



## Plant Production Science

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# EFFECT OF MINERAL NITROGEN LEVELS AND FOLIAR SPRAY WITH (*Spirulina platensis*) EXTRACT ON GROWTH, YIELD AND QUALITY OF LETTUCE PLANTS

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**ABSTRACT:** This study was conducted during the two successive winter seasons of 2022/2023 and 2023/2024 to evaluate the effect of three mineral nitrogen levels (30, 60, and 90 kg N/feddan) and foliar application of *Spirulina platensis* (SP) at rates of 0, 1, 2, and 4 ml/l on the growth, yield, chemical composition, and nitrate accumulation of lettuce (*Lactuca sativa* L., cv. Dark Green). The experiment was set up in a two-factorial design: Factor A involved nitrogen levels as main plot, while Factor B included (SP) as foliar spray as sub plots. Results showed that applying 90 kg N/feddan or spraying spirulina at 4 ml/l individually led to the highest values for most tested traits, including plant height, number of leaves, leaf area, head diameter, fresh weight, dry matter percentage, pigment content, total yield, vitamin C, and total sugars% in both seasons. However, 90 kg N/feddan also caused the highest nitrate accumulation and lowest nitrogen use efficiency, conversely spirulina at 4 ml/l reduced nitrate levels and enhanced nitrogen use efficiency. Certainly, the combination of 90 kg N/feddan with 4 ml/l spirulina showed a clear superiority in most of studied traits while significantly reducing nitrate content and improving nitrogen use efficiency compared to 90 kg N/feddan alone. Interestingly, the treatment of 60 kg N/feddan with 4ml/l (SP) showed no significant differences with 90 kg N/feddan without (SP) in most important traits such as (total yield, ... etc.) in both seasons. This suggests the potential to reduce mineral nitrogen fertilizer by 30 kg N/feddan through partial substitution with (SP), contributing to lower environmental pollution, improving nitrogen efficiency and reducing nitrate accumulation in lettuce leaves. The dual N/Spirulina combination is promising for enhanced lettuce production economy.

**Key words:** Lettuce, nitrogen rates, *Spirulina Platensis* (SP) and sandy soil.

## INTRODUCTION

In Egypt, Lettuce (*Lactuca sativa* L.) is one of the most important vegetable crops for both formers and consumers. For farmers, it is considered a fast cash crop in the winter season. For consumers, lettuce is eaten fresh and salad, where it is rich in vitamins and minerals. Most vegetables growers often over fertilize their crops by mineral fertilizers due to the high relatively return of extra yields (Rubatzky and Tamaguchi, 1997, Stewart *et al.*, 2005;

Schenk, 2006). Vegetable crops require nitrogen in large amounts because it's essential for plant productivity (Marschner, 1995). The extra availability of nitrogen which is not tailored to the plant requirement may reduce product quality through nitrate accumulation in the edible parts (Parente *et al.*, 2006). And may causing environmental pollution due to the excessive accumulation and leaching of harmful elements to the ground water (Reinink, 1991; Premuzic *et al.*, 2004; Ju *et al.*, 2007; Prasad and Chetty, 2008). The nitrogen fertilization

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process in lettuce fields must take into accounts not only farming economics but also the consumer preference and human health as well as environmental issues (Schenk, 2006).

Currently, there is an increased advantage of using natural products such as algae in agriculture (Ramya, *et al.*, 2010; Calvo *et al.*, 2014; Dwivedi *et al.*, 2014; Narasimha Rao and Reshmi Chatterjee, 2014; Du Jardin, 2015 and Michalak *et al.*, 2016).

Positive influence of bio-stimulant treatment on yield parameters was observed. The overall increase in pigments content of leaves after bio-stimulants application agrees well with better total and commercial yields of treated vegetable cultivars in comparison with their controls. In addition, bio-stimulants improved antioxidant activity, vitamin C and higher pigment levels in leaves of vegetables compared to non-treated plants. Thus, the application of bio-stimulants is considered as a good production strategy for obtaining high yields of nutritionally valuable vegetables with lower impact on the environment Sazetak, (2011). Bio-stimulants able to promote vegetative growth mineral nutrients uptake and improve the productivity of many plants Dwivedi *et al.*, 2014 reported that seaweed extracts not only increase the vegetative growth of the plant but also triggers the early flowering, fruiting in crops and ultimately on seed yields. The main objective of these study was to increase the yield and quality of onion through investigating the effect of different potassium fertilization rates and foliar application of bio stimulants (algae extract) as well as their interaction.

Algae extracts (*Spirulina platensis*) (SP) are an excellent source of chemical fertilizers (NPK) and plant growth promoters such as auxins, cytokinins, gibberellins, amino acids, macronutrients, microelements, and organic matter that improve plant productivity Al-Najjar (2022). Using (SP) as a foliar organic spray is a part of the solution to decrease nitrate accumulation, produced by mineral fertilizers in vegetables, hence improving the quality of salad vegetables and improving human health (Saleh *et al.*, 2010; Shams, 2012; Zaki *et al.*, 2012 and Saleh *et al.*, 2013).

Biofertilizers are organic products containing live or latent cells of different types of microorganisms (bacterial, fungal or algal). The main advantages of biofertilizers are cheap nutrient sources; suppliers of microelements; suppliers of organic matter; counteracting the negative effects of chemical fertilizers; secretion of growth hormones; no adverse effects to ecosystem and longer shelf-life Pallabi and Debiprasad (2014). *Spirulina platensis* (SP) is a rich source of mineral nutrient, which hence stimulates root establishment, root elongation and promotes vegetative growth of plants Shalaby and El Ramady (2014). Foliar application of algae extract induces many positive effects, as it improved crop yield and quality, increased nutrient uptake, resistance to frost and stress conditions especially those grown under semi-arid and desert conditions Manrich *et al.*, (2014).

(SP) is a blue-green microalgae, including high-antioxidant components, organic and amino acids, high-quality proteins, Fe and Ca, unsaturated fatty acids, many types of vitamins (including A, B2, B6, B8, E, and K) chlorophyll, carotenes, phycocyanin and plant hormones (Gyenis *et al.*, 2005 and Spolaore *et al.*, 2006). (SP) contains 18 of the 20 known amino acids, all essential minerals, and trace elements, as well as enzymes (Fox, 1993).

The application of (SP) can reduce chemical fertilizers' use and positively affect their growth and physiology (Shaheen *et al.*, 2013). Antimicrobial action of (SP) against different bacteria has been demonstrated due to large levels of acrylic acid and organic acids such as propionic, benzoic, and mandelic (Balloni *et al.*, 1980). Phycocyanin is the major pigment in (SP) that promotes anthocyanin production in secondary metabolites and inhibits oxidative damage in plant DNA Bhat and Madyastha 2001 Phycocyanin is a powerful antioxidant that is scavenging free radical.

The main objective of this study was to increase the yield and quality of lettuce through investigating the effect of different nitrogen fertilization rates and foliar application of bio stimulants (SP extract) as well as their interaction.

## MATERIALS AND METHODS

This experiment was carried out during the two consecutive winter seasons of 2022/2023 and 2023/2024 on lettuce plants (*Lactuca sativa*, L.) cv. Dark green planted in a privet farm, located at Bilbies district, Sharkia Governorate, Egypt to evaluate the effect of mineral nitrogen rates and foliar spray with *Spirulina Platensis* (SP) in different concentrations on the growth, chemical composition and yield of lettuce plants under the conditions of sandy soil using drip irrigation system.

The experiment consisted of (12) treatments which were the combination of three mineral nitrogen rates (30,60 and 90Kg N/ Feddan) and four concentrations foliar spray with (SP), 0ml/l (control), 1 ml/l, 2ml/l and 4 ml/l. These treatments were randomly arranged in a split plot design system with three replicates; mineral nitrogen rates were randomly arranged in the main plots while (SP) doses were randomly distributed in the sub plots of this experiment.

Ammonium sulphate (AS) (20.5%N) was used as a source of mineral nitrogen and was added as a soil application according to treatments distribution in three doses started after 15 days intervals from transplanting. On the other hand, (SP) concentrations were added as a foliar application to the plants three times after 15, 30 and 45 days from transplanting. Transplants of lettuce cv. Dark green were transplanted on the first week of December in both seasons of the study. The area of the experimental unit was (12.6) m<sup>2</sup>. It contained three lines, each of 6 m length and 0.7 m width. Lettuce transplants were planted at 25 cm a part on both sides of the drippers. The other normal agricultural treatments for growing lettuce plants were practiced as commonly.

### Preparation of Algae Extract

The used algae in the present study *Spirulina platensis* is photosynthetic and multicellular blue-green microalgae that grow in a wide range fresh, marine and brackish water (Marrezet *et al.*, 2014). The fresh material was chopped into small pieces, with an approximate weight of one kilogram. The sample was first blended and then ground using a mortar and pestle. The resulting

mixture was filtered through a double layer of muslin cloth to eliminate any solid residues. The resulting filtrate was labeled as 100% SWC and from these different concentrations (1ml/l, 2ml/l, and 4ml/l) were prepared by adding distilled water. As the liquid fertilizer contained organic matter, it was refrigerated between 0 - 4°C until use. **Pise and Sabal (2010)**. The cyanobacterium *Spirulina platensis* was obtained from the Algae Biotechnology Unit of The National Research Center, Giza, Egypt (www.nrc.sci.eg), grown in the Zarrouk medium (**Zarrouk 1966**).

### Data Recorded

#### Plant growth measurements

At 75 days from transplanting a random sample of (five) plants were taken from each experimental unit for measuring plant height, number of leaves per plant, and leaf area /plant(cm<sup>2</sup>) was calculated according to the formula of **Koller (1972)**.

#### Leaf chlorophyll content

Chlorophyll a, b, and carotenoids (mg/g FW) were measured in disc samples from the fourth upper leaf taken from five plants at harvesting time in every plot by using the technique outlined by **Saric et al. (1976)**.

#### Yield, head diameter and weight

At harvest time (100 days after transplanting) the fresh heads of lettuce plants in each experimental plot were harvested then weighed to determine head diameter (cm), head fresh weight (gm), total yield (ton/Feddan) and nitrogen use efficiency (kg yield / kg N) according to **Clark (1982)**.

#### N, P and K %

Nitrogen, Phosphorus and Potassium in leaves were determined at (75) days from transplanting in the dry matter of lettuce plant leaves according to the methods as described by to both methods described by **AOAC (1995)**.

#### nitrate content

nitrate content in lettuce leaves was determined at 75 days from transplanting by using Brucine methods as described by **Cheng and Tsang (1998)**.

**Table 1. Physical and chemical properties of experimental soil (average of the two seasons).**

Physical analysis				Soil texture							
Clay (%)	Silt (%)	Sand (%)		sandy							
8.88	15.23	75.89									
Chemical analysis											
pH	EC dSm-1	Organic matter (%)	CaCO <sub>3</sub> (%)	Soluble cations (meq./ L)				Soluble anions (meq. /L)			
				Ca <sup>++</sup>	Mg <sup>++</sup>	Na <sup>+</sup>	K <sup>+</sup>	CO <sub>3</sub> <sup>-</sup>	HCO <sub>3</sub> <sup>-</sup>	Cl <sup>-</sup>	SO <sub>4</sub> <sup>-</sup>
8.03	2.56	0.52	0.54	11.13	8.79	2.11	2.9	0.00	10.78	2.89	11.26
Available nutrients (mg kg-1soil)											
N	P	K	Fe	Zn	Cu	Mn					
45.62	8.25	150.35	1.87	0.75	0.68	0.55					

**Dry matter (%)**

Known weight of fresh leaves (100 gm) was taken and dried at 105°C to determine dry matter percentage.

**Total sugar**

were determined in the dried samples of shoots and grains of all treatments photometrically according to **Bernfeld (1955)** and **Miller (1959)** methods with some modifications.

**Statistical Analysis**

All the data obtained were statistically analysis using Statistic 9 program and means separation were done by least significant value (L.S.D.) at 0.05 level of probability according to **Snedecor and Cochran (1980)**.

**RESULTS****Vegetative Growth Characters**

Data in **Table 2** is clear that the tested nitrogen levels significantly affected plant height, number of leaves /plants, and leaf area/plant in both tested seasons. As such, the highest fertilization rate (90 Kg N/feddan) recorded the highest plant height (33.07 and 31.47 cm); number of leaves /plant (36.05 and 35.27) and leaf area / plant (7838.8 and 7488.9cm<sup>2</sup>) in the first and second season respectively. compared to (30 Kg N/feddan)

which recorded the lowest values of the previous tested in both tested seasons.

Also, the same table clear that the tested spraying (SP) levels significantly affected the lettuce plants height, number of leaves and leaf area/ plant in both tested seasons. The highest level of spraying treatment (4ml/l) recorded the highest plant height (30.98 and 29.96 cm), (leaves number / plant (34.40 and 34.06), and leaf area/plant (6886.9 and 6609.1 cm<sup>2</sup>) as compared to the control (spraying with distilled water only) which recorded the lowest values of the previous tested treats in both tested seasons.

In addition, the interaction between the two tested factors affected significantly all the tested treats, as such, the highest level of N fertilization combined with the highest concentration of (SP) ( 4 ml/l), recorded the highest plant height (33.55 and 31.99 cm), leaves number/ plant (38.41 and 36.04) and leaf area / plant (8152.9 and 8103.6 cm<sup>2</sup>) for both seasons respectively. Without significant differences with those treated with (90 Kg N/feddan + spraying with (SP) at concentration of (2 ml/l.). In the second rank came those treated with (90 Kg N/feddan + spraying with (SP) at concentration of 1ml/l.). While the lowest values of all tested treats were recorded by (30 Kg N/feddan + spraying (SP) at 0ml/l (with tap water)).

**Table 2: Effect of nitrogen fertilization rates, foliar spraying with *Spirulina platensis* extract (SP), and the interaction between them on the plant growth characters of lettuce during the 2022/2023 and 2023/2024 seasons.**

Properties	Plant height (cm)		No. of leaves/plant		Leaf area/plant (cm <sup>2</sup> )		
	2023/2024 season	2024/2025 season	2023/2024 season	2024/2025 season	2023/2024 season	2024/2025 season	
<b>Effect of nitrogen rates</b>							
<b>30 N (Kg/ fed)</b>	26.69 c	27.14 c	29.96 c	29.80 c	4587.4 c	4778.3 c	
<b>60 N (Kg/ fed)</b>	31.12 b	29.76 b	32.22 b	33.60 b	6291.6 b	5902.3 b	
<b>90 N (Kg/ fed)</b>	33.07 a	31.47 a	36.05 a	35.27 a	7838.8 a	7488.9 a	
<b>Effect of foliar spray with <i>Spirulina platensis</i> (SP)</b>							
<b>0 ml/l</b>	29.57 d	28.74d	31.25 d	32.12 d	5598.1 d	5394.7 d	
<b>1 ml/l</b>	30.21 c	29.35c	31.69 c	32.41 c	6022.1 c	5883.2 c	
<b>2 ml/l</b>	30.41 b	29.76b	33.64 b	32.96 b	6450.1 b	6339.1 b	
<b>4 ml/l</b>	30.98 a	29.96a	34.40 a	34.06 a	6886.9 a	6609.1 a	
<b>Effect of interaction</b>							
<b>nitrogen rates</b>	<b>foliar spray (SP)</b>						
	<b>0 ml/l</b>	25.93 g	29.66 e	28.79 g	28.86 g	4143.1 i	4320.8 i
<b>30 N (Kg/ fed)</b>	<b>1 ml/l</b>	26.81 f	26.76 h	28.95 g	29.02 g	4289.4 h	4665.5 h
	<b>2 ml/l</b>	26.95 f	26.87 h	30.61 f	29.61 f	4582.7 g	4960.0 g
	<b>4 ml/l</b>	27.08 f	27.31 g	31.48 e	31.70 e	5334.2 f	5167.0 f
<b>60 N (Kg/ fed)</b>	<b>0 ml/l</b>	30.29 e	27.61 g	31.64 e	32.86 d	5406.4 f	5237.7 f
	<b>1 ml/l</b>	30.87 d	28.97 f	31.74 e	33.52 c	5911.8 e	5790.7 e
	<b>2 ml/l</b>	31.01 d	30.12 d	32.21 d	33.56 c	6675.0 d	6024.2 d
	<b>4 ml/l</b>	32.30 c	30.29 cd	33.30 c	34.45 b	7173.5 c	6556.7 c
<b>90 N (Kg/ fed)</b>	<b>0 ml/l</b>	32.49 c	30.48 c	33.31 c	34.63 b	7244.7 c	6625.7 c
	<b>1 ml/l</b>	32.96 b	31.53 b	34.38 b	34.67 b	7865.1 b	7193.4 b
	<b>2 ml/l</b>	33.27 ab	31.86 a	38.12 a	35.72 a	8092.6 a	8033.0 a
	<b>4 ml/l</b>	33.55 a	31.99 a	38.41 a	36.04 a	8152.9 a	8103.6 a

Data in **Table 3** reveal that the tested nitrogen levels significantly affected the lettuce plants head diameter (23.61 and 23.91 cm), head fresh weight (449.54 and 451.80g) and dry matter % (5.93 and 5.92%) in the first and second seasons respectively. While nitrogen fertilization at 30 Kg N/feddan recorded the lowest values. Whereas nitrogen fertilization at 60 Kg N/feddan recorded intermediate values.

As for the effect of (SP) foliar spray the data also indicated that the spraying at 4ml/l recorded the highest head diameter (19.99 and 20.95 cm), head fresh weight (424.35 and 414.65 g) and Dry matter % (5.41 and 5.40%) in the first and second seasons respectively. Whereas spraying treatment at a concentration of 2ml/l came in the second rank. While the control (0 ml/l) recorded the lowest values.

Regarding the interaction between the two tested factors, it was found that the highest N fertilization rate at 90Kg N + highest rate of foliar (SP) (4ml/l) recorded the highest head diameter, head fresh weight and dry matter % of the lettuce plants. In this respect, N

fertilization rate at 90Kg N + foliar spray (SP) at 1 or 2 ml/l came in the second rank without significant differences between them. The lowest values were recorded by 30 Kg N + foliar spray (SP) at 0 ml/l (control). The other tested treatments came in-between.

**Table 3: Effect of nitrogen fertilization rates, foliar spraying with *Spirulina platensis* extract (SP), and the interaction between them on the head traits of the lettuce plant during the 2022/2023 and 2023/2024 seasons.**

Properties	Head diameter (cm)		Head fresh weight (g)		Dry matter (%)		
	2022/2023 season	2023/2024 season	2022/2023 season	2023/2024 season	2022/2023 season	2023/2024 season	
<b>Treatments</b>							
<b>Effect of nitrogen rates</b>							
<b>30 N (Kg/ fed)</b>	15.16 c	15.23 c	349.99 c	330.89 c	4.39 c	4.44 c	
<b>60 N (Kg/ fed)</b>	17.93 b	19.61 b	404.01 b	389.03 b	5.19 b	5.15 b	
<b>90 N (Kg/ fed)</b>	23.61 a	23.91 a	449.54 a	451.80 a	5.93 a	5.92 a	
<b>Effect of foliar spray with <i>Spirulina platensis</i> (SP)</b>							
<b>0 ml/l</b>	17.44 d	18.15 d	377.06 d	372.54 d	4.93 d	4.93 d	
<b>1 ml/l</b>	18.74 c	19.31 c	399.41 c	385.83 c	5.09 c	5.10 c	
<b>2 ml/l</b>	19.42 b	19.93 b	403.90 b	389.27 b	5.24 b	5.25 b	
<b>4 ml/l</b>	19.99 a	20.95 a	424.35 a	414.65 a	5.41 a	5.40 a	
<b>Effect of interaction</b>							
<b>nitrogen rates</b>	<b>foliar spray (SP)</b>						
	<b>0 ml/l</b>	14.27 i	13.71 i	318.19 j	321.23 i	4.27 i	4.28 i
<b>30 N (Kg/ fed)</b>	<b>1 ml/l</b>	15.04 hi	14.99 h	347.30 i	325.49 i	4.32 hi	4.32 i
	<b>2 ml/l</b>	15.46 gh	15.78 g	356.22 h	330.06 h	4.41 hi	4.50 hi
	<b>4 ml/l</b>	15.86 fg	16.45 g	378.25 g	346.80 g	4.55 gh	4.65 gh
	<b>0 ml/l</b>	16.53 f	18.17 f	388.80 f	375.67 f	4.80 g	4.89 fg
<b>60 N (Kg/ fed)</b>	<b>1 ml/l</b>	17.55 e	19.20 e	404.08 e	384.36 e	5.12 f	5.08 ef
	<b>2 ml/l</b>	18.61 d	19.79 e	407.86 e	388.69 e	5.32 ef	5.22 de
	<b>4 ml/l</b>	19.01 d	21.29 d	415.29 d	407.38 d	5.51 de	5.39 cd
<b>90 N (Kg/ fed)</b>	<b>0 ml/l</b>	21.51 c	22.56 c	424.17 c	420.73 c	5.70 cd	5.61 c
	<b>1 ml/l</b>	23.63 b	23.75 b	446.85 b	447.64 b	5.82 bc	5.89 b
	<b>2 ml/l</b>	24.20 b	24.23 b	447.62 b	449.06 b	6.00 ab	6.03 ab
	<b>4 ml/l</b>	25.11 a	25.11 a	479.52 a	489.79 a	6.18 a	6.17 a

Data in Table 4 is clear that the tested nitrogen levels, foliar (SP) level, and their interaction significantly affected leaf pigment contents of the lettuce plants during the two tested seasons. As such, the highest fertilization rate (90 Kg N/feddan) recorded the highest

chlorophyll a (3.73 and 3.59 mg/g FW), chlorophyll b (1.72 and 1.78 mg/g FW), and carotenoids (1.48 and 1.40 mg/g FW) in the first and second season respectively. compared to the lowest nitrogen treatment (30 Kg N/feddan) which recorded the lowest values of the previous

tested treats in both tested seasons. Meanwhile, (60 Kg N/feddan) occupied the second rank.

As for the effect of foliar spray with (SP) the data in **Table 4** also indicated that spraying (SP) at 4ml/l recorded the highest chlorophyll a (3.45 and 3.36 mg/g FW), chlorophyll b (1.64 and 1.63 mg/g FW), and carotenoids (1.33 and 1.28 mg/g FW) in the first and second season respectively, compared to the control (0 ml/l) which recorded the lowest values of the previous tested treats in both seasons. The other tested treatments came in-between.

Regarding the interaction between soil N

fertilization rate and foliar (SP) rates, it was found that the highest N fertilization rate at 90Kg N + highest rate of foliar (SP) (4ml/l) recorded the highest chlorophyll a (3.81 and 3.80 mg/g FW), chlorophyll b (1.77 and 1.85 mg/g FW), and carotenoids (1.53 and 1.52 mg/g FW) in the first and second season respectively. Without significant differences in most cases with those treated by 90 kg N + foliar (SP) at (2ml/l). The lowest values of the previously tested treat in both seasons were recorded by (30 Kg N+ foliar (SP) at (0 ml/l). The other tested treatments recorded intermediate values.

**Table 4: Effect of nitrogen fertilization rates, foliar spraying with *Spirulina platensis* extract (SP), and the interaction between them on the leaf pigments of the lettuce plant during the 2022/2023 and 2023/2024 seasons.**

Properties	Chlorophyll a (mg/g FW)		Chlorophyll b (mg/g FW)		Carotenoides (mg/g FW)		
	2022/2023 season	2023/2024 season	2022/2023 season	2023/2024 season	2022/2023 season	2023/2024 season	
<b>Treatments</b>							
<b>Effect of nitrogen rates</b>							
<b>30 N (Kg/ fed)</b>	2.84 c	2.82 c	1.44 c	1.30 c	1.08 c	1.06 c	
<b>60 N (Kg/ fed)</b>	3.26 b	3.19 b	1.57 b	1.53 b	1.23 b	1.17 b	
<b>90 N (Kg/ fed)</b>	3.73 a	3.59 a	1.72 a	1.78 a	1.48 a	1.40 a	
<b>Effect of foliar spray with <i>Spirulina platensis</i> (SP)</b>							
<b>0 ml/l</b>	3.12 d	3.06 d	1.51 d	1.46 d	1.19 d	1.15 d	
<b>1 ml/l</b>	3.21 c	3.15 c	1.55 c	1.50 c	1.24 c	1.18 c	
<b>2 ml/l</b>	3.32 b	3.25 b	1.60 b	1.57 b	1.29 b	1.24 b	
<b>4 ml/l</b>	3.45 a	3.36 a	1.64 a	1.63 a	1.33 a	1.28 a	
<b>Effect of interaction</b>							
<b>nitrogen rates</b>	<b>foliar spray (SP)</b>						
	<b>0 ml/l</b>	2.71 f	2.67 h	1.37 g	1.21 f	1.05 g	1.04 g
<b>30 N (Kg/ fed)</b>	<b>1 ml/l</b>	2.81 f	2.76 gh	1.42 fg	1.27 ef	1.06 fg	1.05 fg
	<b>2 ml/l</b>	2.83 f	2.90 fg	1.47 ef	1.32 e	1.07 fg	1.07 fg
	<b>4 ml/l</b>	3.02 e	2.95 f	1.48 ef	1.41 d	1.11 f	1.08 fg
<b>60 N (Kg/ fed)</b>	<b>0 ml/l</b>	3.03 e	3.06 ef	1.49 e	1.44 d	1.11 f	1.10 f
	<b>1 ml/l</b>	3.11 e	3.18 de	1.53de	1.51 cd	1.18 e	1.18 e
	<b>2 ml/l</b>	3.38 d	3.19 de	1.57 d	1.54 c	1.27 d	1.19 e
<b>90 N (Kg/ fed)</b>	<b>4 ml/l</b>	3.50 cd	3.32 cd	1.66 c	1.63 b	1.36 c	1.24 d
	<b>0 ml/l</b>	3.64 bc	3.43 c	1.67 c	1.72 b	1.40 c	1.31 c
	<b>1 ml/l</b>	3.71 ab	3.50 c	1.70 bc	1.72 b	1.46 b	1.31 c
<b>2 ml/l</b>	<b>2 ml/l</b>	3.75 ab	3.64 b	1.75 ab	1.84 a	1.51 a	1.46 b
	<b>4 ml/l</b>	3.81 a	3.80 a	1.77 a	1.85 a	1.53 a	1.52 a

Data in **Table 5** clear that the tested nitrogen levels, foliar (SP) level, and their interaction significantly affected total yield (ton/fed.), and nitrogen use efficiency (kg yield / kg N) of the lettuce plants during the two tested seasons. As such, the highest fertilization rate (90 Kg N/feddan) recorded the highest total yield (19.92 and 19.97 ton/fed.) in the first and second seasons, respectively. Whereas the lowest fertilization rate (30 Kg N/feddan) recorded the lowest values. While (60 Kg N/feddan) recorded intermediate values.

As for the effect of foliar spray with (SP) the data in **Table 5** also indicated that spraying (SP) at 4ml/l recorded the highest total yield (18.36

and 18.03 ton/fed.) in the first and second seasons, respectively. While the control (0 ml/l) recorded the lowest values. Moreover, spraying treatment at a concentration of 1 or 2ml/l recorded intermediate values.

Regarding the interaction between the two tested factors, it was found that the highest N fertilization rate at 90 Kg N + highest rate of foliar (SP) (4ml/l) recorded the highest total yield (21.24 and 21.42 ton/fed.) in the first and second seasons, respectively. The lowest total yield (ton/fed.) in both seasons were recorded by (30 Kg N+ foliar (SP) at (0 ml/l). The other tested treatments recorded intermediate values.

**Table 5: Effect of nitrogen fertilization rates, foliar spraying with *Spirulina platensis* extract (SP), and the interaction between them on the total yield and nitrogen use efficiency of the lettuce plant during the 2022/2023 and 2023/2024 seasons.**

Properties	Total yield (ton/fed.)		Nitrogen use efficiency (kg yield / kg N)		
	2022/2023 season	2023/2024 season	2022/2023 season	2023/2024 season	
<b>Effect of nitrogen rates</b>					
<b>30 N (Kg/ fed)</b>	14.36 c	13.66 c	478.63 a	455.19 a	
<b>60 N (Kg/ fed)</b>	17.18 b	16.63 b	286.28 b	276.93 b	
<b>90 N (Kg/ fed)</b>	19.92 a	19.97 a	221.36 c	221.92 c	
<b>Effect of foliar spray with <i>Spirulina platensis</i> (SP)</b>					
<b>0 ml/l</b>	15.98 d	15.71 d	303.80 d	298.41 d	
<b>1 ml/l</b>	16.81 c	16.36 c	321.55 c	309.78 c	
<b>2 ml/l</b>	17.47 b	16.91 b	335.75 b	320.58 b	
<b>4 ml/l</b>	18.36 a	18.03 a	353.94 a	343.29 a	
<b>Effect of interaction</b>					
<b>nitrogen rates</b>	<b>foliar spray (SP)</b>				
<b>30 N (Kg/ fed)</b>	<b>0 ml/l</b>	13.00 j	12.78 h	433.24 d	425.96 c
	<b>1 ml/l</b>	13.98 i	13.22 gh	465.99 c	440.71 c
	<b>2 ml/l</b>	14.75 i	13.72 g	491.66 b	457.44 b
	<b>4 ml/l</b>	15.71 h	14.90 f	523.64 a	496.67 a
<b>60 N (Kg/ fed)</b>	<b>0 ml/l</b>	16.19 gh	15.75 ef	269.76 g	262.53 f
	<b>1 ml/l</b>	16.88 fg	16.22 de	281.30 fg	270.27 ef
	<b>2 ml/l</b>	17.51 ef	16.78 d	291.88 ef	279.71 de
	<b>4 ml/l</b>	18.13 de	17.78 c	302.20 e	295.21 d
<b>90 N (Kg/ fed)</b>	<b>0 ml/l</b>	18.76 cd	18.61 c	208.40 i	206.73 i
	<b>1 ml/l</b>	19.56 bc	19.65 b	217.37 i	218.36 hi
	<b>2 ml/l</b>	20.13 b	20.21 b	223.70 hi	224.60 gh
	<b>4 ml/l</b>	21.24 a	21.42 a	235.97 h	237.99 g

Data in **Table 5** also shows that the tested nitrogen levels, foliar (SP) levels, and their interaction significantly affected nitrogen use efficiency (kg yield / kg N). The highest nitrogen use efficiency (478.63 and 455.19 kg yield / kg N) were recorded by (30 Kg N/feddan) in the first and second seasons, respectively. While the lowest nitrogen use efficiency (221.36 and 221.92 kg yield / kg N) were recorded by (90 Kg N/feddan) in the first and second seasons, respectively. Whereas (60 Kg N/feddan) recorded intermediate nitrogen use efficiency.

As for the effect of (SP) foliar spray the data also indicated that spraying (SP) at 4ml/l recorded the highest nitrogen use efficiency (353.94 and 343.29 kg yield / kg N). While the lowest nitrogen use efficiency (303.80 and 298.41 kg yield / kg N) were recorded by the control SP at (0 ml/l). Moreover, spraying (SP) at a concentration of 1 or 2ml/l recorded intermediate values.

Regarding the interaction between the two factors, it was found that (30 Kg N+ foliar (SP) at (4 ml/l) recorded the highest nitrogen use efficiency (523.64 and 496.67 kg yield / kg N). While the lowest nitrogen use efficiency (208.40 and 206.73 kg yield / kg N) was recorded by (90 kg N / feddan + control SP at 0 ml/l). The other tested combinations induced intermediate nitrogen use efficiency.

Data in **Table 6** shows that the tested nitrogen levels, foliar (SP) levels, and their interaction significantly affected the lettuce leaves content from N%, P% and K% during the two tested seasons. As such, the highest fertilization rate (90 Kg N/feddan) recorded the highest N% (2.55 and 2.46%), P% (0.47 and 0.49%) and K% (2.34 and 2.22%) in the first and second seasons, respectively, compared to the lowest nitrogen treatment (30 Kg N/feddan) which recorded the lowest values.

As for the effect of foliar spray with (SP) the data in **Table 6** also indicated that the highest level at 4ml/l recorded the highest N% (2.19 and 2.17%), P% (0.42 and 0.44%) and K% (2.10 and 2.06%) in the first and second seasons, respectively, compared to the lowest nitrogen treatment (30 Kg N/feddan) which recorded the lowest values. In this respect, spraying (SP) at a

concentration of 1 or 2ml/l recorded intermediate values.

Regarding the interaction between the two factors, it was found that highest N fertilization rate at 90 Kg N + highest rate of foliar (SP) (4ml/l) recorded the highest leaves N% (2.75 and 2.63 %), P% (0.52 and 0.52%) and K% (2.48 and 2.46%) in the first and second seasons, respectively, in the second rank came those treated by (90 Kg N/feddan +SP at 2ml/l without significant difference between them in most cases. Meanwhile, 30 Kg N+ foliar (SP) at (0 ml/l) recorded the lowest values. The other tested combinations induced intermediate values.

Data in **Table 7** shows that the tested nitrogen levels, foliar (SP) levels, and their interaction significantly affected the lettuce leaves content from nitrate (ppm), vitamin C (mg/100g F.W.) and Total sugar (%) during the two tested seasons. As such, the highest fertilization rate (90 Kg N/feddan) recorded the highest nitrate content (237.66 and 238.35 ppm), vitamin C content (4.05 and 3.91 mg/100g F.W.) and Total sugar content (6.45 and 6.51%) in the first and second seasons, respectively, compared to the lowest nitrogen treatment (30 Kg N/feddan) which recorded the lowest values. While (60 Kg N/feddan) recorded intermediate values.

As for the effect of foliar spray with (SP) the data in **Table 7** indicated that the highest level at 4ml/l recorded the lowest nitrate content (204.71 and 202.99 ppm) in the first and second seasons, respectively. While the control (0 ml/l) recorded the highest values. Whereas the second and third treatments (1 and 2 ml/l) recorded intermediate values.

Regarding the interaction between the two factors, it was found that the highest N fertilization rate at 90 Kg N + the control of foliar (SP) (0ml/l) recorded the highest leaves of nitrate content (247.16 and 248.40 ppm) without significant differences with those treated by highest N fertilization rate at 90 Kg N + foliar (SP) (1 ml/l) in the first and second seasons, respectively. Whereas the lowest values were recorded by the lowest N fertilization rate at 30Kg N + highest level of foliar (SP) (4ml/l). The other tested treatments recorded intermediate values.

**Table 6: Effect of nitrogen fertilization rates, foliar spraying with *Spirulina platensis* extract (SP), and the interaction between them on nitrogen (N), phosphorus (P) and potassium (K) Percentage of the lettuce plant during the 2022/2023 and 2023/2024 seasons.**

Properties	N%		P%		K%		
	2022/2023 season	2023/2024 season	2022/2023 season	2023/2024 season	2022/2023 season	2023/2024 season	
<b>Effect of nitrogen rates</b>							
<b>30 N (Kg/ fed)</b>	1.42 c	1.49 c	0.28 c	0.29 c	1.66 c	1.65 c	
<b>60 N (Kg/ fed)</b>	2.00 b	1.99 b	0.38 b	0.40 b	1.97 b	1.86 b	
<b>90 N (Kg/ fed)</b>	2.55 a	2.46 a	0.47 a	0.49 a	2.34 a	2.22 a	
<b>Effect of foliar spray with <i>Spirulina platensis</i> (SP)</b>							
<b>0 ml/l</b>	1.79 d	1.78 d	0.34 d	0.36 d	1.86 d	1.78 d	
<b>1 ml/l</b>	1.91 c	1.91 c	0.36 c	0.37 c	1.96 c	1.87 c	
<b>2 ml/l</b>	2.07 b	2.06 b	0.38 b	0.41 b	2.04 b	1.93 b	
<b>4 ml/l</b>	2.19 a	2.17 a	0.42 a	0.44 a	2.10 a	2.06 a	
<b>Effect of interaction</b>							
<b>nitrogen rates</b>	<b>foliar spray (SP)</b>						
	<b>0 ml/l</b>	1.25 i	1.22 i	0.25 h	0.25 i	1.52 g	1.56 h
<b>30 N (Kg/ fed)</b>	<b>1 ml/l</b>	1.36 hi	1.35 i	0.26 gh	0.26 i	1.64 f	1.64 gh
	<b>2 ml/l</b>	1.50 gh	1.62 h	0.29 g	0.31 h	1.69 ef	1.66 g
	<b>4 ml/l</b>	1.58 fg	1.74 gh	0.32 f	0.34 g	1.77 e	1.71 g
<b>60 N (Kg/ fed)</b>	<b>0 ml/l</b>	1.75 ef	1.82fg	0.36 e	0.36 fg	1.88 d	1.72 fg
	<b>1 ml/l</b>	1.94 de	1.97 ef	0.37 e	0.38 f	1.97 cd	1.81 f
	<b>2 ml/l</b>	2.07 cd	2.04 e	0.39 e	0.41 e	1.99 c	1.91 e
<b>90 N (Kg/ fed)</b>	<b>4 ml/l</b>	2.25 bc	2.13 de	0.41 d	0.45 d	2.04 c	2.01 d
	<b>0 ml/l</b>	2.38 b	2.29 cd	0.43 cd	0.47 cd	2.19 b	2.06 cd
	<b>1 ml/l</b>	2.42 b	2.41 bc	0.45 c	0.48 bc	2.27 b	2.15 bc
<b>(Kg/ fed)</b>	<b>2 ml/l</b>	2.63 a	2.51 ab	0.48 b	0.50 ab	2.42 a	2.22 b
	<b>4 ml/l</b>	2.75 a	2.63 a	0.52 a	0.52 a	2.48 a	2.46 a

As for the effect of foliar spray with (SP) the data in **Table 7** also clear that the highest level at 4ml/l recorded the highest vitamin C content (3.66 and 3.41 mg/100g F.W.) and Total sugar content (5.85 and 6.00 %) in the first and second seasons, respectively. While the control (0 ml/l) recorded the lowest values. Whereas the second and third treatments (1 and 2 ml/l) recorded intermediate values.

Regarding the interaction between the two factors, it was found that the highest N fertilization rate at 90 Kg N + highest level of

foliar (SP) (4ml/l) recorded the highest vitamin C content (4.38 and 4.40 mg/100g F.W.) and total sugar content (6.77 and 6.92 %) without significant differences with those treated by highest N fertilization rate at 90 Kg N + foliar (SP) (2 ml/l) in most cases, in the first and second seasons, respectively. Whereas the lowest values were recorded by the lowest N fertilization rate at 30Kg N + the control of foliar (SP) (0ml/l). The other tested treatments recorded intermediate values.

**Table 7: Effect of nitrogen fertilization rates, foliar spraying with *Spirulina platensis* extract (SP), and the interaction between them on the head quality of the lettuce plant during the 2022/2023 and 2023/2024 seasons.**

Properties		nitrate (ppm)		vitamin C (mg/100g F.W)		Total sugar (%)	
Treatments		2022/2023 season	2023/2024 season	2022/2023 season	2023/2024 season	2022/2023 season	2023/2024 season
<b>Effect of nitrogen rates</b>							
30 N (Kg/ fed)		190.75 c	190.83 c	2.68 c	2.40 c	4.51 c	4.73 c
60 N(Kg/ fed)		216.93 b	216.40 b	3.41 b	3.04 b	5.39 b	5.61 b
90 N (Kg/ fed)		237.66 a	238.35 a	4.05 a	3.91 a	6.45 a	6.51 a
<b>Effect of foliar spray with <i>Spirulina platensis</i> (SP)</b>							
0 ml/l		224.82 a	225.89 a	3.09 d	2.76 d	5.05 d	5.24 d
1 ml/l		218.55 b	219.28 b	3.27 c	3.03 c	5.28 c	5.49 c
2 ml/l		212.38 c	212.61 c	3.52 b	3.26 b	5.61 b	5.75 b
4 ml/l		204.71 d	202.99 d	3.66 a	3.41 a	5.85 a	6.00 a
<b>Effect of interaction</b>							
nitrogen rates	foliar spray (SP)						
	0 ml/l	202.60 gh	202.77 f	2.27 i	2.12 j	4.10 i	4.27 g
30 N (Kg/ fed)	1 ml/l	195.48 hi	195.26 fg	2.60 h	2.33 ij	4.32 i	4.51 g
	2 ml/l	186.05 ij	186.80 gh	2.85 g	2.52 hi	4.72 h	4.95 f
	4 ml/l	178.88 j	178.49 h	3.02 fg	2.63 gh	4.89 gh	5.18 ef
60 N (Kg/ fed)	0 ml/l	224.72 de	226.49 cd	3.22 ef	2.78 fg	4.92 gh	5.23 ef
	1 ml/l	219.87 de	220.18 de	3.36 de	3.00 ef	5.18 fg	5.58 de
	2 ml/l	215.30 ef	215.78 e	3.49 d	3.15 de	5.57 ef	5.76 d
90 N (Kg/ fed)	4 ml/l	207.85 fg	203.13 f	3.58 cd	3.22 d	5.88 de	5.88 cd
	0 ml/l	247.16 a	248.40 a	3.77 bc	3.37 d	6.13 cd	6.23 bc
	1 ml/l	240.30 ab	242.40 ab	3.85 b	3.77 c	6.35 bc	6.37 b
2 ml/l	235.78 bc	235.26 bc	4.21 a	4.13 b	6.53 ab	6.53 ab	
	4 ml/l	227.41 cd	227.34 cd	4.38 a	4.40 a	6.77 a	6.92 a

## Discussion

The presented study reported that the maximum growth of lettuce and its productivity as well as highest of most chemical contents are associated to the application of mineral source of N fertilizer at highest dose (90 Kg N/feddan). This is due to the high essentiality of N as a component of amino acids, proteins, nucleic acids, pigments and many enzymes (Marschner, 1995). So, N is critical for growth

and development of plants, especially during cell division and cell enlargement phase of growth. Haque and Jakhro (1996) reported that N promotes vigorous growth, and its deficiency leads to stunted growth and low production. The observed enhancement in lettuce productivity under higher dose of nitrogen condition agrees with those findings of Shams, 2012 and Zaki *et al.*, 2012, who reported that plant productivity increased as N dose increased. The maximum yield under

higher supply of mineral nitrogen due to increasing the photosynthetic rates and the assimilation rates, which leading to increase the vegetative growth and heads yield of lettuce (Saleh *et al.*, 2010). With the increase of nitrogen dose, growth and productivity increased due to reciprocal relationship between nitrogen and carbohydrates exist within the plant (Patil *et al.*, 2003) leading to more accumulation of carbohydrates and proteins which are the final products of photosynthesis and metabolic activities. Although lettuce growers are seeking to increase productivity, the consumers are extremely preferred high product quality (FAO, 2002). McCall and Willumsen (1998) mentioned that higher doses of N are unlikely to achieve more gain in yield but risk increasing the nitrate content of the crop to a non-acceptable rate. Increasing the use of mineral-N causes environmental problems due to the excessive nitrate accumulation in the edible parts (Parente *et al.*, 2006). The application of nitrogen fertilizer in appropriate mode (source and dose) is important to get optimum lettuce productivity and to improve its quality.

Algae extracts (Spirulina extract) are an excellent source of chemical fertilizers (NPK) and plant growth promoters such as auxins, cytokinins, gibberellins, amino acids, macronutrients, microelements, and organic

matter that improve plant productivity (Al-Najjar, 2022). Spraying lettuce plants with (SP) is a part of the solution to decrease nitrate accumulation, therefore it can be used to reduce the amount of nitrate produced by mineral fertilizers in vegetables, hence improving the quality of salad vegetables produced as well as human health (Fawzy *et al.*, 2006; Uddin *et al.*, 2009; Saleh *et al.*, 2010; Shams, 2012; Zaki *et al.*, 2012 and Saleh *et al.*, 2013). The presented study reported that lettuce that sprayed with (SP) resulted in the highest product quality (the highest vitamin C and dry matter as well as less nitrate content) compared to mineral-N source. Also, the superiority in vitamin C and dry matter in heads tissues plus less nitrate content were associated to medium application dose of N-fertilizer. The slight decrease in lettuce yield by spraying (SP) is acceptable and may partly be compensated for by saving of the mineral fertilizers cost and the positive environmental effect as well as the possible higher price for higher nutritional quality of the yield (Uddin *et al.*, 2009). So, it is possible to reach remarkable yield even by application of moderate mineral-N dose (Parente *et al.*, 2006) and using (SP) spraying (Al-Najjar, 2022). Consequently, there is double advantage by increasing product quality (vitamin C and dry matter) and reducing nitrate accumulation (Premuzic *et al.*, 2004). In conclusion, it is possible to achieve an acceptable yield and more quality by providing moderate dose of N and using (SP) spraying.

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## تأثير مستويات النيتروجين المعدني والرش الورقي بمستخلص (الإسبيرولينا بلاتنيسيس) على النمو والمحصول وجودة نباتات الخس

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أجريت هذه الدراسة خلال موسمي الشتاء المتتاليين 2023/2022 و 2024/2023 لتقييم تأثير ثلاثة مستويات من النيتروجين المعدني (30، 60، و90 كجم نيتروجين/فدان) والرش الورقي بـ (الإسبيرولينا) بمعدلات 0، 1، 2، و4 مل/لتر على النمو، والإنتاج، والتركيبة الكيميائي، وتراكم النترات في الخس (*Lactuca sativa L., cv. Dark Green*) نُفذت التجربة باستخدام نظام ذات عاملين، حيث تضمن العامل (أ) مستويات النيتروجين في القطع الرئيسية، بينما تضمن العامل (ب) الإسبيرولينا كرش ورقي في القطع تحت الرئيسية. أظهرت النتائج أن التسميد بـ 90 كجم نيتروجين/فدان أو الرش الورقي بالإسبيرولينا بتركيز 4 مل/لتر كل على حدة أدى إلى أعلى القيم لمعظم الصفات المدروسة، والتي شملت: طول النبات، عدد الأوراق، المساحة الورقية، قطر الرأس، الوزن الطازج، نسبة المادة الجافة، محتوى الصبغات، المحصول الكلي، فيتامين C، ونسبة السكريات الكلية في كلا الموسمين. ومع ذلك، فإن المعاملة بـ 90 كجم نيتروجين/فدان تسببت أيضاً في أعلى تراكم للنترات وأقل كفاءة في استخدام النيتروجين، بينما على العكس، فإن الرش بالإسبيرولينا بتركيز 4 مل/لتر أدى إلى خفض مستويات النترات وتحسين كفاءة استخدام النيتروجين. بالطبع، أظهر التداخل بين 90 كجم نيتروجين/فدان مع 4 مل/لتر إسبيرولينا تفوقاً واضحاً في معظم الصفات المدروسة، مع انخفاض معنوي في محتوى النترات وتحسن في كفاءة استخدام النيتروجين مقارنةً باستخدام 90 كجم نيتروجين/فدان فقط. ومن المثير للاهتمام أن المعاملة بـ 60 كجم نيتروجين/فدان مع 4 مل/لتر من الإسبيرولينا لم تُظهر فروقاً معنوية مقارنةً بمعاملة 90 كجم نيتروجين/فدان بدون إسبيرولينا في معظم الصفات المهمة مثل (المحصول الكلي، ..... إلخ) في كلا الموسمين. وهذا يشير إلى إمكانية تقليل السماد النيتروجيني المعدني بمقدار 30 كجم/فدان من خلال الاستبدال الجزئي بالإسبيرولينا، مما يسهم في تقليل التلوث البيئي، وتحسين كفاءة استخدام النيتروجين، وتقليل تراكم النترات في أوراق الخس. ويُعد التداخل بين النيتروجين والإسبيرولينا واعداً لتعزيز اقتصاديات إنتاج الخس.

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