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Efficiency of spraying with *spirulina* and variety on the growth of bean plant under drip irrigation system

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ABSTRACT: The utilization of bio-stimulants in agricultural settings has emerged as a potentially fruitful technique for enhancing the development and production of crops. Microalgae extracts are a form of bio-stimulant that has recently garnered much attention. These extracts are well-known for their high metabolic activity, bioactive chemicals, and capacity to boost plant growth and development. The experiment aimed to determine the efficiency of *Spirulina platensis* extracts by foliar spraying on bean plants. The experiments were carried out in the autumn of 2023 in Diyala Governorate to study the efficiency of spraying with *spirulina extract* and its physiological effect on bean plant growth. The experiment was carried out in RCBD design with three replications, using four concentrations of *spirulina* (0, 0.25, 0.50, and 1.0) % and two varieties of bean plants (Barcelona and luz de otono). The results showed a superiority concentration of 1.0% in all studied traits. Which record in 73.28 cm,51,43 SPAD,1.41,42.03 g/ plant,3.90%,0.156%, and 1.41% for the high plant, chlorophyll index, leaf area index, dry matter, N%, P%, and K% respectively, Barcelona variety superiority in all characteristics except leaf area index (LAI). The results also showed that the binary interaction between spray and variety significantly affected the studied factors.

Keywords: Spraying, Bean plant, *spirulina*, chlorophyll

1. INTRODUCTION

The common bean, belonging to the *Fabaceae family*, is a herbaceous vegetable crop that is widely consumed across the globe. It is considered one of the most significant leguminous crops regarding human nutrition and has a higher commercial value than other types of beans. It is the second most important provider of proteins in the human diet and the third most significant source of calories (1). The bean plant (*Vicia faba L.*) is one of the most important vegetable leguminous plants with a high protein percentage. It is considered the meat of the poor in third world countries, where malnutrition with protein energy is regarded as a major nutritional problem. It is an important part of human food, especially those with low income. It is also important in improving soil fertility through the biological fixation of nitrogen in the soil through The way of coexistence with Rhizobium bacteria (2). It is a cost-effective protein source in comparison to animal-derived protein. His plants serve as green manure for soils, enhancing the production of many field crops by atmospheric nitrogen fixation (3). It is widely recognized that the build-up of sodium ions in leaf tissues leads to a decline in enzyme activity and protein synthesis, which is closely associated with elevated levels of salinity (4). Applying plant

growth regulators, osmoprotectants, and fertilizers externally can help mitigate the negative effects of salt stress (5). Algae has been effectively employed as a biofertilizer in soil restoration. *It* is a kind of photosynthetic cyanobacteria. Today, it is manufactured on a large scale to be used as a food source, animal feed, and bio-fertilizer due to its significant nutritional content. It has been utilized as a biofertilizer for various crops through diverse application methods, on its own, or in conjunction with other organic fertilizers. The biomass of is composed of around 62% amino acids and also contains a complete range of mixed natural carotene and xanthophyll phytopigments. These phytopigments are a valuable natural source of vitamin B-12 and antioxidants. *It*a type of cyanobacterium, is highly regarded as an optimal nutritional supplement and an effective remedy for addressing malnutrition issues in underdeveloped nations.

The foliar application is one of the methods used to compensate for the plant spray on vegetative parts and penetration into the leaves and then move on to parts of the plant's deep, depending on species, fertilizer form, concentration, frequency of application, and the stage of plant growth. It's also the most efficient way to supply plant nutrients in the case of shortages, which cannot compensate for roots, as in the case of obstacles to uptake, such as drought or a case of worms. The foliar spraying of nutrients is more effective as compared to soil applied. Therefore, using cyanobacteria species as biofertilizers instead of expensive chemical alternatives is advisable. This is because chemical fertilizers contribute to the contamination of soil and water. Recent research has demonstrated that bio-stimulants derived from microalgae are a potential, ecologically friendly, and sustainable agricultural approach for enhancing crop output and sustainability(6, 7). Khalil and Hamza (2012) found in a field experiment that when spraying the extract of the seaweed known as Marvel at a concentration of 1 ml/liter on green bean plants, there was a significant increase in plant height, chlorophyll, and dry color, as the averages reached 42.53 cm, SPAD 36.41 and 42.35 g, respectively, compared to the treatment. The comparison that gave the lowest averages was 38.52 cm each, SPAD 33.62 and 38.21 g, respectively. Al-Khafaji and Al-Jubouri (2023) indicated in a field experiment that when spraying spirulina on lettuce plants at a concentration of 5 g/l, there was a significant increase in plant height, dry weight, and nitrogen concentration, and the averages reached 40.89 cm, 31.22 g, and 1.657 mg/g, respectively, compared to the comparison treatment, which gave less. Averages reached 37.78 cm 24.38 g and 1.264 mg/g, respectively(8)

Aziz (2016) observed, in a field experiment, when spraying Jamex seaweed extract on two varieties of beans, Aquadulce (A1) and A2 (Primato), at a concentration of 3 ml/liter, that there was a significant increase in plant height for the A1 variety, with an average of 101.66 cm, while the A2 variety gave An average of 93.66 cm, and the leaf area of the A1 variety exceeded an average of 135.28 cm^2, while the A2 variety gave an average of 133.66 cm^2. Chlorophyll also outperformed the A1 variety with an average SPAD of 35.36, while the A2 variety gave an average of 51.86 SPAD. As for the dry matter, the A2 variety outperformed with an average of 19.34%, while the A1 variety gave an average of 17.06% (9). Based on the above information, the aim of this study was to Efficiency of spraying with *spirulina* extract on the growth of bean plant.

2. MATERIALS AND METHODS

2.1 Study area

A field experiment was conducted in the autumn of 2023 in the fields of a farmer in Diyala Governorate in clay soil to study the efficiency of spraying with spirulina extract, Spirulina was obtained from the Malaysian company DXN, and its physiological effect on the growth of bean plants. A random compound sample from different regions of the field was used before planting to determine the physical and chemical properties of the soil, as shown in Table (1), in the

laboratories of the College of Agriculture / University of Diyala. The field was fertilized with phosphorus (20% P_2O_5) at 120 kg/ha, nitrogen with (urea 46% N) at 44 kg/ha, and potassium (Potassium sulfate 41.5% K) with 75 kg/ha. The experiment was designed according to randomized complete block design (RCBD) with three replicates, and using two factors, spraying four concentrations of ***spirulina*** (0,0.25,0.5,1) % and two varieties of bean Turkish cultivar (luz de otono) and Spanish cultivar (Barcelona).

Table 1. Some chemical and physical properties of the study soil before planting.

pH	Ec Dsm. m^{-1}	Available N $mg.kg^{-1}$	Available P $mg.kg^{-1}$	Available K $mg.kg^{-1}$	Available Zn $mg.kg^{-1}$	Sand g. kg^{-1}	Silt g. kg^{-1}	Clay g. kg^{-1}	Texture
7.02	3.69	41.0	22.3	172.3	0.21	189	310	501	clay

Spraying after 20 days from planting on the vegetation parts in the early morning during the elongation using a washing solution to break the surface tension of water, and two sprays after 14 days from the first spray. The distance between the lines was 50 cm, and the variable distances between the plants were 20 cm, with a plant density of 100000 plants. ha-1, The number of experimental units at 8 units and the area unit (1.5m * 2 m). Seeds of the bean plant were obtained from the Diyala Agriculture Department / Agricultural Production Department and planted on 25 October 2023. The field was controlled and fertilized symmetrically, and thinning, grafting, and crop service were carried out whenever needed. The drip irrigation system was installed by extending an amin tube with a diameter of 5 cm connected to the pump on the water. Small branch tubes were installed from it so that they were equal to the number of lines present in the experimental unit, and then drippers were placed to charge 2,0 L/ hour at each plant. The physical and chemical characteristics of the water are analyzed in Table (2).

Table 2. Some characters of the water

pH	Ec Dsm. m^{-1}	Available Ca m.mole. $^{-1}$	Available HCO3 m.mole. $^{-1}$
7.1	1.39	3.11	2.70

Calculated according to methods mentioned in (10)

Five plants were randomly selected for all experimental units for the measurement of growth parameters after one week of flowering , Plant height (cm) measured by means of a measuring tape inserted from the surface of the soil up to the node (11). Chlorophyll intex (SPAD) measuring chlorophyll in leaves using the manual SPAD-502 meter in the field directly (Felix et al., 2000) .Leaf area index (LAI) measured by LAI = Leaf area (LA)/ Ground area (GA)(12) .Shoot Dry matter. The material was dried in paper bags at about 70 ◦C in a force-draft oven to a constant weight(13). Concentration of NPK% in leaves according to (14).

Statistical Analysis

The statistical analysis was done using the Statistical Analysis System- SAS program, and the significant differences between the averages were compared with the least significant difference test LSD (15).

3. RESULTS AND DISCUSSION

3.1 Plant height (cm)

Table (3) shows that the height of the plant increased significantly with the increase in the foliar application of *spirulina*; the concentration of 1.0% gave the highest plant height, 73.28cm higher than the control which gave lowest average 48.08cm, due to the *spirulina* consists of about 62% amino acids, auxin hormone(IAA), carotene, antioxidants, nitrogen and xanthophyl phytopigments are considered as a rich natural source of vitamin B-12 and which encouraged increased vegetative growth in plant including the plant height(16).

The variety Barcelona was also significantly superior, with the highest average in this trait 36.05 cm compared to luzde otono variety, which gave 58.10 cm. The reason is that the plant height can be influenced by genotype (17).

The interaction between spraying and varieties were significant differences in this trait; the treatment of the concentration of 1.0% with the Barcelona variety had the highest height of 77.26 cm compared to the lowest treatment of the concentration of 0% with the luz de otono variety, which was 46.83 cm.

Table 3: Efficiency of spraying with *spirulina* and variety of bean and interaction between them in Plant height (cm)**.**

Spray / **Variety**	**Concentration *of spirulina*%**				**Mean**
	0	**0.25**	**0.5**	**1.0**	
luzde otono	46.83	49.33	62.56	69.30	58.10
Barcelona	53.70	57.23	68.40	77.26	63.05
Mean	48.08	55.46	65.48	73.28	
LSD	S		V	V*S	
0.05	0.289		0.205	0.410	

The results of the current study are consistent with the study, which indicated the role of foliar application of *C. vulgaris* extract *i*n enhancing plant height, both in the vegetative and fruiting stages of *P. vulgar compared* to the control (18). This can be attributed to the effect of protein, carbohydrates, fats, and plant hormones in *C. vulgaris* extract on Growth, development, cell division, and plant elongation. Our results are similar to those obtained by. *platensis* foliar spraying of the red beat (6), and, lettuce (19).

3.2 **Chlorophyll content**

able (4) shows that each of the concentrations of spraying *spirulina*, variety, and the interaction between them have a significant effect in increasing chlorophyll in the bean plant leaves. The percentage increase when spraying *spirulina* at 15.74, 38.59, and 67.91 % for concentrations of 0.25, 0.50, and 1.0 % respectively. The concentration of 1.0% was superior highest mean in chlorophyll index at 51.43 (SPADunite) compared with the control treatment, which gave the lowest mean at 30.63 (SPADunit) due to the role of *spirulina* containing amino acids, selenium, and vitamins (e.g., A, B, and E) that helps absorption of iron and this is reflected on synthesis of(3).

Table (4) also showed Barcelona a significant difference in the Uzde Otono variety in this trait, giving an average of 41.31 and 38.66 SPAD units for the two varieties, respectively. Perhaps this is due to its genetic nature. The interaction S1.0 * V 2 had a significant effect on the chlorophyll index, leading to a higher mean of 53.53 (SPADunite) compared with the control treatment, which had the lowest mean of 29.16 (SPADunit).

Table 4: Efficiency of spraying with spirulina and variety of bean and interaction between them in chlorophyll (SPAD).

Spray / Variety	Concentration of spriulina%				Mean
	0	0.25	0.5	1.0	
luzde otono	29.16	32.10	41.53	49.33	38.66
Barcelona	34.63	36.26	43.36	53.53	41.31
Me	30.63	35.45	42.45	51.43	
LSD	S		V	V*S	
0.05	0.548		0.387	0.775	

Several studies have documented increased chlorophyll levels in plants treated with cyanobacterial extracts. This rise can be ascribed to the enhanced utilization of nutrients by plants or the protective effect of the extract on chlorophyll (4). This protective effect lowers chlorophyll's breakdown and slows the aging process in plants (20, 21). The treated plant leaves would absorb the nutritious contents of the cyanobacterial foliar spray through the stomata.

On the other hand, the application of algal extract enhanced chlorophyll levels in tuberose plants. The present findings align with the results of (22). found that applying algal extract on sugar beet's surface resulted in significantly higher levels of photosynthetic pigments and vegetative growth indices (23). found that the application of algal extract at a rate of 1.5 ml/l enhanced the leaf content of chlorophyll in freesia plants(24).

3.3 Leaf area index (LAD)

The leaf area index quantifies the amount of leaf surface area about the area of land it covers, indicating the level of photosynthetic capacity (25). Table (5) shows that both the spray concentrations of *spirulina and* the variety and the interaction between them significantly increase the bean plant's leaf area index. The mean increase when sprayed with *spirulina* was 1.163, 1.276, and 1.413 for concentrations of 0.25, 0.50, and 1.0%, respectively. The 1.0% concentration exceeded the highest average in leaf area at 1.413 compared to the control treatment, which gave the lowest average at 0.986. The application of *spirulina* extract through foliar spray resulted in an increase in the micronutrient content (specifically Fe, Zn, Mn, and Cu in parts per million) of plant leaves. Additionally, the extract contains cytokines that stimulate physiological activity and enhance chlorophyll production in leaves, thereby promoting photosynthesis and synthesizing materials that contribute to positive growth characteristics, such as leaf area index(26).

The results shown in Table 5 indicate the luzde otono variety had the highest significant increase (1.265) in leaf area compared to the Barcelona variety (1.155). The reason for this is the increase in the height of the same plant in the Barcelona variety had affected reducing the leaf area index. In contrast, the opposite happened in the Luzde otono variety, with the opportunity to increase the leaf area (Table 3). This is called “plant architecture “The interaction S1.0 * V 1 had a significant effect on the leaf area index, leading to a higher mean of 1.456 compared with the control treatment, which had the lowest mean of 1.086.

Table 5: Efficiency of spraying with *spirulina* and variety of bean and interaction between them in concentration of leaf area index (LAI).

Spray / Variety	Concentration *of spirulina*%				Mean
	0	**0.25**	**0.5**	**1.0**	
luzde otono	1.086	1.203	1.313	1.456	1.265
Barcelona	1.203	1.123	1.240	1.370	1.155
Mean	0.986	1.163	1.276	1.413	
LSD	S		V	V*S	
0.05	0.008		0.006	0.012	

3.4 Dry matter of shoot(g/plant)

In the present study, the dry matter increased significantly with the increase in foliar application of *spirulina* as shown in Table 6, the concentration 1.0% gave the highest average 42.03 g/ plant compared to the control treatment that gave the lowest average 25.75 g/ plant , Perhaps the reason is the increase in plant height, chlorophyll and leaf area index (Tables 3, 4, and 5) which reflected to increase dry matter, So the fact that *spirulina* activated the phosphate transfer enzyme, which was important in the formation of ATP by activating the uptake of phosphorus, to accelerate the transfer and filling of sieve tubes with photosynthesis products such as carbohydrates and proteins, which increased the thickness of the height ,diameter and leaves that reflected to dry matter (26) Shown in Table 6 indicate that the Barcelona variety had the highest significant increase 36.69 g/ plant compared to the luzde otono variety 30.09 g/ plant, The reason for this is the increase in the height plant, chlorophyll and leaf area index(table 3,4,5) in the same of treatments had effected dry matter.

The interaction $S_{1.0} * V_{2}$ was significant effect in dry matter has led to get a higher mean at 44.56 g/ plant compared with control treatment which was the lowest mean at 22.63 g/ plant.

Table 6: Efficiency of spraying with *spirulina* and variety of bean and interaction between them in concentration of dry matter of shoot (g/ plant).

Spray / Variety	Concentration *of spirulina*%				Mean
	0	**0.25**	**0.5**	**1.0**	
luzde otono	22.63	28.86	31.56	39.50	30.09
Barcelona	26.63	32.76	40.20	44.56	36.60
Mean	25.75	29.70	35.88	42.03	
LSD	S		V	V*S	
0.05	0.449		0.317	0.635	

3.5 Concentration of N% in leaves

The results in (Table 7) showed significant differences for foliar spraying of *spirulina* and variety and interaction between them in the characteristic of nitrogen percentage. The spraying of *sprulina*gave the highest average at a concentration of 1.0%, reached 3.90%, followed by a concentration of 0.59 with an average of 3,25%, while the control treatment gave the lowest average of 1.89%, and the reason may be that *spirulina contains* nitrogen element and worked

to increase the efficiency of the plant in absorption of the nitrogen element which is essential in photosynthesis, formation of proteins, nucleic acids, and the activation of enzymes (16).

The Barcelona variety was also significantly superior, with the highest average in this trait, 3.90 %, compared to the luzde otono variety, which gave 2.64%. The reason is that the plant can be influenced by genotype (17).

The interaction between spraying and varieties caused significant differences in this trait. The Barcelona variety's treatment of 1.0% concentration had the highest height of 4.44% compared to the Luz de Otono variety's lowest concentration of 0%, which was 1.88%.

Table 7: Efficiency of spraying with *spirulina* and variety of bean and interaction between them in concentration of N% in leaves.

Spray / **Variety**	**Concentration *of spirulina*%**				**Mean**
	0	**0.25**	**0.5**	**1.0**	
luzde otono	1.88	1.91	3.22	3.36	2.64
Barcelona	2.10	3.16	3.28	4.44	3.19
Mean	1.89	2.63	3.25	3.90	
LSD	S		V	V*S	
0.05	0.005		0.003	0.007	

3.6 Concentration of P% in leaves

The results of the statistical analysis in Table (8) showed that there were significant differences between the averages of the phosphorus percentage in the leaves when spraying *spirulina*. The 1.0% gave a significant superiority at the mean of 0.156 % compared to the control, which gave the lowest average of 0.108%, and this is due to the role of *spirulina* in raising the efficiency of the plant to absorb phosphorous because it contains phosphorus in body and this reflected to activates the processes of carbohydrate representation, respiration, energy formation, cell division, transport and the formation of cell membranes and nucleic acids(27).

The Barcelona variety was also significantly superior, with the highest average in this trait, 0.138 %, compared to the luzde otono variety, which gave 0.126%. The reason is that the plant can be influenced by genotype (17).

The interaction between spraying and varieties caused significant differences in this trait. The treatment of the concentration of 1.0 % with the Barcelona variety had the highest height of 0.162% compared to the lowest treatment of the concentration of 0% with the Luz de Otono variety, which was 0.101%.

Table 8: Efficiency of spraying with spirulina and variety of bean and intraction between them in concentration of P % in leaves.

Spray / **Variety**	**Concentration *of spirulina*%**				**Mean**
	0	**0.25**	**0.5**	**1.0**	
luzde otono	0.101	0.116	0.133	0.151	0.126
Barcelona	0.122	0.131	0.143	0.162	0.138
Mean	0.108	0.126	0.138	0.156	
LSD	S		V	V*S	
0.05	0.0003		0.0002	0.0004	

3.7 Concentration of K% in leaves

It was noted in Table (9) that the potassium concentration has increased significantly with increasing concentrations of *spirulina* spray. It gave a concentration of 1.0%, with the highest mean of 1.41 %, while the control treatment showed the lowest mean of 0.20 %; the reason for the increasing percentage of potassium in the leaves due to the role of foliar spray in the direct processing of *spirulina solution* contained potassium when sprayed, which increased in the leaves and then moved to other parts of the plant (16) .

It was also noted from the table (9) that there were significant differences between bareclona variety and uzde otono in this characteristic. The bareclona gave the highest average of 0.74 % , while the luzde otono gave an average of 0.69 % , the reason is the genotype of each (17).

The interaction between spraying and variety in S1.0* V2 significantly impacted the percentage of potassium obtained, which was the highest mean of 1.43 % compared to the treatment of the control, which was the lowest mean of 0.18 %, which did not differ significantly from S 0.50* V2 and S1.0* V1.

Table 9: Efficiency of spraying with *spirulina* and variety of bean and interaction between them in concentration of K% in leaves.

Spray / **Variety**	**Concentration *of spirulina*%**				**Mean**
	0	**0.25**	**0.5**	**1.0**	
luzde otono	0.18	0.21	0.97	1.38	0.69
Barcelona	0.24	0.28	1.03	1.43	0.74
Mean	0.20	0.26	1.00	1.41	
LSD	S		V	V*S	
0.05	0.333		0.035	0.471	

4. CONCLUSION

The study concludes that spraying *spirulina* is superior in all parameters, except leaf area index, where the Barcelona variety is higher in all other attributes. The interaction between spray and variety significantly influenced all phenotypic measures compared to the control treatment.

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