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Effect of Nitrogen Fertilizer Rates and Microalgae Application on the Vegetative Growth and Biochemical Constituents of Sweet Basil Plants

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Abstract: A field experiment was carried out at Ismailia Agricultural Research Station, Ismailia governorate, during two successive seasons (2017 and 2018) to evaluate the impact of microalgae foliar application with reduced nitrogen fertilizer rates on the vegetative growth and biochemical constituents of sweet basil (*Ocimum basilicum* L.) plants. The microalgae belonging to the strain *Spirulina platensis* and *Chlorella vulgaris* were used as a foliar spray at 7.5 ml/l. The obtained results revealed that the application of *Spirulina platensis* individually or in a mixture with *Chlorella vulgaris* combined with 75% of the recommended dose of N fertilizer improved growth characters, essential oil composition and antioxidant activity of sweet basil plants compared to the full dose of nitrogen fertilizer.

Key words: Sweet basil · Nitrogen fertilizer · Microalgae · Essential oil

INTRODUCTION

Ocimum basilicum L. (sweet basil) is an annual herb which belongs to the Lamiaceae family and grows in several regions all over the world. The plant is widely used in food and oral care products. The essential oil of the plant is also used as perfumery [1]. The leaves and flowering tops of sweet basil are used as carminative, galactogogue, stomachic and antispasmodic in folk medicine [2].

Several studies have demonstrated that extracts of this herb exhibit many properties, including antioxidant activity, immunomodulatory effects, antimicrobial properties and decreases cholesterol synthesis and lipid accumulation [3, 4].

The main phenolics reported in basil are phenolic acids and flavonol glycosides. Rosmarinic acid has been found as the main phenolic acid in basil and has a major contribution to antioxidant activity [5, 6].

Basil herb and essential oil yield are largely affected by nitrogen fertilization and determines its quality as reported by Sifola and Barbieri [7] and Golcz *et al*. [8]. They found that increased nitrogen fertilization contributed to an increase in fresh herb yield and essential oil yield in basil. Inorganic nitrogen fertilizer, though enhance productivity but adversely affect the environment and human health. So, an alternative approach is necessary for enhancing sweet basil production without causing substantial damage to the ecosystem.

Microalgae have emerged as a promising alternative used in conjunction with synthetic fertilizers [9-11]. Microalgae are typically single-cell photosynthetic autotrophic microscopic organisms naturally found in fresh water and marine environment. They produce complex compounds such as lipids, carbohydrates and proteins, using simple substances from their surroundings. The microalgae are divided into four groups: a) *cyanobacteria* (blue-green algae), b) *rhodophytes* (red algae), c) *chlorophytes* (green algae) and d) *chromophytes* (all other algae).

Spirulina (Arthrospira) belongs to the cyanobacteria group. Spirulina is symbiotic, multicellular and filamentous blue-green microalgae that utilises nitrogen from air. It can have two distinctive shapes: spiral rod or disk-like. The main photosynthetic pigment of Spirulina is

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phycocyanin (blue colour). *Spirulina* is rich in proteins, vitamins, essential amino acids, minerals and essential polyunsaturated fatty acid and pigments including phycocyanin, myxoxanthophyl and zeaxanthin. Spirulina contains 46-71% protein, 8-16% carbohydrate and 4-9% lipids of its dry weight and vitamins [12, 13].

Chlorella species belong to the chlorophytes group. They are single-cell, spherical shaped (~2 to 10 µm in diameter) and photoautotrophic green microalgae with no flagella. *Chlorella* contains the green photosynthetic pigments chlorophyll-a and -b in its chloroplast. It multiplies rapidly requiring only CO_2, water, sunlight and a small amount of minerals. The biochemical composition of *Chlorella* shows that it contains 11-58% protein, 12-28% carbohydrate and 2- 46% lipids of its dry weight [12].

The beneficial effects of microalgae application include their ability to secrete growth promoting substances such as auxins, gibberellins, cytokinins, vitamins, polypeptides, amino acids, which promote plant growth [14]. Microalgae applications were previously used in several crops to reduce the use of chemical fertilizers and to improve the plant growth [11, 15]. The application of microalgae in conjunction with nitrogen fertilizer in sweet basil plants has not been discussed so far. Therefore, the objective of the present study was to assess the impact of microalgae foliar application with reduced nitrogen fertilizer rates on the vegetative growth, biochemical constituents and the antioxidant activity of sweet basil plants.

MATERIALS AND METHODS

A field experiment was carried out at the experimental farm of Ismailia Agricultural Research Station, Ismailia governorate, Egypt, during two successive seasons (2017 and 2018). Sweet basil (*Ocimum basilicum* L.) seeds were obtained from Medicinal and Aromatic Plants Research Dept., Horticulture Research Institute, ARC, Egypt. Seeds were sown in a nursery until the seedlings lengths were about10-15 cm and then transplanted in the experimental area on 1st April in both seasons. The plot area was 12 m^2 (3x4 m) with 4 rows, 60 cm apart and 25 cm between seedlings (48 plant /plot).

Soil Analysis: The experimental soil was sandy and the physical and chemical analysis of the soil was determined before conducting the experiment according to Jackson [16] with the following properties: 90% sand, 2% silt, 8% clay, 7.92 pH, 0.40% organic matter, 1.4% calcium carbonate, 17 mg/kg available N, 20 mg/kg available P and 55 mg/kg available K.

Strain Source and Growth Conditions: The fresh microalgae strains belonging to *Spirulina platensis* and *Chlorella vulgaris* were obtained from Algal Biotechnology Unit, National Research Centre, Egypt. *S. platensis* was grown on Zarrouk medium [17] and BG11 medium [18] was used for the growth of *C. vulgaris*. The culture was incubated in growth chamber under continuous illumination (2000 lux) and a temperature of 35°C±2°C for *S. platensis* and 25°C±2°C for *C. vulgaris*. The microalgae were sprayed 4 times during the growing season at a concentration of 7.5 ml/l by one month intervals.

Experimental Layout and Treatments: The experiment was conducted in a complete randomized block design with three replicates and included seven fertilization treatments. The treatments were as follows:

- 100% of the recommended dose of nitrogen (N) fertilizer as a control.
- 75% of the recommended dose of N + *Spirulina platensis* foliar spray (7.5 ml/l).
- 75% of the recommended dose of N + *Chlorella vulgaris* foliar spray (7.5 ml/l).
- 75% of the recommended dose of N + mixture of *Spirulina platensis* and *Chlorella vulgaris* foliar spray (7.5 ml/l).
- 50% of the recommended dose of N + *Spirulina platensis* foliar spray (7.5 ml/l).
- 50% of the recommended dose of N + *Chlorella vulgaris* foliar spray (7.5 ml/l). 50% of the recommended dose of N + mixture of *Spirulina platensis* and *Chlorella vulgaris* foliar spray (7.5 ml/l).

The recommended dose of N fertilizer according to the Ministry of Agriculture, Egypt was applied only in the control treatment (600kg per feddan of ammonium sulfate 20.6 % nitrogen). The recommended dose of both calcium super phosphate (15.5 % P2O5) and potassium sulfate (48 % K2O) fertilizers according to the Ministry of Agriculture were added to all seven treatments equally. Nitrogen fertilizer was added to the soil while the microalgae were sprayed on the herb four times during the growing season, the first dose was applied after one month from transplanting and the other three doses were applied at one month intervals. All plants received normal agricultural practices whenever they needed.

At harvest, two cuts were taken; the first cut on 15th June and the second one on 30 thJuly during both seasons and the vegetative parameters were taken as follows: Plant height (cm), number of branches per plant, fresh and dry weights (g) per plant, essential oil percentage and yield.

Essential Oil Extraction: Essential oil was isolated by hydro-distillation using a Clevenger type apparatus according to Guenther [19] and essential oil percentage was calculated based on dry weight and the essential oil yield was thus calculated by multiplication of herb dry weight (g) x oil (%). The essential oil was dried with anhydrous sodium sulphate and subjected to gas chromatography analysis.

Gas Chromatography Analysis: The Gas chromatography analysis of the essential oil samples was carried out in the Laboratory of Medicinal and Aromatic plants Research department, Horticulture Research Institute, (ARC) using Ds Chrom 6200 Gas Chromatograph apparatus, fitted with capillary column BPX-5, 5 phenyl (equiv.) polysillphenylene-siloxane 30 x 0.25 mm ID x 0.25µ film. The temperature program varied in the range 70° - 200°C, at a rate of 10° C/min. Flow rates of gases were nitrogen at 1 ml / min, hydrogen at 30 ml / min and 330 ml / min for air. Detector and injector temperatures were 300°C and 250°C respectively. The identification of the compounds was done by matching their retention times with those of authentic samples injected under the same conditions.

Nitrogen Content in Leaves: Total nitrogen was determined using the modified micro-Kjeldahl method according to AOAC [20] on a dry weight basis.

Free Radical Scavenging Activity: The antioxidant activity was determined by DPPH free radical scavenging assay according to Brand-Williams *et al*. [21]. The inhibition percent of DPPH free radical (I) was calculated by the formula:

$$(I\ \%) = [(A_{blank} - A_{sample}) / A_{blank}] \times 100$$

where, A_{blank} is the absorbance of the control reaction (DPPH alone) and A_{sample} is the absorbance of DPPH solution in the presence of the test compound.

Statistical Analysis: All measurements were conducted in triplicate and analysis of significant differences among means were tested by one-way ANOVA followed by L.S.D to compare treatments means at a probability level of 0.05 as illustrated by Snedecor and Cochran [22].

RESULTS AND DISCUSSION

Vegetative Growth Parameters of Sweet Basil Plants: Data presented in Tables (1 and 2) indicated that the foliar application of *Spirulina platensis, Chlorella vulgaris* individually or in a mixture at 7.5 ml/l with N fertilizer rates had a significant effect on plant height, number of branches per plant, fresh weight per plant as well as dry weight per plant compared to control treatment. Reducing nitrogen fertilizer up to 75% of recommended dose and using Spirulina as a foliar spray at 7.5 ml/l resulted in the highest mean values of plant height in the 1st and 2nd cuts during the two growing seasons, whereas reducing nitrogen fertilizer up to 75% of recommended dose and using Spirulina as a foliar spray combined with Chlorella recorded maximum values of number of branches per plant in the 1st and 2nd cuts during the two growing seasons. Also, data revealed that maximum values of fresh and dry weights per plant were obtained with the application of 75% recommended dose of N + *S. platensis* in the 2nd cut during both growing seasons. In contrast, minimum values of growth characters were obtained from plants received 50% recommended dose of N + *C. vulgaris* or N + a mixture of *S. platensis* and *C. vulgaris* and was followed by 100% N fertilizer application. This could be due to the presence of essential nutrients in a readily available form through foliar application and was not lost from soils through leaching. Similar results were reported by Masoud [11] and Abdelaziz *et al.* [15] who confirmed the stimulatory role of *Spirulina platensis* on grapevine and their effect in reducing the doses of chemical fertilizer and improving the growth characteristics, yield, cluster weight and quality of the berries. The presented results also agree with Grzesik *et al.* [10] who indicated that foliar biofertilization with cyanobacteria and *Chlorella sp*. limited the doses of chemical fertilizers by as much as 50%, without significant reduction of growth and biomass yield of willow. The enhanced growth characters of sweet basil plants in the present work could be attributed to the importance of microalgae in providing the plants with complex compounds produced by these organisms. Spirulina and Chlorella are rich in proteins, vitamins,

Table 1: Effect of nitrogen fertilizer rates and microalgae foliar application on plant height and number of branches/ plant of sweet basil during 2017 and 2018 seasons

	Plant height (cm)				Number of branches/plant			
	First season		Second season		First season		Second season	
Treatments	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
100% N	46.43	43.11	46.67	43.56	9.27	15.33	10.27	16.33
75% N + *S. platensis*	51.56	48.78	52.33	50.00	15.33	17.30	15.89	17.67
75% N + *C. vulgaris*	50.44	47.89	51.11	49.56	16.78	17.67	17.11	18.41
75% N + *S. platensis* and *C. vulgaris*	50.22	47.78	51.45	49.11	17.11	17.89	17.67	19.00
50% N + *S. platensis*	51.44	45.89	52.00	46.22	16.22	17.56	15.45	18.55
50% N + *C. vulgaris*	50.11	43.33	50.67	44.33	13.78	15.78	14.11	16.67
50% N + *S. platensis* and *C. vulgaris*	46.56	44.78	47.00	45.22	13.33	16.44	13.67	17.44
L.S.D. at 0.05	1.77	1.39	0.81	1.27	1.06	1.48	1.00	0.62

Table 2: Effect of nitrogen fertilizer rates and microalgae foliar application on fresh and dry weights (g)/ plant of sweet basil during 2017 and 2018 seasons

	Fresh weight (g)/plant				Dry weight (g)/plant			
	First season		Second season		First season		Second season	
Treatments	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
100% N	128.01	181.49	131.57	184.93	35.73	40.67	36.64	42.00
75% N + *S. platensis*	220.28	256.95	225.84	262.50	46.53	65.53	47.90	63.76
75% N + *C. vulgaris*	193.79	200.39	199.32	205.68	35.78	54.21	36.10	57.27
75% N + *S. platensis* and *C. vulgaris*	221.64	223.46	229.83	225.21	39.46	58.57	40.57	59.84
50% N + *S. platensis*	215.01	204.66	209.49	209.09	37.25	63.49	37.86	64.49
50% N + *C. vulgaris*	205.51	178.93	207.53	172.58	33.29	55.85	34.21	58.54
50% N + *S. platensis* and *C. vulgaris*	167.18	180.79	171.14	184.79	36.05	48.62	36.88	51.37
L.S.D. at 0.05	9.88	9.10	5.32	4.74	1.42	5.46	0.833	3.10

Table 3: Effect of nitrogen fertilizer rates and microalgae foliar application on essential oil % and yield (ml)/ plant of sweet basil during 2017 and 2018 seasons

	1st season				2nd season			
	1st cut		2nd cut		1st cut		2nd cut	
Treatments	Essential oil (%)	Essential oil yield (ml/plant)	Essential oil (%)	Essential oil yield (ml/plant)	Essential oil (%)	Essential oil yield (ml/plant)	Essential oil (%)	Essential oil yield (ml/plant)
100% N	0.60	0.21	1.79	0.72	0.70	0.25	1.72	0.72
75% N + *S. platensis*	0.90	0.42	1.53	1.00	0.86	0.41	1.67	1.06
75% N + *C. vulgaris*	0.49	0.17	1.71	0.92	0.55	0.19	1.67	0.95
75% N + *S. platensis* and *C. vulgaris*	0.90	0.35	1.37	0.80	0.87	0.35	1.44	0.86
50% N + *S. platensis*	0.88	0.32	1.65	1.04	0.84	0.31	1.56	1.00
50% N + *C. vulgaris*	0.78	0.26	1.20	0.67	0.82	0.28	1.48	0.86
50% N + *S. platensis* and *C. vulgaris*	0.86	0.31	1.75	0.85	0.85	0.31	1.64	0.84
L.S.D. at 0.05	0.21	0.08	0.16	0.23	0.11	0.09	0.13	0.18

essential amino acids, minerals and essential polyunsaturated fatty acids and pigments, all these metabolites are needed for plant growth and development [23]. In addition, they provide growth-promoting substances such as indole acetic acid, gibberellins and cytokinins [14].

When comparing between the two microalgae under study, it was noticed that the addition of *S. platensis* to 50% and 75% recommended dose of N was more effective in improving growth characters than using *C. vulgaris* with N levels. This could be attributed to a higher protein content (64%) found in the chemical composition of *S. platensis* compared to *C. vulgaris* as indicated in an earlier study conducted by Gaese [24]. Accordingly, it could be concluded that using the microalgae *S. platensis* individually or a mixture of *S. platensis* and *C. vulgaris* with 75% nitrogen fertilizer enhanced growth parameters compared to the application of the recommended dose of nitrogen. Thus, reducing the quantity of inorganic fertilizers and saving up to 25%.

Table 4: Effect of nitrogen fertilizer rates and microalgae foliar application on essential oil composition of sweet basil during the 2nd season (2018)

	Treatments													
	100 % N		75% N + *S. platensis*		75% N + *C. vulgaris*		75% N + *S. platensis* and *C. vulgaris*		50 % N + *S. platensis*		50 % N + *C. vulgaris*		50 % N + *S. platensis* and *C. vulgaris*	
Compound (%)	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
α-pinene	0.34	-	-	0.85	-	2.80	-	-	-	-	-	-	-	-
β-pinene	1.51	3.03	0.69	0.22	1.84	-	1.09	0.42	2.16	3.11	1.89	0.45	1.62	0.49
Myrcene	0.16	-	2.26	-	0.21	-	2.17	2.32	2.05	-	0.17	2.41	0.22	2.26
1, 8-cineol	11.54	16.91	11.92	17.02	15.96	16.07	15.55	15.23	11.60	17.40	19.65	14.92	14.48	14.19
Linalool	48.08	53.72	52.19	53.49	52.01	51.93	41.85	53.46	47.37	48.85	37.46	53.13	40.51	51.43
Methyl chavicol	3.89	2.43	3.85	3.14	3.82	2.65	3.88	3.52	7.34	3.09	5.79	4.08	6.01	3.73
Eugenol	6.38	7.06	7.37	7.04	5.63	6.44	5.21	6.90	6.85	5.98	10.80	8.56	7.43	6.76
Identified compounds	71.90	83.15	78.28	81.76	79.47	79.89	69.75	81.85	77.37	78.43	75.76	83.55	70.27	78.86

Essential Oil Content and Yield: Essential oil percentage ranged from 0.49 % to 0.90 % in the 1st cut and from 1.20% to 1.79 % in the 2nd cut, on a dry weight basis during both seasons (Table, 3). The highest mean values of essential oil percentage were obtained by applying 75% N+ *S. platensis* and 100% recommended dose of N in the 1st and 2nd cuts during the 1st season, respectively. Moreover, essential oil yield was significantly affected by different treatments; the maximum essential oil yield was obtained by applying 75% N+ *S. platensis* in the 2nd cut during the second growing season. The obtained results were in agreement with Abd El-Aleem *et al*. [25] on fennel plants.

Essential Oil Composition: The essential oil of sweet basil plants from different treatments were analyzed by gas chromatography. The main components were linalool (37.46% to 53.72%), 1, 8-cineol (11.54% to 19.65%), eugenol (5.21% to 10.80%) and methyl chavicol (2.43% to 7.34%).

The results presented in Table (4) show that the highest percentage of linalool (53.72%) was recorded in the treatment that received the full dose of nitrogen fertilizer in the 2nd cut. The percentage of 1, 8-cineol decreased in the treatment that received 100% nitrogen fertilizer compared to other treatments, whereas, reducing nitrogen fertilizer up to 50% of recommended dose and spraying plants with chlorella resulted in the highest values of this compound (19.65%) as well as eugenol percentage (10.80%). Methyl chavicol, a compound with probable carcinogenic effect as indicated De Vincenzi *et al*. [26], recorded a maximum value (7.34%) in the treatment that received 50% N + *S. platensis* in the 1st cut, whereas minimum values (2.43%) were found in the treatment that received the full dose of nitrogen fertilizer in the 2nd cut. Accordingly, the abovementioned results showed that the chemical composition of sweet basil oil were altered and depended on the nitrogen rate and microalgae application, which is in agreement with Sifola and Barbieri [7] and Nurzyñska-Wierdak [27].

Nitrogen Content in Sweet Basil Leaves: Nitrogen content in sweet basil leaves ranged in the 1st cut from 3.11% to 3.25% and from 3.09% to 3.21% for the 1st and 2nd season, respectively (Fig. 1, a and b). As for the 2nd cut, it ranged from 3.12% to 3.20% and from 3.06% to 3.15% for the 1st and 2nd season, respectively. The highest values of nitrogen content in the 1st and 2nd seasons as 3.25 and 3.21% respectively were obtained in the 1st cut by applying 75% N+ *S. platensis*. However, the obtained results showed no significant difference in the leaf nitrogen content among different treatments. Similar findings were reported by Nagy and Pintér [28] on grapevines.

Antioxidant Activity: The antioxidant activity of sweet basil plants ranged from 61.56 % to 63.69% in the 1st cut and from 65.00% to 68.80 % in the 2nd cut, during both seasons as shown in Table (5). The highest values were obtained by applying 75% N+ *S. platensis* and 50% N+ *S. platensis* in the 2nd cut during both growing seasons. These results are in agreement with previous studies on the antioxidant properties of *O. basilicum* [3, 29]. The antioxidant potential of the plants is due to the presence of polyphenolic compounds and flavonoids such as rosmarinic acid, caffeic acid and eugenol [30, 31].

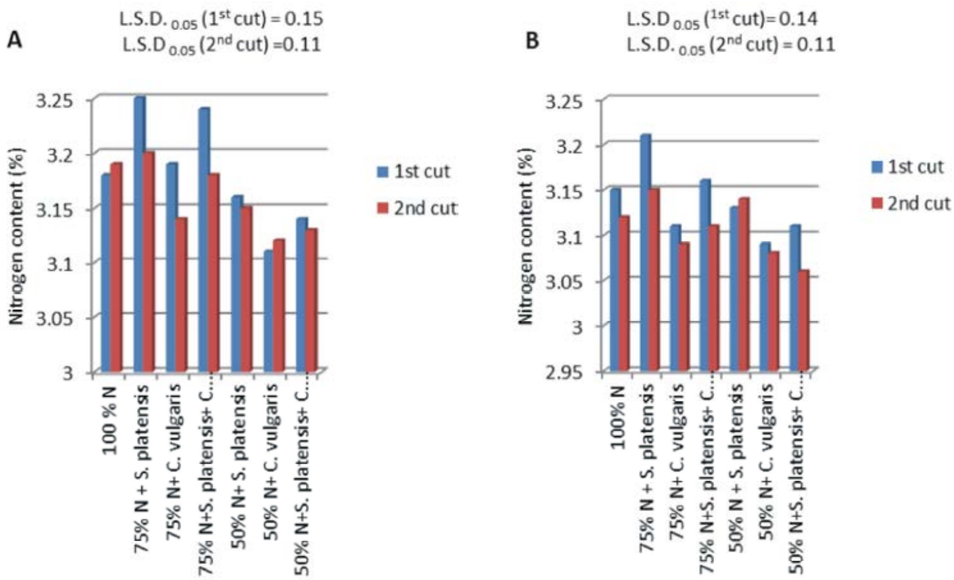

Fig. 1: Nitrogen content (%) as affected by nitrogen fertilizer rates and microalgae foliar application of sweet basil during 2017 (A) and 2018 (B) seasons

Table 5: Effect of nitrogen fertilizer rates and microalgae foliar application on antioxidant activity (%) of sweet basil during 2017 and 2018 seasons

	Antioxidant activity (%)			
	1st season		2nd season	
Treatments	1st cut	2nd cut	1st cut	2nd cut
100% N	63.69	66.18	63.21	66.34
75% N + *S. platensis*	61.04	67.43	62.43	68.80
75% N + *C. vulgaris*	62.88	65.00	63.56	67.49
75% N + *S. platensis* and *C. vulgaris*	61.56	66.75	62.66	66.14
50% N + *S. platensis*	63.54	67.87	62.12	68.32
50% N + *C. vulgaris*	63.12	66.78	63.46	67.70
50% N + *S. platensis* and *C. vulgaris*	62.60	66.85	61.57	66.38
L.S.D. at 0.05	0.97	1.02	0.94	1.17

CONCLUSION

Thus, the present investigation proved the potential of foliar application of *Spirulina platensis* (7.5 ml/l) individually or in a mixture with *Chlorella vulgaris* (7.5 ml/l) along with 75% recommended dose of N in improving the morphological parameters, essential oil content and composition as well as antioxidant activity of sweet basil plants when compared with that of full dose of nitrogen chemical fertilizer.

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