Journal of Horticultural Science & Ornamental Plants 4 (1): 34-49, 2012
ISSN 2079-2158

Effect of Chemical and Bio-Fertilization on Marjoram Plants

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Abstract: The present study was conducted on *Majorana hortensis* L. plants during the two successive seasons 2009 and 2010 at the nursery of Medicinal and Aromatic plants Research Department, Horticulture Research Institute, Agriculture Research Center, Ministry of Agriculture, Dokki, Giza, Egypt. This work was designed in order to investigate the possibility of reducing the need for NPK chemical fertilization as well as improving plant productivity by using bacterial biofertilization as an alternative. The plants of control treatment received only the full recommended rate of NPK chemical fertilization as ammonium sulphate (20.5%N) at the rate of 300 kg/feddan, calcium superphosphate (15.5% P_2O_5) at the rate of 300 kg/feddan and potassium sulphate (48% K_2O) at the rate of 150 kg/feddan. For the other treatments, a single inoculum or mixture of different inoculum combinations of *Azospirillum brasilense* as a nitrogen fixing bacteria (NFB), *Bacillus megatherium* var. *phosphaticum* as a phosphate solubilizing bacteria (PSB) and *Frateuria aurantia* as a potassium solubilization bacteria (KSB) were used in combination with NPK chemical fertilizer as quarter or half dose of the full recommended rate. Results obtained showed that most of the fertilization treatments caused more positive effects on plant height, number of branches per plant, herb fresh weight per plant, herb air dry weight per plant, dry weight of herb per plant, leaves: stems fresh weight ratio, essential oil percentage, oil yield per plant, oil components percentages, total chlorophyll and carotenoids contents in fresh leaves as well as total carbohydrates, N, P and K percentages in the oven (70°C) dry herb. So, partial substitution of chemical NPK fertilizer by bio-fertilizer was recommended. Generally, plants received1/4NPK dose with the biofertilizer gave higher values than those received 1/2NPK with the same biofertilizer during the three cuts of both seasons. Gas chromatography analysis of volatile oil indicated that the main components were terpinen-4-ol, linalool and sabinene. The components were positively affected by different combined treatments between biofertilization and chemical NPK fertilizer. The treatment of 1/4NPK+NFB+PSB was the most efficient among the used treatments for improving most of vegetative characteristics, active constituents and chemical composition of marjoram plants as well as reducing the requirements of NPK chemical fertilizer by 75%.

Key words: Chemical fertilization · Bio-fertilization · Marjoram · *Majorana hortensis*

INTRODUCTION

Marjoram (*Majorana hortensis* L.) plant is a perennial herb, belonging to family Lamiaceae, indigenous to Mediterranean countries. It was known to the ancient Egyptians, Greeks and Romans [1]. Marjoram herb, aerial parts, is used including the stems, leaves and white blossoms. It is cultivated as culinary herbs and garden plants as well. The leaves are used as flavoring. *Majorana hortensis* L. is valued as a medicinal plant for improving antiseptic, antispasmodic, carminative, stimulant and expectorant and nerve tonic rheumatic habits, stimulates moreover the blood circulation, nerve habits, muscle pain, muscle rheumatism, arthritis, flu, cold, bronchitis, stucked cough, asthma, hiccups, slow digestion, bad appetite, menstruation problems, low blood pressure, worm infections, cramps and mould infections [2, 3].

The three essential macronutrients that all plants need for growth are nitrogen (N), phosphorus (P) and Potassium (K). Even only one of the nutrients needed is in short supply, plant growth is limited and yield is reduced especially under harsh growth condition of sandy soil. However, intensive cultivation results in the soil being deficient in these important nutrients. The use of synthetic NPK fertilizers replaces the chemical components that are taken from the soil by growing

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plants. They maintain soil productivity and significantly support food security to improve the quality and quantity of the food available today, although their long-term use is debated by environmentalists. With fertilizers, yield of the plant can often be doubled or even tripled; fertilizers can also be tailored to suit the type of crop that is being grown, creating a better growing environment. Mineral fertilizers are one of the most important tools for agricultural development and will continue to play a decisive role and this irrespective of which new technologies may yet emerge [4].

Biofertilizers are one of the most promising alternatives of supplying nutrients required for the growing plant to substitute chemical fertilizers for safe healthy production for human and the environment. Biofertilization mainly comprises nitrogen fixers (i.e. Rhizobium, Azotobacter, Azospirillum, Azolla or blue green algae), phosphate dissolvers (vesicular arbuscular mycorrhizae) and potassium solubilizers (Bacillus and Pseudomonas, which are termed as silicate bacteria). These organisms may affect their host plant by one or more certain microbial processes such as nitrogen fixation, production of growth promoting substances like auxins and gibberellins, organic acids, changing unavailable forms of nutrients into available ones that can be easily assimilated by plants, synthesis of vitamins, amino acids or elimination of the plant enemies including microbial pathogens, insects and weeds and improving vegetative growth, photosynthetic pigments and chemical composition [5-7].

Although biofertilizers are useful for recycling elements and reserving natural resources many researchers recommended not to use bio-fertilizers alone but they used them to reduce the need for chemical fertilizers which have adverse deleterious environmental effects on public health and national income as well as reduce the cost of fertilizers and labor. In this respect, Kandeel *et al.* [8] reported that dual inoculation of sweet basil plants with Azotobacter+Azospirillum supplemented with half or full dose of the recommended mineral N-fertilizer significantly increased oil percentage and yearly oil yield / plot compared with uninoculated plants. Sakr [9] found that fertilizing senna plants with half dose of N fertilizer (200 Kg ammonium sulphate / fed) in association with biofertilization (*Azospirillum brasilense*, *Bacillus polymyxa*, *Azotobacter chroococcum*, *Klebsiella pneumonia* and *Pseudomonas putida*) increased total herb fresh weight per plant, total carbohydrates as well as N, P and K contents in leaves more than using nitrogenous or bio- fertilizers singly. Kandeel and Sharaf [10] on *Majorana hortensis* L. found that inoculation with *Bacillus circulans* in the presence of half dose inorganic NPK fertilization remarkably increased the different plant growth parameters compared with full dose of NPK (300 Kg/fed ammonium sulphate, 300 Kg/fed calcium super phosphate and 150 Kg/fed potassium sulphate). Al-Qadasi [11] recommended that applying composite culture of Azotobacter, Azospirillum and Bacillus plus quarter dose of NPK fertilizer led to high vegetative growth (plant height, number of branches per plant, herb fresh and dry weights per plant), oil percentage and oil yield per plant as well as contents of total carbohydrates, total chlorophyll, carotenoids, N, P and K of *Ocimum basilicum* herb. Mahfouz and Sharaf-Eldin [12] reported that a mixture of *Azotobacter chroococcum, Azospirillum liboferum* and *Bacillus megatherium* applied with 50% of the recommended dosage of NPK increased vegetative growth of fennel plants (plant height, number of branches per plant and herb fresh and dry weights per plant), total carbohydrates, oil yield per plant and oxygenated compounds in the essential oil as well as nitrogen, phosphorus and potassium levels compared to chemical fertilizer treatment. El-Mekawy [13] on *Thymus capitatus* L. found that plant height, branches number per plant, fresh and dry weights of herb per plant, essential oil percentage, essential oil yield per plant and total carbohydrates content significantly increased as a result of inoculation of *Azotobacter chroococcum* and *Azospirillum lipoferum* with inorganic fertilizer (300 kg Ammonium nitrate /fed equal to 100 kg N) followed by the same m ixture plus 50 kg N/fed and then control (100 kg N/fed) treatment. Azzaz *et al.* [14] on fennel plants indicated that plant height, number of branches per plant, herb dry weight /plant, essential oil percentage and essential oil yield /plant as well as N, P and K percentages in dry herb were augmented as a result of using the recommended NPK + Bio-fertilizer mixtures of *Azotobacter* sp. *Bacillus megatherium* var *Phosphaticum* and *Bacillus circulans*, compared to other treatments including the recommended dose of NPK separately. Abo-Baker and Mostafa [15] on *Hibiscus sabdariffa*, revealed that applying 50% of the recommended dose of NPK plus mixture of *Azospirillum* sp. (nitrogen fixing bacteria, NFB) and *Bacillus polymyxa* (phosphate dissolving bacteria, PDB) improved plant height, number of branches per plant, shoots and roots fresh weights and sepal yield per feddan as well as N, P, K and total chlorophyll contents compared to the control (full recommended dose of NPK alone). Hellal *et al.* [16] on *Anethum graveolens* L., indicated that the highest values of plant height, number of main branches, fresh and dry weights of herb per plant, oil yield and total chlorophyll content as well as N, P and K percentages in dried herb were recorded with the combined treatment of bio-fertilizer (*Azotobacter chroococcum*, *Azospirillum lipoferum*, *Bacillus polymyxa*, *Bacillus megatherium* and *Pseudomonas fluorescens*, mixed in equal parts) plus two third of nitrogen fertilizer recommended dose (200 kg/fed ammonium sulphate) compared to using the biofertilizer in combination with 1/3 or complete recommended dose of mineral nitrogen fertilizer.

The objective of this study was the judicious use of NPK chemical fertilization by investigating the effect of inoculation with bacterial cultures of nitrogen fixing bacteria (*Azospirillum brasilense*); Phosphate solubilizing bacteria (*Bacillus megatherium* var. *phosphaticum*) and potassium solubilization bacteria (*Frateuria aurantia*) as biofertilization, singly or as different combination mixtures, combined with NPK chemical fertilizer as quarter or half dose of the full recommended rate on vegetative traits, active constituents and chemical composition of marjoram (*Majorana hortensis* L.) plants in order to decrease the environmental hazard from chemical fertilizer as well as obtaining a high quality product under the experimental conditions.

MATERIALS AND METHODS

The present study was conducted during the two successive seasons of 2009 and 2010 at the nursery of Medicinal and Aromatic Plants Research Department, Horticulture Research Institute, Agriculture Research Center, Ministry of Agriculture, Dokki, Giza, Egypt. Seeds of *Majorana hortensis* L. were secured from Agriculture Research Center, Ministry of Agriculture, Giza, Egypt. Seeds were sown on November 15th of the two seasons in plastic pots (50 cm diameter) filled with sandy loam soil. Seedlings were transplanted on February 1st of both seasons using plastic pots (30 cm diameter) filled with sandy loam soil (three seedlings per pot). After two weeks, the seedlings were thinned to one plant per pot. Thereafter, the usual agricultural practices were followed as recommended. The mechanical and chemical characteristics of the soil used as growing media during the two seasons (Table 1) were carried out at laboratories of Agriculture Research Center, Ministry of Agriculture, Giza.

The plants of control treatment received only the full recommended rate of NPK chemical fertilization, Egyptian Ministry of Agriculture and Land Reclamation [17], including ammonium sulphate (20.5%N) at the rate of 300 kg/feddan (5.33 g/ pot), calcium super phosphate (15.5% P_2O_5) at the rate of 300 kg/feddan (5.33 g / pot) and potassium sulphate (48% K_2O) at the rate of 150 kg/feddan (2.67 g/ pot). Calcium superphosphate (P) was incorporated with the potting medium of all treatments before transplanting. Ammonium sulphate and potassium sulphate (N and K) were added at three equal doses, the first addition was applied 45 days after transplanting (on March 15th), the second one was added on May 15th (after two weeks of the first cut) and the third dose was added on July15th (after two weeks of the second cut).

Biofertilizers (singly or as different combination mixtures) were used in combination with NPK chemical fertilizer (quarter or half dose of the full recommended rate). Biofertilizer inoculations liquid bacterial cultures of *Azospirillum brasilense* as a nitrogen fixing bacteria (NFB), 5.5 x 10^6 cell /ml; *Bacillus megatherium* var. *phosphaticum* as a phosphate solubilizing bacteria (PSB), 7.2 x 10^6 cell /ml and *Frateuria aurantia* as a potassium solubilization bacteria (KSB), 6 x 10^6 cell/ml were obtained from the Bio Agriculture Research Center, Faculty of Agriculture, Ain Shams University. For the biofertilization treatments, roots of transplants were soaked for 30 minutes in the liquid suspension contains single inoculum or mixture of different inoculum combinations in equal proportions, then plants were directly transferred to the prepared pots. Additional boost (five ml of each inoculum used according to the treatment) was injected in a hole adjacent to the plants after one week from chemical NK application. The experiment included fourteen treatments in addition to the control. Plants were harvested at three times during each season, the first one at commencement of the flowering on May 1st, the second one on July1st and the third cut was on September 1st.

At harvesting, the studied characteristics were determined on the investigated plants of different treatments. Plant vegetative parameters were recorded as plant height (cm), number of branches per plant, fresh and air dry weights of herb per plant (g), dry weight of herb per plant in gram (oven dried at 70°C) and leaves: stems fresh weight ratio. Chemical analyses were made at laboratory of the Medicinal and Aromatic Plants Department, Horticulture Institute, Agriculture Research Center, Ministry of Agriculture, Dokki, Giza, Egypt. The essential oil percentage was determined in the fresh herb. The distillation and the determination of the essential oil were described in the British Pharmacopoeia [18]. Oil yield per plant was calculated by multiplying the

Table 1: The mechanical and chemical analysis of the soil used as growing media during the two seasons of 2009 and 2010

Physical analysis			
Sand%	Clay%	Silt%	Texture class
55.2	18.5	26.3	Sandy loam

Chemical analysis											
		Soluble cations and anions (meq/L)							Available elements (ppm)		
pH	EC dS/m	HCO_3^-	Cl^-	SO_4^{--}	Ca^{++}	Mg^{++}	Na^+	K^+	N	P	K
7.5	4.45	2	17.14	33.48	18.67	14.4	12.79	6.76	24.3	208	340.7

percentage of the oil by weight of the fresh herb per plant. The essential oil obtained from the fresh herb in the third cut of the second season was analyzed using DsChrom 6200 Gas Chromatograph equipped with a flame ionization detector for separation of volatile oil constituents. The use of GLC in the quantitative determination was achieved by following Hoftman [19] and Buzon *et al.* [20] methods. Total chlorophyll as well as carotenoids contents (mg/g) were determined in fresh leaf samples [21]. Dried herb (oven dried at 70°C) was analyzed to determine percentages of total carbohydrates [22], nitrogen [23], phosphorus [24] and potassium [25].

The experiment layout was arranged in a randomized complete block design (RCBD) with three replicates. Each block contained fifteen fertilization treatments that allocated randomly. Each replicate contained 3 pots. Data collected for vegetative growth characteristics and active constituents were subjected to analysis of variance and the means were compared using the "Least Significant Difference (LSD)" test at the 0.05 level [26].

RESULTS AND DISCUSSION

Effect of Chemical and Bio-fertilization on Vegetative Characteristics

Plant Height: Data presented in Table 2 showed that during the first season most of fertilization treatments significantly increased the height of *Majorana hortensis* L. plants as compared to the control (the full recommended rate of NPK chemical fertilizer). Plants received 1/4NPK+NFB+PSB treatment gave the significantly higher plants in the three cuts. In the first and third cuts, the plants received the recommended NPK rate (control) were the shortest plants, whereas in the second cut, the lowest plant height was recorded with plants received 1/2NPK+NFB + PSB + KSB treatment. As to the second season, in the first and second cuts, some treatments significantly increased plant height of *Majorana hortensis* L. as compared to the control, whereas in the third cut most treatments significantly increased plant height as compared to the control plants. In the three cuts, plants received 1/4NPK+NFB +PSB treatment gave the significantly higher plants. In the second cut, plants received 1/4NPK+NFB + PSB + KSB treatment gave the same highest plant height. Such results are in agreement with those reported on *Ocimum basilicum* [11], fennel [12], *Thymus capitatus* [13], *Hibiscus sabdariffa* [15], guar [27], *Carum carvi* [28, 29], marjoram [30] and sage [31].

In both seasons, the mean of the three cuts revealed that all treatments increased plant height than the control except 1/2NPK+NFB+ PSB + KSB treatment which gave the lowest mean plant height; whereas plants received 1/4NPK+NFB+ PSB gave the highest mean plant height. Generally, in both seasons in all cuts, plants received 1/4NPK with biofertilizer were higher than those received 1/2NPK with the same biofertilizer.

Number of Branches/Plant: Data presented in Table 2 showed that in the three cuts of both seasons, most of fertilization treatments regardless of significancy increased number of branches per plant of *Majorana hortensis* L. plant as compared to the control (the recommended rate of chemical NPK fertilizer). Plants received 1/4NPK+NFB+PSB treatment gave higher number of branches/plant as compared to the other combined treatments. In the first cut of the first season and in the second and third cuts of the second season, plants received 1/2NPK+NFB+ PSB + KSB treatment gave the lowest number of branches/ plant. Plants received 1/2NPK+NFB+ KSB treatment gave the lowest number of branches/ plant in the second cut of the first season and in the first cut of the second season. In the third cut of the first season, control plants gave the lowest number of branches/plant.

In both seasons, the mean of the three cuts revealed that most treatments increased the number of branches/plant as compared to the control. The highest mean number of branches/plant was recorded with plants received 1/4NPK+NFB +PSB. In all cuts of both seasons, plants received 1/4NPK with biofertilizer gave higher number of branches/plant than that received 1/2NPK with the same biofertilizer. The obtained positive effects on number of branches per plant due to applying chemical and biofertilizers are coincided with those reported on *Ocimum basilicum* [11], fennel [12, 32,33], sage [31], *Thymus capitatus* [13] and *Hibiscus sabdariffa* [15, 34].

Leaves Stems Fresh Weight Ratio: Data presented in Table 3 revealed that in the three cuts of the first season, leaves: stems fresh weight ratio was significantly increased by most of fertilization treatments as compared to the control. Plants received 1/4NPK+PSB treatment had significantly higher leaves: stems fresh weight ratios as compare to the other treatments, in most cases.

The lowest leaves: stems fresh weight ratios were recorded with plants received 1/4NPK+NFB+ PSB, 1/2NPK+NFB+ KSB and control treatments, respectively. The mean of the three cuts revealed that all fertilization treatments increased the leaves: stems fresh weight ratio except 1/2NPK+NFB+ KSB, as compared to the control. The highest mean leaves: stems fresh weight ratio was recorded with plants received 1/4NPK+PSB.

As to the second season, in the first and second cuts, few treatments significantly increased leaves: stems fresh weight ratios as compared to the control, whereas most treatments in the third cut significantly increased leaves: stems fresh weight ratios as compared to the control. The significantly higher leaves: stems fresh weight ratios were recorded with plants received 1/4NPK+NFB+ PSB, 1/4NPK+KSB and 1/4NPK+PSB in the first, second and third cuts, respectively as compared to the other treatments, in most cases. The lowest leaves: stems fresh weight ratios were recorded with plants received 1/2NPK+NFB+PSB+KSB in the first and third cuts and with plants received 1/2NPK+ PSB+ KSB treatment in the second cut. The mean of the three cuts revealed that some treatments increased the leaves: stems fresh weight ratio as compared to the control. The highest mean leaves: stems fresh weight ratio was recorded with plants received 1/4NPK+KSB, whereas the lowest mean leaves: stems fresh weight ratio was recorded with plants received 1/2NPK+NFB+ PSB + KSB.

In both seasons, combinations between half dose of chemical NPK fertilization and bio-fertilizer were generally less effective than adding quarter dose of chemical fertilization with the same bio-fertilizer in most cases. Results obtained agreed with Abd El-Azim [31] who found that fertilizing *Salvia officinalis* L. plants with 150 Kg ammonium sulphate, 150 Kg calcium superphosphate and 100 Kg potassium sulphate /fed caused a significant increase in leaves: branches ratio. In this respect, Sakr [9] found that fertilizing senna plants with full dose of N fertilizer (400 Kg ammonium sulphate / fed) gave the highest leaf: stem fresh weight ratio compared to other treatments including biofertilization (*Azospirillum brasilense*, *Bacillus polymyxa*, *Azotobacter chroococcum*, *Klebsiella pneumonia* and *Pseudomonas putida*) either alone or associated with half dose of nitrogen.PK were used as a basal dressing for all treatments at 300 Kg calcium superphosphate / fed and 100Kg potassium sulphate / fed.

Fresh, Air Dry and Dry Weights of Herb/Plant: Data presented in Tables 3 and 4 showed that in both seasons, in the first cut few treatments significantly increased herb fresh, air dry and dry weights of *Majorana hortensis* L. plant as compared to the control. In the second and third cuts most treatments significantly increased herb fresh, air dry and dry weights/plant of *Majorana hortensis* L. as compared to the control. In both seasons, in each cut, the plants received 1/4NPK+NFB+ PSB treatment gave the significantly heavier herb fresh, air dry and dry weights/plant as well as the highest total weights/season. Generally, the total of the three cuts revealed that most treatments increased herb fresh, air dry and dry weights/plant than the control. Such results are in agreement with those reported on senna [9], *Ocimum basilicum* [11], fennel [12], *Thymus capitatus* [13], *Hibiscus sabdariffa* [15], sage [31] and *Euryops pectinatus* [35].

On the other hand, in most cases, plants received 1/2NPK+ NFB + KSB treatment gave the lowest herb fresh, air dry and dry weights/plant as well as total weights/season as compared to the other combined treatments. Generally, in all cuts of both seasons, plants received 1/4NPK with biofertilizer gave heavier herb fresh, air dry and dry weights/plant as well as total weights/season than those received 1/2NPK with the same biofertilizer. Fertilizing marjoram plants with NPK plus biofertilizers resulted in a positive impact on biomass production compared to the control. Plants receiving bio-nitrogen fertilization produced higher number of both branches and leaves giving higher fresh weights and total herb air dry and dry yields.

Effect of Chemical and Bio-fertilization on Active Constituents of Marjoram Plant

Essential Oil Percentage: Data presented in Table 5 showed that in both seasons, most treatments significantly increased essential oil percentage of

Table 2: Effect of chemical and bio-fertilization on plant height and number of branches per plant of marjoram plant during the two growing seasons of 2009 and 2010

	Plant height (cm)								Number of branches/ plant							
	First Season				Second Season				First Season				Second Season			
Treatments	1st cut	2nd cut	3rd cut	Mean	1st cut	2nd cut	3rd cut	Mean	1st cut	2nd cut	3rd cut	Mean	1st cut	2nd cut	3rd cut	Mean
Control(NPK)	42.67	25.22	18.00	28.63	51.00	24.22	19.11	31.44	11.44	15.56	17.22	14.74	11.22	14.33	21.67	15.74
1/4NPK+NFB	46.33	32.22	24.67	34.41	50.78	24.78	19.89	31.81	13.89	16.33	21.22	17.15	13.56	16.11	21.22	16.96
1/4NPK+PSB	51.11	35.89	25.89	37.63	55.67	28.67	27.00	37.11	14.89	18.78	22.33	18.67	13.78	19.00	23.33	18.70
1/4NPK+KSB	46.44	32.56	23.78	34.26	49.22	26.44	26.00	33.89	13.33	16.56	20.67	16.85	12.22	15.44	20.89	16.18
1/4NPK+NFB+ PSB	52.55	36.00	27.78	38.78	57.45	29.56	29.33	38.78	15.89	20.22	23.11	19.74	14.89	19.78	24.56	19.74
1/4NPK+NFB+KSB	47.11	31.67	23.33	34.04	48.67	23.22	23.89	31.93	12.89	15.67	20.00	16.19	12.22	15.33	21.22	16.26
1/4NPK+PSB+ KSB	49.56	32.44	25.11	35.70	53.33	23.44	24.55	33.77	13.00	16.89	20.44	16.78	13.00	16.00	22.44	17.15
1/4NPK+NFB+PSB+KSB	47.89	30.89	24.44	34.41	50.44	29.56	24.33	34.78	13.56	16.44	22.89	17.63	12.78	15.11	22.22	16.70
1/2NPK+NFB	44.89	31.00	20.33	32.07	42.22	22.44	22.89	29.18	13.11	15.56	20.67	16.45	11.56	14.89	20.78	15.74
1/2NPK+PSB	46.89	30.56	24.44	33.96	42.00	25.11	23.00	30.04	13.11	16.56	20.67	16.78	12.11	17.44	22.56	17.37
1/2NPK+ KSB	46.11	31.22	22.33	33.22	40.67	21.33	21.33	27.78	11.44	16.00	20.44	15.96	11.67	14.67	20.22	15.52
1/2NPK+NFB+ PSB	49.44	31.22	25.00	35.22	41.00	25.44	23.33	29.92	13.45	16.56	21.22	17.08	12.56	17.56	23.22	17.78
1/2NPK+NFB +KSB	45.89	30.22	22.56	32.89	43.78	21.11	21.22	28.70	11.11	14.11	18.33	14.52	10.78	14.22	20.11	15.04
1/2NPK+ PSB+ KSB	48.56	31.67	22.56	34.26	40.11	22.33	23.22	28.55	11.48	14.33	19.67	15.16	11.11	15.22	20.67	15.67
1/2NPK+NFB+PSB+KSB	45.33	19.11	20.55	28.33	36.67	22.11	22.44	27.07	11.00	16.11	20.00	15.70	10.94	12.89	19.56	14.46
L.S.D.5%	2.40	3.10	2.60		4.10	1.90	1.50		0.59	1.60	N.S		1.30	0.90	1.70	

Control (NPK): Full recommended rate, 1/4NPK: Quarter of recommended rate, 1/2NPK: Half of recommended rate
NFB: Nitrogen fixing bacteria, PSB: phosphate solubilizing bacteria, KSB: potassium solubilization bacteria

Table 3: Effect of chemical and bio-fertilization on leaves: stems fresh weight ratio and herb fresh weight of marjoram plant during the two growing seasons of 2009 and 2010

	Leaves: stems fresh weight ratio								Herb fresh weight (g/plant)							
	First Season				Second Season				First Season				Second Season			
Treatments	1st cut	2nd cut	3rd cut	Mean	1st cut	2nd cut	3rd cut	Mean	1st cut	2nd cut	3rd cut	Total	1st cut	2nd cut	3rd cut	Total
Control(NPK)	31.35	16.52	8.66	18.84	39.62	24.75	17.88	27.42	43.23	20.82	13.14	77.19	55.27	29.60	23.02	107.89
1/4NPK+NFB	33.48	20.92	18.76	24.39	42.29	23.67	20.55	28.84	47.62	28.47	23.94	100.03	59.18	38.29	26.59	124.06
1/4NPK+PSB	44.56	26.97	23.32	31.62	53.20	29.02	22.14	34.79	54.43	33.74	25.77	113.94	62.04	47.77	29.37	139.18
1/4NPK+KSB	39.03	22.03	16.42	25.83	47.61	36.36	21.83	35.27	47.87	26.47	20.79	95.13	47.83	35.66	25.94	109.43
1/4NPK+NFB+ PSB	26.69	19.47	17.51	21.22	54.24	25.49	20.97	33.57	54.46	35.51	26.28	116.25	65.51	48.18	31.74	145.43
1/4NPK+NFB+KSB	29.34	20.14	22.66	24.05	37.60	16.86	20.84	25.10	40.90	28.03	23.00	91.93	54.78	31.04	26.97	112.79
1/4NPK+PSB+ KSB	39.53	21.93	20.49	27.32	42.33	24.31	21.20	29.28	48.80	29.57	24.89	103.26	58.31	39.91	27.53	125.75
1/4NPK+NFB+PSB+KSB	39.71	19.05	14.38	24.38	36.29	20.64	17.84	24.92	48.42	29.67	20.28	98.37	55.79	37.24	27.86	120.89
1/2NPK+NFB	36.17	20.89	20.86	25.97	35.70	23.01	19.51	26.07	44.90	24.28	22.07	91.25	39.26	29.29	25.96	94.51
1/2NPK+PSB	37.75	23.63	18.69	26.69	25.03	31.49	20.64	25.72	45.22	29.59	21.68	96.49	38.44	39.03	28.37	105.84
1/2NPK+ KSB	38.96	24.48	21.89	28.44	21.74	21.41	20.26	21.14	44.08	25.72	20.29	90.09	31.26	25.28	21.30	77.84
1/2NPK+NFB+ PSB	38.37	18.94	16.44	24.58	22.98	22.08	21.64	22.23	49.49	32.78	22.13	104.40	39.07	42.40	28.79	110.26
1/2NPK+NFB +KSB	29.72	12.26	13.67	18.55	27.18	19.08	20.52	22.26	38.34	21.94	16.68	76.96	30.27	22.30	22.20	74.77
1/2NPK+ PSB+ KSB	31.17	21.99	16.23	23.13	27.40	14.60	19.77	20.59	40.03	26.59	19.02	85.64	38.58	27.96	26.10	92.64
1/2NPK+NFB+PSB+KSB	33.24	23.12	17.11	24.49	21.36	16.28	16.86	18.17	38.80	26.70	20.17	85.67	35.52	29.08	26.44	91.04
L.S.D.5%	4.90	3.80	3.10		4.80	3.50	1.60		5.40	4.30	3.30		7.80	4.60	1.60	

Control (NPK): Full recommended rate, 1/4NPK: Quarter of recommended rate, 1/2NPK: Half of recommended rate
NFB: Nitrogen fixing bacteria, PSB: phosphate solubilizing bacteria, KSB: potassium solubilization bacteria

Table 4: Effect of chemical and bio-fertilization on herb air dry weight and dry weight of marjoram plant during the two growing seasons of 2009 and 2010

	Herb air dry weight (g/plant)								Herb dry weight (g/plant)							
	First Season				Second Season				First Season				Second Season			
Treatments	1st cut	2nd cut	3rd cut	Total	1st cut	2nd cut	3rd cut	Total	1st cut	2nd cut	3rd cut	Total	1st cut	2nd cut	3rd cut	Total
Control(NPK)	20.09	8.86	5.67	34.62	25.44	14.22	10.81	50.47	14.70	7.08	4.47	26.25	18.79	10.06	7.83	36.68
1/4NPK+NFB	21.92	12.62	10.68	45.22	28.01	19.74	13.14	60.89	16.19	9.68	8.14	34.01	20.12	13.02	9.04	42.18
1/4NPK+PSB	26.31	16.19	12.02	54.52	29.82	21.78	13.76	65.36	18.51	11.47	8.76	38.74	21.09	16.24	9.99	47.32
1/4NPK+KSB	23.34	12.11	9.38	44.83	22.48	17.74	12.47	52.69	16.28	9.00	7.07	32.35	16.26	12.12	8.82	37.20
1/4NPK+NFB+ PSB	26.81	16.81	12.08	55.70	33.41	22.80	15.61	71.82	18.52	12.07	8.94	39.53	22.27	16.38	10.79	49.44
1/4NPK+NFB+KSB	19.93	13.36	11.19	44.48	26.15	14.70	12.78	53.63	13.91	9.53	7.82	31.26	18.63	10.55	9.17	38.35
1/4NPK+PSB+ KSB	22.12	13.52	11.94	47.58	29.96	17.55	13.11	60.62	16.59	10.05	8.46	35.10	19.83	13.57	9.36	42.76
1/4NPK+NFB+PSB+KSB	22.58	13.48	9.17	45.23	28.22	16.52	13.20	57.94	16.46	10.09	6.90	33.45	18.97	12.66	9.47	41.10
1/2NPK+NFB	20.98	10.58	10.05	41.61	21.15	12.81	12.67	46.63	15.27	8.26	7.50	31.03	13.35	9.96	8.83	32.14
1/2NPK+PSB	20.54	13.67	10.24	44.45	23.44	17.06	14.85	55.35	15.37	10.06	7.37	32.80	13.07	13.27	9.65	35.99
1/2NPK+ KSB	19.77	11.62	9.19	40.58	15.89	11.21	10.23	37.33	14.99	8.74	6.90	30.63	10.63	8.60	7.24	26.47
1/2NPK+NFB+ PSB	21.79	14.70	9.96	46.45	20.30	19.38	14.72	54.4	16.83	11.15	7.52	35.50	13.28	14.42	9.79	37.49
1/2NPK+NFB +KSB	17.07	9.50	7.43	34.00	15.45	10.39	10.84	36.68	13.04	7.46	5.67	26.17	10.29	7.58	7.55	25.42
1/2NPK+ PSB+ KSB	16.56	12.55	9.01	38.12	19.26	12.21	12.29	43.76	13.61	9.04	6.47	29.12	13.12	9.51	8.87	31.50
1/2NPK+NFB+PSB+KSB	17.83	11.65	8.77	38.25	16.25	13.47	12.16	41.88	13.19	9.08	6.86	29.13	12.08	9.89	8.99	30.95
L.S.D.5%	2.10	1.30	0.99		3.30	1.80	0.82		1.27	1.17	0.73		1.36	1.21	0.56	

Control (NPK): Full recommended rate, 1/4NPK: Quarter of recommended rate, 1/2NPK: Half of recommended rate
NFB: Nitrogen fixing bacteria, PSB: phosphate solubilizing bacteria, KSB: potassium solubilization bacteria

Table 5: Effect of chemical and bio-fertilization on essential oil percentage and oil yield of marjoram plant during the two growing seasons of 2009 and 2010

	Essential oil percentage								Essential oil yield (ml /plant)							
	First Season				Second Season				First Season				Second Season			
Treatments	1st cut	2nd cut	3rd cut	Mean	1st cut	2nd cut	3rd cut	Mean	1st cut	2nd cut	3rd cut	Total	1st cut	2nd cut	3rd cut	Total
Control (NPK)	0.45	0.58	0.42	0.48	0.49	0.53	0.54	0.52	0.19	0.12	0.06	0.37	0.27	0.16	0.12	0.55
1/4NPK+NFB	0.52	0.58	0.47	0.52	0.54	0.63	0.71	0.63	0.25	0.17	0.11	0.53	0.32	0.24	0.19	0.75
1/4NPK+PSB	0.65	0.60	0.53	0.59	0.74	0.53	0.58	0.62	0.35	0.20	0.14	0.69	0.46	0.25	0.17	0.88
1/4NPK+KSB	0.67	0.59	0.54	0.60	0.61	0.52	0.45	0.53	0.32	0.16	0.11	0.59	0.29	0.19	0.12	0.60
1/4NPK+NFB+ PSB	0.73	0.71	0.61	0.68	0.82	0.75	0.75	0.77	0.40	0.25	0.16	0.81	0.54	0.36	0.24	1.14
1/4NPK+NFB+KSB	0.67	0.60	0.57	0.61	0.57	0.53	0.59	0.56	0.27	0.17	0.13	0.57	0.31	0.16	0.16	0.63
1/4NPK+PSB+ KSB	0.70	0.67	0.49	0.62	0.54	0.73	0.72	0.66	0.34	0.20	0.12	0.66	0.31	0.29	0.20	0.80
1/4NPK+NFB+PSB+KSB	0.63	0.58	0.42	0.54	0.64	0.72	0.60	0.65	0.31	0.17	0.09	0.57	0.36	0.27	0.17	0.80
1/2NPK+NFB	0.49	0.50	0.37	0.45	0.53	0.53	0.70	0.59	0.22	0.12	0.08	0.42	0.21	0.16	0.18	0.55
1/2NPK+PSB	0.73	0.65	0.75	0.71	0.85	0.74	0.74	0.78	0.33	0.19	0.16	0.68	0.33	0.29	0.21	0.83
1/2NPK+ KSB	0.74	0.65	0.63	0.67	0.61	0.56	0.45	0.54	0.33	0.17	0.13	0.63	0.19	0.14	0.10	0.43
1/2NPK+NFB+ PSB	0.56	0.57	0.59	0.57	0.75	0.54	0.55	0.61	0.28	0.19	0.13	0.60	0.29	0.23	0.16	0.68
1/2NPK+NFB +KSB	0.72	0.57	0.52	0.60	0.57	0.52	0.56	0.55	0.28	0.13	0.09	0.50	0.17	0.12	0.12	0.41
1/2NPK+ PSB+ KSB	0.57	0.52	0.35	0.48	0.54	0.72	0.72	0.66	0.23	0.14	0.07	0.44	0.21	0.20	0.19	0.60
1/2NPK+NFB+PSB+KSB	0.61	0.51	0.33	0.48	0.55	0.73	0.60	0.63	0.24	0.14	0.07	0.45	0.20	0.21	0.16	0.57
L.S.D.5%	0.04	0.03	0.07		0.05	0.04	0.05		0.04	0.03	0.03	_	0.05	0.03	0.02	_

Control (NPK): Full recommended rate, 1/4NPK: Quarter of recommended rate, 1/2NPK: Half of recommended rate

NFB: Nitrogen fixing bacteria, PSB: phosphate solubilizing bacteria, KSB: potassium solubilization bacteria

Majorana hortensis L. fresh herb as compared to the control. 1/2NPK+KSB treatment resulted in the highest essential oil percentage in the first cut of the first season, while 1/4NPK+NFB+ PSB treatment resulted in the highest values in the second cut of the first season as well as the second and third cuts of the second season. The treatment included 1/2NPK+PSB resulted in the highest essential oil percentage in the third cut of the first season and in the first cut of the second season. In both seasons, the plants received 1/2NPK+PSB gave the highest mean of essential oil percentage. In many cases, in both seasons, plants received 1/4NPK with biofertilizer gave higher essential oil percentage than those received 1/2NPK with the same biofertilizer. These results are consisting with those reported on *Ocimum basilicum* plants [8, 11], *Thymus capitatus* [13] and fennel plants [14].

Essential Oil Yield per Plant: Data presented in Table 5 showed that in both seasons, most treatments significantly increased essential oil yield of *Majorana hortensis* L. plant as compared to the control. In all cuts of both seasons, plants received 1/4NPK+NFB+ PSB gave higher essential oil yield as compared to other combined treatments. In the first season, the plants received recommended NPK treatment (control plants) gave the lowest essential oil yield, whereas plants received 1/2NPK+NFB +KSB gave the lowest essential oil yield in the first and second cuts of the second season. These results are in agreement with those reported on fennel plants [12, 14], *Thymus capitatus* [13] and *Anethum graveolens* [16].

In both seasons, most treatments increased total essential oil yield per plant as compared to the control. Also, plants received 1/4NPK+NFB+ PSB treatment gave the highest total essential oil yield per plant in both seasons. Generally, in both seasons, plants received 1/4NPK with biofertilizer gave higher essential oil yield per plant than those received 1/2NPK with the same biofertilizer.

Essential Oil Components: The percentages of the essential oil components according to the gas chromatography (GC) analysis of oil samples are shown in Table 6. The GC profile of the essential oil of the plants showed sixteen compounds (presenting approximately 94.60-98.75 % of essential oil composition), namely α-thujene, α-pinen, sabinene, β-myrcene, α-terpine, α-phyllandrene, β-phyllandrene, limonene, linalool, linalyl acetate, terpinen-4-ol, thuyanol, α-terpineol, thuyan-4-ol and β-caryophyllene as well as one unknown components (U.K). The main components were terpinen-4-ol (the most important essential oil component), linalool, limonene, β-Phyllandrene and sabinene. This agrees with prior studies, as it was reported that essential oil components of marjoram are monoterpenoids: α-pinene, beta-pinene, sabinene, myrcene, α-terpinene, y-terpinene, paracymene, terpinolene α-phellandrene, β-phellandrene. Sesquiterpenoids: β-caryophyllene, α-humulene. Monoterpenols: linalool, terpine-4-ol, α-terpineol, cisthuyanol- 4, trans-thuyanol-4. Terpenic esters: linalyl-acetate, terpenyl-acetate, geranyl-acetate. Phenol-methyl-ethers: trans-anethol [3]. Also, Edris *et al.*

[36] stated that essential oil components of marjoram are terpinen-4-ol, trans-sabinene hydrate, linalool, thujanol, terpinolene and thymol. El-Ghorab *et al*. [37] estimated the volatile extract compositions of marjoram (Majorana hortensis Moench) leaves by gas chromatography-mass spectrometry (GC-MS). The major compounds were terpinen-4-ol, γ-terpinene, trans-sabinene hydrate, linalool, trans-sabinene hydrate acetate, thujanol, terpinolene and thymol.

Data presented in Table 6 and Figs. 1-4 showed that the effect of different fertilization treatments on percentages of the essential oil components differed according to each treatment. The highest terpinen-4-ol content (26.29%) was found in the oil of plants fertilized with 1/4NPK+NFB+ PSB, followed by plants received 1/4NPK+KSB (24.72%) then the treatment 1/2NPK+NFB +PSB+ KSB (22.22%), compared to the control (18.91%). For linalool, 1/2NPK+NFB +PSB treatment gave the highest components percentage (34.98%), followed by 1/4NPK+ KSB (33.83%) followed by 1/2NPK+NFB +PSB (31.95%) then 1/4NPK+NFB + KSB (31.33%). Regarding to limonene content, the result showed that most fertilization treatments decreased limonene except 1/2NPK+NFB+ KSB. On the contrary, most fertilization treatments resulted in higher sabinene percentage as compared to the control (5.56%). 1/4NPK+NFB+ PSB treatment gave the highest β-Phyllandrene content (11.27%) compared to other treatments. All fertilization treatments decreased the α-phyllandrene content compared to the control (5.43%) except the treatment consisting of 1/4NPK+NFB which increased the percentage to 6.40%. The highest value of sabinene content (8.47%) was detected in oil of plants fertilized with 1/4NPK+NFB.

Among the different fertilization treatments, 1/2NPK+NFB+PSB was the most effective treatment for increasing the total content of main components (giving a value of 98.75%) followed by 1/2NPK+PSB treatment (98.62%), while the lowest total content of main components (94.60%) was noticed with 1/4NPK+NFB + KSB treatment whereas the control recorded 95.99%.

Effect of Chemical and Bio-fertilization on Pigments, Total Carbohydrates and Elements Contents of Marjoram Plant

Leaf Pigments Contents: Data presented in Table 7 revealed that in the three cuts of both seasons the synthesis and accumulation of total chlorophyll and carotenoids in fresh leaves of *Majorana hortensis* L. plants were increased by the application of most fertilization treatments, as compared to the control

Table 6: Effect of chemical and bio-fertilization on essential oil components of marjoram plant during the two growing seasons of 2009 and 2010

	Treatments														
oil components%	Control (NPK)	1/4NPK +NFB	1/4NPK +PSB	1/4NPK +KSB	1/4NPK+ NFB+ PSB	1/4NPK+ NFB+ KSB	/4NPK+ PSB+ KSB	1/4NPK+NFB +PSB+KSB	1/2NPK +NFB	1/2NPK + PSB	1/2NPK + KSB	1/2NPK+ NFB+ PSB	1/2NPK+ NFB+ KSB	1/2NPK+ PSB+ KSB	1/2NPK+NFB +PSB+KSB
α-Thujene	0.53	0.93	0.69	0.36	0.71	0.59	0.66	0.57	0.51	0.54	0.63	0.46	0.54	0.43	0.60
α-pinen	0.49	0.87	0.78	0.40	0.67	0.55	0.63	0.68	0.71	0.66	0.60	0.74	0.60	0.57	0.73
Sabinene	5.56	8.47	7.61	4.62	8.43	5.56	6.65	6.27	6.33	6.24	6.87	6.69	6.35	6.64	6.50
β-Myrcene	2.48	3.21	2.97	1.91	2.92	2.76	2.72	3.02	2.62	2.86	2.69	2.93	2.69	2.81	2.77
α-Terpine	3.82	3.88	4.22	2.90	5.84	3.87	4.94	4.41	4.13	4.13	4.45	3.69	4.22	4.14	4.28
α-Phyllandrene	5.43	2.80	2.95	2.99	6.40	2.41	2.50	5.08	4.90	4.69	2.49	4.11	4.75	2.81	4.68
β-Phyllandrene	8.00	7.13	7.41	5.17	11.27	7.32	9.08	8.59	7.93	8.08	8.27	7.05	8.18	7.71	8.60
limonene	8.55	7.40	7.66	8.22	7.98	7.97	7.34	7.99	7.33	7.45	7.45	7.76	8.99	8.57	7.56
Linalool	29.44	31.95	29.19	33.83	15.27	31.11	26.29	28.80	29.74	30.11	29.32	34.98	27.65	26.94	26.64
Linalyl acetate	1.81	1.47	1.57	2.00	1.50	1.49	1.87	1.69	1.95	2.06	1.48	1.88	1.91	1.71	1.95
U.K	0.61	0.50	0.52	0.70	0.65	0.46	0.85	0.80	1.10	0.95	0.52	0.89	0.91	0.54	0.70
Terpinen-4-ol	18.91	17.06	20.62	24.72	26.29	17.73	21.12	19.78	19.53	20.13	19.85	16.83	20.06	21.41	22.22
Thuyanol	4.37	4.38	5.35	5.76	6.10	6.03	5.62	4.64	5.64	6.53	5.57	6.22	5.40	6.03	6.15
α-Terpineol	0.89	1.04	1.10	1.24	0.82	1.01	0.62	0.81	0.57	0.70	0.55	0.78	0.84	0.71	0.66
Thuyan-4-ol	2.32	2.30	1.92	1.61	2.29	2.85	2.02	2.21	2.39	2.14	2.62	2.12	2.54	2.65	2.24
β-Caryophyllene	2.78	2.29	1.53	1.32	0.89	2.89	2.33	1.68	2.09	1.35	3.04	1.62	1.96	2.47	2.02
Total components	95.99	95.68	96.09	97.75	98.03	94.60	95.24	97.02	97.47	98.62	96.40	98.75	97.59	96.14	98.30

Control (NPK): Full recommended rate, 1/4NPK: Quarter of recommended rate, 1/2NPK: Half of recommended rate

NFB: Nitrogen fixing bacteria, PSB: phosphate solubilizing bacteria, KSB: potassium solubilization bacteria

Table 7: Effect of chemical and bio-fertilization on total chlorophyll and carotenoids contents of marjoram plant during the two growing seasons of 2009 and 2010

	Total chlorophyll content (mg/g fresh weight)						Carotenoids content (mg/g fresh weight)					
	First Season			Second Season			First Season			Second Season		
Treatments	1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut
Control(NPK)	1.20	1.20	1.75	0.76	0.57	0.95	0.36	0.21	0.25	0.14	0.18	0.20
1/4NPK+NFB	1.62	1.66	1.92	1.83	1.27	1.15	0.61	0.24	0.85	0.31	0.28	0.58
1/4NPK+PSB	1.60	1.53	1.94	1.61	1.10	1.14	0.46	0.25	0.65	0.22	0.24	0.44
1/4NPK+KSB	1.46	1.66	1.95	1.48	1.00	1.13	0.37	0.24	0.73	0.23	0.23	0.48
1/4NPK+NFB+ PSB	2.01	2.03	2.39	1.98	1.36	1.19	0.62	0.30	1.03	0.49	0.39	0.76
1/4NPK+NFB+KSB	1.63	2.00	1.90	1.87	1.04	1.11	0.66	0.30	0.81	0.21	0.25	0.51
1/4NPK+PSB+ KSB	1.38	1.35	1.97	1.49	0.91	1.37	0.50	0.26	0.60	0.16	0.21	0.38
1/4NPK+NFB+PSB+KSB	1.91	1.74	2.28	1.58	1.10	1.30	0.49	0.25	0.62	0.23	0.24	0.43
1/2NPK+NFB	1.32	1.24	1.76	0.96	0.62	0.99	0.44	0.22	0.43	0.15	0.19	0.29
1/2NPK+PSB	1.62	1.25	1.92	1.57	1.06	1.12	0.61	0.26	0.96	0.20	0.23	0.58
1/2NPK+ KSB	1.55	1.57	1.82	1.40	0.92	1.13	0.60	0.24	0.82	0.20	0.22	0.51
1/2NPK+NFB+ PSB	1.43	1.63	1.81	1.51	1.07	1.06	0.51	0.24	0.50	0.21	0.22	0.35
1/2NPK+NFB +KSB	1.60	1.78	2.13	1.46	1.25	1.27	0.60	0.22	0.63	0.18	0.19	0.40
1/2NPK+ PSB+ KSB	1.84	1.85	2.07	1.68	1.21	1.20	0.65	0.22	0.69	0.21	0.21	0.45
1/2NPK+NFB+PSB+KSB	1.39a	1.53	1.98	1.21	0.74	1.14	0.41	0.22	0.56	0.16	0.20	0.36

Control (NPK): Full recommended rate, 1/4NPK: Quarter of recommended rate, 1/2NPK: Half of recommended rate

NFB: Nitrogen fixing bacteria, PSB: phosphate solubilizing bacteria, KSB: potassium solubilization bacteria

Table 8: Effect of chemical and bio-fertilization on total carbohydrates and nitrogen contents of marjoram plant during the two growing seasons of 2009 and 2010

	Total carbohydrates (% of dry weight)						N (% of dry weight)					
	First Season			Second Season			First Season			Second Season		
Treatments	1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut
Control(NPK)	14.37	15.36	14.33	15.13	16.37	16.33	1.46	1.45	1.39	1.23	1.22	1.25
1/4NPK+NFB	17.28	22.22	18.77	21.23	23.21	24.68	2.15	2.07	2.09	1.89	1.76	2.02
1/4NPK+PSB	22.72	18.27	19.26	17.78	25.07	25.51	2.13	1.99	2.00	1.85	1.70	1.90
1/4NPK+KSB	19.75	15.81	24.69	17.84	16.79	17.83	1.51	1.64	1.39	1.24	1.38	1.38
1/4NPK+NFB+ PSB	25.19	24.20	26.15	25.53	26.43	25.98	2.37	2.35	2.41	2.16	2.00	2.17
1/4NPK+NFB+KSB	18.77	15.84	24.69	25.68	24.56	24.63	2.26	2.09	2.30	1.99	1.80	2.11
1/4NPK+PSB+ KSB	17.04	16.79	17.28	24.20	16.84	17.28	1.94	1.66	1.56	1.66	1.39	1.86
1/4NPK+NFB+PSB+KSB	24.69	23.70	15.33	21.73	19.26	22.27	2.28	2.06	2.20	1.99	1.76	2.01
1/2NPK+NFB	17.28	19.75	19.26	18.27	18.30	19.26	2.18	1.83	1.93	1.89	1.55	1.84
1/2NPK+PSB	17.28	16.32	15.80	17.35	24.64	17.31	1.91	1.67	1.88	1.63	1.39	1.66
1/2NPK+ KSB	14.81	16.33	16.30	16.84	17.32	17.83	1.45	1.38	1.36	1.19	1.11	1.22
1/2NPK+NFB+ PSB	16.30	23.70	18.77	17.28	20.25	24.63	2.19	1.99	2.03	1.88	1.70	1.86
1/2NPK+NFB +KSB	15.31	15.81	15.80	16.81	19.26	23.59	1.89	1.91	1.07	1.60	1.63	1.54
1/2NPK+ PSB+ KSB	20.25	21.68	20.25	15.41	17.32	17.78	1.73	1.62	1.83	1.46	1.37	1.55
1/2NPK+NFB+PSB+KSB	14.62	15.41	15.83	15.89	17.36	17.32	1.93	1.74	1.64	1.66	1.44	1.77

Control (NPK): Full recommended rate, 1/4NPK: Quarter of recommended rate, 1/2NPK: Half of recommended rate

NFB: Nitrogen fixing bacteria, PSB: phosphate solubilizing bacteria, KSB: potassium solubilization bacteria

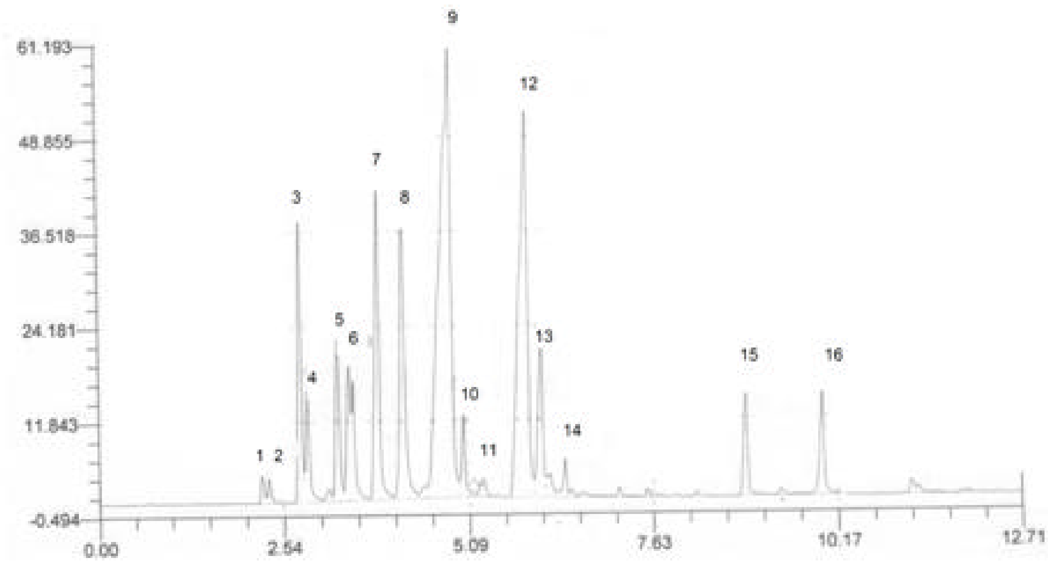

Fig. 1: Chromatogram of marjoram essential oil obtained by hydrodistillation of control
1.a-Thujene, 2.a-Pinene, 3. Sabinene, 4 .ß Myrcene, 5. a – Terpinene, 6. a- phyllandrene 7. ß-phyllandrene ,8. Limonene, 9. Linalool, 10.Linalyl acetate, 11.U.K, 12.Terpine-4-ol, 13. Thuyanol, 14. a-terpineol, 15. Thuyan-4-ol, 16.ß-caryophllene

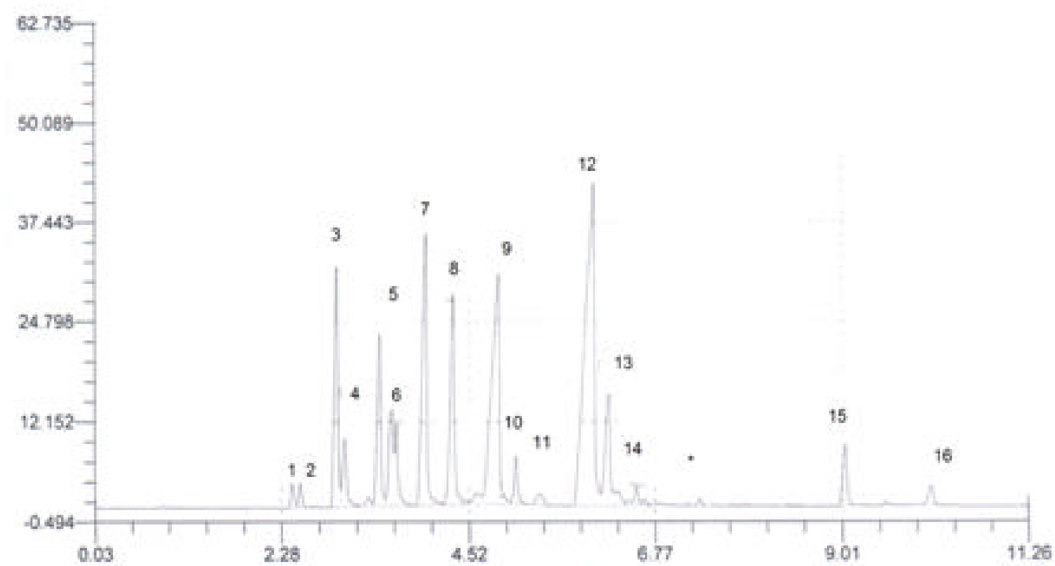

Fig. 2: Chromatogram of marjoram essential oil obtained by hydrodistillation of 1/4 NPK+ NFB +PSB treatment
1.a-Thujene, 2.a-Pinene, 3. Sabinene, 4. ß Myrcene, 5. a – Terpinene, 6. a- phyllandrene 7. ß-phyllandrene ,8. Limonene, 9. Linalool, 10.Linalyl acetate, 11.U.K, 12.Terpine-4-ol, 13. Thuyanol, 14. a-terpineol, 15. Thuyan-4-ol, 16.ß-caryophllene

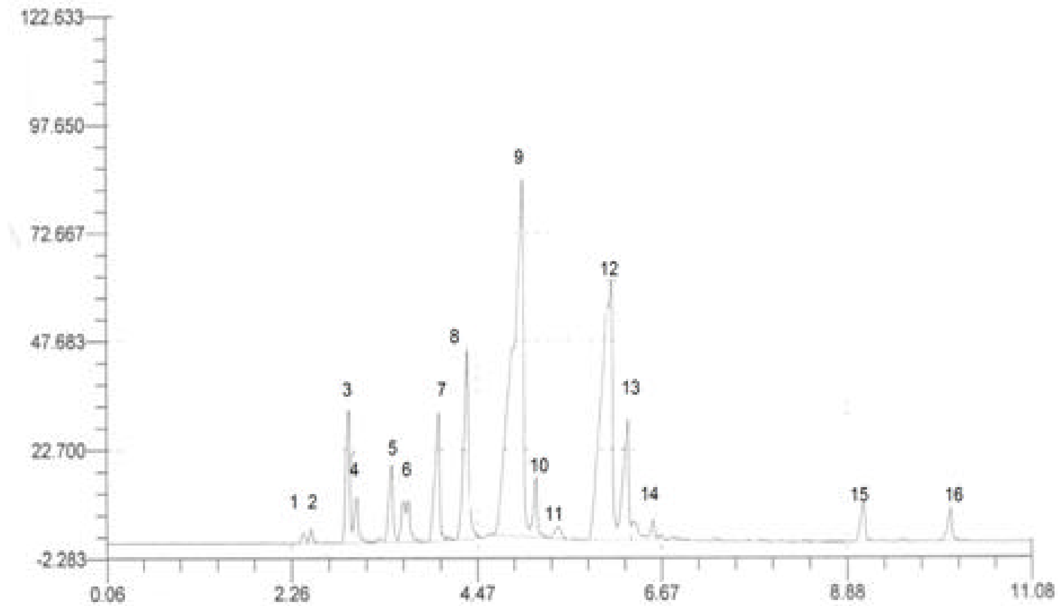

Fig. 3: Chromatogram of marjoram oil obtained by hydrodistillation of 1/4 NPK+ KSB treatment
1.a-Thujene, 2.a-Pinene, 3. Sabinene, 4. ß Myrcene, 5. a – Terpinene, 6. a- phyllandrene 7. ß-phyllandrene ,8. Limonene, 9. Linalool, 10.Linalyl acetate, 11.U.K, 12.Terpine-4-ol, 13. Thuyanol, 14. a-terpineol, 15. Thuyan-4-ol, 16.ß-caryophllene

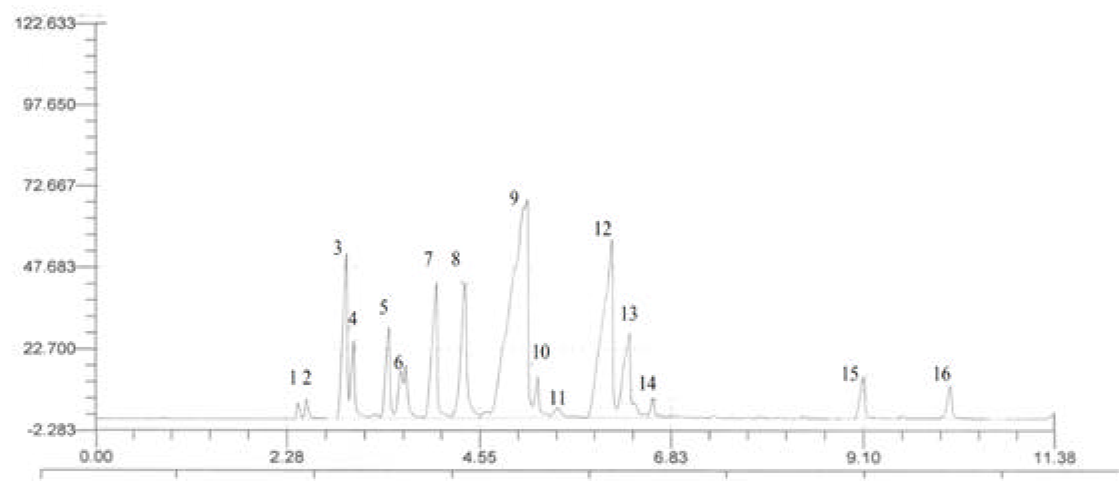

Fig. 4: Chromatogram of marjoram oil obtained by hydrodistillation of 1/2NPK+ NFB+ PSB treatment
1.a-Thujene, 2.a-Pinene, 3. Sabinene, 4 .ß Myrcene, 5. a – Terpinene, 6. a- phyllandrene 7. ß-phyllandrene ,8. Limonene, 9. Linalool, 10.Linalyl acetate, 11.U.K, 12.Terpine-4-ol, 13. Thuyanol, 14. a-terpineol, 15. Thuyan-4-ol, 16.ß-caryophllene

Table 9: Effect of chemical and bio-fertilization on phosphorus and potassium contents of marjoram plant during the two growing seasons of 2009 and 2010

	P (% of dry weight)						K (% of dry weight)					
	First Season			Second Season			First Season			Second Season		
Treatments	1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut
Control(NPK)	0.15	0.14	0.16	0.09	0.08	0.09	1.39	1.34	1.32	1.12	1.09	1.14
1/4NPK+NFB	0.19	0.20	0.21	0.13	0.14	0.11	1.58	1.43	1.50	1.30	1.14	1.36
1/4NPK+PSB	0.22	0.22	0.16	0.15	0.15	0.15	1.69	1.58	1.62	1.41	1.32	1.42
1/4NPK+KSB	0.22	0.20	0.20	0.15	0.13	0.16	1.68	1.53	1.59	1.39	1.27	1.32
1/4NPK+NFB+ PSB	0.29	0.27	0.25	0.22	0.20	0.18	1.87	1.78	1.85	1.58	1.48	1.46
1/4NPK+NFB+KSB	0.24	0.24	0.25	0.17	0.17	0.18	1.74	1.53	1.72	1.44	1.26	1.55
1/4NPK+PSB+ KSB	0.22	0.21	0.19	0.15	0.14	0.15	1.74	1.68	1.64	1.45	1.41	1.42
1/4NPK+NFB+PSB+KSB	0.22	0.22	0.20	0.15	0.15	0.14	1.44	1.43	1.41	1.18	1.15	1.14
1/2NPK+NFB	0.18	0.17	0.17	0.11	0.11	0.10	1.33	1.29	1.39	1.06	1.02	1.03
1/2NPK+PSB	0.21	0.20	0.19	0.14	0.13	0.14	1.64	1.38	1.58	1.37	1.11	1.42
1/2NPK+ KSB	0.18	0.16	0.17	0.11	0.09	0.12	1.69	1.73	1.77	1.41	1.46	1.32
1/2NPK+NFB+ PSB	0.19	0.18	0.19	0.12	0.12	0.13	1.79	1.53	1.64	1.51	1.25	1.71
1/2NPK+NFB +KSB	0.18	0.18	0.19	0.12	0.11	0.12	1.83	1.69	1.79	1.54	1.39	1.60
1/2NPK+ PSB+ KSB	0.19	0.18	0.17	0.12	0.11	0.13	1.77	1.57	1.69	1.48	1.30	1.67
1/2NPK+NFB+PSB+KSB	0.21	0.21	0.22	0.15	0.14	0.14	1.64	1.36	1.45	1.35	1.13	1.46

Control (NPK): Full recommended rate, 1/4NPK: Quarter of recommended rate, 1/2NPK: Half of recommended rate
NFB: Nitrogen fixing bacteria, PSB: phosphate solubilizing bacteria, KSB: potassium solubilization bacteria

(the recommended rate of chemical NPK fertilizer) which recorded the lowest values. In both seasons, the highest total chlorophyll and carotenoids contents in fresh leaves of *Majorana hortensis* L. plants were generally recorded with plants received 1/4NPK+ NFB + PSB treatment. Such results are in agreement with those reported on *Ocimum basilicum* [11], *Hibiscus sabdariffa* [15] and *Anethum graveolens* [16].

In both seasons in most cases, plants received 1/4NPK with biofertilizer gave higher values of total chlorophyll and carotenoids in fresh leaves of *Majorana hortensis* L. than those received 1/2NPK with the same biofertilizer and this trend of effect was more obvious with carotenoids content.

Total Carbohydrates (%): Data presented in Table 8 revealed that in the three cuts of both seasons the synthesis and accumulation of total carbohydrates in *Majorana hortensis* L. plants was increased by the application of all fertilization treatments, as compared to the control which recorded the lowest values. In the three cuts of both seasons,the highest total carbohydrates in dry herb of *Majorana hortensis* L. plants was generally recorded with plants received 1/4NPK+ NFB + PSB treatment. Such results are in line with those reported on senna plants [9], *Ocimum basilicum* [11], fennel plants [12] and *Thymus capitatus* [13].

Generally, plants received 1/4NPK with biofertilizer gave higher percentages of total carbohydrates in dry herb of *Majorana hortensis* L. plants than those received 1/2 NPK with the same biofertilizer in the three cuts of both seasons.

Elements Content (N, P and K %): Data presented in Tables 8 and 9 revealed that in the three cuts of both seasons, N; P and K% of herb dry matter of *Majorana hortensis* L. plants were increased as a result of most fertilization treatments, compared to the control. In both seasons, the highest N, P and K percentages of dry herb were in general recorded with plants received 1/4NPK+NFB+ PSB treatment. Only one exception to this general trend of effect was recorded with plants received 1/4NPK+NFB+ KSB treatment giving the same highest P% that was recorded in the third cut of both seasons. In most cases, the lowest N % of herb dry matter of *Majorana hortensis* L. plants was recorded with plants received 1/2NPK+ KSB treatment, whereas the lowest P % of dry herb was recorded with plants received the recommended rate of NPK (control plants). Mostly, the lowest K % of dry herb was recorded with plants received 1/2NPK+NFB. Such results are in agreement with those reported on senna plants [9], *Ocimum basilicum* [11], fennel plants [12 and 14], *Hibiscus sabdariffa* [15] and *Anethum graveolens* [16].

Generally, in both seasons, plants received 1/4NPK with biofertilizer gave higher values of N, P and K% of dry herb of *Majorana hortensis* L. plants than those received 1/2NPK with the same biofertilizer in both seasons.

DISCUSSION

The favorable effect on the vegetative characteristics, in respect of producing taller and heavier plants with an increase in number of branches per plant as well as fresh; air dry and dry yields of marjoram herb, especially with feeding plants with chemical and biofertilizer can be explained by the important role of N, P and K in the different physiological processes within the plant, which in turn affect the plant growth. Nitrogen is present in the structure of purines, pyrimidines found in the nucleic acids RNA and DNA which are essential for synthesis of protein molecules. Besides, N is present in coenzymes which are essential to the function of many enzymes that play roles in the synthesis of all metabolic intermediates, cellular structure components and storage components which constitute the plant body and are required for the meristmatic activity and growth of cells and organs. N is biologically combined with C, H, O and S to create amino acids, which are the building blocks of proteins and form protoplasm (the site for cell division) needed for plant growth and development. N is found in cytochromes which are a major part of the chlorophyll molecule and is therefore necessary for photosynthesis and respiration. N is a necessary component of several vitamins. Phosphorus is an essential constituent of nucleic acids and phospholipids and has a positive effect on photosynthesis and respiration; it plays a major role in energy storage and transfer as ADP, ATP, DPN and TPN. It is also required in large quantities in young cells such as shoots and root tips, it also aids in root development, flower initiation. Unlike N and P, K does not form any vital organic compounds in the plant, but the presence of K is vital for plant growth because it is known to be an enzyme activator that promotes metabolism, it is associated with many enzymes involved in photosynthesis, organic compound synthesis and translocation of photosythates (sugars) for plant growth or storage in roots and assists in regulating the plant's use of water by controlling the opening and closing of leaf stomates. Through its role assisting ATP production, K involves in protein synthesis and has been shown to improve disease resistance in plants [40-43].

The positive effect of bio-fertilization on plant height can be explained, at least in part, by that Azospirillum species are plant growth-promotive bacteria whose beneficial effects have been postulated to infiuence the hormonal balance of the plant and to secrete phytohormones (e.g. indole-3-acetic acid (IAA), cytokinins and gibberellins) which could stimulate plant growth, absorption of nutrients and photosynthesis process. Gibberellins have many direct demonstrated effects on shoot elongation. In addition, Azotobacter and Azospirillum fix N_2, reduce membrane potential of the root and synthesize some enzymes such as ACC deaminase that modulate the level of plant hormones. Also, phosphobacteria improve plant growth due to biosynthesis of plant growth substances rather than their action in solubilizing inorganic phosphate by secreting phosphatase enzyme and liberating phosphorous from organic compounds which make phosphorus available to the plants [44-57].

The effect of biofertilizer on increasing the essential oil synthesis in the herb might be attributed to their enhancing effect on increasing the uptake of nutrients by plant roots especially phosphorus element as phosphate group one linked by pyrophosphate bonds is adenosine triphosphate (ATP). Essential oils are terpenoids based on integral C5 units (isopernoid). Biologically active isopernoid requires acetyl-COA, ATP and Nicotinamide adenine dinucleotide phosphate (NADPH) for synthesis. Hence, the biosynthesis of essential oil is dependent on inorganic P content in the plant [58]. The increase in oil yield might be due to changes in leaf oil gland population and monoterpenes biosynthesis [59]. The response of volatile oil content to nitrogen fertilization might be attributed to that nitrogen increases the ability of the plant to produce new meristemic cells as well as their metabolism to produce dry matter with essential oil production. These results agree with those of Omer [60] and Omer *et al.* [61] who found a positive correlation between nitrogen fertilizer and essential oil content in herbage of *Origanum syriacum* and *Ocimum americanum*, respectively.

The increase in chlorophylls content is attributed to the existence of nitrogen (either from chemical or bio source) in the structure of the porphyrin which is found in chlorophyll pigments. The favorable effect of the different fertilization treatments on the synthesis and accumulation of carbohydrates may be attributed to the increase in the contents of chlorophylls and cytochrome enzymes results in an increment in the photosynthetic rate and a promotion in carbohydrate synthesis and accumulation. The increase in the contents of nutrients in the dry matter of *Majorana hortensis* as a result of the fertilization treatments is reasonable, since raising NPK levels as a result of fertilization treatments in the root medium led to more root growth. This may be accompanied by converting the unavailable forms of nutrient elements to available forms by the microorganisms in biofertilizer, more absorption of essential elements from the soil and their accumulation in plant tissues [40, 62]. In addition, the non symbiotic N_2-fixing bacteria (Azospirillum) produces adequate amounts of IAA and cytokinins with increasing the surface area per unit root length and enhancing the root hair formation with an eventual increase on the uptake of nutrients from the soil [53, 63]. In this regard, Belimov *et al.* [64] reported that the inoculation with bacterial mixtures provide a more nutrition for the plants and improvement in root uptake of both nitrogen and phosphorus as a balance result of mechanism of interaction between nitrogen fixing and phosphate solubilizing bacteria. Phosphate solubilizing bacteria (*Bacillus polymyxa*) release organic and inorganic acids which reduce soil pH leading to change of phosphorus and other nutrients to available forms ready for uptake by plants [65].

CONCLUSION AND RECOMMENDATION

It can be concluded that, under the conditions of the experiment, marjoram plants significantly responded to different combination treatments between NPK chemical fertilizer and bio-fertilizers, which positively improved vegetative characters, active constituents and chemical composition of marjoram plants. Also, plants received 1/4NPK with biofertilizer gave higher values for most characteristics than those received 1/2NPK with the same biofertilizer. Generally, 1/4NPK+NFB+ PSB was the best treatment. So, this work may be considered as an applied work to replace at least partly biofertilizers instead of chemical NPK fertilizers to reduce the costs of fertilizers and labor as well as to avoid the hazard of environmental pollution and improving the plant productivity.

REFERENCES

1. Tainter, D.R. and A.T. Grenis, 1993. Spices and Seasonings: A Food Technology Hand Book. VCH Publishers Inc. New York.
2. Sivropoulou, A., E. Papanicolaou, C. Nicolaou, S. Kokkin, T. Lanaras and M. Arsenakis, 1996. Antimicrobial and cytotoxic activities of origanum essential oils. J. Agric. Food Chem., 44: 1202-1205.
3. Kumar, B.V.N.S., M.R. Kumar, T. Tamizhmani, O.M.F. Rahiman and K.M. Niyas, 2011. *Majorana hotensis:* A review update. pharma science monitor, an international J. Pharmaceutical Sci., 2: 59-74.
4. FAO and IFA, 2000. Fertilizers and their use. Fourth edition, Rome.
5. Barker, A.V. and D.J. Pilbeam, 2007. Handbook of plant nutrition. CRC Press, USA.
6. Lugtenberg, B.J.J., L.A. Weger, J.W. Bennett and L.A. De Weger, 1991. Microbial stimulation of plant growth and protection from disease. Current Opinion in Biotechnol., 2: 457-464.
7. Subba Rao, N.S., 1981. Biofertilizers in Agriculture. Oxford and IBH Publishing Co. New Delhi, pp: 6-160.
8. Kandeel, A.M., N.S. Abo-Taleb and A.A. Sadek, 2002. Effect of biofertilizers on the growth, volatile oil yield and chemical composition of *Ocimum basilicum* L. plant. Annals Agric. Sci. Ain Shams Univ. 47: 351-371.
9. Sakr, W.R.A., 2005. Effect of organic-and bio-fertilization on growth and active constituents production of senna plant. Ph.D. Thesis, Faculty of Agriculture, Cairo University, Egypt.
10. Kandeel, A.M. and M.S. Sharaf, 2003. Productivity of *Majorana hortensis*, L. plants as influenced by the interaction between mineral and biological fertilization. J. Agric. Sci. Mansoura Univ., 28: 1373-1389.
11. Al-Qadasi, A.S.S., 2004. Effect of biofertilization on *Ocimum basilicum* L. plants. M.Sc. Thesis, Faculty of Agriculture, Cairo University, Egypt.
12. Mahfouz, S.A. and M.A. Sharaf-Eldin, 2007. Effect of chemical vs. biofertilizer on growth, yield and essential oil content of fennel (*Foeniculum vulgare* Mill.). International Agrophysics, 21: 361-366.
13. El-Mekawy, M.A.M., 2009. Effect of irrigation intervals and biofertilization on growth and oil yield of *Thymus capitatus* L. under El-Arish conditions. J. Agric. Res. Kafr Elsheikh Univ., 35: 109.
14. Azzaz, N.A., E.A. Hassan and E.H. Hamad, 2009. The Chemical Constituent and Vegetative and Yielding Characteristics of Fennel Plants Treated with Organic and Bio-fertilizer Instead of Mineral Fertilizer. Australian J. Basic and Applied Sci., 3: 579-587.
15. Abo-Baker, A.A. and G.G. Mostafa, 2011. Effect of bio- and chemical fertilizers on growth, sepals yield and chemical composition of *Hibiscus sabdariffa* at new reclaimed soil of South Valley Area. Asian J. Crop Sci., 3: 16-25.
16. Hellal, F.A., S.A. Mahfouz and F.A.S. Hassan, 2011. Partial substitution of chemical nitrogen fertilizer by bio-fertilizer on *Anethum graveolens* L. plant. Agriculture and Biology J. North America, 2: 652-660.
17. Egyptian Ministry of Agriculture and Land Reclamation, 1993. Marjoram. Central administration of horticulture, Developing Horticultural Crops Production Program.
18. British Pharmacopoeia, 1963. Determination of volatile oils in drugs. The Pharmaceutical Press, London.
19. Hoftman, E., 1967. Chromatography. Reinhold publ. corp. 2nd Ed. pp: 208-515.
20. Buzon, J., N. Guichard, J.Lebbe, A.Prevot, J. Serpinet and J. Tranchant, 1969. Practical Manual of Gas Chromatography. Elsevier Publishing Co. New York.
21. Porra, R.J., W.A. Thompson and P.E. Kriedemann, 1989. Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls a and b extracted with four different solvents: verification of the concentration

of chlorophyll standards by atomic absorption spectroscopy. Biochim. et Biophys. Acta, 975: 384-394.

22. Herbert, D., P.J. Phillips and R.E. Strange, 1971. Chemical analysis of microbial cells. In: Methods in microbiology vol. 58, Norris, J. R. and D. W. Ribbons (eds.). Academic Press, New York, pp: 204-344.
23. A.O.A.C, 1995. Methods of Analysis. Association of Official Agriculture Chemists. Washington D.C. 16th Ed. USA.
24. Goodwin, J.F., 1970. Quantification of serum inorganic phosphorus, phosphatase and urinary phosphate without preliminary treatment. Clinical Chemistry, 16: 776-780.
25. Huang, C.Y.L. and E.E. Schulte, 1985. Digestion of plant tissue for analysis by ICP emission spectroscopy. Communications in Soil Science and Plant Analysis, 16: 943-958.
26. Steel, R.G.D. and S.H. Torrie, 1980. Principles and Procedure of Statistics. McGrow Hill Inc. 2nd Ed. New York.
27. Ali, A.F., E. Osman and M.R. Khater, 2001. Effect of Phosphorine and potassium sulphate on guar, *Cyamposis tetragonoloba*, L.. Egypt. J. Appl. Sci., 16: 217-228.
28. Khater, R.M.R., 2001. Effect of some fertilizer treatments on the growth and volatile oil yield of *Carum carvi* plants. M. Sc. Thesis, Faculty of Agriculture, Zagazig University, Egypt.
29. Abdou, M.A.H. and A.A. EL-Sayed, 2002. Effect of planting date and biofertilization treatments on growth and yield characters of caraway crop (*Carum carvi,* L.).Proc. 2nd Inter. Conf. Hort. Sci. 10-12 Sept.,Kafr EL-Sheikh, Tanta University, Egypt, 28: 423-433.
30. Eid, M.I. and E.O. EL-Ghawwas, 2002. Study on the responsibility of marjoram plant to biofertilizer in sandy soil. Egypt. J. Appl. Sci., 17: 163-175.
31. Abd El-Azim, W.M., 2003. Production of *Salvia officinalis* L. plant under Sinai conditions. Ph.D. Thesis, Faculty of Agriculture, Cairo University, Egypt.
32. Ibrahim, S.M.M., 2000. Effect of seeding rate and bio-fertilization on fennel plants. M.Sc. Thesis, Faculty of Agriculture, Minya University, Minia, Egypt.
33. Tanious, C.T.S., 2008. Effect of some organic and bio fertilization treatments on fennel plants. M.Sc. Thesis, Fac.Agric. Minia Univ. Egypt.
34. Eid, M.I., 2001. Effect of potassin and phosphorein on roselle plant (*Hibiscus sabdariffa*). J.Agric. Sci. Mansoura Univ., 26: 7879 -7885.
35. Swaefy, H.M.F.A. and S.M.N. Milad, 2006. Effect of chemical and bio-fertilization on growth and chemical composition of *Euryops pectinatus*. J. Agric. Sci. Mansoura Univ., 31: 7329-7345.
36. Edris, A.E., A. Shalaby and H.M. Fadel, 2003. Effect of organic agriculture practices on the volatile aroma components of some essential oil plants grown in Egypt II: Sweet marjoram (*Origanum marjorana*, L.) essential oil. Flavour and Fragrance J., 18: 345-351.
37. El-Ghorab, A.H., A.F. Mansour and K.F. El-Massry, 2004. Effect of extraction methods on the chemical composition and antioxidant activity of Egyptian marjoram (*Majorana hortensis* Moench). Flav.Fragr. J., 19: 54-61.
38. Kandeel, A.M. and M.S. Sharaf, 2003. Productivity of *Majorana hortensis*, L. plants as influenced by the interaction between mineral and biological fertilization. J. Agric. Sci. Mansoura Univ., 28: 1373-1389.
39. Mohamed, F.S., 2007. The effect of growth regulators and partial replacement of mineral fertilizers by bio-fertilizers on botanical characters of caraway (*Carum carvi,* L.) and anise (*Pimpinella anisum*, L.) plants. Ph.D. Thesis, Fac. Agric. Fayoum Univ. Egypt, pp: 147-153.
40. Devlin, R.M., 1975. Plant Physiology. Affiliated East-West Press Pvt. Ltd. 3rd Ed. New Delhi, pp: 221-240, 284-298, 353.
41. Lawlor, D.W., G. Lemaire and F. Gastal, 2001. Nitrogen, plant growth and crop yield. In: Plant Nitrogen, Lea, P. J. and J. Morot-Gaudry (eds). Springer, New York.
42. Mengel, K. and E.A. Kirkby, 1982. Principles of Plant Nutrition. International Potash Institute, Bern, Switzerland.
43. Uchida, R., 2000. Essential Nutrients for Plant Growth: Nutrient Functions and Deficiency Symptoms. In: Plant Nutrient Management in Hawaii's Soils, Approaches for Tropical and Subtropical Agriculture, Silva, J. A. and R. Uchida (eds). College of Tropical Agriculture and Human Resources, University of Hawaii, Manoa.
44. Unno, Y., K. Okubo, J. Wasaki, T. Shinano and M. Osaki, 2005. Plant growth promotion abilities and microscale bacterial dynamics in the rhizosphere of lupin analysed by phytate utilizationability. Environ Microbiol, 7: 396-404.
45. Costacurta, A. and J. Vanderleyden, 1995. Synthesis of phytohormones by plant-associated bacteria. Crit Rev Microbiol, 21: 1-18.

46. Okon, Y. and C.A. Labandera-Gonzalez, 1994. Agronomic applications of Azospirillum: an evaluation of 20 years of worldwide field inoculation. Soil Biol. Biochem, 26: 1591-1601.
47. Janzen, R., S. Rood, J. Dormar and W. McGill, 1992. *Azospirillum brasilense* produces gibberellins in pure culture and chemically-medium and in co-culture on straw. Soil Biol. Biochem, 24: 1061-1064.
48. Lucangeli, C. and R. Bottini, 1997. Effects of *Azospirillum* spp. on endogenous gibberellins content and growth of maize (*Zea mays* L.) treated with uniconazole. Symbiosis, 23: 63-72.
49. Piccoli, P., O. Masciarelli and R. Bottini, 1999. Gibberellin production by *Azospirillum lipoferum* cultured in chemically defined medium as affected by water status and oxygen availability. Symbiosis, 27: 135-146.
50. Davies, P.J. and P.J. Davies, 1995. The plant hormones: their nature, occurrence and functions. In: Plant Hormones: Physiology, Biochemistry and Molecular Biology, Davies, P.J. (ed).Kluwer Academic Publishers, Dordrecht, The Netherlands, pp: 1-12.
51. Piccoli, P. and R. Bottini, 1994. Metabolism of 17,17-[2H2]-gibberellin A20 to 17,17-[2H2] gibberellin A1 by *Azospirillum lipoferum* cultures. Agri. Scientia, 11: 13-15.
52. Piccoli, P. and R. Bottini, 1996. Gibberellin production in *Azospirillum lipoferum* cultures is enhanced by light. Biocell, 20: 185-190.
53. Ponmurugan, P.C. and C. Gopi, 2006. In vitro production of growth regulators and phosphates activity by phosphate solubilizing bacteria. African J. Biotechnol., 5: 348-350.
54. Rodriguez, H. and R. Fraga, 1999. Phosphate solubilizing bacteria and their role in plant growth promotion. Biotech. Advan., 17: 319-339.
55. Fayez, M., N.F. Emam and H.E. Makboul, 1985. The possible use of nitrogen fixing Azospirillum as biofertilizer for wheat plants. Egypt. J. Microbiol, 20: 190-206.
56. Abd-El Latif, M.R., A.A. El-Bana and A.A. Galal, 2001. Effect of biofertilizers Microbein and Phosphorine on bacterial pod blight of guar and black cumin damping off root rot and with diseases. Proc. of the fifth Arabian Horticulture Conference, Ismailia, Egypt, March 24-28, pp: 133 -140.
57. Gharib, F.A., L.A. Moussa and O.N. Massoud, 2008. Effect of compost and bio-fertilizers on growth, yield and essential oil of sweet marjoram (*Majorana hortensis*) plant. International J. Agriculture and Biol., 10: 381-387.
58. Kapoor, R., B. Giri and K.G. Mukerji, 2004. Improved growth and essential oil yield and quality in *Foeniculum vulgare* Mill on mycorrhizal inoculation supplemented with P-fertilizer. Bioresour. Technol. J., 93: 307-311.
59. Croteau, R., 1977. Site of monoterpene biosynthesis in *Majorana hortensis* leaves. Plant Physiol., 59: 519-520.
60. Omer, E.A., 1998. Response of wild Egyptian oregano to nitrogen fertilization in sandy soil. Egypt J. Hort., 25: 295-307.
61. Omer, E.A., A.A. Elsayed, A. El-Lathy, A.M.E. Khattab and A.S. Sabra, 2008. Effect of the nitrogen fertilizer forms and time of their application on the yield of herb and essential oil of *Ocimum americanum* L. Herba Polonica, 54: 34-46.
62. Cocking, E.C., 2003. Endophytic colonisation of plant roots by nitrogen fixing bacteria. Plant Soil, 252: 169-75.
63. Gomaa, A.O. and H.E. Abou-Aly, 2001. Efficiency of bioferitlization in the presence of both inorganic and organic fertilizers on growth, yield and chemical constituents of anise plant (*Pimpinella anisum* L.). Proc. of the fifth Arabian Hort. Conf. Ismailia, Egypt, March. 24-28, pp: 73-80.
64. Belimov, A.A., A.P. Kojemiakov and C.V. Chuvarliyeva, 1995. Interaction between barley and mixed cultures of nitrogen fixing and phosphate-solubilizing bacteria. Plant and Soil, 173: 29-37.
65. Singh, S. and K.K. Kapoor, 1999. Inoculation with phosphate-solubilizing microorganisms and visicular-arbuscular mycorrhizal fungus improves dry matter yield and nutrient uptake by wheat grown in sandy soil. Biol. Ferti. Soils, 28: 139-144.