

Article

Spirulina platensis Biofertilization for Enhancing Growth, Photosynthetic Capacity and Yield of *Lupinus luteus*

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Abstract: Cyanobacteria can play an important role in sustainable agriculture by improving crop growth and production, as well as environmental quality. The current study was conducted to evaluate the role of *Spirulina platensis* extract in the improvement of growth, photosynthetic capacity and yield of *Lupinus luteus*. Different concentrations (0.25, 0.5 and 1.0%) of *S. platensis* methanolic extract were used for priming seeds and foliar spraying applications. Growth, photosynthetic pigment content and activity were recorded at 35 days of cultivation. The yield measurements and protein profile of seeds were performed at 60 days. For both treatments, the lower concentration (0.25%) of the extract was the most successful in improving all growth metrics and the phytohormonal level of *L. luteus*. Photosynthetic pigment and capacity, nutrient content and yield components of *L. luteus* were improved with the extract of 0.25% concentration. However, the growth was significantly inhibited at the higher concentration (1.0%), recording lower values of the measured characters than the control. A positive correlation was observed between the total chlorophyll, photosynthetic rate, yield parameters and carbohydrate accumulation by the application of the *S. platensis* extracts. The protein profile of seeds revealed that spraying treatment triggered the appearance of a new band of 30.56 KDa. The promotion of growth could be attributed to the nutrients, bioactive molecules and phytohormones in the extract. The use of *Spirulina*-based stimulators is reliable with the concept of sustainable agriculture by enhancing photosynthetic pigment content and rate ensuring the correlation between the yield and those measured parameters of *L. luteus*.

Keywords: microalgae; priming; foliar spraying; yield



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1. Introduction

Agricultural development and management are broadly dependent on the growth promoters' effects for crop production enhancement. However, chemical growth promoters represent a great threat to the environment and human health [1]; so, to cover the global demand for food and maximize the yield of cultivated plants without threatening the environment is a challenge. Microalgae and cyanobacteria have attracted great interest for using as promising fertilizers or enhancers for plant growth and production [1]. Today, bio-fertilizers industry insight points to the eco-friendly microalgae approach for sustainable plants [2]. Several perspectives on the stimulation actions of microalgae by affecting plant performance were proposed for aiding in advanced food production. Some of the microalgal advantages are quicker growth rates, increased biomass productivity, and the capacity to manufacture complex metabolites with minimum inputs. Microalgal species have a great taxonomic and biochemical variety, making them an excellent source of numerous biomolecules with industrial and medicinal applications [3].

The naturally effective molecules that produced by cyanobacteria, including phenolics, proteins, vitamins, carbohydrates, amino acids, polysaccharides and phytohormones, may work out in cooperation to enhance plant growth [4]. Cyanobacteria are involved in the signaling pathway of gene expression for plant metabolism regulation [5]. Moreover, cyanobacteria improve the rhizosphere community, leading to the modulation of the root system and mineral nutrition [6]. Cyanobacterial extracts can be used to improve seed germination, plant growth, flowering and fruit production, as well as the life shelf of post-harvested fruits [7] by improving mineral nutrient utilization. Microalgal/cyanobacterial metabolites can stimulate a variety of plant metabolic responses, including as respiration, photosynthesis, nucleic acid synthesis, chlorophyll formation and ion absorption [8]. Cyanobacteria can reduce stressors through crop management [9].

Several application strategies have been used to provide cyanobacterial to crops. According to Santini et al. [5], the most common method of cyanobacterial application is foliar spraying (54%), followed by basal application (26% in soil or inert hydroponic substrates) and seed coating. Spraying practices allow the use lower dosages of extract [10], therefore developing economic sustainability. Plant responses to nutrients and bioactive compounds included in extracts are often faster with foliar treatments than with application as a soil amendment [11]. Moreover, foliar biofertilizers with essential elements such as Optysil, Metalosate Potassium, Bolero Bo, ADOB 2.0 Zn IDHA, ADOB B, ADOB 2.0 Mo improved the chlorophyll content and yield traits (for example, number of pods and thousand seed weight) of white lupine [12], indicating that foliar fertilization is more economic and efficient. The concentration of an algal stimulator appears to be an important determinant in its efficacy on plants [13]. Godlewska et al. [14] reported that the enhancement in radish fresh weight after foliar spraying with *A. platensis* growth medium was positively correlated with the applied doses up to a maximum concentration of 15%, beyond which a decreasing biostimulator activity was observed. The increase in radish fresh weight by foliar spraying with *A. platensis* growth medium was connected with the applied dosages up to a maximum concentration of 15%.

Botanists categorize *Spirulina* as a microalga belonging to Cyanophyceae class; but it is categorized as a bacterium owing to its prokaryotic structure [15]. *Spirulina* has significant growth potential, particularly as a small-scale crop for nutritional enhancement and environmental mitigation [16]. According to Ramírez-Rodriguez et al. [17], the *Spirulina* biomass has a high level of protein (47%), γ -Linolenic acid ($24.45 \text{ g } 100 \text{ g}^{-1}$ of fat), iron ($16.27 \text{ mg } 100 \text{ g}^{-1}$), calcium ($207 \text{ mg } 100 \text{ g}^{-1}$) and potassium ($1675 \text{ mg } 100 \text{ g}^{-1}$). *S. platensis* was used as a fertilizer to improve the agronomic bio-fortification of *Amaranthus dubius* (red spinach) with carbohydrates, proteins, essential macronutrients, micronutrients and vitamin A [18]. By adding *Spirulina* sp. to the soil, it prevents the malnourishment of macro and micro-elements for cultivated plants [19]. In two seasons, the application of an *A. platensis* extract alone or in combination with the inoculation of the nitrogen-fixing bacteria *Pseudomonas stutzeri* in the presence of varied dosages of nitrogen fertilizer increased onion growth and production [20].

Lupin is a hopeful legume crop, belongs to the Fabaceae family [21]. Lupin is significant in agriculture due to its capacity to fix nitrogen and yield seeds high in protein, fiber and mono- and polyunsaturated fatty acids [22]. Different species of *Lupinus* have proteins of high value and prospective advantages for human health and ecological productivity. This means that *Lupinus* sp. natural legumes can be used as an alternative to soya beans in Europe [23]. We hypothesized that *S. platensis* will improve the growth, phytohormonal level, nutrient supply, photosynthetic pigments content and activity of *L. luteus*, which would be positively reflected and correlated with the yield. Accordingly, our objectives were first to assess the effects of different *S. platensis* extracts (0, 0.25, 0.5 and 1.0%) on external growth parameters and endogenous levels of phytohormones, nutrients, pigments and photosynthetic capacity of *L. luteus*; and second, to determine the correlation between different measurements.

2. Materials and Methods

2.1. Materials

Lupinus luteus (L. luteus) seeds were received from Agriculture Research Center, Dokki, Giza, Egypt.

2.2. Method

2.2.1. *Spirulina platensis* Biomass Preparation

S. platensis was cultivated on a standard Zarrouk culture medium [24]. Each culture vessel (2L) was inoculated with 200 mL of *S. platensis* culture, then incubated at 28 ± 2 °C and illuminated (fluorescent light tubes at $45 \mu\text{Em}^{-2}\text{s}^{-1}$). The culture was supplied with an air (97% O₂ and 3% CO₂) pump to accelerate *S. platensis* growth. The biomass was harvested by centrifugation at 5000 rpm for 15 min. The cell pellets were washed three times and resuspended in sterilized distilled water to remove traces of growth medium. The suspension was then centrifuged at 5000 rpm for 15 min. The collected biomass of *S. platensis* was dried in air for 3 days, powdered by manual mortar and stored at 4 °C until used.

2.2.2. *Spirulina* Extract Preparation

Extraction of air-dried powder of *S. platensis* was conducted by methanol (PIOCHEM) (80%) according to Pant et al. [25]. Evaporation of the solvent occurred at 40 °C under reduced pressure by a rotatory evaporator. Dried extracts were dissolved in a polar solvent, and completed with distilled water in w (residue)/v (distilled water) ratio according to the concentration.

2.2.3. Experimental Design

The field experiment was conducted in 2020/2021 at Ain Helwan, Cairo (Latitude: 29°50'59.99" Longitude: 31°19'60.00" E). The seeds were sterilized (by 5% solution of sodium hypochlorite) and divided into two sets. The first set of seeds were sown in a field block that was divided into four lines. Each line was sprayed (first spray) by one *S. platensis* extract (SE) concentration (0 (control), 0.25%, 0.5% and 1.0%) after 14 days of cultivation. The second foliar spraying was carried out two weeks after the first one. The second set of seeds were soaked or primed in the different concentrations of SE for 12 h. After soaking, seeds were sown in a second field block which was divided into more 4 lines. The plants were irrigated twice a week. After 35 days of cultivation, the plants were collected and divided into roots and shoots. Growth parameters were measured (in 5 replicates) as length, dry and fresh weight of shoots, roots and leaves. After 60 days of cultivation, the yield was collected and measured by counting pods per plant, seeds per pod, measuring the length of pod and the weighing of 100 seeds.

2.2.4. Measurement of Chlorophyll Content Index and Gas Exchange

The chlorophyll content index (CCI) was measured after 35 days of cultivation with a chlorophyll meter (CCM-200; ADC Bioscientific, Hoddesdon, UK) by clipping the sensor onto the *L. luteus* leaf (ten leaves were measured for each treatment). The absorbance was measured at two different wavelengths 653 nm (Chlorophyll) and 931 nm (Near Infra-Red).

Gaseous exchange of each control and treated *L. luteus* plant (3 leaves were used, 9 records for each) was measured by a portable photosynthesis system (LCpro-SD, ADC BioScientific, Hoddesdon, UK) with a standard $2 \times 3 \text{ cm}^2$ leaf chamber. The pots were well watered a day before measurements. The measurements were carried out on fully expanded leaves. The measurements were carried out at ambient light and leaf temperature 23 °C. The photosynthetic parameters were measured after 35 days of cultivation. The measured attributes include the photosynthetic rate (A), stomatal conductance (G_s), internal CO₂ (C_i) and evaporation rate (E).

2.3. Chemical Analysis

Determination of photosynthetic pigment of a leaf (chlorophyll a (Chl a), chlorophyll b (Chl b) and carotenoids) was performed as described by the Metzener et al. [26] method. The extraction of 0.5 g was carried out with 85% acetone (PioMed) from usually two collected small green leaves. The previous extract was assessed against a blank of pure 85% acetone at wavelengths of 452.5, 644 and 663 nm. Determinations were performed in triplicate ($n = 3$).

$$\text{Chlorophyll a } (\mu\text{g/g}) = 10.3 \text{ E663} - 0.918 \text{ E644}$$

$$\text{Chlorophyll b } (\mu\text{g/g}) = 19.7 \text{ E644} - 3.87 \text{ E663}$$

$$\text{Carotenoids } (\mu\text{g/g}) = 4.2 \text{ E452.5} - (0.264 \text{ Chlorophyll a} + 0.426 \text{ Chlorophyll b})$$

Total soluble sugar and total carbohydrates (after hydrolysis of dry plant material in 1 N H_2SO_4 (Fluka) at 80–90 °C for 24 h, filtration and filtrate was used for total carbohydrate concentration by anthrone) were determined by anthrone (PIOCHEM) reagent according to Umbriet et al. [27]. One milliliter of ethanolic leaf extract was mixed with 3.0 mL of fresh anthrone reagent. This mixture was put in a boiling water bath for 5 min. After cooling, the absorbance of the developed color was determined at 625 nm using a spectrophotometer. Determinations were performed in triplicate ($n = 3$).

The concentration of potassium (K), sodium (Na), magnesium (Mg) and K/Na ratio in dry leaves was determined according to Essa et al. [28] using a microwave plasma atomic emission spectrometer (MP-AES, Agilent, Switzerland). The leaf ash was mixed with 1:0.2 (v/v) of nitric acid (HNO_3)-hydrogen peroxide (H_2O_2) (BIOCHEM) in digestion vessels. Then, samples were incubated at 85 °C for 90 min, the digestion followed with filtration by a 0.45 μm nylon filter membrane. The filtrate used MP-AES analysis for elements.

Protein electrophoresis analysis was performed in the central labs of Ain-Shams University. Sodium dodecyl sulfate (SDS) polyacrylamide gel (SDS-PAGE) was used according to the Laemmli [29] method. The gel was captured to count and differentiate bands for each treatment.

The extraction for phytohormone determination was conducted by the Wasfy and Orrin [30] technique. Plant material (5 g) was grounded in a cold 80% methanol. The methanolic mixture was transferred to a flask with a methanol volume adjusted to 100 mL. The previous mixture incubated for 24 h at 0 °C. After incubation, the mixture was filtered using Whatman filter paper number 42 under vacuum. The residue was resuspended with fresh methanol and stirred up for 30 min. Then, the mixture was filtrate and the method was repeated once more. We then evaporated the combined extracts with the rotatory evaporator to obtain the 10 to 30 aqueous phase.

The aqueous phase was divided into two parts. For part one, the aqueous phase pH was adjusted to 8.6 with NaOH and partitioned three times with an equal volume of ethyl acetate. The ethyl acetate fraction was evaporated. The combined alkaline ethyl acetate phase (fraction I) was used for high-performance liquid chromatography (HPLC) injection for zeatin determination. For part two, the aqueous phase pH was adjusted to 2.8 with hydrochloric acid (HCl) and partitioned three times with an equal volume of ethyl acetate. The ethyl acetate fraction was evaporated. The combined acidic ethyl acetate phase (fraction II) was used for HPLC injection for auxins and gibberellins determination.

2.4. Statistical Analysis

One-way ANOVA (Duncan, post-hoc) was applied to assess the difference between results. This difference was considered as significant at $p \leq 0.05$ using SPSS (SPSS base 15.0 user's guide, Chicago: SPSS 523 Inc.). Pearson correlation analysis was used to test the relationships between the measured variables using Excel and SPSS.

3. Results

3.1. Growth Parameters and Phytohormonal Levels

The growth of *L. luteus* was measured after 35 days of cultivation to assess the impact of different concentrations of the *S. platensis* extract (Table 1).

Table 1. Effect of *S. platensis* extract (0, 0.25, 0.5 and 1.0%), by priming or spraying, on shoot, root and leaves growth parameters (lengths (centimeter [cm]), fresh (gram [g]) and dry weights (milligram [mg]) on *L. luteus* after 35 days of cultivation.

Treatment		Root			Shoot			Leaves	
		Length (cm)	Fresh wt (g)	Dry wt (mg)	Length (cm)	Fresh wt (g)	Dry wt (mg)	Number	Dry wt (g)
Priming in ¹ SE (%)	0	6.63 ± 0.28 ^{bc}	0.31 ± 0.03 ^d	50 ± 1 ^{cd}	32.1 ± 2.20 ^c	3.50 ± 0.25 ^b	446 ± 75 ^b	13 ± 0.570 ^{bc}	0.52 ± 0.01 ^b
	0.25	8.06 ± 0.72 ^a	0.58 ± 0.09 ^b	70 ± 5 ^b	39.1 ± 1.70 ^{ab}	4.81 ± 0.76 ^a	593 ± 85 ^a	15 ± 1.150 ^a	0.70 ± 0.04 ^a
	0.5	7.50 ± 0.25 ^{ab}	0.45 ± 0.02 ^c	60 ± 10 ^{bc}	32.2 ± 2.00 ^c	3.07 ± 0.25 ^b	330 ± 34 ^{bc}	12 ± 1.150 ^c	0.47 ± 0.05 ^{bc}
	1.0	5.30 ± 0.87 ^d	0.32 ± 0.01 ^d	20 ± 5 ^e	24.7 ± 2.70 ^d	2.26 ± 0.21 ^c	226 ± 37 ^c	11 ± 1.000 ^c	0.35 ± 0.04 ^c
Spraying with ¹ SE (%)	0	6.63 ± 0.02 ^{bc}	0.59 ± 0.01 ^b	60 ± 5 ^{bc}	37.5 ± 0.76 ^b	3.53 ± 0.48 ^b	450 ± 34 ^b	12 ± 1.000 ^c	0.38 ± 0.08 ^c
	0.25	8.00 ± 0.23 ^a	0.73 ± 0.07 ^a	90 ± 5 ^a	40.5 ± 1.50 ^a	5.19 ± 0.54 ^a	620 ± 115 ^a	15 ± 1.700 ^{ab}	0.78 ± 0.13 ^a
	0.5	7.23 ± 0.60 ^{ab}	0.56 ± 0.01 ^b	50 ± 5 ^c	33.3 ± 1.00 ^c	3.34 ± 0.22 ^b	373 ± 47 ^b	13 ± 1.000 ^{abc}	0.43 ± 0.06 ^{bc}
	1.0	6.20 ± 0.98 ^c	0.25 ± 0.08 ^d	40 ± 9 ^d	31.6 ± 1.00 ^c	3.30 ± 0.47 ^b	440 ± 50 ^b	13 ± 0.057 ^{abc}	0.35 ± 0.04 ^c

Means with the same letters are not significant according to Duncan test. Each value means ± standard deviation, ¹ SE is *S. platensis* extract.

The lowest concentration of the extract (0.25%) was the most efficient application at priming or spraying treatment. It significantly ($p \leq 0.05$) increases the shoot and root fresh, dry weight and length compared to the control. The 0.5% SE showed no significant changes in all growth parameters by priming or spraying application comparable with the control. The highest concentration of the extract (1.0%) was acting as a growth inhibitor leading to the decline of the shoot, root length and weight. The inhibition of growth at 1.0% SE by priming treatment was higher than the spraying one (Table 1).

The level of phytohormones was altered by *S. platensis* extract treatment as represented in Table 2. The application of 0.25% SE by priming or foliar spraying has significantly increased the level of auxins, gibberellins and benzyl adenine compared to control. Zeatin level showed no change in treated plants by the spraying with 0.25% SE. Moreover, 0.5% SE was non-significantly increased the levels of gibberellins and zeatin for spraying treatments.

Table 2. Effect of *S. platensis* extract (0, 0.25 and 0.5%), by priming or spraying, on the hormonal levels (auxin, gibberellins, zeatin and benzyl adenine) [mg 100g⁻¹ fresh weight] of *L. luteus* leaves after 35 days of cultivation.

Treatment		Auxin	Gibberellins	Zeatin	Benzyl-Adenine
Priming in ¹ SE (%)	0	41.61 ± 0.8 ^e	119.89 ± 1.70 ^e	1.19 ± 0.11 ^c	1.05 ± 0.10 ^e
	0.25	65.53 ± 0.4 ^b	215.55 ± 1.83 ^b	2.41 ± 0.16 ^a	1.63 ± 0.08 ^c
	0.5	24.52 ± 0.1 ^f	149.45 ± 1.09 ^c	2.16 ± 0.04 ^b	1.44 ± 0.03 ^d
Spraying with ¹ SE (%)	0	48.61 ± 0.9 ^d	164.53 ± 2.02 ^e	1.21 ± 0.02 ^c	1.33 ± 0.04 ^d
	0.25	68.38 ± 2.1 ^a	941.25 ± 2.26 ^a	1.32 ± 0.02 ^c	6.33 ± 0.15 ^a
	0.5	52.26 ± 2.8 ^c	213.55 ± 2.02 ^e	1.30 ± 0.07 ^c	2.19 ± 0.10 ^b

Means with the same letters are not significant according to Duncan test. Each value means ± standard deviation, ¹ SE is *S. platensis* extract.

3.2. Yield Parameters

The results in Figure 1 revealed the impact of *S. platensis* extract on the yield parameters. The number of pods per plant, seeds per pod and weight of 100 seeds were significantly increased by priming or spraying applications at 0.25% SE, while these parameters were decreased at 1.0% SE compared to the control.

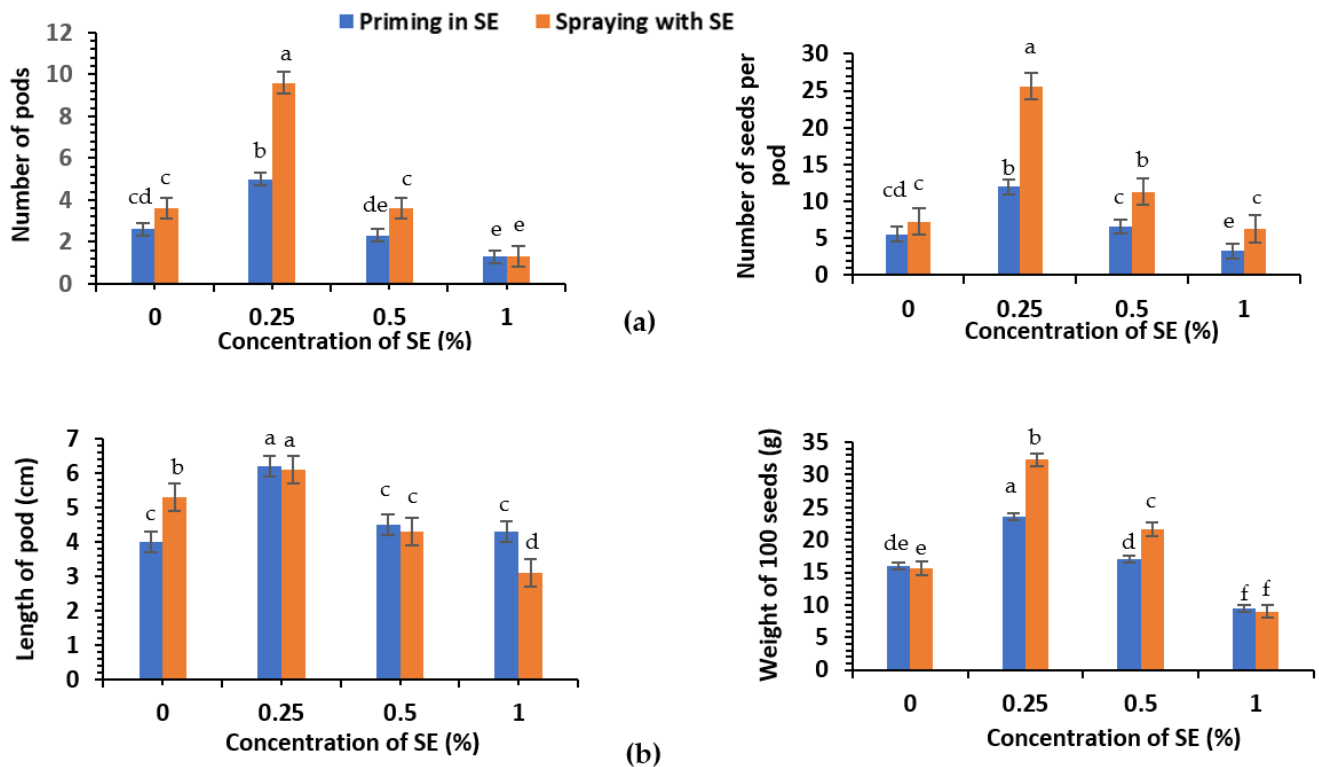


Figure 1. Effect of *S. platensis* extract (0, 0.25, 0.5, 1.0%), by priming or spraying, on yield parameters of *L. luteus* after 60 days of cultivation: (a) no of pods per plant, no of seeds per pod; and (b) length of pod (cm) and weight of 100 seeds (g) [Standard deviation was represented for each column]. Means with the same letters are not significant according to Duncan test.

The foliar spraying treatment was the most efficient to increase the number of seeds per pod and the weight of 100 seeds at 0.5% SE. However, all yield parameters were negatively affected by 1.0% SE applied to the plants by both priming or spraying.

3.3. Photosynthetic Pigments and Activity

For the priming or spraying treatment, the *S. platensis* extract of 0.25% significantly ($p \leq 0.05$) increased the Chl a, Chl b and carotenoids content compared to control (Table 3). However, 1.0% SE had no effect on the pigments content of treated plants. The total chlorophyll pigments (Chl a + Chl b) content was only enhanced by the lower concentration (0.25%) of the extract compared with the control. The chlorophyll content index (CCI) value was significantly increased at 0.25% SE and decreased at 1.0% SE, respectively, by priming or spraying.

Table 3. Effect of *S. platensis* extract (0, 0.25, 0.5, 1.0%), by priming or spraying, on the photosynthetic pigments content (Chl a, Chl b, carotenoids and total chlorophyll) [mg g^{-1} fresh weight] and chlorophyll content index (CCI) of *L. luteus* after 35 days of cultivation.

Treatment		Chl a	Chl b	Carotenoids	Total Chl	Chl a/Chl b	CCI
Priming in ¹ SE (%)	0	0.682 ± 0.001 ^{cd}	0.339 ± 0.002 ^e	0.066 ± 0.001 ^c	1.022 ± 0.003 ^c	2.0 ± 0.01 ^b	19.96 ± 2.9 ^c
	0.25	0.720 ± 0.014 ^b	0.443 ± 0.024 ^b	0.092 ± 0.005 ^b	1.163 ± 0.030 ^b	1.6 ± 0.08 ^d	42.06 ± 4.9 ^a
	0.5	0.620 ± 0.004 ^e	0.406 ± 0.010 ^c	0.072 ± 0.002 ^{bc}	1.027 ± 0.013 ^c	1.4 ± 0.04 ^{de}	32.12 ± 0.49 ^b
	1.0	0.540 ± 0.008 ^f	0.371 ± 0.021 ^d	0.070 ± 0.003 ^{bc}	0.911 ± 0.025 ^d	1.4 ± 0.08 ^e	13.65 ± 1.70 ^d
Spraying with ¹ SE (%)	0	0.684 ± 0.001 ^{cd}	0.329 ± 0.010 ^e	0.079 ± 0.003 ^{bc}	1.014 ± 0.011 ^c	2.0 ± 0.08 ^b	22.18 ± 2.2 ^c
	0.25	0.867 ± 0.009 ^a	0.469 ± 0.017 ^a	0.137 ± 0.028 ^a	1.334 ± 0.021 ^a	1.8 ± 0.07 ^c	45.30 ± 2.2 ^a
	0.5	0.695 ± 0.001 ^c	0.324 ± 0.004 ^e	0.078 ± 0.023 ^b	1.019 ± 0.005 ^c	2.1 ± 0.03 ^a	33.02 ± 1.3 ^b
	1.0	0.679 ± 0.001 ^d	0.325 ± 0.003 ^e	0.059 ± 0.004 ^c	1.005 ± 0.004 ^c	2.0 ± 0.02 ^{ab}	20.70 ± 2.2 ^c

Means with the same letters are not significant according to Duncan test. Each value means ± standard deviation, ¹ SE is *S. platensis* extract.

The ratio between Chl a and b decreased by the application of 0.25% concentration of the extract by priming or spraying. The chlorophyll a/b ratio tend to decrease due to greater increase in Chl b compared to Chl a. Chl b is produced from Chl a via Chl b oxygenase.

The photosynthetic rate (A), stomatal conductance (Gs), evaporation rate (E) and internal carbon dioxide (Ci) measurements under control and application of *S. platensis* extract are represented in Table 4. Priming as well spraying application of 0.25% SE maximized the photosynthetic capacity (A), stomatal conductance (Gs) and evaporation rate (E) of fully expanded leaves to exceed the value of the control plants.

Table 4. Effect of *S. platensis* extract (0, 0.25, 0.5, 1.0%), by priming or spraying, on the photosynthetic rate [A] ($\mu\text{mol m}^{-2} \text{s}^{-1}$), stomatal conductance [Gs] ($\mu\text{mol m}^{-2} \text{s}^{-1}$), transpiration rate [E] ($\text{mol m}^{-2} \text{s}^{-1}$), internal CO₂ [Ci] ($\mu\text{mol mol}^{-1}$) of *L. luteus* after 35 days of cultivation.

Treatment		Photosynthetic Rate (A)	Stomatal Conductance (Gs)	Transpiration Rate (E)	Internal CO ₂ (Ci)
Priming in ¹ SE (%)	0	2.29 ± 0.730 ^d	0.020 ± 0.008 ^d	0.532 ± 0.01 ^{cd}	243.0 ± 71.8 ^b
	0.25	3.39 ± 0.250 ^b	0.040 ± 0.009 ^{bc}	0.990 ± 0.01 ^b	268.2 ± 35.9 ^{ab}
	0.5	2.79 ± 0.150 ^c	0.027 ± 0.009 ^{cd}	0.820 ± 0.03 ^{bc}	240.2 ± 16.3 ^b
	1.0	1.07 ± 0.380 ^e	0.020 ± 0.011 ^d	0.480 ± 0.01 ^d	312.5 ± 59.0 ^a
Spraying with ¹ SE (%)	0	2.19 ± 0.090 ^d	0.030 ± 0.008 ^{cd}	0.960 ± 0.02 ^b	293.2 ± 23.4 ^{ab}
	0.25	4.52 ± 0.104 ^a	0.050 ± 0.001 ^a	1.420 ± 0.03 ^a	242.2 ± 4.1 ^b
	0.5	3.52 ± 0.137 ^b	0.047 ± 0.005 ^{ab}	1.480 ± 0.01 ^a	280.2 ± 9.7 ^{ab}
	1.0	1.28 ± 0.061 ^e	0.020 ± 0.001 ^d	0.710 ± 0.08 ^{bcd}	295.5 ± 3.6 ^{ab}

Means with the same letters are not significant according to Duncan test. Each value means ± standard deviation, ¹ SE is *S. platensis* extract.

Spraying treatment was more effective than priming one to reinforce the photosynthetic capacity. The concentration 1.0% SE showed an inhibitory and stimulatory effects on the photosynthetic rate (A) and internal CO₂ (Ci), respectively (Table 4).

3.4. Total Carbohydrate and Nutrient Content

Leaves of the treated plants with 0.25% SE by priming or spraying showed a higher content of insoluble and total carbohydrate content, while these leaves showed lower content of soluble sugar compared to control (Table 5).

Table 5. Effect of *S. platensis* extract (0, 0.25, 0.5 and 1.0%), by priming or spraying, on the level of the soluble and insoluble carbohydrate and total carbohydrates in leaves of *L. luteus* (mg g^{−1} dry weight) after 35 days of cultivation.

Treatment		Soluble Sugar	Insoluble Carbohydrates	Total Carbohydrates
Priming in ¹ SE (%)	0	18.74 ± 0.09 ^c	34.66 ± 0.53 ^c	53.41 ± 0.44 ^c
	0.25	16.37 ± 0.02 ^f	40.78 ± 1.91 ^b	57.15 ± 1.89 ^b
	0.5	19.39 ± 0.25 ^b	25.70 ± 1.86 ^{de}	45.09 ± 2.05 ^{de}
	1.0	19.95 ± 0.30 ^a	23.07 ± 0.67 ^e	43.03 ± 0.36 ^e
Spraying with ¹ SE (%)	0	19.90 ± 0.33 ^a	33.32 ± 0.13 ^c	53.22 ± 0.43 ^c
	0.25	17.38 ± 0.16 ^e	46.51 ± 2.14 ^a	63.89 ± 2.17 ^a
	0.5	17.77 ± 0.18 ^d	36.42 ± 2.88 ^c	54.20 ± 3.00 ^{bc}
	1.0	18.64 ± 0.08 ^c	28.69 ± 3.11 ^d	47.33 ± 3.03 ^d

Means with the same letters are not significant according to Duncan test. Each value means ± standard deviation, ¹ SE is *S. platensis* extract.

However, insoluble and total carbohydrate were negatively affected by 1.0% SE applying to the plants by spraying or soaking (Table 5). Spraying treatment was more effective than priming, to increase the total carbohydrate and insoluble sugar content in treated leaves compared to the control.

By priming and spraying at 0.25% SE, potassium (K⁺), magnesium (Mg⁺²) and potassium/ sodium ratio (K⁺/Na⁺ ratio) were significantly ($p \leq 0.05$) increased, but sodium (Na⁺) was decreased. The concentration of 1% SE decreased K⁺, Mg⁺² and K⁺/Na⁺ ratio, while Na⁺ did not affect the priming application (Figure 2).

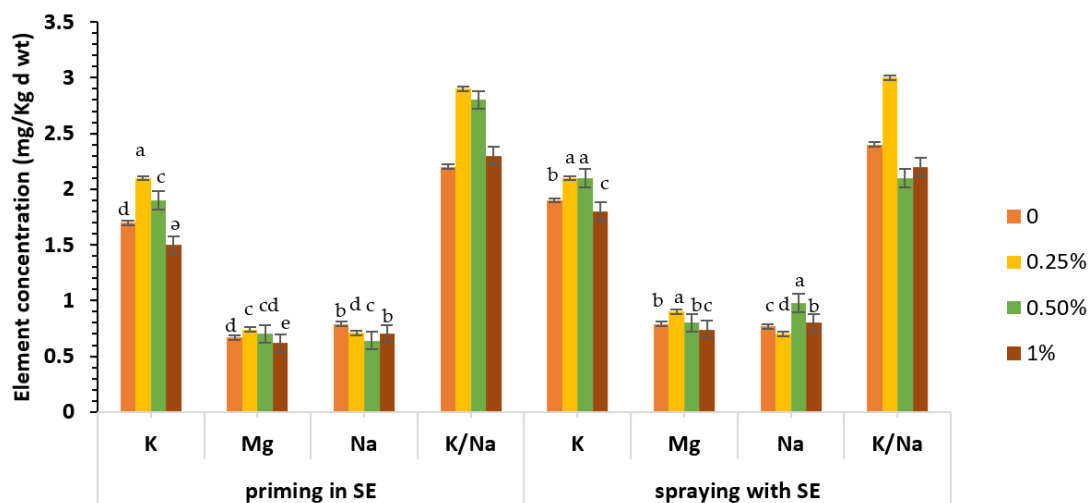


Figure 2. Effect *S. platensis* extract (0, 0.25, 0.5 and 1.0%), by priming or spraying on the content of potassium (K), sodium (Na), K/Na ratio and magnesium (Mg) (mg Kg^{−1} dry weight) of *L. luteus* leaves after 35 days of cultivation [Standard deviation was represented for each column]. Means with the same letters are not significant according to Duncan test.

3.5. The Correlation between the Yield Parameters and Photosynthetic Measurements

A highly significant ($p \leq 0.01$) positive correlation was observed between the yield parameters such as number of pods, number of seeds and weight of 100 seeds, CCI, photosynthetic pigments (total chlorophyll content) and rate (A) of *L. luteus* plants treated by SE (Figure 3). The length of the pod was negatively correlated with total chlorophyll content, CCI and photosynthetic rate. In addition, all yield parameters (except length of pod) were positively and negatively correlated with total carbohydrate and soluble sugar content, respectively.

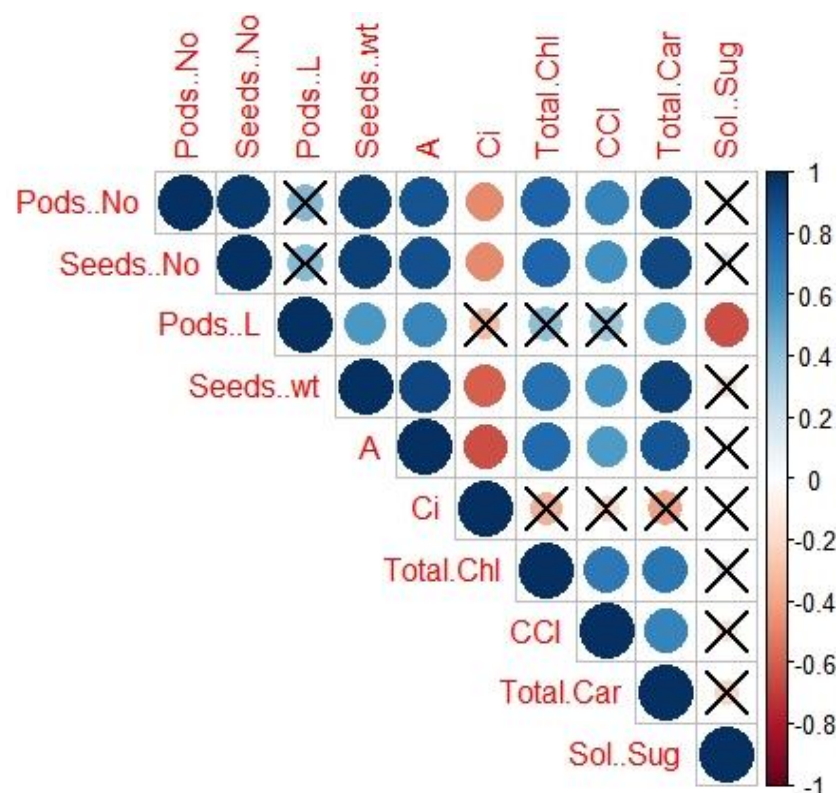


Figure 3. The correlation between yield parameters (number of pods plant⁻¹, number of seeds pod⁻¹, length of pod and weight of 100 seeds), photosynthetic pigments (total chlorophyll and chlorophyll content index [CCI]) and activity (photosynthetic rate [A], internal CO₂[Ci]) and Total carbohydrate.

3.6. Protein Profile

The protein profiling of *L. luteus* seeds of control showed 14 bands ranges from 28 to 142.95 and 158.40 KDa at spraying and priming treatments, respectively. The maximum number of bands was 15 at spraying treatment with lower concentration 0.25% SE. No obvious changes in the banding pattern were detected, except for the appearance of a new band by the spraying treatment at 0.25 and 0.5% of molecular weight of 30.56 KDa.

4. Discussion

Spirulina is the base of many microalgal products, as it is the most spread algae worldwide. The enhancement of plant growth by *S. platensis* and many other microalgae was previously studied [11,20,31,32]. Promotion of growth (shoot, root and leaves length, fresh and dry weight) and yield (pods and seeds number and weight of 100 seeds) of *L. luteus* by *S. platensis* extract may be mainly attributed to the phytohormonal and micro-macro nutrients enrichment of *S. platensis* extract. In agreement with the data in Table 1, Godlewska [15] confirmed that the microalga *S. platensis* increased the length of radish plants compared to the control. The longest aerial parts of radish were in the group treated with 15% of spray, soaked for 6 h in 15% of filtrate and coated with 300 µL of homogenate per 1.5 g of seeds.

There was an enrichment of all phytohormonal levels in the *L. luteus* leaves by the application of *S. platensis* extract. This enhancement of phytohormonal levels was greatly correlated with the growth and the yield parameters improvement. Recent research suggests that cyanobacteria's potential to enhance plant development is connected not only to their hormonal content, but also to their ability to induce endogenous hormone production in treated plants [33]. This agrees with Haroun and Hussein [34] study on *Lupinus termis*. The *Cylindrospermum muscicola* filtrate enhanced the auxin, gibberellins and cytokinins content of the *L. termis* shoots. Inoculation of rice plant with different cyanobacterial strains

such as *Anabaena oryzae*, *Anabaena doliolum*, *Phormidium fragile* and *Oscillatoria acuta* accumulated phenolic acids, flavonoids and phytohormones such as indole acetic acid (IAA) and indole butyric acid (IBA) in leaves [35]. The main pathway for auxin biosynthesis in cyanobacteria is the indole dependent pathway, and the main enzyme (indole-3-pyruvate decarboxylase) of this pathway is coded by *ipdC* gene [36]. Such a gene was detected in lots of unicellular and filamentous cyanobacteria [37]. The most common hypothesis for the effects of cyanobacterial extracts as bio-stimulator or bio-fertilizers is attributed to the direct or indirect effect of phytohormones on plants. Cyanobacterial extracts may contain phytohormones or other substances which trigger signaling paths [38]. Cyanobacterial *Nostoc muscorum* extracts increase the seedlings' growth parameters of presoaked seeds of cotton, wheat, sorghum, maize and lentil probably by the presence of auxin and other active substances in the extract [39]. The stimulation impact of cyanobacteria on plant growth may be linked to high amounts of Gibberellic acid (GA), which inhibit chlorophyllase activity [40].

In addition, cyanobacterial extracts have demonstrated to trigger biochemical processes that led to increase plant productivity and yield. In a field experiment, *Anabaena* and *Calothrix* sp. were assessed for their role in improving the nutritional quality of wheat grains in terms of protein content and essential micronutrients (Fe, Cu, Zn and Mn) [41]. It was postulated that cyanobacteria growth regulators have a function in nitrogen feeding and the expression level of zinc (Zn) and iron (Fe) transporter proteins in root cells. Osman et al. [42] have reported an improvement values 6%, 11%, 6% and 7% for the number of pods/plants, number of seeds/pods, number of seeds/plant and the weight of 100 seeds (g), respectively, for broad bean seeds primed in *Nostoc muscorum*. Salvi et al. [43] reported that *Arthrospira platensis* may enhance the biosynthesis of the compounds that increase the weight of treated berries. *Spirulina* and *Oscillatoria* extracts increased the number of pods/plants, reaching 21.7 pods in comparison to 14.3 in the control [44]. Agwa et al. [45] concluded that *Chlorella vulgaris* is an effective and non-expensive bio-fertilizer to improve soil nutrients and increase the yield of *Hibiscus esculentus*.

An increment of the chlorophyll a, b and carotenoids content by cyanobacterial extracts was reported. Data in Table 3 were in harmony with Puglisi et al.'s [46] study, which found that *Scenedesmus quadricauda* methanolic extract increased the chlorophyll a, b and carotenoids level in lettuce seedlings. Furthermore, microalgal extract treatment for spinach and strawberry increases chlorophyll levels [47,48]. Microalgae contain a wide diversity of beneficial compounds such as vitamins, amino acids and exopolysaccharides [49], and such compounds enhance the internal biosynthesis of chlorophyll and consequently improve plant development. Chlorophyll synthesis depends on the availability of macro-nutrients as nitrogen (N), phosphorous (P) in soil [50]. So, the increasing of the concentration of these nutrients through the application of cyanobacterial extract may be one of the stimulatory factors for endogenous chlorophyll biosynthesis. Some studies have reported increased chlorophyll content in plants treated with cyanobacterial extracts; this effect can be attributed to improved nutrient use by plants or to the extract protective effect on chlorophyll, which reduces chlorophyll degradation and delays plant senescence [20,32]. Elements availability for plants were higher for spraying treatment rather soil application especially potassium (K^+), iron (Fe^{+2}), magnesium (Mg^{+2}) and calcium (Ca^{+2}) [51]. The nutritional elements of the cyanobacterial foliar spray would be absorbed by the treated plant leaves through stomata. Therefore, the spraying is more effective in the morning when the leaves are fully expanded [11]. Elevation of carotenoids content by *S. platensis* extract indicating their vital role for plants with various functions in photosynthesis machine such as photoprotection, phytohormone synthesis and signaling [52].

The primary impact of the cyanobacterial extract on the chlorophyll, followed by the secondary effect on the photosynthetic rate, improving the growth parameters (as shoot and root weight and length), biomass and yield measurements. Chl a is the major photosynthetic pigment; thus, its increment is consequently enhancing photosynthetic potential by maximizing the light capture [53]. The enhancement of photosynthetic capacity

by algal extract application was also reported by Mikiciuk and Dobromilska [54] in tomato and Xu and Leskovar [55] in spinach. It has been clear that the diffusion of gas via stomatal conductance G_s is a basic factor for the rate of CO_2 assimilation (A) [56]. Moreover, the high-water use efficiency (WUE) needs a rapid stomatal response [57].

Microalgal potassium is a key element in the explanation of how algal extracts improves photosynthesis by regulating the water level in plants promoting the photosynthesis and the growth of meristems [58]. The enhancement of the stomatal conductance by *S. platensis* may increase the photosynthetic rate (A) and mass production of *L. luteus* plants (Tables 3 and 4). High G_s leads to a greater transpiration rate and lower water use efficiency (WUE), which can affect the value of enriching photosynthetic capacity and biomass production [59]. In accordance with our results, Haroun and Hussein [34] found that the stomatal conductance of the treated *Lupinus* leaves with *Cylindrospermum* sp. filtrate was higher than the control. The increment of photosynthetic rate was followed by an increment of building compounds such as carbohydrates. Inversely, the soluble sugar decreased which was correlated with the improvement of the photosynthetic process. Thus, there is a positive correlation between the carbohydrate content, yield components and photosynthetic rate (Figure 3). Pego et al.'s [60] studies proved the inhibition of the expression of the photosynthetic genes when soluble sugars accumulated and consequently the photosynthetic process stopped. Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) was also repressed by the high sugar content in leaves, where its content was reduced due to *de novo* synthesis repression of its protein in sugar-treated leaves. Moreover, the decline of the carbon fixation by RuBP may be related to the suppression of the photosynthetic process at the increase of soluble sugar content [61].

A new band of 30.56 KDa was appeared by gel electrophoresis of sprayed leaves of *L. luteus* by *S. platensis* compared to control (Figure 4). This data was in harmony with Haroun and Hussein [34] for *L. termis* plants results who stated that two new bands appeared in seed gel protein profile, one of 129 KDa appeared upon the treatment with algal filtrate of *Cylindrospermum muscicola* and the other one (18 KDa) appeared with algal filtrate of *Anabana Oryzae*. Additionally, Osman et al. [62] reported that the protein profile of the pea seeds showed the appearance of new protein bands in response to the bio-fertilization by *Nostoc entophytum* and *Oscillatoria angustissima* compared to the control. The appearance of such protein bands may be specific for this treatment [61], due to the gene expression alteration [63]. According to Duranti et al. [64] on *Lupinus albus*, the protein bands of molecular weight from 25 to 46 KDa representing α -conglutin in seeds [65]. Seed vigor and viability is correlated to the physiological role of these proteins (α -conglutin) such as their role in the scavenging of free radicals [66].

Although the cyanobacteria such as *S. platensis* has a stimulative effect on the yield of *L. luteus* and other plants, the cost of the *S. platensis* extract production on small or large scale will be the determinant factor for the future use of cyanobacterial in agriculture [67]. The cost of production will be based on the culture technique used (natural or artificial light, photobioreactors, or open ponds). The cost of production in commercial closed reactors averages 50 € kg^{-1} , with the productivity and requirements of the species cultivated [68]. However, production costs on a large scale in closed systems under natural light and suitable climatic conditions might range from 3.2 to 12.4 € kg^{-1} [68].

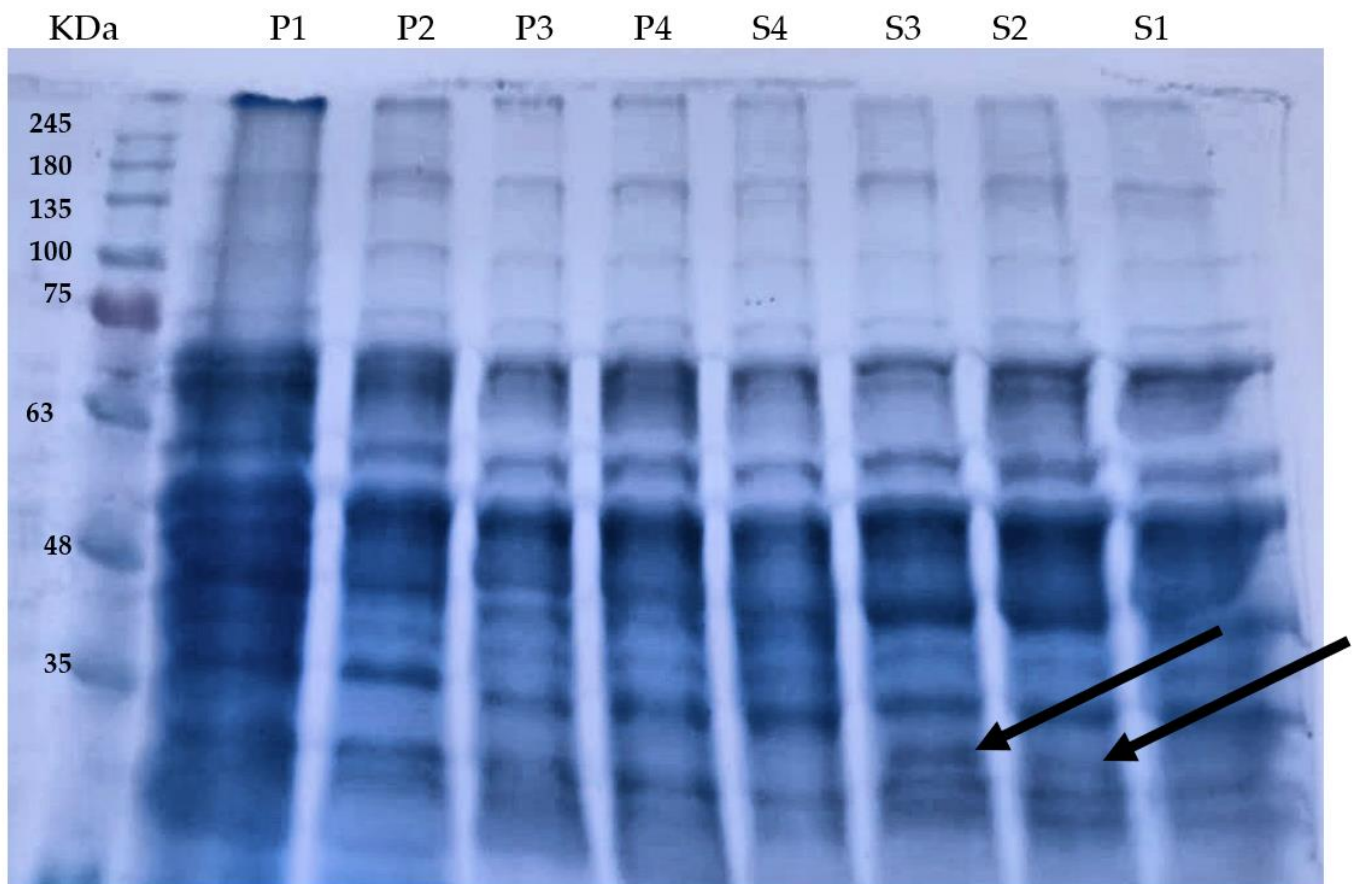


Figure 4. Effect of *S. platensis* extract (0, 0.25, 0.5 and 1.0%) on the protein banding analysis of *L. leuteus* seeds after 60 days of cultivation. P1 represents the control of priming treatment, P2, P3 and P4 are the treatments of priming at 0.25, 0.5 and 1% SE, respectively. S1 is the control of the spraying treatment, S2, S3 and S4 are the treatments of spraying by 0.25, 0.5 and 1% SE, respectively. The black arrows pointed at a new band.

5. Conclusions

This study proved that the priming or spraying of *L. leuteus* with *S. platensis* extract increased growth indices, yield measurements, photosynthetic pigment content and capacity. This improvement was linked to the biologically active compounds generated by *S. platensis* such as phytohormones (elicitor molecules). Phytohormones participate in signaling pathways that promote plant development and productivity. Furthermore, the elevation of macronutrients such as K^+ and Mg^{+2} in *L. leuteus* leaves may have a role in increasing the photosynthetic rate and hence growth and yield. The spraying procedure had superior effect than the priming treatment on all assessed criteria. In contrast, a greater concentration of *S. platensis* had a suppressive effect on growth, yield and photosynthetic characteristics. The number of pods, seeds and weight of 100 seeds were all positively correlated to photosynthetic chlorophyll content and rate. The use of *S. platensis* on plants offers several agricultural and environmental advantages, but the cost of cyanobacterial extract production is still a question that needs an answer.

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