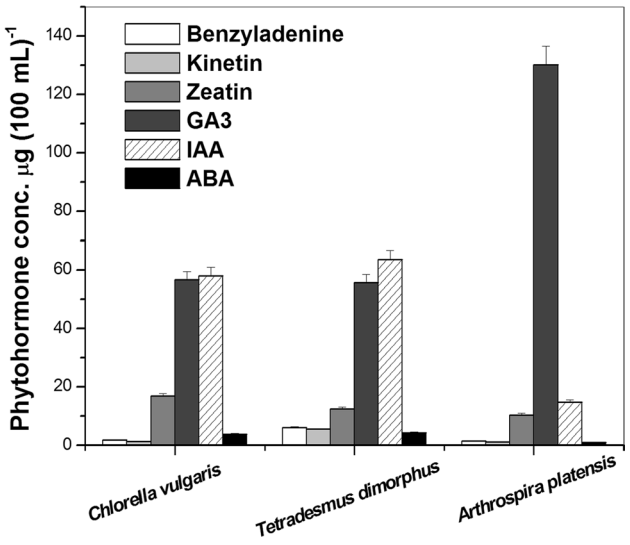


**Fig. 2** Variation in phytohormones content (benzyladenine, kinetin, zeatin, GA3, IAA, and ABA) of different tested microalgae. Data represent mean ± SD, $n = 3$

Phytohormone content was assessed at the end of the stationary phase of growth of each microalgae species. Figure 2 shows that phytohormone content is species dependent. For instance, *T. dimorphus* followed by *C. vulgaris* had the highest content of cytokinins (19.99 ± 0.87 µg (100 mL)$^{-1}$), auxins (IAA) (58.08 ± 2.7 µg (100 mL)$^{-1}$), and abscisic acid (ABA) (3.79 ± 0.18 µg (100 mL)$^{-1}$), while *A. platensis* had the highest content of gibberellins ($GA_3$) (130.05 ± 6.50 µg (100 mL)$^{-1}$).

## Effect of microalgae treatments on the fresh and dry weight, length, and number of leaves of *Phaseolus vulgaris* plant during vegetative and fruiting stage growth

The changes in the estimated growth parameters (shoot length, number of leaves, shoot fresh weight, shoot dry weight, root length, root fresh weight, root dry weight) of *P. vulgaris* plant in response to different treatments during vegetative and fruiting stages are displayed in Tables 1 and 2. Compared with the control, a noticeable significant increase in all growth parameters were observed in the case of T2 (foliar spraying with *C. vulgaris* but soil without N-urea addition), T4 (foliar spraying with *A. platensis* but soil without N-urea addition), T5 (foliar spraying with *C. vulgaris* and soil amended with N-urea), T6 (foliar spraying with *T. dimorphus* and soil amended with N-urea), and T7 (foliar spraying with *A. platensis* and soil amended with N-urea) at the vegetative stage, except for T4 which showed a non-significant increase in shoot dry weight and root length. T1 (soil amended with N-urea but no foliar

**Table 1** Effect of microalgae treatments on the fresh and dry weights (g), length and growth, and number of leaves of *Phaseolus vulgaris* during vegetative stage. Data represent mean ± SD, $n = 10$. Different letters indicate significant differences at $P \leq 0.05$

| Treatment | Fresh and dry weight (g plant$^{-1}$) | | | | | | | Length (cm) | | | | Number of Leaves |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Fresh weight | | | | Dry weight | | | | | | | |
| | Shoot | Root | whole plant | % increase in fresh wt. | Shoot | Root | whole plant | % increase in dry wt. | Shoot | Root | Total length | % increase in length | |
| Control | 12.86±0.41$^{f}$ | 0.643±0.08$^{d}$ | 13.504±0.6$^{e}$ | - | 1.502±0.3$^{d}$ | 0.116±0.02$^{e}$ | 1.618±0.1$^{c}$ | - | 11.6±2.1$^{d}$ | 2.93±0.8$^{d}$ | 14.53±0.72$^{d}$ | - | 3±0.94$^{c}$ |
| T1 | 13.047±3.7$^{f}$ | 0.782±0.06$^{cd}$ | 13.829±0.6$^{e}$ | 2.35 | 1.574±0.5$^{d}$ | 0.128±0.01$^{de}$ | 1.702±0.1$^{bc}$ | 4.9 | 12.75±2.9$^{c}$ | 3.31±0.65$^{cd}$ | 16.08±0.8$^{cd}$ | 9.6 | 3.1±0.84$^{abc}$ |
| T2 | 15.192± 1.8$^{d}$ | 0.943±0.07$^{b}$ | 16.135±0.8$^{c}$ | 16.31 | 1.864±0.3$^{bc}$ | 0.151±0.01$^{c}$ | 2.015±0.1$^{ab}$ | 19.7 | 14.97±2.6$^{bc}$ | 3.54±0.9$^{bc}$ | 18.51 ±0.93$^{c}$ | 21.5 | 3.9±1.15$^{ab}$ |
| T3 | 13.593±0.1$^{f}$ | 0.767±0.08$^{cd}$ | 14.36±0.72$^{de}$ | 5.96 | 1.645±0.2$^{d}$ | 0.125±0.02$^{de}$ | 1.77±0.09$^{bc}$ | 8.58 | 13.78±1.4$^{c}$ | 3.1±0.9$^{d}$ | 16.88±0.84$^{cd}$ | 13.9 | 3.3±1.5$^{bc}$ |
| T4 | 14.308± 0.8$^{e}$ | 0.89±0.19$^{bc}$ | 15.198±0.7$^{d}$ | 11.15 | 1.73±0.17$^{cd}$ | 0.139±0.04$^{cd}$ | 1.86±0.09$^{bc}$ | 13.01 | 14.58±2.5$^{c}$ | 3.43±0.62$^{cd}$ | 18.01 ±0.9$^{c}$ | 19.3 | 3.7±0.9$^{ab}$ |
| T5 | 17.362± 2.9$^{a}$ | 1.317±0.16$^{a}$ | 18.679±0.9$^{a}$ | 27.7 | 2.391±0.2$^{a}$ | 0.194±0.03$^{a}$ | 2.58±0.13$^{a}$ | 37.28 | 15.91±2.8$^{a}$ | 3.98±0.77$^{a}$ | 19.89 ±0.99$^{a}$ | 26.9 | 4.3±1.1$^{a}$ |
| T6 | 15.512±1.2$^{c}$ | 1.022±0.18$^{b}$ | 16.534±0.8$^{c}$ | 18.33 | 1.985±0.1$^{b}$ | 0.176±0.01$^{b}$ | 2.15±0.11$^{ab}$ | 24.7 | 15.12±3.6$^{b}$ | 3.68±0.61$^{b}$ | 18.8 ±0.94$^{bc}$ | 22.7 | 4±0.69$^{ab}$ |
| T7 | 16.529±4.5$^{b}$ | 1.253±0.16$^{a}$ | 17.764±0.8$^{b}$ | 23.98 | 2.25±0.11$^{a}$ | 0.180±0.01$^{b}$ | 2.43±0.12$^{ab}$ | 33.4 | 15.61±2.3$^{a}$ | 3.78±0.65$^{a}$ | 19.39 ±0.97$^{b}$ | 25.06 | 4.2±0.67$^{a}$ |
| LSD 0.05 | 0.30506 | 0.12026 | 0.86282 | | 0.16668 | 0.01209 | 0.46981 | | 1.5757 | 0.58949 | 3.79134 | | 0.47915 |

**Table 2** Effect of microalgae treatments on the fresh and dry weights (g) and length and growth of *Phaseolus vulgaris* during fruiting stage. Data represent mean ± SD, $n = 10$. Different letters indicate significant differences at $P \leq 0.05$

| Treatment | Fresh and dry weight (g plant$^{-1}$) | | | | | | | Length (cm) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Fresh weight | | | | Dry weight | | | Shoot | Root | Total length | % increase in length |
| | Shoot | Root | whole plant | % increase in fresh wt. | Shoot | Root | whole plant | % increase in dry wt. | | | | |
| Control | 60.17±5.5$^{d}$ | 4.478±0.79$^{e}$ | 64.65±3.2$^{d}$ | - | 11.612±2.6$^{g}$ | 1.337±0.25$^{d}$ | 12.949±0.65$^{g}$ | - | 27.6±3.5$^{h}$ | 10.37±2.6$^{d}$ | 37.97±1.8$^{g}$ | - |
| T1 | 63.31±7.3$^{d}$ | 6.047±1.6$^{cd}$ | 69.357±3.4$^{d}$ | 9.19 | 11.677±4.9$^{g}$ | 1.792 ±0.5$^{bc}$ | 13.469±0.67$^{g}$ | 15.66 | 30.16±6.3$^{g}$ | 12.19 ±2.2$^{bc}$ | 42.35±2.1$^{f}$ | 11.9 |
| T2 | 75.69±0.8$^{c}$ | 6.354 ±0.1$^{cd}$ | 82.044±4.1$^{c}$ | 19.53 | 17.031±3.1$^{d}$ | 1.957±0.01$^{bc}$ | 18.988±0.95$^{d}$ | 25.7 | 34.4±4.2$^{d}$ | 12.73 ±1.9$^{bc}$ | 47.13±2.3$^{d}$ | 20.69 |
| T3 | 64.27±8.8$^{d}$ | 5.159±1.1$^{de}$ | 69.429±3.4$^{d}$ | 7.535 | 14.584±0.1$^{f}$ | 1.739 ±0.23$^{c}$ | 16.323±0.81$^{f}$ | 16.11 | 31.25±5.5$^{f}$ | 11.11±1.4$^{cd}$ | 42.36±2.1$^{f}$ | 11.73 |
| T4 | 66.989±4.9$^{d}$ | 6.155 ±0.7$^{cd}$ | 73.144±3.6$^{d}$ | 15.26 | 15.775±1.7$^{e}$ | 1.849±0.22$^{bc}$ | 17.624±0.88$^{e}$ | 20.68 | 32.55±3.5$^{e}$ | 12.27± 1.8$^{bc}$ | 44.82±2.2 | 18.18 |
| T5 | 90.933±6.7$^{a}$ | 9.941 ±0.1$^{a}$ | 100.874±5$^{a}$ | 36.49 | 20.707±3.1$^{a}$ | 2.28±0.13$^{a}$ | 22.987±1.15$^{a}$ | 39.22 | 37.33±7.7$^{a}$ | 14.64± 2.5$^{a}$ | 51.97±2.5$^{a}$ | 28.08 |
| T6 | 81.06±9.8$^{b}$ | 7.323±0.1 $^{bc}$ | 88.383±4.4$^{bc}$ | 24.06 | 17.72±0.6$^{c}$ | 2.083 ±0.16$^{abc}$ | 19.803±0.99$^{c}$ | 30.16 | 35.5±4.3$^{c}$ | 13.1 ±2.9$^{b}$ | 48.6±2.4$^{c}$ | 22.15 |
| T7 | 83.52±8.7$^{b}$ | 8.265 ±0.6$^{b}$ | 91.785±4.5$^{b}$ | 30.07 | 18.703±3.1$^{b}$ | 2.116±0.12$^{ab}$ | 20.819±1.04$^{b}$ | 34.97 | 36.64±5.3$^{b}$ | 13.25± 3.45$^{b}$ | 49.89±2.4$^{b}$ | 23.87 |
| LSD 0.05 | 5.0328 | 1.1926 | 7.4607 | | 0.32611 | 0.23146 | 0.65275 | | 0.31045 | 1.1926 | 0.32611 | |

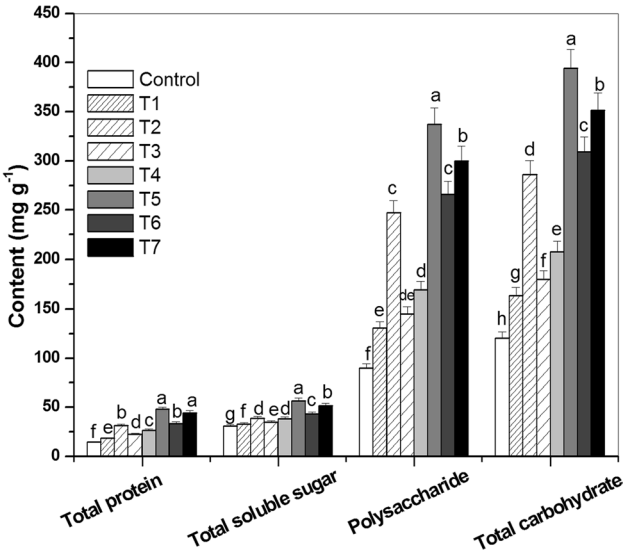


**Fig. 3** Effect of microalgal treatments on biochemical analyses of *Phaseolus vulgaris* shoot during vegetative stage. Data represent mean ± SD, $n = 3$. Different letters indicate significant differences at ($P \leq 0.05$)

spraying with microalgae) and T3 (foliar spraying with *T. dimorphus* but soil without N-urea addition) showed non-significant increase in all parameters except shoot length which increased significantly. During fruiting stage, all parameters increased significantly except T1 in shoot fresh and dry weight, T3 in shoot and root fresh weight and root length, and T4 in shoot fresh weight. The foliar application of *C. vulgaris* with chemical fertilizer treated plants (T5) caused the maximum increase in shoot fresh weight, root fresh weight, shoot dry weight, root dry weight, shoot length, and root length during both vegetative and fruiting stages (Tables 1 and 2). In addition to the previous growth parameters, number of leaves was highest in the T5 treatment (4.3 ± 1.1) during the vegetative stage (Table 1).

## Effect of microalgae treatments on biochemical composition of *Phaseolus vulgaris* shoot during vegetative growth

Figure 3 illustrates the levels of total protein, total soluble sugar, polysaccharides, and total carbohydrates of *P. vulgaris* shoot during vegetative growth. There was significant increase in all parameters of *P. vulgaris* leaves in response to treatments (T1 to T7) compared to the control during early-stages *P. vulgaris* growth. T5 had the highest protein, total soluble sugar, polysaccharide, and total carbohydrate content during the vegetative stage.

Figure 4 shows a significant increase in chlorophyll *a*, chlorophyll *b*, and carotenoids content in the case of treatments T2 to T7 compared to the control. T1 treatment

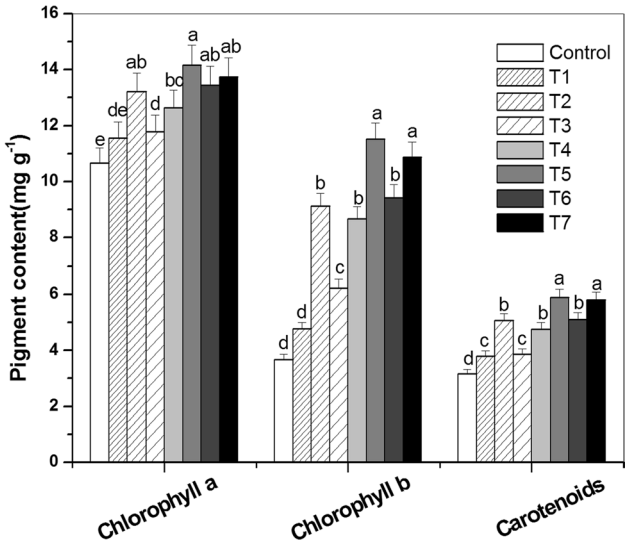


**Fig. 4** Effect of microalgal treatments on pigments content of *Phaseolus vulgaris* leaves during vegetative stage. Data represent mean ± SD, $n = 3$. Different letters indicate significant differences at ($P \leq 0.05$)

showed a non-significant increase in chlorophyll *a* and chlorophyll *b* but a significant increase in carotenoids. The highest value in chlorophyll *a*, chlorophyll *b*, and carotenoids content was recorded in the treatment with *C. vulgaris* and chemical fertilizer (T5).

### Seed yield characteristics and biochemical composition of *Phaseolus vulgaris* after harvest

The yield attributes of *P. vulgaris* are given in Table 3. The seed yield characters including number of pods, number of seeds, and pods fresh and dry weight increased significantly with treatments T2, T5, T6, T7 compared to control. T3 showed non-significant increase in number of seeds and pods fresh weight, and T4 showed non-significant increase in pods fresh weight. T1 showed significant increase in number of pods and non-significant increase in number of seeds and pods fresh and dry weight. The maximum of these parameters observed in the T5 treated plants.

Figure 5 shows the levels of protein, total soluble sugar, polysaccharides, and total carbohydrates of *P. vulgaris* seeds during fruiting growth. There was significant increase in the biochemical parameters of *P. vulgaris* seeds in the case of treatments T1 to T7 compared to the control during late stage of *Phaseolus vulgaris* growth except T1 in total soluble sugar which increase non-significantly. T5 exhibited the highest protein content (159.25 ± 7.962 mg $g^{-1}$ fresh wt.), total soluble sugar (128.65 ± 6.43 mg $g^{-1}$ dry wt.), polysaccharide (379.58 ± 18.979 mg $g^{-1}$ dry wt.), and total carbohydrate (508.23 ± 25.41 mg $g^{-1}$ dry wt.) during fruiting stage.

## Discussion

The use of microalgae biostimulants has become a global approach for obtaining environmentally friendly, high-yield crops with good quality that are safe for human. It is evident from the results obtained (Figs. 1 and 2) that *C. vulgaris*, *T. dimorphus*, and *A. platensis* biomass are rich in protein, carbohydrate, lipid, and phytohormones. In this context, identifying and selecting microalgae which are high in primary metabolites (carbohydrates, proteins, and lipids) and natural phytohormones particularly auxins and cytokinins is considered a key factor for plant biostimulants to enhance plant growth and productivity (Du Jardin 2015; Chiaiese et al. 2018; Ronga et al. 2019). Thus, the potential of *C. vulgaris*, *T. dimorphus*, and *A. platensis* as plant biostimulants on the vegetative growth and yield production of *P. vulgaris* was investigated in the present study.

Several studies have revealed foliar spray application as an effective technique alternative to soil application in fertilization for satisfying plant with nutrients sprays required for achieving high yield crops through leaf surface where they can penetrates easier and faster via cuticular cracks, stomata, trichomes, or lenticles reaching target cells where nutrients are required (Fernández et al. 2013; Battacharyya et al. 2015; Dias et al. 2016; Plaza et al. 2018).

**Table 3** Effect of microalgal treatments on yield attributes of *Phaseolus vulgaris* plant during fruiting stage. Data represent mean ± SD, $n = 10$. Different letters indicate significant differences at $P \leq 0.05$

| Treatment | No. of pods/plant | No. of seeds/pod | Pods fresh wt (g) | Pods dry wt (g) |
|---|---|---|---|---|
| Control | $3.4 \pm 0.17^{c}$ | $2.6 \pm 0.13^{c}$ | $2.3 \pm 0.12^{d}$ | $0.67 \pm 0.03^{d}$ |
| T1 | $4.2 \pm 0.21^{b}$ | $2.8 \pm 0.14^{bc}$ | $2.5 \pm 0.125^{cd}$ | $0.7 \pm 0.03^{d}$ |
| T2 | $4.5 \pm 0.22^{b}$ | $3.3 \pm 0.165^{ab}$ | $2.9 \pm 0.145^{bc}$ | $0.84 \pm 0.042^{bc}$ |
| T3 | $4.3 \pm 0.21^{b}$ | $3 \pm 0.15^{bc}$ | $2.6 \pm 0.13^{cd}$ | $0.75 \pm 0.03^{bc}$ |
| T4 | $4.4 \pm 0.22^{b}$ | $3.2 \pm 0.16^{b}$ | $2.7 \pm 0.123^{cd}$ | $0.81 \pm 0.04^{bc}$ |
| T5 | $5.2 \pm 0.26^{a}$ | $3.5 \pm 0.17^{a}$ | $3.3 \pm 0.165^{a}$ | $0.95 \pm 0.047^{a}$ |
| T6 | $4.6 \pm 0.23^{b}$ | $3.4 \pm 0.17^{a}$ | $3.18 \pm 0.15^{ab}$ | $0.85 \pm 0.042^{bc}$ |
| T7 | $5 \pm 0.25^{a}$ | $3.45 \pm 0.17^{a}$ | $3.2 \pm 0.16^{a}$ | $0.88 \pm 0.044^{ab}$ |
| LSD 0.05 | 0.32612 | 0.47974 | 0.54386 | 0.07648 |

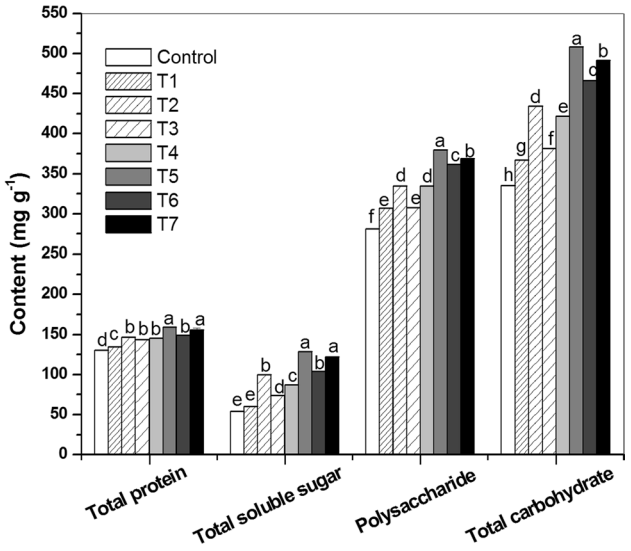


**Fig. 5** Effect of microalgal treatments on biochemical analyses of *Phaseolus vulgaris* yield seeds during fruiting stage. Data represent mean ± SD, $n = 3$. Different letters indicate significant differences at ($P \leq 0.05$)

In the present study (Tables 1 and 2), foliar application treatments with *C. vulgaris*, *T. dimorphus*, and *A. platensis* suspensions significantly enhanced the plant height, fresh weight, dry weight, and number of leaves at both vegetative growth and fruiting stage of *P. vulgaris* compared with the control. These obvious positive changes in both vegetative growth and yield of *P. vulgaris* can be outcome to the effect of protein, carbohydrate, lipid, and phytohormones of the test microalgae biomass singly or in combination. It has become evident that the effects of mixed ingredients are the sum synergetic, antagonistic, and/or addition interactions including obvious stimulation in growth and yield of *P. vulgaris*.

Additionally, the phytohormones present in *C. vulgaris*, *T. dimorphus*, and *A. platensis* biomass particularly auxins, cytokinins, and gibberellins are contributing to several plant growth and development aspects. It has been reported (Plaza et al. 2018; Bayona-Morcillo et al. 2020; Chookalaii et al. 2020) that both auxins and cytokinins play a prominent role in cell division, cell elongation, and root and shoot development. Gibberellins regulate seed germination, stem elongation, leaf expansion, early flowering, seed development, and inhibition of seed dormancy.

Our results are similar to those obtained by *A. platensis* foliar spraying of red beat (Ronga et al. 2019), tomato and pepper (Elarroussia et al. 2016), aubergine (*Solanum melongena*) (Dias et al. 2016), lettuce (Mogor et al. 2018), and *Petunia x hybrida* (Plaza et al. 2018) as well as *C. vulgaris* foliar application of grapes (Nagy and Pintér 2015), tomato and cucumber (Bumandalai and Tserennadmid 2019), and rice and maize (Dineshkumar et al. 2018, 2019).

The considerable significant increase in root and shoot fresh weight and dry weight of *P. vulgaris* in response to the application of *C. vulgaris* and chemical fertilizer (T5) among other treatments during both vegetative and fruiting stages could be due to the combined effect of both biostimulants along with chemical fertilizer in increasing nutrients use efficiency of *P. vulgaris* for macro and micronutrients added by the test microalgae that are essential for growth and development (Osman et al. 2010; Garcia-Gonzalez and Sommerfeld 2016; Barone et al. 2019).

The significant increase in total pigment content of *P. vulgaris* leaves during vegetative growth (Fig. 4) with the *C. vulgaris* and chemical fertilizer (T5) treatment compared with other treatments may contribute to the growth regulators added by *C. vulgaris*, particularly cytokinins, which play a crucial role in increasing the leaf chlorophyll content and consequently decreasing senescence or it may be due to the increase the number of leaves and/or the changes in the pigment biosynthesis in response to the used treatment. These results are in accordance with other findings which shown increase in chlorophyll content of grapevine and strawberry plants treated with algae (Dineshkumar et al. 2019; Puglisi et al. 2020).

Additionally, the treatment with *C. vulgaris* suspension and chemical fertilizer (T5) induced the highest and most significant increase in total protein and total carbohydrate contents of *P. vulgaris* leaves during vegetative growth (Fig. 3) and seeds during fruiting stage (Fig. 5). These results are similar to those obtained by Puglisi et al. (2020). The high protein content in *P. vulgaris* plant may be due to the increase in nitrogen uptake by *P. vulgaris* roots which is then translocated to shoot and other plant parts. It has been reported (Gorelova 2006) that microalgae can play an important role in symbiosis with other organisms, including higher plants as well as microalgal extracts, when applied as foliar spray, showed an increased N-content in root and shoot tissues (Kim et al. 2018). Moreover, the enhancement of pigments production is reflected on carbohydrate synthesis, whereas the increase in carbohydrate contents may contribute to the increase in photosynthesis rate of *P. vulgaris*.

The overall increase in *P. vulgaris* yields attribute in response to combined application of biostimulant (*C. vulgaris*) and chemical fertilizer (T5) may be largely attributed to an adequate and balanced provision of nutrients to the plant throughout the growth period from both the microalgal biomass and the chemical fertilizer resulting in the maximum number of pods per plant, seeds per pods, and pods weight. Our findings confirmed the observations of Osman et al. (2010) who noted an increased yield of pea plants using a combined dose of nitrogen fertilizer and

microalgae, as well as Dineshkumar et al. (2018) who found higher yield in rice growth and seed yield and Dineshkumar et al. (2019) who found higher yield in maize using microalgae and cow dungs.

In conclusion, results show that *C. vulgaris*, *T. dimorphus*, and *A. platensis* biomass are potential candidate plant biostimulants. The combination of *C. vulgaris* and chemical fertilizer is the most effective in enhancing the plant height, fresh weight, dry weight, and number of leaves at both vegetative growth and fruiting stage of *P. vulgaris* as well as yield compared with other treatments. Thus, foliar spraying with *C. vulgaris* can be proposed as a plant biostimulant enhancing plant growth and crop production. More in-depth future studies are required to study the effect of *C. vulgaris* inoculation on soil quality hoping to reduce further the use of chemical fertilizers by biofertilizers.

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**Availability of data** The authors confirm that all the data are available within the article.

## Declarations

**Conflict of interest** The authors declare no competing interests.

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