451
Annals
Agric. Sci.,
Ain Shams Univ.,
Cairo, 52(2), 451-463, 2007

EFFECT OF SOME CHEMICAL AND BIO – FERTILIZERS ON PEPPERMINT PLANTS GROWN IN SANDY SOIL
1. EFFECT ON VEGETATIVE GROWTH AS WELL AS ESSENTIAL OIL PERCENTAGE AND CONSTITUENTS

[37]

Swaefy Hend[1], M.F.; Weaam R.A. Sakr[1]; A.Z. Sabh[2] and A.A. Ragab[3]

1. **Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, Giza, Egypt**
2. **Agricultural Botany Department, Faculty of Agriculture, Cairo University, Giza, Egypt**
3. **Agricultural Microbiology Department, Soil, Water and Environment Research Institute, Agricultural Research Center, Giza, Egypt**

Keywords: Chemical fertilizers, Biofertilizers, Peppermint, Sandy soil

ABSTRACT

This investigation was carried out at the Experimental Nursery of the Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, Giza, during the two successive seasons of 2005 and 2006, with the aim of investigating the effect of chemical fertilizers (N and P) and / or some microbial strains (*Rhizobium leguminosarum* bv. *phaseoli*, *Azotobacter chroococcum* and *Bacillus megatherium* var. *phosphaticum*) as a bio-fertilizers or as a plant growth promoting rhizobacteria (PGPR) on the population of rhizospher microorganism (RMO) as well as the vegetative characteristics, the yield of fresh and dry herb, essential oil percentage, and oil constituents of *Mentha piperita*, L. plants grown in sandy soil.

The results showed the ability of the tested microbial strains to produce IAA, hydrocyanic acid (HCN), siderophore production and solubilized phosphorus as a qualitative assessment. The most effective treatment in promoting total herb fresh weight was the application 50% of the recommended amount of chemical fertilizers [175 Kg/fed ammonium sulphate (20.5% N) and calcium super-phosphate (15.5% P_2O_5) at 100 Kg/fed] in addition to *Azotobacter sp.* as a bio-fertilizer. *Mentha piperita*, L. plants inoculated with *Rhizobium* only gave the significantly higher rhizomes fresh and dry weights in both seasons, compared to the other treatments. According to the results of GLC analysis, it could be noticed that using most of the bio-fertilizer treatments either alone or in combination with chemical fertilizer (NP at 50%) increased the oxygenated compounds percentage in the volatile oil of *Mentha piperita*, L. plants.

The combined treatments of 175 Kg/fed ammonium sulphate (20.5% N) and calcium super-phosphate (15.5% P_2O_5) at 100 Kg/fed (50% of the recommended amount of chemical fertilizers) and *Azotobacter* or *Rhizobium* can be recommended because of producing the same oil percentage and resulted in insignificantly different herb fresh yield compared to that recorded using the full amount of the recommended chemical fertilizers, but with much more oxygenated compounds and healthier environment

(Received September 17, 2007)
(Accepted October 22, 2007)

INTRODUCTION

Peppermint belongs to the genus *Mentha*, family: Labiatae (Lamiaceae) which includes about 25 species **(Pandey, 1982 and Bown, 1995).** Peppermint has been used extensively for a variety of complaints, including sore throat, diarrhea, toothaches, cramp and in indigestion. The oil is used as flavouring agent in foods and pharmaceuticals especially in massage lotions for aching muscles and rheumatic joints. Aroma-therapists recommend a footbath of diluted peppermint oil for tired feet. It is mildly anesthetic, giving the cooling, numbing sensation experienced when smelling or tasting **(Bown, 1996).**

Chemical fertilization is an important culture practice that has a considerable influence on growth, yield and chemical composition of different crops especially in sandy soil which may have less fertility in general, and less availability of some elements such as phosphorus and nitrogen.

In bio-organic farming systems, chemical fertilization is often replaced (at least partly) by bio-fertilization, using different N-fixing and P-solubilizing bacteria **(Burris, 1976)** and using some plant growth promoting rhizobacteria (**Antoun *et al* 1998** and **Ragab *et al* 2005**). The efforts to decrease chemical fertilizers by using bio-fertilizers might reduce the high costs and environmental pollution. Hence biological N_2-fixation has great attention to cover N-requirements and improve the soil fertility **(Okon and Lanabdera, 1994)**. Plant growth promoting rhizobacteria (PGPR) improve plant growth and increase yield productivity, suggesting that N_2-fixing bacteria may be considered as PGPR and can benefit plants growth and yield through different mechanisms of action including: a) the production of secondary metabolites such as antibiotic, hydrogen cyanide and plant hormones like substances, b) the production of siderophors, c) antagonism to soil borne root pathogens, d) phosphate solubilization and e) dinitrogen fixation (**Gilic, 1995, Antoun *et al* 1998 and Ragab & Rashed, 2003**).

Remarkable effects of bio-fertilizers have been reported by **Paramaguru and Natarajan (1993)** on chilli (*Capsicum annuum,* L.), they recorded significant enhancement in plant growth due to application of *Azospirillum* as a bio-fertilizer and stated that bio-fertilizers saved about 25 to 50% of the used mineral fertilizers especially nitrogen and phosphorus. Also, **Radwan and Farahat (2002)** found that application of chicken manure combined with bio-fertilizers (phosphate dissolving bacteria, *Azospirillum spp.+ Pseudomonas spp.*) significantly increased fruits yield of coriander plant compared to the control (NPK) and **Sharaf and Khattab (2004)** on fennel plant, found that bio-fertilizer (*Bacillus polymyxa*) combined with inorganic fertilizer (60 kg N/fed.) produced the highest values of umbels number, umbels weight and fruits yield/plant in comparison to unfertilized plants. Inoculation with *Azotobacter*, combined with a moderate N-fertilization rate had a beneficial effect on plant growth and productivity. In this respect, **Hassan *et al* (2006)** found that moghat plants obtained from inoculated seeds by bio-fertilizers then received 50 or 100% of the recommended dose of NP as mineral fertilizers showed significant increase in all morphological and yield characters under investigation when compared with moghat plants obtained from un-inoculated seeds

This study was aimed to investigate the effect of some chemical and bio-fertilizers on vegetative characteristics, herb yield and oil composition of *Mentha piperita*, L. plants as well as the population of rhizospher microorganism (ROM) to assess the possibility of using these bio-fertilizers to reduce the need for chemical NP fertilization and act as a plant growth promoting rhizobacteria (PGPR).

MATERIALS AND METHODS

This study was conducted at the Experimental Nursery of the Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, Giza, during the two successive seasons of 2005 and 2006.

The strains of *Rhizobium leguminosarum* bv. *phaseoli*, *Azotobacter chroococcum* and *Bacillus megatherium* var. *phosphaticum* were purified and maintained on yeast extract mannitol agar medium **(Vincent, 1970)**, Ashby's medium **(Hegazi and Neimela,1976)** and Bunt and Rovira medium **(Bunt and Rovira, 1955)**, respectively and stored at – 70°C in a liquid media containing 20% glycerol.Microbial strains were checked for the ability to produce hydrocyanic acid (HCN), **(Bakker and Schippers, 1987)**, phytohormones like indole acétic acid (1AA) **(De-Britto Alvarez *et al* 1995)**, siderophore production **(Alexander and Zubeter, 1991)** and phosphate solubilization **(Goldstein, 1986)**. Microbial inocula were prepared in this study by adding 100 ml liquid culture (Ca 10^9/ml) from each microorganisms to 200 g vermiculite as a carrier material to be used as a bio-fertilizers.

On March 15[th], (in both seasons), rhizomes of *Mentha piperita*, L. were obtained from the Experimental Farm of Medicinal and Aromatic Plants, Faculty of Pharmacy, Cairo University, Giza. The rhizomes (12 cm long, with 8–10 leaves) were planted in 30-cm clay pots filled with a sandy soil. The mechanical and chemical analyses of the sandy soil **(Table 1)** were carried out before planting according to **Chapman and Pratt (1961)**.

Table 1. Physical and chemical characteristics of the soil used for growing *Mentha piperita*, L plant during 2005 and 2006 seasons.

Physical characteristics					
Coarse sand (%)	Fine sand (%)	Silt (%)	Clay (%)	Soil texture	Field capacity (% V)
32.5	62.1	1.7	3.7	Sandy	15.8

Chemical characteristics							
pH	Organic matter (%)	$CaCO_3$ (%)	EC (dS/m) (1:2.5)	CEC (meq/100 g)	Available macro - nutrients (ppm)		
					N	P	K
7.4	1.23	0.43	0.76	5.1	19.8	3.6	98.1

Prior to planting, the rhizomes used for planting 108 pots (6 treatments) were inoculated with *Rhizobium*, *Azotobacter* or *Bacillus* [each at 10^8 colony forming units (CFU)/g carrier] at the rate of 300 g/fed as bio-fertilizers. The rhizomes were inoculated by wetting their bases, then immersing them in the bio-fertilizers. A basal dressing of the previous bio-fertilizers were repeated after 90 days from planting, at the rate of 300 g/fed, and the pots were irrigated immediately thereafter, while vermiculite (carrier material) was added alone at the same rate to pots receiving no bio-fertilization.

Chemical N and P fertilizations were added using ammonium sulphate (20.5% N) and calcium super-phosphate (15.5% P_2O_5). Ammonium sulphate at 350 Kg/fed and calcium super-phosphate at 200 Kg/fed (100% NP, a recommended rate reported by **Swaefy Hend, 1996**) were added to 18 un-inoculated pots. Also, 50% of the recommended rate (ammonium sulphate at 175 Kg/fed and calcium super-phosphate at 100 Kg/fed) were applied to 18 un-inoculated pots. The half number of inoculated pots (54 pots) received 50% of chemical fertilizers (NP). The above rates were divided into two doses: the first dose was added after one month from planting, and the second one was added two months later (two weeks after the first cut). Eighteen un-inoculated pots were left without NP chemical fertilization (control). All pots were supplied with potassium sulphate (48% K_2O) at the rate of 60 Kg/fed, divided into two equal doses. The first dose was applied as a basal dressing one month after planting and the second one was added two weeks after the first cut.

The layout of the experiment was a randomized complete blocks design. The experiment included 9 treatments, with 3 blocks (replicates), each consisting of 54 planting pots (six pots for each treatment).

In each season, two cuts were taken on 1st June and 15th August by cutting the vegetative parts of all plants, 5 cm above the soil surface. In both cuts of each season, the plant height (cm), fresh and air dry weights of herb (g/plant), leaf area (cm^2) of the fifth leaf from the shoot apex, leaves/stems fresh weight ratio and rhizomes fresh and air dry weights (g/plant) were recorded.

Essential oil% was determined in the fresh herb for each treatment according to the **British pharmacopoeia (1963)**. Also, samples taken from the oil obtained in the second cut of the second season were analyzed using Gas Liquid Chromatography (GLC), at the Central Laboratory of Faculty of Agriculture, Cairo University, according to the method of **Hoftman (1967)**. The qualitative identification of the oil components was achieved by comparing the retention time (R_t) of their peaks with those of the expected authentic chromatographed under the same conditions.

Data (collected on vegetative characteristics and oil percentage) were subjected to an analysis of variance, and the means were compared using the "Least Significant Difference (LSD)" test at the 0.05 level according to **Snedecor and Cochran (1982)**.

Qualitative assessments of indole acetic acid (IAA), siderophore, hydrocyanic acid (HCN) and phosphate solubilization were carried out before the beginning of the experiment. A change of color from yellow to orange-brown of filter papers impregnated with 0.5% picric acid - 2% $NaCO_3$ indicated the production of cyanide (**Bakker and Schippers, 1987**). A bacteria forming an orange halo on chrome azurol S (CAS) agar plates or growing on TSA (10%) agar plates containing 50 mgL^{-1} of 8-hydroxyquinoline was considered as positive siderophore producers (**Alexander and Zubeter, 1991**). IAA producing bacteria were separated from organisms producing other indoles (Yellow to Yellow-brown pigment) by their characteristics pink to red color produced after exposure to Salkowski reagent for 0.5-3.0 h. (**De-Britto Alvarez *et al* 1995**). The bacterial colonies forming clarification halos on dicalcium phosphate agar plates (**Goldstein, 1986**); were considered as phosphate solubilizers.

Also, total counts of bacteria, actinomycetes and fungi were estimated at the two cuts of the second season in soil rhizospher samples according to (**Wollum, 1982**).

RESULTS AND DISCUSSION

Bioassay of the bacterial strains

Data presented in **Table (2)** indicated that *Rhizobium leguminosarum* bv. *Phaseoli* and *Azotobacter chroococcum* showed positive reaction for IAA, siderophore, HCN and P- solubilizers, while the strain of *Bacillus megatherium* var. *phosphaticum* showed negative reaction on the siderophore and HCN tests. These results are in agreement with those obtained by **Antoun *et al* (1998), Arora *et al* (2001), Ragab and Rashed (2003); Ragab *et al* (2005), and Ragab *et al* (2006)**, they found that some rhizobia and *Azotobacter* produce IAA, siderophore, HCN and soluble phosphate.

Table 2. Qualitative assessments of IAA, siderophores, cyanagens (HCN) and soluble phosphate produced by different microbial strains

Strains relative reaction	Microbial strains: *Rhizobium leguminosarum* (ARC315)	*Azotobacter chroococcum* (AZ3)	*Bacillus megaterium* (BM5)
IAA	+ +	+ + +	+ +
Siderophores	+ + +	+ + +	-
Cyanagens (HCN)	+	+	-
P-solubilizers	+ +	+	+ + +

(-) no, (+) low, (+ +) moderate and (+ + +) high reaction.

Microbial status

Soil microbial status during the growth stage of the two cuts in the second season was evaluated. Data presented in **Table (3)** showed that NP-fertilizers at the rate of 100% of the recommended dose (ammonium sulphate at 350 Kg/fed and calcium super-phosphate at 200 Kg/fed) had a negative effect on rhizospher microorganism (RMO) (bacteria, fungi and actinomycetes). This treatment scored the lowest values of log number of total bacteria, fungi and actinomycetes as compared to all the other treatments.

In generally, in both cuts, inoculation with *Azotobactar or Rhizobium or Bacillus* inocula gave higher values of log number of total bacteria, fungi and actinomycetes as compared to other treatments contain NP-fertilizers. In most cases, *Azotobacter* inoculation recorded highest values of log number for RMO.

Generally, the first cut gave higher values of RMO as compared to the second cut among all tested treatments.

Table 3. Effect of some chemical and bio-fertilizers on log number of total bacteria, fungi and Actinomycetes of peppermint rhizospher area at the first and second cuts during the second season, 2006

Treatments	Total bacteria Log number 1st cut	2nd cut	Fungi log number 1st cut	2nd cut	Actinomycetes Log number 1st cut	2nd cut
Control	5.02	4.63	4.72	4.53	3.84	3.64
NP 50%	5.64	4.71	4.25	4.10	3.21	3.09
NP 100%	4.32	3.25	4.01	3.89	3.05	3.02
Rhizobium (Rh)	7.41	6.90	4.63	4.34	3.95	3.71
Rh+NP 50%	6.85	5.70	4.52	4.42	3.79	3.45
Azotabacter (Az)	7.52	6.51	4.75	4.32	4.53	4.12
Az+NP50%	7.14	5.92	4.34	4.01	4.50	3.92
Bacillus (B)	6.94	6.23	4.50	4.37	3.86	3.70
B + NP 50%	6.30	5.82	4.42	4.30	3.72	3.61
Means	6.36	5.51	4.46	4.25	3.82	3.58

NP100% = Ammonium sulphate at 350 Kg/fed and calcium super-phosphate at 200 Kg/fed
NP50% = Ammonium sulphate at 175 Kg/fed and calcium super-phosphate at 100 Kg/fed
Rh= *Rhizobium leguminosarum* bv. *phaseoli*
Az = *Azotobacter chroococcum*
B = *Bacillus megatherium* var. *phosphaticum*

These results are in harmony with those obtained by **Pondy *et al* 1998, Mona *et al* 2000; Abotaleb *et al* 2002** and **Ragab *et al* 2006**, they reported that inoculation with diazotrophic bacteria had stimulation effect on the population of rhizoshper microorganism (RMO) and increased their numbers by more than 50% at the end of the experiment compared with the numbers recorded before planting.

Vegetative characteristics

Plant height

Regarding the effect of fertilization treatments on plant height, the data recorded in **Table (4)** showed that plant height of *Mentha piperita*, L. was generally increased as a result of fertilization treatments, compared to the control plants. In most cases, it is evident that plants received bio-fertilizers only gave plant height values insignificantly different than those recorded for unfertilized control plants. Using chemical fertilization at 175 Kg/fed ammonium sulphate and calcium super-phosphate at 100 Kg/fed (50% of chemical fertilizers), in addition to each bio-fertilizers increased the plant height, compared to the plants received bio-fertilizer only. In the first cut of the first season and in the second cut of the second season, the most effective treatment in promoting plant height was the application of 350 Kg/fed ammonium sulphate and calcium super-phosphate at 200 Kg/fed (100% of chemical fertilizers) giving 75.7 and 85.3 cm, respectively. In the second cut of the first season and the first cut of the second season, the most effective treatment in promoting plant height was the application of 175 Kg/fed ammonium sulphate and calcium super-phosphate at 100 Kg/fed in addition to *Azotobacter* as a bio-fertilizer giving 70.3 and 75.7 cm, respectively.

The obtained results are in agreement with the **Swaefy Hend (1996)**, **Sakr Weaam (2001)** on *Mentha piperita* and **Dessouky (2002)** on *Borago officinalis,* they found that nitrogen bio-fertilizer treatment (*Azotobacter chroococcum*) increased plant height. Also, **Swaefy Hend and Milad (2006)** on *Euryops pectinatus*, found that there was a significant increase in plant height by using the combination of microbein and 50% of the soil chemical fertilizers and nofatrein.

Leaf area

Regarding the effect of fertilization treatments on leaf area, the data recorded in **Table (4)** showed that the leaf area of *Mentha piperita*, L. plants was significantly increased as a result of fertilization treatments, in most cases, compared to the control plants. The most effective treatment in increasing leaf area differed according to the cut and the season.

Table 4. Effect of some chemical and bio- fertilizers on plant height and leaf area of *Mentha piperita*, L. plants during 2005 and 2006 seasons

Treatments	Plant height (cm)				Leaf area (cm ^{2})			
	First season (2005)		Second season (2006)		First season (2005)		Second season (2006)	
	First cut	Second cut	First cut	Second cut	First cut	Second cut	First cut	Second cut
Control	63.3	53.3	61.7	61.7	4.39	5.90	4.24	6.28
NP50%	74.0	66.7	65.7	82.0	4.96	8.79	5.50	7.50
NP100%	75.7	69.3	70.0	85.3	7.80	9.68	8.08	8.72
Rhizobium (Rh)	62.0	52.3	60.0	57.0	4.66	7.29	4.98	7.41
Rh + NP50%	69.3	62.0	68.3	73.7	6.37	10.44	6.83	9.44
Azotabacter (Az)	71.0	60.0	69.0	71.0	7.99	9.17	7.69	8.41
Az + NP50%	73.0	70.3	75.7	84.7	6.15	9.83	5.73	8.79
Bacillus (B)	69.3	65.3	63.0	77.7	7.96	10.23	7.64	10.56
B + NP50%	70.3	67.3	68.3	79.7	4.84	8.92	4.47	9.36
LSD $_{0.05}$	7.1	7.1	8.4	10.0	0.76	0.64	0.47	0.50

NP100% = Ammonium sulphate at 350 Kg/fed and calcium super-phosphate at 200 Kg/fed
NP50% = Ammonium sulphate at 175 Kg/fed and calcium super-phosphate at 100 Kg/fed
Rh= *Rhizobium leguminosarum* bv . *phaseoli* Az = *Azotobacter chroococcum*
B = *Bacillus megatherium* var. *phosphaticum*

In the first season, plants inoculated with *Azotobacter* only had the highest leaf area (7.99 cm^2) in the first cut, whereas in the second cut the treatment included *Rhizobium* and 50% of chemical fertilizers was the most effective treatment in increasing leaf area (10.44 cm^2). In the first cut of the second season, plants received 100% of chemical fertilizers had the highest leaf area (8.08 cm^2), whereas in the second cut the treatment included *Bacillus* only was the most effective treatment in increasing leaf area (10.56 cm^2). The present results are in harmony with those obtained by **Milad (2003),** who found that roselle plants treated with 50% NPK + bio-fertilization or bio-fertilization only gave significantly larger leaves than those receiving 100% NPK. Generally, unfertilized control plants had the least leaf area in both cuts of the two seasons.

Herb fresh weight

The data shown in **Table (5)** revealed that, in both seasons, most of the treatments significantly increased the herb fresh weight compared to the control plants. Applying 100% of chemical fertilizers caused significant increase compared with applying 50% of chemical fertilizers in the first cut in both seasons, while it caused insignificant increase in the second cut of both seasons.

It is worth mentioning that, in most cases, using chemical fertilization at 175 Kg/fed ammonium sulphate and 100 Kg/fed calcium superphosphate in addition to each biofertilizer gave heavier fresh herb than the biofertilizer only.

In the first cut of the first season, the treatment of 100% of the chemical fertilizers produced the highest herb fresh weight (281.70 g/plant) followed by the treatment including 50% of chemical fertilization in addition to *Rhizobium* (265.53 g/plant). In the second cut of the first season, the highest herb fresh weight (475.47 g/ plant) was achieved by using *Azotobacter* + 50% NP. This treatment gave the highest total herb fresh weight in the first season (699.90 g/plant).

Table 5. Effect of some chemical and bio-fertilizers on herb fresh and dry weights of *Mentha piperita*,L. plants during the 2005 and 2006 seasons

Fertilization treatments	Herb fresh weight (g / plant)					
	First season (2005)			Second season (2006)		
	First cut	Second cut	Total	First cut	Second cut	Total
Control	87.93	216.00	303.90	102.58	241.77	344.30
NP50%	249.20	394.03	643.20	205.07	421.53	626.60
NP100%	281.70	410.43	692.10	252.50	467.33	719.80
Rhizobium (Rh)	131.63	322.50	454.10	131.21	284.67	405.90
Rh + NP50%	265.53	396.67	662.20	258.32	483.53	741.90
Azotabacter (Az)	153.97	271.33	425.30	142.57	397.20	539.80
Az + NP50%	226.39	475.47	699.90	258.10	551.61	809.70
Bacillus (B)	131.78	336.97	468.70	135.56	385.97	521.50
B + NP50%	214.55	371.77	586.32	201.12	394.73	595.80
$LSD_{0.05}$	20.12	53.96	55.76	27.51	54.04	60.29
	Herb dry weight (g / plant)					
Control	20.65	47.92	68.57	22.89	50.65	73.54
NP50%	48.80	74.38	123.18	39.84	70.55	110.39
NP100%	59.59	62.17	121.76	48.85	85.58	134.43
Rhizobium (Rh)	25.93	57.68	83.61	25.94	46.51	72.45
Rh + NP50%	50.25	56.49	106.74	43.25	84.37	127.62
Azotabacter (Az)	29.51	41.62	71.13	27.26	65.24	92.50
Az + NP50%	44.21	73.23	117.44	45.94	91.74	137.68
Bacillus (B)	30.73	49.88	80.61	30.62	63.82	94.44
B+ NP50%	43.30	52.84	96.14	40.43	69.30	109.73
$LSD_{0.05}$	8.01	17.96	20.41	8.87	23.57	26.38

NP100% = Ammonium sulphate at 350 Kg/fed and calcium super-phosphate at 200 Kg/fed
NP50% = Ammonium sulphate at 175 Kg/fed and calcium super-phosphate at 100 Kg/fed
Rh= *Rhizobium leguminosarum* bv. *phaseoli* Az = *Azotobacter chroococcum*
B = *Bacillus megatherium* var. *phosphaticum*

In the first cut of the second season, the treatment of *Rhizobium* + 50% NP and *Azotobacter* + 50% NP gave approximately the same herb fresh weight (258.32 and 258.10 g/plant, respectively), followed by that recorded with the treatment of 100% chemical fertilization (252.50 g/plant) with no significant difference among them. In the second cut of the second season, using *Azotobacter* + 50% NP caused superiority in herb fresh weight (551.61 g/plant) and consequently produced the highest total herb fresh weight (809.70 g/plant) in the second season. These results mean that using *Azotobacter* + 50% NP produced the highest total herb fresh weight in both seasons.

Herb air dry weight

Data presented in **Table (5)** revealed that, herb dry weight was generally increased as a result of using different fertilization treatments as compared to the unfertilized control plants in the two cuts of each season. In most cases, using bio-fertilizers only insignificantly increased herb dry weight compared to unfertilized control plants. Generally, in both seasons, using 50% of chemical fertilizers in addition to each bio-fertilizer gave heavier total dry herb than the bio-fertilizer only. The highest herb dry weight resulted from different treatments depending on the cut and the season. In the first cut of each season, the plants received 100% of chemical fertilizers gave the highest herb dry weight (59.59 and 48.85 g/plant, respectively). In the second cut of the first season, the plants received 50% of chemical fertilizers gave the highest herb dry weight (74.38 g/plant) and the highest total herb dry weight (123.18 g/plant). In the second cut of the second season, the plants received 50% of chemical fertilizers in addition to *Azotobacter* as a bio-fertilizer gave the highest herb dry weight (91.74 g/plant) and the highest total herb dry weight (137.68 g/plant). It is worth mentioning that, in both seasons, using 50% of chemical fertilizers in addition to *Rhizobium* as a bio-fertilizer gave values insignificantly lower than the highest herb dry weight, in most cases.

The present findings are in harmony with those reported by **Dessouky (2002)** on *Borago officinalis*, who found that bio-fertilizer treatment (*Azotobacter chroococcum*) increased dry weight of leaves, **AL-Qadasi (2004)** on basil plants, who found that inoculation with biofertilizers (*Azotobacter chroococcum* + *Azospirillum lipoferium* + *Bacillus megatherium* var. *phosphaticum*) at 150 ml/plant increased herb dry weight. Furthermore, **Swaefy Hend and Milad (2006)** on *Euryops pectinatus*, found that the treatment of 75% of the recommended chemical fertilizers combined with microbein gave the heaviest fresh and dry weights of plants.

Leaves/ stems fresh weight ratio

Concerning the effect of chemical and bio-fertilization treatments on leaves/ stems fresh weight ratio, the data presented in **Table (6)** showed that plants received 50% or 100% of the chemical fertilizers gave the lowest leaves/stem fresh weight ratio in both seasons. In most cases, using each bio-fertilizer only gave higher leaves/stems fresh weight ratio than unfertilized control plants. Addition of 50% of chemical fertilizers to each bio-fertilizer decreased leaves/stems fresh weight ratio, in most cases, compared to that recorded with plants received each bio-fertilizer only.

In the first season, using *Bacillus* only as a bio-fertilizer resulted in the highest leaves/stems fresh weight ratios (1.47 and 1.51 in the first and second cut, respectively). In the second season, plants received *Rhizobium* only gave the highest leaves/stems fresh weight ratio in the first cut (1.63), whereas plants received 50% of chemical fertilizers in addition to *Azotobacter* gave the highest leaves/stems fresh weight ratio in the second cut (1.64). Such results are in agreement with **AL-Qadasi (2004)**, who found that inoculation of basil plants with a mixture of bio-fertilizers at 150 ml/plant plus NPK (25%, 50% and full dose) increased leaves/branches ratio.

Fresh and air dry weights of Rhizomes

In both seasons, *Mentha piperita*, L. plants received *Rhizobium* only gave the significantly higher rhizomes fresh and air dry weights, compared to the other treatments **(Table 6)**. The favourable effect of *Rhizobium* in improving plant growth and increasing yield productivity may be attributed to different mechanisms of action including: a) the production of secondary metabolites such as antibiotic, hydrogen cyanide and plant hormones like substances, b) the production of siderophors, c) antagonism to soil borne root pathogens, d) phosphate solubilization and e) dinitrogen fixation **(Gilic, 1995, Antoun *et al* 1998 and Ragab & Rashed, 2003).**

Table 6. Effect of some chemical and bio-fertilizers on leaves/stems fresh weight ratio and rhizomes fresh and air dry weights of *Mentha piperita*, L. plants during 2005 and 2006 seasons.

Fertilization treatments	Leaves / stems fresh weight ratio				Rhizomes fresh weight (g / plant)		Rhizomes dry weight (g / plant)	
	First season (2005)		Second season (2006)					
	First cut	Second cut	First cut	Second cut	First season (2005)	Second season (2006)	First season (2005)	Second season (2006)
Control	1.28	0.95	1.35	1.34	58.0	157.4	31.6	75.1
NP50%	1.00	0.89	0.75	1.08	138.5	316.7	58.2	94.6
NP100%	0.98	0.97	0.91	1.03	142.6	232.7	46.4	81.2
Rhizobium (Rh)	1.31	1.22	1.63	1.19	301.8	370.7	111.8	127.6
Rh + NP50%	1.22	1.12	1.13	1.28	119.4	226.2	47.7	96.2
Azotabacter (Az)	1.25	1.12	1.62	1.36	185.6	273.7	56.4	83.4
Az + NP50%	1.03	1.21	1.32	1.64	140.4	295.5	41.5	94.6
Bacillus (B)	1.47	1.51	1.58	1.52	163.4	255.5	53.8	101.2
B+NP50%	1.34	1.33	1.02	1.49	143.5	282.8	51.0	102.4
LSD $_{0.05}$	**0.20**	**0.17**	**0.16**	**0.13**	**38.5**	**39.0**	**9.9**	**12.9**

NP100% = Ammonium sulphate at 350 Kg/fed and calcium super-phosphate at 200 Kg/fed.
NP50% = Ammonium sulphate at 175 Kg/fed and calcium super-phosphate at 100 Kg/fed
Rh= *Rhizobium leguminosarum* bv . . *phaseoli* Az = *Azotobacter chroococcum*
B = *Bacillus megatherium* var. *phosphaticum*

In most cases, unfertilized control plants recorded the significantly lower fresh and air dry weights in both seasons, compared to the other treatments. Plants that received 50% of chemical fertilizers gave significantly higher rhizomes air dry weight compared to the plants which received 100% of chemical fertilizers.

Essential oil percentage

Data presented in **Table (7)** revealed that in the first cut of each season, the plants inoculated with *Bacillus* only gave the significantly higher essential oil percentages (0.29 and 0.35% in the first and second seasons, respectively) compared to the other fertilization treatment. In the second cut of the first season, plants received 50% of chemical fertilizers gave the significantly higher essential oil percentage (0.42%), compared to the other fertilization treatments. In the second cut of the second season, plants received 100% of chemical fertilizers as well as plants received 50% of chemical fertilizers in addition to *Rhizobium* gave the highest essential oil percentage (0.44%) compared to the other treatments.

In the first cut of the first season, plants received full chemical fertilization as well as that received 50% of chemical fertilizers alone and that received 50% of chemical fertilizers + *Rhizobium* or *Azotobacter* gave the lowest essential oil percentage (0.20%), compared to the other treatments.

Table 7. Effects of some chemical and bio-fertilizers on essential oil percentage of *Mentha piperita*, L. plants during 2005 and 2006 seasons.

Fertilization treatments	Essential oil%			
	First season (2005)		Second season (2006)	
	First cut	Second cut	First cut	Second cut
Control	0.23	0.23	0.22	0.25
NP50%	0.20	0.42	0.24	0.42
NP100%	0.20	0.40	0.33	0.44
Rhizobium (Rh)	0.23	0.34	0.22	0.37
Rh + NP50%	0.20	0.40	0.19	0.44
Azotabacter (Az)	0.23	0.38	0.23	0.38
Az + NP50%	0.20	0.40	0.22	0.41
Bacillus (B)	0.29	0.31	0.35	0.30
B+NP50%	0.21	0.37	0.27	0.43
LSD $_{0.05}$	**0.02**	**0.02**	**0.02**	**0.04**

NP100% = Ammonium sulphate at 350 Kg/fed and calcium super-phosphate at 200 Kg/fed
NP50% = Ammonium sulphate at 175 Kg/fed and calcium super-phosphate at 100 Kg/fed
Rh = *Rhizobium leguminosarum* bv . *phaseoli*
Az = *Azotobacter chroococcum*
B = *Bacillus megatherium* var. *phosphaticum*

In the first cut of the second season, plants received 50% of chemical fertilizers in addition to *Rhizobium* gave the lowest essential oil percentage

(0.19%), compared to the other treatments. In the second cut of each season, unfertilized control plants gave the lowest essential oil percentage (0.23 and 0.25% in the first and second seasons, respectively), compared to the other treatments.

The previous results are in agreement with that reported by **Gad (2001)** who reported that applying bio-fertilizers to fennel and anise plants increased the volatile oil percentage in the seeds and **AL-Qadasi (2004)** who found that the highest oil percentage was obtained from basil plants received full NPK treatment plus mixture of bio-fertilizers at 150 ml/plant. Also, **Mohamed (2007)** found that bio-fertilizers applied alone had a stimulation effect on the oil percentage of caraway and anise fruits as compared to the unfertilized plants. The application of two third dose of mineral combined with bio-fertilizers to caraway plants, gave essential oil percentage similar to that produced from plants received full chemical fertilizers.

Essential oil constituents

Concerning the effect of chemical and bio-fertilization treatments on the oil constituents, the data presented in **Table (8)** show that, from GLC analysis of essential oil samples, the components separated were β-Pinene, menthone, iso-menthone, menthol and menthyl acetate.

Regarding the effect of fertilization treatments on β-pinene , the treatment of 350 kg/fed ammonium sulphate and 200 kg/fed calcium super-phosphate (100% NP) produced the highest β-pinene (9.4%) followed by *Rhizobium* inoculation alone (5.6%) then *Azotobacter* inoculation alone (5.5%). *Bacillus* inoculation alone gave the same result of control plants (5.1%). It is clear that using the combination between each bio-fertilizer and chemical fertilizers (50% NP) resulted in lower β-pinene percentage as compared to that recorded with each biofertilizer only or that recorded with the control plants.

Plants received 50% NP in addition to *Rhizobium* resulted in the highest menthone percentage (78.1%) followed by that recorded with plants inoculated with *Azotobacter* only (76.4%) and *Bacillus* alone (74.3%). On the other hand, plants inoculated with *Rhizobium* only gave the lowest menthone percentage (46.6%).

The plants received 50% of chemical fertilizers in addition to *Azotobacter* produced the highest Iso-menthone percentage (16.3%). Generally, plants received bio-fertilizer alone produced higher menthol% compared with those received bio-fertilizer plus chemical fertilizers. The plants inoculated with *Rhizobium* only resulted in the highest menthol percentage (26.8%).

Among the different treatments, it could be observed that plants received 50% NP in addition to *Rhizobium* gave the best results in terms of oxygenated compounds which reached 98.5%. Also, the plants received 50% NP in addition to *Azotobacter* resulted 95.4% oxygenated compounds. These two treatments gave approximately the same mean oil percentage and, generally, gave the heaviest herb fresh weight.

Table 8. Effect of some chemical and bio-fertilizers on the percentage of essential oil constituents of *Mentha piperita*, L. plants in the second cut of the second season (2006).

Fertilization treatments	Essential oil constituents%					
	β-Pinene	Menthone	Iso-menthone	Menthol	Menthyl acetate	other
Control	5.1	52.9	14.3	7.2	10.0	10.5
NP50%	3.3	51.2	15.8	22.9	2.6	4.2
NP100%	9.4	66.1	6.9	10.7	2.1	4.8
Rhizobium (Rh)	5.6	46.6	11.7	26.8	3.7	5.6
Rh+ NP50 %	1.9	78.1	7.0	9.6	1.9	1.5
Azotabacter (Az)	5.5	76.4	6.1	8.1	1.8	2.1
Az + NP50%	2.1	62.9	16.3	11.8	2.3	4.6
Bacillus (B)	5.1	74.3	7.0	7.8	2.2	3.6
B + NP50%	3.3	63.9	13.1	6.2	8.9	4.6

NP100% = Ammonium sulphate at 350 Kg/fed and calcium super-phosphate at 200 Kg/fed
NP50% = Ammonium sulphate at 175 Kg/fed and calcium super-phosphate at 100 Kg/fed
Rh= *Rhizobium leguminosarum* bv . . *phaseoli*
Az = *Azotobacter chroococcum*
B = *Bacillus megatherium* var. *phosphaticum*

In this respect, **AL-Qadasi (2004)** found that inoculation with bio-fertilizers at 150 ml/plant led to formation of the highest content of methyl chavicol and inoculation at 300 ml/plant gave the highest percent of cineol in the *Ocimum basilicum* oil. Applying 50% NPK with inoculation with bio-fertilizers at 150 ml/plant significantly increased eugenol (32.2%) compared with full NPK (27.9%)

and with control (11.9%). Also, **Mohamed (2007)** found that all major essential oil constituents were increased as a result to applying two thirds dose of chemical fertilizers combined with bio-fertilizers to caraway and anise plants in comparison to those produced from plants received full chemical fertilizers.

CONCLUSION

It could be advised to apply 175 kg/fed ammonium sulphate and 100 kg/fed calcium superphosphate (50% of the recommended amount of chemical fertilizers) in addition to inoculation with *Azotobacter chroococcum* or *Rhizobium leguminosarum* bv. *phaseoli* as a biofertilizers because of producing the same oil percentage and resulted in insignificantly different herb fresh yield compared to that recorded using the full amount of the recommended chemical fertilizers, but with much more oxygenated compounds and healthier environment.

REFERENCES

Abotaleb, H.H.; Mona A.M. Soleman and N.M. Mahrous (2002). The enhancement of wheat productivity by inoculation with associative diazotrophs and foliar application of methanol under different N-fertilizer levels. **Assiut J. Agric. Sci., 33:113-127.**

Alexander, D. and D.A. Zubeter (1991). Use of chrome azural S reagents to evaluate siderophore production by rhizosphere bacteria. **Biol. Fertil. Soils, 12:3945.**

AL-Qadasi, A.S.S. (2004). Effect of Biofertilization on *Ocimum basilicum* L. Plant. PP. 115-119. M.Sc. Thesis , Fac. Agric., Cairo Univ., Egypt.

Antoun, H.C.; J. Beauchamp; N. Houssard; R. Chabat and M. Lolande (1998). Potential of *Rhizobium* and *Bradyrhizobium* species as plant growth promoting rhizobacteria on non-legumes: Effect on radishes (*Rophanous sativus*, L). **Plant and Soil, 204:762 - 767.**

Arora, N.K.; S.C. Kang and D.K. Maheshwari (2001). Isolation of siderophore-producing strains of *Rhizobium meliloti* and their biocontrol potential against *Macrophomina phaseolina* that causes charcoal rot of groundnut. **Curr. Sci, 81 (6): 673-677.**

Bakker, A.W. and M. Schippers (1987). Microbial cyanide production in the rhizosphere in relation to potato yield reduction and *Pseudomonas spp*. Mediated plant growth- stimulation. **Soil Biol. Biochem., 19:451-457.**

Bown, D. (1995). Growing Herbs, P. 77. Dorling Kindersley, London.

Bown, D. (1996). Encyclopedia of Herbs and Their Uses, pp. 311-314. Dorling Kindersley, London.

British pharmacopoeia (1963). Determination of Volatile Oil in Drugs, pp. 29 – 38. The pharmaceutical Press, London.

Bunt, J.S. and A.D. Rovira (1955). Microbiological studies of some subantarctic soils. **J. Soil, 6: 119 -128.**

Burris, R.H. (1976). Nitrogen Fixation. **In: Plant Biochemistry, pp. 887-908.** Academic Press, New York.

Chapman, H.D. and P.F. Pratt (1961). Methods of Soil, Plants and Water Analysis, pp.60-69. University of California, Division of Agricultural Sciences.

De-Britto Alvarez, M.A.; S. Gagne and H. Antoun (1995). Effect of compost on rhizosphere microflora of the tomato and on the incidence of plant growth-promoting rhizobacteria. **Appl. Environ. Microbiol., 61:194-199.**

Dessouky, M.M.M. (2002). A comparative response of *Borago officinalis*, L. plant to the bio., chemical fertilization and Adinosine-tri-Phosphate (ATP) treatments. **Bull. Fac. Agric., Cairo Univ., 53 (4): 613-638.**

Gad, W.M. (2001). Physiological Studies on *Foeniculum vulgare* Mill. and *Anethum graveolens*, L. pp. 98-110. M.Sc. Thesis, Fac. Agric., Kafr El-Sheikh, Tanta Univ., Egypt.

Gilic, C. (1995). The enhancement of plant growth by free-living bacteria. **Can. J. Microbiol., 41:109 - 117.**

Goldstein, A.H. (1986). Bacterial solubilization of mineral phosphates: historical perspective and future prospect. **Amer. J. Altern. Agric., 1: 51-57.**

Hassan, H.R.; Nassar Dalia, M.A. and M.H.A. Abou Bakr (2006). Effect of mineral and bio-fertilizers on growth, yield components, chemical constituents and anatomical structure of moghat plant (*Glossostemon bruguieri*, Desf.) grown under reclaimed soil conditions. **J. Agric. Sci. Mansoura Univ., 31 (3): 1433-1455.**

Hegazi, N. A. and S. Neimela (1976). A note on the estimation of Azotobacter densities by membrane filters technique. **J. Appl. Bacteriol, 41: 311-313.**

Hoftman, E. (1967). Chromatography, 2nd Ed., pp. 208-515. John Wiley & Sons, Inc., New York.

Milad, S.M.N. (2003). A Study of Some Agricultural Practices Affecting Growth, Yield, and Chemical Composition of Roselle (*Hibiscus sabdariffa*, L.). PP. 136-147. Ph.D. Thesis, Fac. Agric., Cairo Univ., Egypt.

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. pp. 147-153. Ph.D. Thesis, Fac. Agric., Fayoum Univ., Egypt.

Mona, A.M.; H.H. Abo-Taleb; M. M. El-Sawi and Nadia M. A. Ghalab (2000). Growth, Yield and yield components of diazotophs inoculated rape seed as affected by foliar application of methanol and N-Fertilizer. **Proc-10th Conference of Microbiology, Applied Microbial. Soc., Cairo, Egypt, pp. 110-118.**

Okon, Y. and C.A. Lanabdera (1994). Agronomic application of *Azospirillum*: An evaluation of 20 years world wide field inoculation. **Soil Biol. Biochem., 26, (12): 1591-1601.**

Pandey, B.P. (1982). Taxonomy of Angiosperms, pp. 414-422. S. Chand & Company Ltd. New Delhi.

Paramaguru, P. and S. Natarajan (1993). Effect of *Azospirillum* on growth and yield of chilli (*Capsicum annuum*, L.) grown under semi-dry conditions. **South Indian Hort., 41 (2): 80-83 (C.F. Hort. Abst., 65: 3087).**

Pondy, A.; S. Eklabya and P. Lokman (1998). Influence of bacterial inoculation on maize in upland forming system of the Sikkim Himalaya. **Soil Biol. Biochem. 30:379-384.**

Radwan, S.M.A. and M.M. Farahat (2002). Growth and yield of coriander plants as affected by bio-organic fertilization and pix application. **Egypt. J. Appl. Sci., 17: 268-286.**

Ragab, A.A. and M.H. Rashed (2003). Enhancement of nutrients uptake and metabolism efficiency of sorghum (*Sorghum bicolor*, L.) plants by biofertilizers under water stress. **Zagazig J. Agric. Res., 30 (1):147-166.**

Ragab, A.A.; Nadia H. El-Batanony and A.A. Abo El-Soud (2005). Response of three soybean varieties to Mixed-Inoculation by *Bradyrhizobium* strains and *Rhizobium* isolates from wild-legumes in Egypt. **Ecology, Taxonomy, Flora & Cytogenetics, 15B: 27-47.**

Ragab, A.A.; H.H. Abotaleb and Nadia M. A. Ghalab. (2006). Response of Lupine plants to inoculation with *Bradyrhizobium* sp. (lupinus) combined with plant growth promoting Rhizobacteria (PGPR) under newly reclaimed soil condition. **J. Agric. Sci. Mansoura Univ., 31(7): 4613-4622.**

Sakr, Weaam R.A. (2001). Effect of Some Organic and Inorganic Fertilizers on *Mentha piperita*. pp. 42-46. M.Sc. Thesis, Fac. Agric., Cairo Univ., Egypt.

Sharaf, M.S. and M.E. Khattab (2004). Effect of fertilization with inorganic, organic and bio-fertilizers on growth, yield and volatile constituents of fennel (*Foeniculum vulgare*, Mill.). **J. Agric. Sci., Mansoura Univ., 29(9): 5245-5264.**

Snedecor, G.W. and W.G. Cochran (1982). Statistical Methods, p. 507. The Iowa State Univ., Press., Ames., Iowa, USA..

Swaefy Hend, M.F.A. (1996). Effect of Chemical Fertilization and Some Trace Elements on Growth and Productivity of *Mentha piperita*, L. Plant. pp. 31-37. M.Sc. Thesis, Fac. Agric., Cairo Univ., Egypt.

Swaefy Hend, 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(11): 7329-7345.**

Vincent, J.M. (1970). A Manual for the Practical Study of the Root- nodule Bacteria. IB p.15. Prentice Hall International, Ltd., New Jersey, USA.

Wollum, A.G. (1982). Cultural Methods for Soil Microorganisms. pp. 781-802. In: Al-Page (ed). Agronomy series No. 9. Methods for Soil Analysis, part 2. Chemical and Microbiological Properties. Amer. Soc. Agron. Madison. Wisconson, USA.

حوليات العلوم الزراعية
جامعة عين شمس، القاهرة
مجلد(٥٢)، عدد (١)، ٤٥١-٤٦٣، ٢٠٠٧

تأثير بعض الأسمدة الكيماوية والمخصبات الحيوية على نباتات النعناع الفلفلى النامية فى أرض رملية

١- التأثير على النمو الخضرى ونسبة الزيت ومكوناته

[٣٧]

هند مصطفى فهمى سويفى[١]- وئام رمضان عرفة صقر[١]- عاطف زكريا سبع[٢]- عاطف عبد العزيز رجب[٣]

١- قسم بساتين الزينة - كلية الزراعة - جامعة القاهرة - الجيزة - مصر

٢- قسم النبات الزراعى - كلية الزراعة - جامعة القاهرة - الجيزة - مصر

٣- قسم الميكروبيولوجيا الزراعية- معهد بحوث الأراضي والمياه والبيئة - مركز البحوث الزراعية - الجيزة - مصر

أجري هذا البحث بمشتل التجارب بقسم بساتين الزينة، كلية الزراعة، جامعة القاهرة، الجيزة، خلال الموسمين المتتاليين ٢٠٠٥ و ٢٠٠٦. وذلك بهدف دراسة تأثير الأسمدة الكيماوية النتروجينية والفوسفاتية مع أو بدون التلقيح ببعض السلالات الميكروبية التالية *Rhizobium leguminosarum* bv. *phaseoli*, *Azotobacter chroococcum* and *Bacillus megatherium* var. *phosphaticum* والمستخدمة كمخصبات حيوية أو كبكتيريا مشجعة للنمو وذلك على كل من تعداد الكائنات الحية الدقيقة في منطقة نمو الجذور وعلى الصفات الخضرية ومحصول العشب الطازج والجاف والنسبة المئوية للزيت الطيار ومكوناته لنبات النعناع الفلفلي *Mentha piperita*, L. النامية فى تربة رملية.

أظهرت النتائج قدرة السلالات الميكروبية تحت الدراسة علي إنتاج إندول حامض الخليك و حامض الهيدروسيانيك وكذلك انتاج مركبات السيدروفور وكذلك قدرة هذه السلالات علي إذابتها للفسفور غير الميسر كتقديرات وصفية. وقد أشارت النتائج المتحصل عليها إلى أن إضافة ١٧٥ كجم سلفات نوشادر (٢٠,٥% ن)/فدان + ١٠٠ كجم سوبر فوسفات الكالسيوم الأحادي (١٥,٥% فو٢أ٥)/فدان مع التلقيح بالأزوتوباكتر كمخصب حيوى كانت أفضل المعاملات في زيادة الوزن الطازج الكلي للعشب. أدى تلقيح نباتات النعناع الفلفلي بالريزوبيم فقط إلى زيادة معنوية فى الوزن الطازج والجاف للريزومات في كلا الموسمين مقارنة بباقي المعاملات. أوضحت النتائج المتحصل عليها من التحليل الكروماتوجرافي للزيت الطيار أن استخدام معظم معاملات المخصبات الحيوية سواء منفردة أو مع إضافة ٥٠% من التسميد الكيماوي أدت إلى زيادة نسبة المركبات الأكسيجينية في الزيت الطيار لنباتات النعناع الفلفلي.

يمكن التوصية بإضافة ١٧٥ كجم سلفات نوشادر (٢٠,٥% ن) /فدان + ١٠٠ كجم سوبر فوسفات

تحكيم: ا.د عواض محمد قنديل
ا.د عبد الغفور عوض السيد

أحادي الكالسيوم (١٥,٥% فو٢أ٥) /فدان (٥٠% من الكمية الموصى بها من التسميد الكيماوي) مع التلقيح بالازوتوباكتر أو الريزوبيم حيث أعطتا نفس نسبة الزيت الطيار ومحصول عشب طازج عالي لا يختلف معنويا عن المتحصل عليه باستخدام ١٠٠% من التسميد الكيماوي مع أفضلية الحصول على نسبة أعلى من المركبات الأكسيجينية بالإضافة إلى الحفاظ على سلامة البيئة.