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EFFECT OF CYANOBACTERIAL FOLIAR APPLICATION AND DIFFERENT LEVELS OF NPK FERTILIZER ON GROWTH, CHEMICAL COMPOSITION AND ANTIOXIDANT ACTIVITY OF *ORIGANUM MAJORANA* L.

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ABSTRACT: A field experiment was conducted during 2011 and 2012 seasons to evaluate the effect of cyanobacterial foliar application with different levels of chemical fertilizers (25%, 50% and 100% of the recommended dose of NPK fertilizer) on the growth characters, macro-elements content, essential oil percentage, yield and composition, total phenol content, total flavonoid content as well as the antioxidant activity of marjoram (*Origanum majorana* L.) plant. The cyanobacteria (Blue green algae) belonging to the strain *Spirulina platensis* were used as a foliar spray at four concentrations (0, 4.5, 7.5 and 10 g/l). The obtained results revealed that the application of cyanobacteria combined with 50 or 100% of the recommended dose of NPK fertilizer improved growth characters, chemical constituents and essential oil composition than the other treatments. So, using cyanobacterial foliar spray with 50% of the recommended dose of NPK fertilizer could provide a high quality product with reduced harmful agrochemicals and eliminate environmental pollution.

Key words: *Origanum majorana*, cyanobacteria, NPK fertilizer levels, essential oil composition, total phenols, total flavonoids, antioxidant activity.

INTRODUCTION

Origanum majorana L. (sweet marjoram) is an aromatic, perennial, herbaceous and medicinal plant belonging to the family Lamiaceae. The aerial parts of sweet marjoram plant have been widely used in foods and flavor industry. The essential oil extracted from *Origanum majorana* aerial parts contains mainly terpinene-4-ol, cis-sabinene hydrate, α- and γ-terpinene as major components. (Gharib *et al*., 2008 and El-Ghandour *et al*., 2009). The herb contains phenolic acids, flavonoids and glycosides (Zheng and Wang, 2001), and its different extracts possess antioxidant and antimicrobial effects (Dorman *et al*., 2004; Leeja and Thoppil, 2007 and Muchuweti *et al*., 2007). Antioxidants are substances that delay the oxidation process, inhibiting polymerization chain initiated by free radicals, which are considered to be the main cause of numerous degenerative diseases (Halliwell and Aruoma, 1991). Marjoram has a strong antioxidant activity due to its high polyphenolic content (Hossain *et al*., 2010). Phenolic compounds isolated from marjoram herb include rosmarinic acid, ursolic acid, carnosic acid and carnosol, which possess free radical scavenging properties (Vagi *et al*., 2005). The antioxidant activity of phenolics is mainly due to their redox properties which allow them to act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators (Rice-Evans *et al*., 1997).

The production of secondary metabolites by plants is affected by genotype and

environmental conditions. Continuous usage of chemical fertilizers affects soil structure and lead to environmental hazards. Therefore, the current trend is to explore the possibility of supplementing chemical fertilizers with biostimulants. Cyanobacteria (Blue green algae) are oxygenic, photosynthetic, free living organisms commonly found in fresh water, marine water and soil. *Spirulina platensis* is a non-nitrogen fixing cyanobacterium, grows photoautotrophically with simple expense of light, water and inorganic nutrients. The dry biomas of *S. platensis* contains 6.7% N, 2.47% P and 1.14% K on a dry basis (Aly and Esawy, 2008). The protein content is as high as 60-70% of its dry weight (Ciefferi, 1983). Spirulina has a high concentration of vitamins and lipids in the amount of 4-7%. The essential fatty acid γ-linolenic acid and also 13.6% of carbohydrates are present (Mahajan and Kamat, 1995 and Cohen, 1997). *S. platensis* has been previously evaluated as a biostimulant for organic farming systems (Aly and Esawy, 2008).

The beneficial effects of cyanobacterial application include their ability to secrete growth promoting substances such as auxins, gibberellins, cytokinins, vitamins, polypeptides, amino acids, which promote plant growth (Sergeeva *et al*., 2002). Cyanobacterial applications were previously used in several crops to reduce the use of chemical fertilizers and to improve the plant growth (Aly and Esawy, 2008 and Hegazi *et al*., 2010).

The fertilizer impact on vegetative growth and essential oil content is well documented. However, there have been limited studies covering the response of other secondary metabolites and antioxidant activity using different fertilizer sources and levels. Therefore, the aim of the present study was to investigate the influence of cyanobacterial foliar application with chemical fertilizers on growth, essential oil content and chemical composition, total phenol content, total flavonoid content, and antioxidant activity of marjoram plant.

MATERIALS AND METHODS

Plant material:

A field experiment was carried out at the Kasassin Experimental Farm, Horticulture Research Station, Ismailia governorate, during two successive seasons (2011 and 2012). Seedlings of marjoram (*O. majorana*) were obtained from a private nursery, which were grown in plastic pots with a shoot length of 10-15 cm then transplanted in the experimental area at the end of March in both seasons. The plot area was 3x4 m^2 with 4 rows 60 cm apart and the seedlings were distanced at 25 cm (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 (1976) with the following properties: 96.5% sand, 1.7% silt, 1.8% clay, 8.1 pH, 0.03% organic matter, 0.18% calcium carbonate, 5.4 ppm available N, 5.5 ppm available P and 52 ppm available K.

Chemical fertilizers:

The recommended dose of NPK fertilizer for sandy soils was applied as ammonium nitrate (33.5% N) at a rate of 400 kg/feddan, calcium superphosphate (15.5% P_2O_5) at a rate of 300 kg/feddan and potassium sulphate (48% K_2O) at a rate of 100 kg/feddan. The inorganic fertilizer was divided into four equal doses. The first addition was carried out 30 days after transplanting, the second addition was performed after one month, the third addition was carried out after one week from the 1st cut and the fourth addition was carried out after one month from the last addition.

Strain source and growth conditions:

The fresh cyanobacterial strain belonging to *Spirulina platensis* was obtained from Algal Biotechnology Unit, National Research Centre, Egypt. The cyanobacterial strain was grown on Zarrouk medium (Zarrouk, 1966). The culture was incubated in growth chamber under

continuous illumination (2000 lux) and a temperature of 35 ± 2ºC. The amino acids composition of *S. platensis* is listed in Table (1) (Aly and Esawy, 2008).

Table 1. The amino acids composition of *Spirulina platensis*.

Essential amino acids	**%**
Isoleucine	5.6
Leucine	8.7
Lysine	4.7
Methionene	2.3
Phenylalanine	4.5
Threonine	5.2
Tryptophan	1.5
Valine	6.5
Non-essential amino acids	**%**
Alanine	7.6
Arginine	6.9
Aspartic acid	9.8
Cystine	1.0
Glutamic acid	14.6
Glycine	5.2
Histidine	1.6
Proline	4.3
Serine	5.2
Tyrosine	4.8

The cyanobacteria (CB) were sprayed 4 times during the growing season at a concentration of 0, 4.5, 7.5 and 10 g/l. All sprays were applied with NPK fertilizer.

Experimental design:

The experiment was conducted in a complete randomized block design with three replicates and included 12 fertilization treatments. The treatments were as follows:

- 25% of the recommended dose of NPK fertilizer + cyanobacteria at 0 g/l.
- 25% of the recommended dose of NPK fertilizer + cynobacteria at 4.5 g/l.
- 25% of the recommended dose of NPK fertilizer + cyanobacteria at 7.5 g/l.
- 25% of the recommended dose of NPK fertilizer + cyanobacteria at 10 g/l.
- 50% of the recommended dose of NPK fertilizer + cyanobacteria at 0 g/l.
- 50% of the recommended dose of NPK fertilizer + cyanobacteria at 4.5 g/l.
- 50% of the recommended dose of NPK fertilizer + cyanobacteria at 7.5 g/l.
- 50% of the recommended dose of NPK fertilizer + cyanobacteria at 10 g/l.
- 100% of the recommended dose of NPK fertilizer + cyanobacteria at 0 g/l.
- 100% of the recommended dose of NPK fertilizer + cyanobacteria at 4.5 g/l.
- 100% of the recommended dose of NPK fertilizer + cyanobacteria at 7.5 g/l.
- 100% of the recommended dose of NPK fertilizer + cyanobacteria at 10 g/l.

Two cuts were harvested; the first one was on the 15th of July and the second one on the 15th of October during both seasons and the vegetative parameters were taken as follows: Plant height (cm), number of branches per plant, fresh weight (g) per plant and dry weight (g) per plant.

Determination of macro-elements in the herb:

Total nitrogen was determined using the modified micro-Kjeldahl method according to AOAC (1990). Phosphorus was determined according to Chapman and Pratt (1978). Potassium was determined according to Cottonie *et al*. (1982). Macro-elements content was determined in samples of the 1st cut during the 1st season.

Essential oil extraction:

Essential oil was isolated by hydro-distillation using a Clevenger type apparatus according to Guenther (1961) and essential oil percentage was calculated based on dry weight and the essential oil yield was thus calculated by multiplication of herb weight (g) x oil (%). The essential oil was dried with anhydrous sodium sulphate and subjected to gas chromatography analysis.

Gas chromatography analysis:

The essential oil was analyzed using a gas chromatography Hewlett Packard (HP) 6890 series equipped with flame ionization detector and capillary column HP-5 (30 m x 0.25 mm, o.25 µm film thickness). The oven

temperature increased from 70 to 220ºC at a rate of 8ºC/min. The injector and detector temperatures were 220ºC and 250ºC, respectively and Hydrogen was used as the carrier gas. The identification of the compounds was done by matching their retention times with those of authentic samples injected under the same conditions. The essential oil composition was determined in samples of the 1st cut during the 1st season.

Total phenolic content:

Total content of phenolic compounds was determined by the Folin-Ciocalteau method (Singleton and Rossi, 1965). The total amount of phenolic compounds was calculated using gallic acid calibration curve. The results were expressed as mg gallic acid equivalents per gram of dry weight.

Total flavonoid content:

The total flavonoid content was determined according to Dewanto *et al*. (2002). The total amount of flavonoids was calculated using rutin calibration curve. The results were expressed as mg rutin equivalents per gram of dry weight.

Free radical scavenging activity:

The antioxidant activity was determined by DPPH free radical scavenging assay according to Brand-Williams *et al*. (1995). The inhibition percent of DPPH free radical 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 LSD to compare treatment means at a probability level of 0.05 as illustrated by Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Effect of fertilization treatments and cyanobacteria on the growth characters of *Origanum majorana* plants:

In general, the performance of marjoram plant in terms of plant height, number of branches, plant fresh and plant dry weights was enhanced by cyanobacterial application in both cuts and seasons, as shown in Tables (2 and 3). It was clearly observed that the addition of 100% NPK + CB (4.5 g/l) significantly increased growth characters compared to other treatments, this application recorded the maximum plant height, number of branches, fresh and dry weights in the 1st and 2nd cuts during both seasons. This treatment was followed by the application of 50% NPK + CB (4.5 g/l) in the 1st cut which was statistically equal to the above mentioned treatment, and the application of 50% NPK + CB (7.5 g/l) in the 2nd cut during both seasons. This indicates that even after reduction in dose of NPK to 50% with applying CB at a concentration of 4.5 and 7.5 g/l increment in growth characters was obtained. Moreover, it was noticed that the lower and medium dose of CB with 50% NPK and 100% NPK, proved to be more effective in enhancing growth characters than the higher dose. The promoting effect of cyanobacterial application on herb yield could be attributed to the fact that cyanobacteria excrete a great number of substances that influence plant growth and development (Haroun and Hussein, 2003 and Rodriguez *et al*., 2006). These micro-organisms play a valuable role in enhancing the growth of higher plants (El-Ayouty, 1998). Cyanobacteria have been reported to benefit plants by producing growth-promoting regulators (the nature of which is said to resemble gibberellins and auxins), vitamins, amino acids, polypeptides, antibacterial and antifungal substances (Karthikeyan *et al*., 2007).

The major role in plant growth promotion by non-nitrogen fixing cyanobacteria is through enriching phosphorus and potassium contents in soils

Table 2. Effect of fertilization treatments and cyanobacteria on the growth characters of *Origanum majorana* during the first season (2011).

Treatments	1st cut				2nd cut			
	Plant height (cm)	Branches No./plant	Fresh weight (g) /plant	Dry weight (g) /plant	Plant height (cm)	Branches No./plant	Fresh weight (g) /plant	Dry weight (g) /plant
25% NPK + CB (0 g/l)	35.00	23.67	83.43	33.50	32.00	35.00	72.54	17.32
25% NPK + CB (4.5 g/l)	37.33	31.00	82.30	33.79	32.00	32.80	93.04	23.56
25% NPK + CB (7.5 g/l)	35.00	24.67	76.30	34.92	33.40	37.00	98.67	28.12
25% NPK + CB (10 g/l)	38.00	30.00	73.77	33.60	30.80	43.00	82.57	21.26
50% NPK + CB (0 g/l)	38.33	31.33	71.57	30.97	35.60	47.00	94.16	24.43
50% NPK + CB (4.5 g/l)	43.67	34.33	98.1	41.3	31.00	28.60	101.9	25.68
50% NPK + CB (7.5 g/l)	42.67	30.67	66.27	32.80	38.20	42.40	124.2	35.92
50% NPK + CB (10 g/l)	43.33	27.33	81.33	34.20	37.40	52.60	119.7	34.84
100% NPK + CB (0 g/l)	40.67	25.67	92.23	38.3	36.60	51.60	105.9	29.63
100% NPK + CB (4.5 g/l)	44.00	41.33	104.2	44.37	43.00	53.80	140.9	43.11
100% NPK + CB (7.5 g/l)	40.00	19.00	61.40	28.64	38.40	38.40	88.63	21.07
100% NPK + CB (10 g/l)	39.33	26.67	81.97	34.32	34.20	42.20	94.80	22.56
L.S.D. at 0.05	4.12	6.36	11.78	5.72	5.17	9.97	10.69	2.68

Table 3. Effect of fertilization treatments and cyanobacteria on the growth characters of *Origanum majorana* during the second season (2012).

Treatments	1st cut				2nd cut			
	Plant height (cm)	Branches No./plant	Fresh weight (g) /plant	Dry weight (g) /plant	Plant height (cm)	Branches No./plant	Fresh weight (g) /plant	Dry weight (g) /plant
25% NPK + CB (0 g/l)	39.00	28.00	75.20	32.93	30.75	29.50	70.67	16.92
25% NPK + CB (4.5 g/l)	40.33	29.67	76.77	31.50	34.25	29.50	97.43	25.67
25% NPK + CB (7.5 g/l)	39.33	28.00	67.50	31.40	32.50	37.25	92.73	26.32
25% NPK + CB (10 g/l)	38.33	28.00	78.07	35.22	35.75	40.75	96.77	23.83
50% NPK + CB (0 g/l)	37.67	30.00	79.20	33.79	35.25	44.50	97.87	26.47
50% NPK + CB (4.5 g/l)	40.67	32.67	93.57	38.68	34.50	29.50	108.4	27.08
50% NPK + CB (7.5 g/l)	41.33	31.33	68.77	32.81	37.50	46.00	126.9	33.37
50% NPK + CB (10 g/l)	40.33	31.33	72.43	33.23	36.50	53.75	121.9	32.73
100% NPK + CB (0 g/l)	39.33	28.67	82.50	37.63	35.75	50.00	108.3	26.42
100% NPK + CB (4.5 g/l)	44.33	43.33	101.2	41.81	39.00	58.00	141.5	38.03
100% NPK + CB (7.5 g/l)	39.67	22.00	79.40	31.04	35.25	39.75	77.77	18.58
100% NPK + CB (10 g/l)	39.33	26.67	71.00	31.54	35.00	53.25	100.3	25.76
L.S.D. at 0.05	3.32	7.13	8.72	3.57	4.03	10.04	10.42	2.69

(Selvarani, 1983). Moreover, the increase in growth characters by applying cyanobacteria as a foliar spray may be due to that the sprayed solution of nutrients is readily absorbed by the leaves and not lost through fixation, decomposition or leaching (Abdel-Hadi *et al*., 1985). It is worthy to mention that the efficiency of foliar nutrition under field conditions does not only depend on the concentrations and combination of nutrients, but also on carriers of the nutrient as well as the soil chemical properties (Kariem *et al*., 1991).

These findings were in agreement with Adam (1999) who studied the effect of cyanobacteria on seed germination and related processes on wheat, sorghum, maize and lentil. It was observed that growth parameters were significantly increased compared to control. The increases were attributed to the amino acids and peptides produced in the algal filtrate and/or other compounds that stimulate growth of crop plants. Also, Aly and Esawy (2008) found that the first collection of pepper was higher in the presence of *Spirulina platensis* compared to those of compost and NPK, which could be due to high content of the free amino acids of the *Spirulina* product, as well as its content of macro and micro elements. They also found that the growth promoting substances were absorbed by the leaves rather faster than the nutrients from the soil by using mineral fertilization or compost. Moreover, Hegazi *et al*. (2010) studied the influence of cyanobacterial application with different levels of nitrogen fertilizer on common bean growth and yield and they found that reducing chemical nitrogen fertilizer up to 50 or 75% of the recommended dose and using cyanobacterial biofertilizer enhanced growth characters of common bean plant. Also, Ahmed *et al*. (2011) found that treating flame seedless grapevines with inorganic nitrogen (60%) + fulvic acid and *Spirulina platensis* algae each at 10 cm^3/ vine was beneficial in improving the yield. So, it could be concluded that the promoting effects of cyanobacteria on growth characters could be due to that cyanobacteria as a biostimulant facilitated the absorption and transport of nutritional macro and micro-elements.

Effect of fertilization treatments and cyanobacteria on nitrogen, phosphorus and potassium contents (NPK) of *Origanum majorana* plants:

The NPK contents of *O. majorana* plants treated with different levels of NPK fertilizers and cyanobacteria are shown in Fig. (1 A, B and C, respectively). Nitrogen content varied from 1.55% to 1.94%, phosphorus content varied from 0.33% to 0.48% and potassium content varied from 2.30% to 2.90%. The maximum NPK contents were recorded in the treatment of 100% NPK + CB (4.5 g/l) followed by the treatment of 50% NPK + CB (4.5 g/l), whereas, the minimum NPK contents were recorded in the application of 25% NPK + CB (0 g/l). The increase in NPK contents of *Origanum majorana* plants treated with different levels of NPK and cyanobacteria may be due to the availability of adequate amounts of N, P and K in the Spirulina foliar application, as it reached 6.7% N, 2.47% P and 1.14% K on a dry weight basis (Aly and Esawy, 2008). Similar trend of increase in nutrient concentration was observed by Hegazi *et al*. (2010) and Ahmed *et al*. (2011) on common bean plants and grapevines, respectively. This increase of nutrient concentration was in response to the application of Spirulina which are in line with the findings of this study. Joubert and Lefranc (2008) mentioned that the algal active ingredients may stimulate nitrate reductase and other plant enzymes responsible for absorbing minerals and their transformation in the plant.

Effect of fertilization treatments and cyanobacteria on essential oil percentage and yield:

The presented data in Table (4) indicates the effect of fertilizer treatments and cyanobacteria on the essential oil percentage and essential oil yield per plant of marjoram. It was observed that the 1st cut had higher

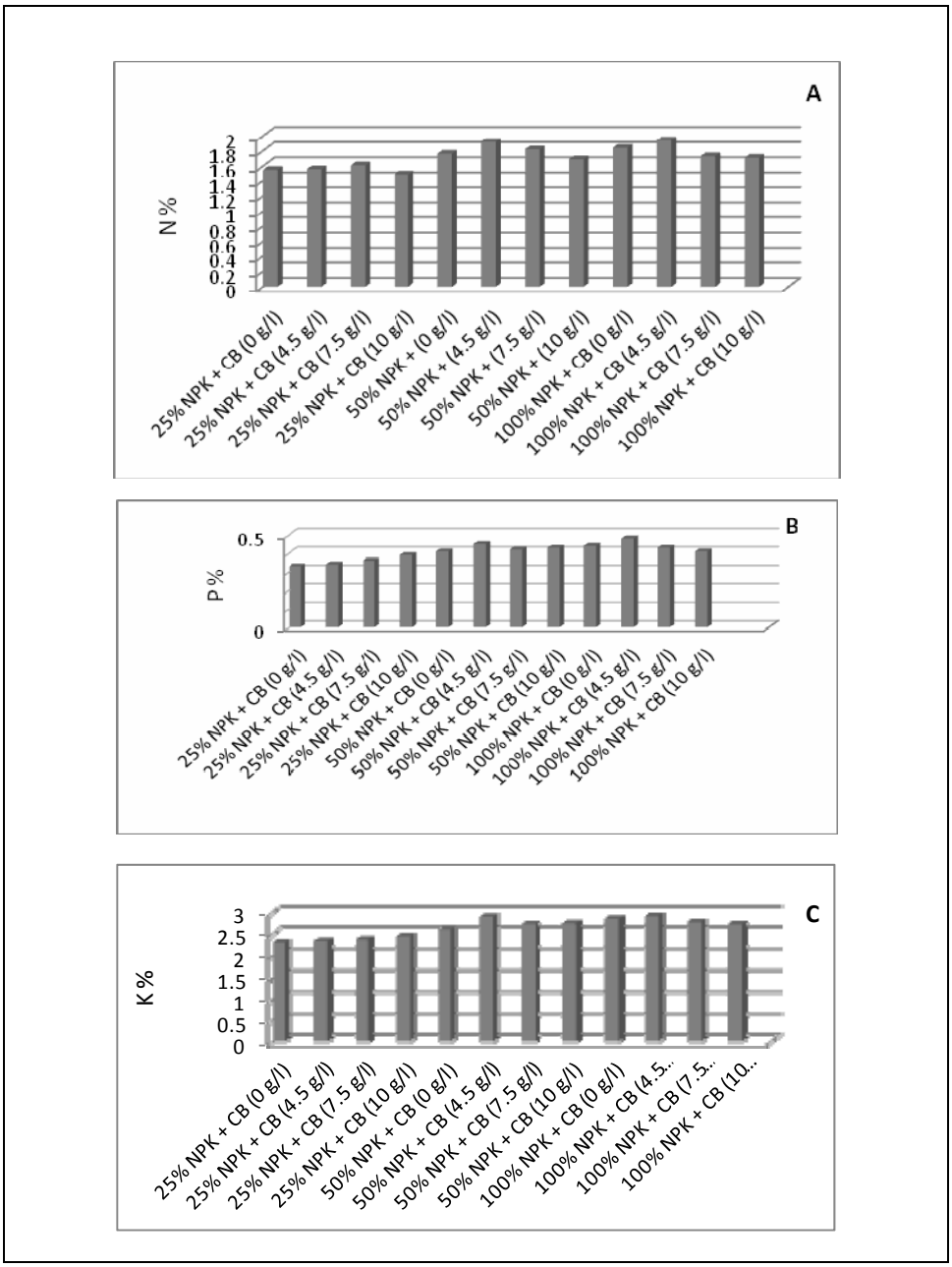

Fig. 1. Effect of fertilization treatments and cyanobacteria on Nitrogen (A), Phosphorus (B) and Potassium (C) contents of *Origanum majorana* plants.

Table 4. Effect of fertilization treatments and cyanobacteria on the essential oil percentage and yield of *Origanum majorana* during 2011 and 2012 seasons.

Treatments	1st season				2nd season			
	1st cut		2nd cut		1st cut		2nd cut	
	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)
25% NPK + CB (0 g/l)	2.41	0.80	2.22	0.33	2.46	0.81	2.19	0.37
25% NPK + CB (4.5 g/l)	2.45	0.82	2.27	0.57	2.46	0.84	2.37	0.60
25% NPK + CB (7.5 g/l)	2.38	0.83	2.31	0.65	2.35	0.73	2.44	0.64
25% NPK + CB (10 g/l)	2.51	0.84	2.48	0.48	2.42	0.85	2.42	0.57
50% NPK + CB (0 g/l)	2.45	0.81	2.12	0.41	2.50	0.84	2.24	0.61
50% NPK + CB (4.5 g/l)	2.61	1.07	2.50	0.64	2.66	1.02	2.37	0.64
50% NPK + CB (7.5 g/l)	2.90	0.95	2.45	0.88	3.01	0.98	2.42	0.73
50% NPK + CB (10 g/l)	2.42	0.82	2.28	0.79	2.50	0.88	2.17	0.71
100% NPK + CB (0 g/l)	2.53	0.96	2.11	0.62	2.60	0.97	2.17	0.57
100% NPK + CB (4.5 g/l)	2.84	1.26	2.24	1.01	2.64	1.21	2.34	0.84
100% NPK + CB (7.5 g/l)	2.86	0.82	2.28	0.48	2.78	0.86	2.15	0.40
100% NPK + CB (10 g/l)	2.81	0.93	2.20	0.43	2.76	0.87	2.23	0.57
L.S.D. at 0.05	0.47	0.41	0.26	0.16	0.30	0.24	0.46	0.19

essential oil percentage than the 2nd cut. Similarly, Bottcher *et al*. (1999) and Gharib (2006) found that *Origanum majorana* plant (1st cut) contained 22% more essential oil than the 2nd cut. The maximum mean values of essential oil percentage in the 1st cut were obtained by applying 50% NPK + CB (7.5 g/l) with values of 2.90 % in the 1st season and 3.01% in the 2nd season. As for the second cut, it was observed that the plants treated with 50% NPK + CB (4.5 g/l) recorded the highest values (2.50%) as shown in Table (4). Moreover, essential oil yield was affected by different treatments; the maximum essential oil yield was obtained when applying 100% NPK + CB (4.5 g/l) in the 1st and 2nd cuts during both experimental seasons. These results ensure the beneficial effects of cyanobacterial foliar application on enhancing oil percentage and oil yield.

Effect of fertilization treatments and cyanobacteria on essential oil composition:

The essential oil composition of marjoram plants as analyzed by gas chromatography treated with different levels of NPK chemical fertilizers and cyanobacterial foliar application are shown in Table (5). The GC profile of the essential oil of all treatments showed fifteen compounds from the identified compounds. The main components were terpinene-4-ol (28.34% to 35.54%), γ-terpinene (11.63% to 19.49%), sabinene (7.70% to 9.23%) and cis-sabinene hydrate (5.00% to 8.69%). Other components were present in amounts of 2% and less. These results are in agreement with the findings of Gharib *et al*. (2008) who found that terpinene-4-ol is the main component in marjoram oil grown in Egypt. Marjoram oil is found in three chemotypes. In the first chemotype, monoterpene alcohol such as cis-abinene hydrate, trans-sabinene hydrate and the acetate of cis-sabinene hydrate are the main constituents of the volatile fraction. These unstable compounds rearrange during hydro-distillation, forming terpinen-4-ol, α-terpineol, α-and γ-terpinene (Lawrence, 1989). For that reason, terpinene-4-ol alone or along with cis-and trans-sabinene hydrate, α-terpineol, α-and γ-terpinene were found to be main constituents of the essential oil found in Egypt. The second chemotype is rich in linalool or linalyl acetate and the third chemotype is characterized by a high concentration of carvacrol (78-80%) and found in Turkey (Baser *et al*., 1993).

Data presented in Table (5) showed that the application of 50% NPK + CB (7.5 g/l) recorded the highest percent of terpinene-4-ol (35.54%), whereas it was accompanied by a decrease in the percentage of cis-sabinene hydrate (5.01%). In addition, application of 50% NPK + CB (4.5 g/l) recorded the highest percent of γ-terpinene (19.49%) and α-terpineol (5.78%), while it was accompanied by a decrease in the percentage of terpinene-4-ol (28.35%). A maximum percentage of sabinene (9.23%) was obtained by the application of 50% NPK + CB (10 g/l). Also, the highest content of cis-sabinene hydrate was obtained when plants received 100% NPK + CB (7.5 g/l), while the highest content of α-terpinene occurred when applying 100% NPK + CB (4.5 g/l). Hence, it could be suggested from the obtained results that applying the cyanobacterial foliar spray to 50% or 100% of the recommended dose of NPK chemical fertilizers increased the essential oil components percent as a result of the improvement of growth characters of the treated plants.

Effect of fertilization treatments and cyanobacteria on total phenol, total flavonoid contents and antioxidant activity:

The effect of fertilizer treatments on the total phenol, total flavonoid contents and antioxidant activity of marjoram plant are shown in Tables (6 and 7). The total phenol content was significantly influenced by the application of NPK fertilizers and cyanobacterial foliar application. Among the various NPK levels and CB concentrations, the application of 50% NPK + CB (10 g/l) recorded the highest values of total phenol

Table 5. Effect of fertilization treatments and cyanobacteria on essential oil composition in the 1st cut of the first season (2011).

Components (%)	25% NPK + CB (0 g/l)	25% NPK + CB (4.5g/l)	25% NPK + CB (7.5g/l)	25% NPK + CB (10 g/l)	50% NPK + CB (0 g/l)	50% NPK + CB (4.5g/l)	50 % NPK + CB (7.5g/l)	50% NPK + CB (10 g/l)	100% NPK + CB (0 g/l)	100% NPK + CB (4.5g/l)	100% NPK + CB (7.5g/l)	100% NPK + CB (10 g/l)
α-Thujene	0.69	0.81	0.70	0.62	0.57	0.55	0.82	0.79	0.60	0.76	0.64	0.55
α-Pinene	1.01	0.94	0.99	1.07	1.20	0.97	0.91	1.01	1.13	1.07	1.03	1.19
Sabinene	8.78	8.90	8.37	8.10	7.70	8.27	8.56	9.23	8.57	8.94	8.20	7.85
Myrcene	2.88	2.52	2.87	3.37	3.82	2.35	2.43	2.75	3.56	3.08	3.47	3.73
α-Terpinene	5.89	7.24	6.34	6.02	5.11	5.96	7.37	7.12	5.29	7.42	5.34	5.98
ρ-cymene	3.10	2.45	2.53	2.58	3.12	1.60	1.90	2.49	2.58	2.00	3.59	2.39
γ-terpinene	12.02	13.40	12.53	12.60	11.72	19.49	13.68	13.34	11.63	14.08	11.78	12.77
Trans-sabinene hydrate	3.34	2.63	3.31	3.24	3.49	1.35	1.71	2.74	3.58	3.17	3.79	3.53
α-terpinolene	2.84	2.97	2.93	2.96	2.79	3.37	3.05	3.05	2.80	3.15	2.87	3.07
Cis-sabinene hydrate	8.26	6.77	7.61	6.20	7.63	8.19	5.00	6.73	7.89	7.02	8.69	8.13
Trans-ρ-menthenol	2.10	2.02	2.18	2.12	2.05	2.31	2.09	2.15	2.15	1.96	2.18	2.13
Cis-ρ-menthenol	1.77	1.69	1.86	1.79	1.78	2.65	0.93	1.76	1.81	1.35	2.04	1.88
Terpinene-4-ol	30.96	31.28	31.96	31.86	30.55	28.34	35.54	33.01	30.8	31.96	30.74	30.84
α-terpineol	4.46	4.74	4.68	4.59	4.83	5.78	4.88	4.62	5.19	4.422	5.11	4.94
β-caryophyllene	0.86	0.87	0.83	0.86	0.99	0.64	0.92	0.88	0.91	0.86	0.92	1.01

Table 6. Effect of fertilization treatments and cyanobacteria on the total phenols, total flavonoids contents and antioxidant activity of *Origanum majorana* during the first season (2011).

Treatments	Total phenol (mg/g)		Total flavonoid (mg/g)		RSA (%) at 100 µg/ml	
	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
25% NPK + CB (0 g/l)	8.87	8.87	8.86	14.24	82.17	80.46
25% NPK + CB (4.5 g/l)	8.87	8.65	12.25	13.36	83.27	81.78
25% NPK + CB (7.5 g/l)	8.59	9.04	8.88	9.72	83.21	81.36
25% NPK + CB (10 g/l)	9.17	9.27	9.89	10.53	82.22	81.29
50% NPK + CB (0 g/l)	9.22	10.47	15.28	8.90	82.28	82.28
50% NPK + CB (4.5 g/l)	8.75	8.95	25.49	13.64	84.19	85.71
50% NPK + CB (7.5 g/l)	8.72	9.13	10.28	17.43	83.17	85.27
50% NPK + CB (10 g/l)	9.71	12.16	19.14	23.55	85.33	86.52
100% NPK + CB (0 g/l)	9.42	10.45	11.38	11.82	82.62	82.36
100% NPK + CB (4.5 g/l)	9.26	11.70	20.39	19.21	83.41	85.20
100% NPK + CB (7.5 g/l)	9.68	11.42	19.52	19.49	82.29	83.37
100% NPK + CB (10 g/l)	8.67	10.92	18.34	13.13	82.75	83.55
L.S.D. at 0.05	0.84	1.37	1.46	1.65	1.22	1.27

Table 7. Effect of fertilization treatments and cyanobacteria on the total phenols, total flavonoids contents and antioxidant activity of *Origanum majorana* during the second season (2012).

Treatments	Total phenol (mg/g)		Total flavonoid (mg/g)		RSA (%) at 100 µg/ml	
	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
25% NPK + CB (0 g/l)	9.03	8.99	8.20	13.49	82.27	81.71
25% NPK + CB (4.5 g/l)	8.62	8.46	12.23	11.62	82.25	81.15
25% NPK + CB (7.5 g/l)	8.10	8.73	7.43	8.64	80.14	82.39
25% NPK + CB (10 g/l)	9.15	9.45	10.33	10.20	81.64	80.26
50% NPK + CB (0 g/l)	9.02	10.92	16.32	8.62	83.29	80.13
50% NPK + CB (4.5 g/l)	9.09	9.37	21.12	13.26	83.41	85.76
50% NPK + CB (7.5 g/l)	8.66	9.78	11.86	18.66	85.06	83.42
50% NPK + CB (10 g/l)	10.25	14.41	22.41	22.35	85.61	87.61
100% NPK + CB (0 g/l)	9.25	10.87	11.62	11.25	80.19	82.54
100% NPK + CB (4.5 g/l)	9.17	11.87	18.36	19.79	84.36	84.17
100% NPK + CB (7.5 g/l)	9.45	11.52	18.55	20.03	84.14	85.46
100% NPK + CB (10 g/l)	9.02	10.91	19.68	12.83	83.12	82.13
L.S.D. at 0.05	0.92	1.29	1.49	1.67	1.28	1.21

content in the 1st cut and 2nd cut compared to other treatments during both seasons. Concerning the effect of different levels of NPK fertilizers and CB concentrations on total flavonoid content, data in Tables (6 and 7) showed that the highest significant increase in total flavonoid content of the 1st cut were obtained as a result of applying 50% NPK + CB (4.5g/l) during the 1st season and applying 50% NPK + CB (10 g/l) during the 2nd season.

As for the 2nd cut, it was observed that treating marjoram plants with 50% NPK + CB (10 g/l) recorded the highest significant values (23.55, 22.35 mg/g) during the 1st and 2nd seasons, respectively. Phenolic compounds and flavonoids are recognized as possessing potent antioxidant activities and are strong free radical scavengers; free radicals may cause many coronary diseases in human. Many plants extracts containing bioactive compounds including phenolics and flavonoids exhibit efficient antioxidant properties and prevent from free radical damage (Larson, 1998). Therefore, the effect of fertilizer treatments on marjoram's free radical scavenging activity against the synthetic free radical DPPH$^{\bullet}$ was determined. Substances capable of donating electrons or hydrogen atoms are able to convert 1,1-diphenyl-2-picrylhydrazyl radicals into 1,1-diphenyl-2-picrylhydrazine. The effect of different fertilizer treatments on the radical scavenging activity (RSA) of *Origanum majorana* plant is shown in Tables (6 and 7). The results showed that there was a significant increase in the radical scavenging activity of marjoram plants of different fertilizer treatments compared to control. The percentage inhibition of DPPH$^{\bullet}$ free radical ranged from 80.13 to 87.61%. The highest percentage inhibition of DPPH$^{\bullet}$ free radical was obtained by the application of 50% NPK + CBB (10 g/l) in the 1st and 2nd cuts during both seasons.

It could be observed from the results that the treatments which performed the highest antioxidant activity, also exhibited the highest concentration of total phenols (Tables 6 and 7). Numerous investigations of the antioxidant activity of plants have confirmed a positive relationship between the values of phenol concentration and antioxidant activity. Phenolic compounds are very important plant constituents because of their scavenging ability on free radicals due to their hydroxyl groups. Rosmarinic acid is considered to be the main phenolic compound of marjoram plant. The antioxidant activity of rosmarinic acid has been studied extensively (Chen and Ho, 1997). Rosmarinic acid has four hydroxyl groups in its structure. The antioxidant activity of polyphenols is related to their hydroxyl group and the presence of a second hydroxyl group in the *ortho* or *para* position is known to increase the antioxidant activity due to additional resonance ability and *0*-quinone or *p*-quinone formation (Chen and Ho, 1997). According to the previous results, it could be concluded that *Origanum majorana* plants exhibited high antioxidant activity, which is in agreement with (Dorman *et al*., 2004; Muchuweti *et al*., 2007 and Moussaid *et al*., 2011). Generally, the increased levels of phytochemical constituents and antioxidant activity in plants treated with NPK fertilizer and enriched with cyanobacteria could be attributed to improved nutrient uptake and availability.

CONCLUSION

The present investigation revealed that improvement of the growth, biochemical parameters and antioxidant activity of marjoram plant might be due to the presence of amino acids, micro and macro elements, growth hormones and vitamins found in *Spirulina platensis*. Further, the study also emphasizes that the cyanobacteria as a foliar application can be effectively used to reduce the chemical fertilizer dosage and to produce high quality plants.

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تأثير إستخدام الرش بالسيانوبكتيريا ومستويات مختلفة من التسميد بـ NPK على النمو والتركيب الكيماوي والنشاط المضاد للأكسدة في نبات البردقوش

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تم إجراء تجربة حقلية خلال موسمين متتاليين هما ٢٠١١ و ٢٠١٢ لدراسة تأثير إستخدام الرش بالسيانوبكتيريا مع مستويات مختلفة من التسميد المعدني بسماد NPK (٢٥، ٥٠ و ١٠٠%) من الجرعات الموصى بها للفدان على النمو والتركيب الكيماوي من حيث محتوى العناصر الكبرى، نسبة ومحصول ومكونات الزيت الطيار، محتوى الفينولات الكلية، محتوى الفلافونويدات الكلية بالإضافة إلى النشاط المضاد للأكسدة في نبات البردقوش. و قد تم رش السيانوبكتيريا (طحالب خضراء مزرقة) التابعة لسلالة *Spirulina platensis* باستخدام ٤ تركيزات (٠، ٤,٥ ، ٧,٥ و ١٠ جم/لتر). أوضحت النتائج أن النمو الخضري، التركيب الكيماوي ومكونات الزيت الطيار قد زادت نتيجة إستخدام الرش بالسيانوبكتيريا مع التسميد المعدني بسماد NPK عند ٥٠% و ١٠٠% من الجرعة الموصى بها. وبالتالي إستخدام الرش بالسيانوبكتيريا مع التسميد المعدني عند ٥٠% من الجرعة الموصى بها يؤدي إلى الحصول على منتج عالي الجودة مع إستخدام أقل من الأسمدة المعدنية بالإضافة إلى الحد من التلوث البيئي.