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PRODUCTIVITY OF *Majorana hortensis* L. PLANTS AS INFLUENCED BY THE INTERACTIONS BETWEEN MINERAL AND BIOLOGICAL FERTILIZATION

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ABSTRACT

A field experiment was carried out to investigate the effect of pre-sowing inoculation of marjoram with *Azotobacter chroococcum*, *Bacillus circulans*, vesicular arbuscular mycorrhizae(VAM) or their mixture in the presence of full or half of the recommended field rate of the inorganic NPK fertilization on the plant growth, microbial activities in the rhizosphere, volatile oil yield and chemical composition. Parameters related to the above- mentioned measures of performance were recorded. Data of this study showed that mixed inoculation with the three biofertilizers supplemented with half dose of NPK gave the highest and significant increases in the growth parameters, oil % and oil yield feddan^{-1}, as well as, the N, P and K % in plant herb . The above-mentioned treatment increased the concentration of the constituents volatile oil, geranyl acetate, cineole, linalool, limonene and α-terpenolene, while decreased the concentrtion of α-terpineol compared with the control. Mixed inoculation also, raised total microbial densities and also those of azotobacters and *B.circulans* in marjoram rhizospheric soil and increased CO_2 evolution, N_2-ase activity and mycorrhizal root infection, as well as, spore production .On the other hand, inoculation with either of *A.chroococcum* or *B.circulans* amended with half dose of NPK decreased the above-mentioned parameters compared with uninoculated plants supplemented with the full dose of NPK fertilization, but VAM applied alone or in conjugation with the bacterial partners significantly enhanced those parameters .

INTRODUTION

Recently, there has been an increasing awareness of the undesirable impact of mineral fertilizers on the environment, as well as, the potentially dangerous effects of chemical residues in plant tissues on the human health. As a result of this awareness, strict regulations have been imposed in several countries (especially in the European markets) prohibiting the import of "chemically-grown" products. These restrictions are also, applied for medicinal plants as the addition of heavy doses of chemical fertilizers adversely affects extraction of active constituents . This finding is faced by the high requirement of many medicinal plants for NPK mineral fertilizers . This has led growers of crop, medicinal and aromatic plants in many countries to adopt organic and biological agricultural practices Biofertilization is known to compensate apart of the mineral fertilizers to minimize high rates of mineral fertilizers and consequently reduce agricultural costs, as well as, soil pollution. *Majorana hortensis* L. plant is considered as one of the most important medicinal and aromatic crop in Egypt for local and foreign markets with high production and great applications (foods, medicine and perfumes). Many trials have been conducted in this concern

for raising the productivity of this plant . In this respect, El-Gadban *et al.* (2002) reported that marjoram plants amended with the highest level of compost (15 m^3 $feddan^{-1}$) either alone or in conjugation with a mixture of N_2-fixing bacteria showed considerable increments in growth characters and contents of N,P and K, as well as ,essential oil (%) and also components of oil (α-terpineol, α-terpenolene, cineole and linalool) compared with mineral fertilization treatment. Also, Abo El-Ala (2002) in his study on marjoram and basil showed that root colonization by N_2-fixers and P-dissolving bacteria was mostly stimulated in the rhizosphere of inoculated plants with a mixture of these microorganisms . This inoculation treatment, also significantly improved plant growth and oil yield. Similarly, Kandeel *et al.* (2002) reported that dual inoculation of sweet basil with *Azotobacter* + *Azospirillum* supplemented with half or full dose of the recommended mineral N-fertilizer, significantly increased plant growth, oil % and yearly oil yield $plot^{-1}$compared with uninoculated plant given full dose of nitrogen . Balabel (1997) showed highly significant yield responses of potato to K fertilization and inoculation with *B.circulans.* Concerning the effect of VAM, Gautam and Sharma (1996) found that VAM colonization varied in different medicinal plant roots and spores count ranged between 200 to 8900 spores 100 $g^{-1}$of soil . Higher spore count supported good growth . Abdul-Khaliq and Janardhanan (1998) studied the influence of 3 VA mycorrhizal fungi, *Glomus aggregatum*, *G. fasciculatum* and *G. mosseae* on 6 cultivated mints and found that inoculation with VAM fungi increased the shoot biomass of all species . Azcon and Barea (1998) demonstrated that mycorrhizal inoculation largely improved N,P and K uptake by lavender plant roots . EL-Sawy *et al.* (1998) stated that inoculation with a mixture of *Azotobacter*, *Azospirillum* and VAM amended with the full dose of P as rock-phosphate and inorganic N-fertilization, in combination with VAM inoculation, remarkably increased the growth of *Ammi visnaga* plant and its production of khellin . This increase, was accompanied by a high percentage of mycorrhizal root infection and rhizospheric N_2-ase activity .

This study aimed to evaluate the effect of inoculation with *A.chroococcum*, *B.circulans* and VAM or their mixture in the presence of half or full dose of the recommended field rate mineral NPK-fertilizers, on the growth, oil yield and chemical composition of marjoram plant. The positive responses of marjoram plants to increased levels of mineral fertilization was considered in this study. Therefore, inoculation with either of 3 biofertilizers, i.e. *A.chroococcum*, *B.circulans* and VAM or their mixture was conjugated with the full and the half of the recommended field of NPK-fertilization . The 2 above-mentioned rates were splitted into 3 doses to: a) minimize the adverse effect of the batch application of mineral nutrients on N_2-fixation or mycorrhizal colonization . b) stimulate a more prolonged effect of those biofertilizers . Moreover, the sustained effectiveness of biofertilizers under the above-mentioned conditions, will accommodate the high nutrient requirements of marjoram plants.

MATERIALS AND METHODS

A field experiment was conducted at the Experimental Farm, Faculty of Agriculture, Ain Shams University, Shobra El-Kheima, for two successive seasons (2000/2001 and 2001/2002). The experiment aimed to study the effect of the interaction between NPK fertilization and single or combined inoculation with 3 biofertilizers (*A.chroococuum*, *B.circulans* and vesicular arbuscular mycorrhizae) on the growth, oil yield and chemical composition of marjoram plant .

Seeds and soil

Seeds of marjoram (*Majorana hortensis* L., Family:Lamiaceae) were obtained from Medicinal and Aromatic Research Department of Horticulture Institute, Dokky, Cairo, Egypt, to be used in this study. The soil of the Experimental Farm of Fac. Agric.,Ain Shams Univ., was used for experimentation. The chemical properties of the soil were determined before cultivation using a method described by Page (1982) . The data of the analysis are in Table (1) .

Table (1): Physical and chemical analysis of the soil used for growing marjoram plant .

Physical analysis	Sand %		24.5
	Silt %		22.7
	Clay %		52.6
	Soil texture		Clay
Chemical analysis	Electrical conductivity (dSm^{-1})		1.3
	PH		7.3
	Soluble anions (meq/L)	CO_3^{--}	---
		HCO_3^-	4.0
		Cl^-	3.8
		SO_4^{--}	2.7
	Soluble cations (meq/L)	Ca^{++}	3.8
		Mg^{++}	2.6
		Na^+	2.2
		K^+	1.9
	$CaCO_3$ %		1.1
	Available micronutrients (ppm)	Fe	91.4
		Mn	35.6
		Zn	18.3
		Cu	16.4
	Available phosphorus ($mg\ g^{-1}$)		0.3
	Total nitrogen ($mg\ 100g^{-1}$)		102
	Organic matter %		1.22

Organic manure ,mineral and bio-fertilizers

a)-A farm yard manure was provided from the Milk Replacer Center, Fac. Agric, Ain Shams Univ., Shobra El-Kheima, Cairo, Egypt. It has an organic carbon of 24.3 and 22.8%, total nitrogen of 1.3 and 1.1%, C/N ratio of

(18.69 :1) and (20.73:1), organic matter of 45.5 and 47.2%, moisture content of 5.6 and 7.4% ,P%= 0.4 and 0.3, K%= 1.7 and 1.5 and pH were 6.7and 6.6 for the first and second seasons, respectively, and chemically analyzed according to Page (1982) .

b)-Ammonium nitrate (33.5 % N) ,calcium superphosphate (15.5 % P_2O_5) and potassium sulphate (48 % K_2O) were used for NPK fertilization, respectively. The inorganic NPK were used at two rates:

1-Full of the recommended field rate ,i.e. 300,200 and 100 kg fed.$^{-1}$of ammonium nitrate, calcium superphosphate and potassium sulphate equal (100, 31 and 48 kg fed.$^{-1}$) of the N, P_2O_5 and K_2O respectively .

2-Half of the recommended dose ,i.e. 150,100 and 50 kg fed.$^{-1}$ equal (50,15.5 and 24 kg fed.$^{-1}$) of N, P_2O_5 and K_2O respectively . Calcium superphosphate was added during soil preparation, while N and K were added into 3 equal doses as follows: a) 30 days after transplantation b) 2 weeks after the first harvest, and c) 2 weeks after the second harvest . Nine treatments were represented in complete randomized block design with 3 replicates as follows

1- Uninoculated(Control) + full dose of NPK .
2- *A.chroococccum* + half dose of NPK .
3- *A.chroococccum* + full dose of NPK .
4- *B.circulans* + half dose of NPK .
5- *B.circulans* + full dose of NPK .
6- VAM + half dose of NPK .
7- VAM + full dose of NPK.
8-A mixture of *A.chroococcum* +*B.circulans* +VAM+half dose of NPK.
9-A mixture of *A.chroococcum* +*B.circulans* +VAM+ full dose of NPK.

c)-*Azotobacter chroococcum* Az.M15 originated from the rhizosphere of *Majorana hortensis* L. and the silicate bacterium (*Bacillus circulans* ATCC 4513) which was provided from Cairo Mircen, Fac. Agric., Ain Shams Univ., Shoubra El-Kheima, Egypt, were used in this investigation . The two strains were grown separately on modified Ashby's medium (Abd El-Malek and Ishac,1968) and modified Alexandrov's medium (Zahra, 1969) for 7 and 4 days at 28 ± 2 °C, respectively. Vesicular arbuscular mycorrhizae (VAM) spores extracted from the rhizosphere of marjoram plants by wet-sieving and decanting technique (Gerdemann and Nicolson ,1963) .and were propagated on roots of maize plants under green house conditions for 12 weeks and then used in this study. The spores represented two main genera of VAM,i.e. *Glomus* and *Gigaspora* .

Experimental techniques

Seeds and seedlings inoculation

Seeds and developed seedlings of marjoram used for inoculation treatments were inoculated prior to sowing in nursery (mid November 2000 or 2001) and directly before transplantation in the field. Inoculation was carried out by soaking of seeds or immersing the seedling roots in cell suspension of either *A chroococcum* or *B.circulans* (contained about 10^8 cells ml^{-1})or their mixtures for 30 minutes. Cell culture prepared for bacterial inoculation was supplemented with arabic gum (16%) as an adhesive agent. Inoculated

seeds were dried at room temperature for 1 hour before sowing in nursery . VAM spores manipulated as a single biofertilization treatment or in combination with bacterial mixture, was applied by pipetting 3 ml of spore suspension (contained about 100 spores ml^{-1}) in seedbed in nursery .

Layout of experiment

The farm yard manure was added to the whole soil area used for experimentation one month before cultivation (January) at a rate of 25 m^3 fed^{-1} At the beginning of February, the soil was prepared into 27 plots . The area of each plot was 4m^2(2×2 m) which contained 3 rows. Ten weeks old seedlings were transplanted in the soil with a distance of 30 cm between transplants. The total number of transplants was 18 $plot^{-1}$. Ammonium nitrate, calcium superphosphate and potassium sulphate were used for NPK fertilization .

At full blooming (during June, August and October), the plants were harvested 3 times and a range of parameters were recorded as follows:

(1)Growth parameters

Plant growth parameters of marjoram, i.e. plant height, number of branches $plant^{-1}$ and herb fresh and dry weights $plant^{-1}$ or $fed.^{-1}$ for each harvest during both seasons were recorded .

(2)Microbiological parameters

Microbiological parameters of marjoram rhizosphere were evaluated at the 1st, 2nd and 3rd cut by determining total microbial count and counts of *B.circulans* on modified Bunt and Rovira (Abd El-Hafez, 1966)and modified Alexandrov (Zahra ,1969) media, respectively, using the decimal plate count technique. Estimates of *Azotobacter* populations were determined by MPN technique using Cochran's Tables (Cochran, 1950) .The rates of CO_2 evolution were determined at each cut according to the method of Pramer and Schmidt (1964) modified by Shehata (1972). Nitrogenase activity in the rhizosphere of plants was estimated by acetylene reduction assay (ARA) according to the method of Scholihorn and Burris (1967) .

Mycorrhizal infection (%) in roots of marjoram plants was recorded in root samples collected from plants grown under different treatments according to the method described by Phillips and Hayman (1970) and mycorrhizal spore densities was also carried out according to Gerdemann and Nicolson (1963).

(3)Quantification and analysis of volatile oil

The volatile oil percentage of plant herb at each harvest was determined according to Guenther (1961) .Gas liquid chromatography (GLC) analysis was also performed on oil samples taken from the first harvest during 2000/2001 season in the Central Lab., Fac. Agric., Ain Shams Univ , Shobra El-Kheima, Cairo, Egypt . The volatile oil constituents of marjoram oil were identified according to Bunzen *et al.* (1969) .

(4)Plant chemical analysis

NPK contents in dry plant herb were determined during the first growing season after wet digestion . Nitrogen % was determined according to A.O.A.C.(1990) and P as well as K were determined according to the method described by Chapman and Pratt (1978) .

Statistical analysis

Data of the 3 harvests of either of the 2 seasons were statistically analyzed according to the procedure described by Snedecor and Cochran (1981) and Duncan' s Multiple Range Test was used to differentiate means of CO_2 evolution and N_2–ase activity by using the method of Waller and Duncan (1969).

RESULTS AND DISCUSSION

1-Influence of biological and NPK fertilizers on the microorganisms number and microbial activities in rhizosphere of marjoram plant:

1.1.Densities of total microbial flora, silicate bacteria and azotobacters:

Data presented in Table (2) showed that densities of total microbial flora, silicate bacteria and azotobacters were considerably affected by inoculation, cutting time and NPK mineral fertilization. The highest densities of total microbial flora and azotobacters were 157.8 x 10^6 and 70.3 x 10^4 cfu g^{-1} dry rhizospheric soil, respectively at the second cut of the plants inoculated with a mixture of the three biofertilizers in the presence of half dose of NPK fertilizers. Density of silicate bacteria was reached to level (108.2 x 10^3cfu g^{-1}dry rhizospheric soil) at the third cut in mixed inoculation treatments in the presence of full dose of NPK mineral fertilizers .This may be due to the maximal production of biologically fixed nitrogen during the maturity stage of plant growth (Nelson, 1983) . Monib *et al.* (1979) found that inoculation of barley grains with *A.chroococcum*, significantly increased azotobacters population, especially in the rhizosphere .

Table (2): **Effect of inoculation with *A.chroococcum*, *B.circulans*, VAM or their mixture in the presence of full or half dose of NPK fertilizers on counts of total microbial flora, silicate bacteria and azotobacters population in rhizosphere soil of marjoram plant at 2000/2001 seasons.**

Treatments		TM[5]	SB[6]	AZ[7]	TM	SB	AZ	TM	SB	AZ
Inoculation	NPK rate	First cut			Second cut			Third cut		
Uninoculated	fd[1]	24.5	12.3	9.3	54.5	19.2	16.3	32.6	35.1	14.2
A.chroococcum	hd[2]	45.8	16.4	23.4	86.6	25.5	67.3	73.4	45.2	49.5
	fd	32.8	20.5	12.2	77.2	34.6	45.6	54.7	51.6	38.4
B.circulans	hd	74.9	34.6	16.5	122.3	52.7	22.9	105.0	71.8	20.4
	fd	63.8	53.8	14.3	111.2	65.8	18.6	81.3	92.3	16.0
VAM[3]	hd	46.6	21.5	17.4	72.4	38.6	27.9	53.2	61.6	23.1
	fd	33.2	23.8	15.9	68.3	44.8	20.7	52.1	68.3	18.6
Mixture[4]	hd	98.3	57.6	47.2	157.8	71.4	70.3	124.5	97.5	58.2
	fd	84.7	62.3	35.7	145.2	86.2	52.6	115.3	108.2	47.8

1 fd:full dose. 2 hd:half dose 3 VAM: Vesicular arbuscular mycorrhizae.
4 Mixture: *A.chroococcum* + *B.circulans* + VAM.
5 TM : Total microbial flora $\times 10^6$ cfu g^{-1}dry soil.
6 SB : Silicate bacteria $\times 10^3$ cfu g^{-1} dry soil.
7 AZ:*Azotobacter* population $\times 10^4$ cfu g^{-1}dry soil.

1.2CO_2 evolution and nitrogenase activity

CO_2 evaluation and nitrogenase activity in marjoram rhizosphere were periodically estimated to evaluate microbial activities in the root zone as affected by application of bacterial and VAM inoculants. Data in Table (3)

Table (3): Effect of Inoculation with *A.chroococcum*, *B.circulans*, VAM or their mixture in the presence of full or half dose of NPK fertilizers on CO_2 evolution and nitrogenase activity in the rhizosphere of marjoram plant at 2000/2001 season

Treatments		CO_2 emitted[5]	N_2-ase activity[6]	CO_2 emitted	N_2-ase activity	CO_2 emitted	N_2-ase activity
Inoculation	NPK Rate	First cut		Second cut		Third cut	
Uninoculated	fd[1]	31.5	14.2	51.4	28.4	38.3	18.6
A.chroococcum	hd[2]	42.6	35.3	64.3	77.5	55.2	41.4
	Fd	36.3	29.5	58.5	61.2	51.6	35.2
B.circulans	Hd	57.2	26.8	71.3	55.6	67.5	32.8
	Fd	50.5	25.6	66.2	43.2	62.4	29.5
VAM[3]	Hd	34.4	24.4	60.8	84.6	54.8	34.6
	Fd	29.2	22.5	53.0	52.7	41.7	30.3
Mixture[4]	Hd	62.2	40.2	90.6	110.4	73.7	58.6
	Fd	51.5	37.3	78.2	84.3	64.1	49.8

1 fd: full dose. 2 hd:half dose
3 VAM: Vesicular arbuscular mycorrhizae 4 Mixture: *A.chroococcum* + *B.circulans* + VAM
5 in mg CO_2 $100g^{-1}$ dry soil
6 in n moles C_2H_4 h^{-1} g^{-1} dry soil

Main effects	Co_2 emitted	N_2-ase activity
Inoculation		
Uninoculated	47.06 d	15.07 d
A. chroococcum	61.29 c	30.02 b
B. circulans	72.35 b	20.41 c
VAM	52.81 a	19.10 cd
Mixture	81.38 a	40.09 a
NPK rate		
Full dose	65.11 a	23.82 b
Half dose	64.30 a	28.81 a
Cutting time		
1st cut	53.95 c	19.19 c
2nd cut	75.77 a	31.71 a
3rd cut	64.53 b	27.19 b
Significance		
Inoculation	**	**
NPK rate	**	*
Cutting time	**	**
Interaction	NS	NS

a,b,c,d : Means with the same letter are not significantly different .
** = ($P < 0.01$) * = ($P < 0.05$) NS = Not significant

showed that CO_2 evolution and N_2-ase activity, significantly increased in the rhizosphere of marjoram plants to reach their maximal levels at the second cut then decreased at the third cut. Under that conditions the maximum level of CO_2 evoluted and N_2-ase activity were recorded with a mixed inoculation in the presence of half dose of NPK fertilizers, being, 90.6 mg CO_2 $100g^{-1}$ dry rhizospheric soil and 110.4 n moles C_2H_4 $h^{-1}g^{-1}$ dry rhizospheric soil, respectively . Rhizosphere soil of uninoculated marjoram plants gave the lowest levels of CO_2 evolution. These results were in accordance with those of Abo El-Ala (2002) who reported that statistical main effects on the rate of CO_2 evolution due to application of multi-biofertilizer inoculant in the presence of half or full dose of mineral N and P was significantly higher, compared with that of uninoculated marjoram plants at the second cut . Heavy application of

inorganic N-fertilizers is known to inhibit N_2-ase activity of N_2–fixers (Pedersen *et al.*, 1978). Low doses of N-fertilizers, on the other hand, promote the establishment of well functional N_2-fixing association (Neyra and Dobereiner,1977) .

1. 3.Mycorrhizal root infection and spore production

Data in Table (4) revealed that mycorrhizal infection of marjoram roots, remarkably increased by inoculation with a mixture of azotobacters, silicate bacteria and VAM and supplementation with half dose of inorganic NPK fertilizers, being 48.83% at the 3rd cut. The corresponding spores density produced under this condition was 7.62×10^3 spores g^{-1} soil. Data, also show that inoculation with VAM appeared to strengthen the stimulatory effect of inoculation with diazotrophs. These findings confirm in part with those obtained by Bagyaraj and Menge (1978) who reported that tomato plants inoculated with both *Glomus fasciculatus* and *A.chroococcum* gave a highest mycorrhizal root infection and spore production . The positive effect noticed on VAM inoculated plants, may be due to that mycorrhizae promote absorption of nutrients from soil by the growing plants . This fact is demonstrated by Ross and Harper (1970) and Allen(1991)

Table(4): Effect of inoculation with *A.chroococcum*, *B.circulans*, VAM or their mixture in the presence of full or half dose of NPK fertilizers on mycorrhizal root infection of marjoram plant at 2000/2001 season .

Treatments		Root infection %	Spore number ($\times 10^3$ g^{-1} soil)	Root infection %	Spore number ($\times 10^3$ g^{-1} soil)	Root infection %	Spore number ($\times 10^3$ g^{-1} soil)
Inoculation	NPK rate	First cut		Second cut		Third cut	
Uninoculated	fd[1]	1.21	0.21	7.12	1.10	13.83	2.23
	hd[2]	3.92	0.77	15.71	1.93	19.82	3.43
A.chroococcum	fd	2.75	0.45		1.62	16.76	2.25
B.circulans	hd	2.43	0.63	14.62	1.45	18.63	2.26
	fd	1.82	0.34	12.32	1.32	17.34	2.23
VAM[3]	hd	7.60	1.23	22.27	2.43	37.55	6.25
	fd	5.20	1.14	19.35	2.21	35.00	5.87
Mixture[4]	hd	11.22	1.71	29.71	4.60	48.83	7.62
	fd	9.37	1.55	27.65	2.54	42.60	6.45

1 fd: full dose
2.hd: half dose
3 . VAM: Vesicular arbuscular mycorrhizae
4. Mixture: *A.chroococcum* + *B.circulans* + VAM

2.Influence of biological and NPK fertilizers on plant growth, oil yield and chemical composition of marjoram plant :

2.1. Plant growth parameters :

Data of growth performance of marjoram plants are expressed in plant height (Table 5), number of branches $plant^{-1}$ (Table 6) and herb fresh and dry weights (Tables 7 and 8) during the two seasons . It is clear from these Tables that, inoculation with *A.chroococcum* or *B.circulans* in the presence of half dose of inorganic NPK-fertilization, remarkably decreased the different plant growth parameters compared with the uninocualted control

given the full dose of NPK. In the meantime, addition of full dose of NPK to *Azotobacter* inoculated plants, increased the recorded values over the control However, increases were insignificant in the first season, and reached to significant levels during the second one compared with the control. Also, plants inoculated with *B.circulans* and amended with full dose of NPK showed insignificant stimulation in different plant growth characters against the control. VAM inoculation in the presence of half or full dose of inorganic NPK, significantly increased vegetative growth compared with the control. However, the most significant increments in different plant growth parameters were detected during both seasons with mixed inoculation treatment in the presence of half dose of NPK compared with the uninoculated plants given full dose of NPK (control). Application of the triple inocula with full dose of NPK reduced growth parameters compared with those obtained from plants amended with half dose of NPK and grown under the same condition . This finding may be due to the effect of higher dose of the mineral fertilizer that inhibited the activity of bacterial and fungal mixture. The adverse effect may also extend to the ability of the N_2- fixing bacteria to release phytohormones which are known to stimulate plant growth, absorption of nutrients and photosynthesis (Fayez *et al.* , 1985) . The silicate bacteria are also known to decompose aluminosilicate minerals, thus releasing potassium in an available form and enhance K uptake by the plants (Lauwers and Heinen, 1974) . The VAM colonization also enhance nutrients uptake of nitrogen, phosphorus, potassium, calcium and water (Lampkin,1994) . The overall results of plant growth reported in this study, were in harmony with those obtained by El-Sawy *et al.* (1998) on *Ammi visnaga*, Abdul Khaliq and Janardhanan (1998) on mint, El-Gadban *et al.* (2002) on marjoram, Abo El-Ala (2002) on marjoram and sweet basil and Kandeel *et al.* (2002) on sweet basil plants .

Table(5): Effect of inoculation with *A.chroococcum*, *B.circulans*, VAM or their mixture in the presence of full or half dose of NPK fertilizers on marjoram plant height.

Treatments		Plant height (cm))					
		2001 season/First 2000			Second 2001/2002 season		
Inoculation	NPK rate	1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut
Uninoculated	fd[1]	45.67	50.17	43.17	48.17	49.67	40.67
A.chroococcum	hd[2]	48.50	49.33	41.83	50.50	47.33	40.17
	fd	49.17	56.17		53.83	52.83	48.33
B.circulans	hd	46.33	48.83	41.17	47.50	44.33	38.67
	fd	47.67	53.33	44.33	54.33	49.67	46.83
VAM[3]	hd	51.83	55.33	45.17	53.50	53.67	47.83
	fd	52.83	59.83	47.83	57.33	56.83	50.33
Mixture[4]	hd	55.17	58.67	48.50	56.67	58.33	51.83
	fd	47.17	53.33	42.33	57.50	48.33	43.33
L.S.D. at 5 %		5.85	2.72	3.59	4.47	6.04	6.97
L.S.D. at 1 %		8.05	3.75	4.95	6.16	8.32	9.60

1 fd: full dose
2.hd: half dose
3 . VAM: Vesicular arbuscular mycorrhizae
4. Mixture: *A.chroococcum* + *B.circulans* + VAM

Table (6): Effect of inoculation with *A.chroococcum*, *B.clrculans*, VAM or their mixture in the presence of full or half dose of NPK fertilizers on number of branches marjoram plant .

Treatments		Branches number plant^{-1}					
		2001 season/First 2000			Second 2001/2002 season		
Inoculation	NPK rate	1stcut	2ndcut	3rdcut	1stcut	2ndcut	3rdcut
Uninoculated	fd[1]	7.67	11.33	17.67	8.33	12.67	16.67
A.chroococcum	hd[2]	8.67	10.67	17.67	7.33	11.00	15.67
	fd	8.33	14.33	19.33	8.67	14.67	19.33
B.circulan	hd	8.33	10.33	17.33	8.00	11.33	15.67
	fd	7.00	12.67	18.67	8.33	13.33	17.67
VAM[3]	hd	8.00	13.67	21.33	8.33	14.33	19.00
	fd	8.67	14.33	22.67	8.67	15.67	21.33
Mixture[4]	hd	9.33	15.67	23.33	8.33	15.33	22.33
	fd	7.67	13.67	20.33	8.00	12.67	19.00
L.S.D. at 5 %		1.38	2.09	1.73	NS	1.41	1.24
L.S.D. at 1 %		1.89	2.88	2.38	NS	1.95	1.70

1 fd: full dose
2.hd: half dose
3 . VAM: Vesicular arbuscular mycorrhizae
4. Mixture: *A.chroococcum* + *B.circulans* + VAM

Table (7): Effect of inoculation with *A.chroococcum*, *B.circulans*, VAM or their mixture in the presence of full or half dose of NPK fertilizers on the herb fresh and dry weights of marjoram plant during 2000/2001 season

Treatments		FW[5] of herb (g)plant^{-1}			DW[6] of herb(g)plant^{-1}			SY[7] of herb (ton)fed^{-1}	
Inoculation	NPK rate	1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut	FW	DW
Uninoculated	fd[1]	110.33	183.50	153.50	27.50	47.50	43.17	8.052	2.127
A.chroococcum	hd[2]	116.17	169.83	149.17	28.17	44.83	42.17	7.833	2.073
	fd	120.17	194.83	155.83	29.50	49.83	44.67	8.475	2.232
B.circulans	hd	106.67	166.67	153.50	26.33	44.17	43.67	7.683	2.055
	fd	117.33	185.67	164.33	29.33	48.67	45.67	8.412	2.226
VAM[3]	hd	126.50	208.50	167.83	31.83	51.17	47.83	9.051	2.355
	fd	125.33	263.33	182.17	31.17	61.50	52.50	9.789	2.613
Mixture[4]	hd	139.33	257.17	192.33	35.83	66.83	55.50	10.608	2.847
	fd	121.83	210.33	162.50	30.67	54.33	54.67	8.904	2.352
L.S.D. at 5 %		15.22	24.24	8.83	3.90	5.76	2.42	0.434	0.106
L.S.D. at 1 %		20.97	33.38	12.16	5.37	7.94	3.34	0.597	0.145

1fd: full dose
2 hd: halfdose
3 VAM: Vesicular arbuscular mycorrhizae
4 Mixture: *A.chroococcum* + *B.circulans* + VAM
5.FW: Fresh weight of herb plant^{-1} (g) .
6 DW: Dry weight of herb plant^{-1} (g)
7 SY: Stimated yield of herb fed^{-1} (ton)

Table (8): Effect of inoculation with *A.chroococcum*, *B.circulans*, VAM or their mixture in the presence of full or half dose of NPK fertilizers on the herb. fresh and dry weights of marjoram plant during 2001/2002 season.

Treatments		FW[5] of herb (g)plant^{-1}			DW[6] of herb(g)plant^{-1}			SY[7] of herb (ton)fed^{-1}	
Inoculation	NPK rate	1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut	FW	DW
Uninoculated	fd[1]	114.83	195.67	150.50	29.17	51.17	42.50	8.298	2.211
A.chroococcum	hd[2]	115.50	186.83	146.83	29.50	49.83	41.33	8.085	2.172
	fd	130.33			33.67	57.83	46.17	9.366	2.478
B.circulans	hd	102.50	172.67	144.67	26.50	45.00	40.83	7.557	2.022
	fd	118.33	203.83	156.50	30.33	52.33	43.67	8.616	2.274
VAM[3]	hd	123.67	223.33	168.67	32.17	57.17	46.67	9.282	2.445
	fd	131.17	240.50	184.83	34.17	61.33	52.83	10.017	2.670
Mixture[4]	hd	144.67	243.50	192.33	37.83	63.67	54.50	10.449	2.808
	fd	125.33	201.67	153.67	32.17	52.00	43.83	8.652	2.304
L.S.D. at 5 %		6.78	27.56	17.95	2.25	6.61	3.98	0.569	0.096
L.S.D. at 1 %		9.33	37.95	24.72	3.09	7.73	5.44	0.783	0.132

1fd: full dose
2 hd: halfdose
3 VAM: Vesicular arbuscular mycorrhizae
4 Mixture: *A.chroococcum* + *B.circulans* + VAM
5.FW: Fresh weight of herb plant^{-1} (g).
6 DW: Dry weight of herb plant^{-1} (g)
7 SY: Stimated yield of herb fed^{-1} (ton)

2.2.Herb contents of volatile oil and its constituents :

2.2.1.Volatile oil content

Data presented in Table (9) clearly showed that volatile oil percentage of marjoram herb was considerably influenced by different inoculation treatments combined with inorganic NPK–fertilization . The least records were generally observed in uninoculated control plants in all harvests during the two growing seasons . The highest oil percentage, on the other hand, was produced by plants treated with the triple inocula in the presence of half dose of NPK, in the second rank plants inoculated with VAM given full dose of NPK lied of magnitude .The increases in oil % obtained from these two above-mentioned treatments were significant when compared with the control. However, the differences between these two treatments were not significant . Inoculation with *Azotobacter* or *Bacillus* given half dose of NPK showed insignificant increases in oil percentages of marjoram compared with the control . On the other hand, inoculation with *A.chroococcum*, *B.circulans* ,and their mixture with VAM in the presence of full dose of NPK or inoculation with VAM alone with half dose of NPK, significantly increased the oil % over the control . Plants treated with the mixed inoculation treatment and amended with the half dose of NPK gave the highest significant increases compared with other treatments. This treatment yielded about 1.5 fold of oil yield in comparison with the control at both seasons . The second higher value was recorded for plants inoculated with VAM given full dose of NPK. However, plants inoculated with *A.chroococcum* or *B.circulans* amended with half dose of NPK gave lower level of the yearly oil yield fed^{-1} compared with the control, but those inoculated with either *A.chroococcum*, *B.circulans*, or their mixture plus VAM given full dose of NPK or inoculation with VAM alone given half dose of NPK , significantly increased oil yield over the control . Similar findings were obtained by El Gadban *et al.* (2002) on marjoram, Kandeel *et al.* (2002) on basil and Abo El-Ala (2002) on marjoram and basil plants.

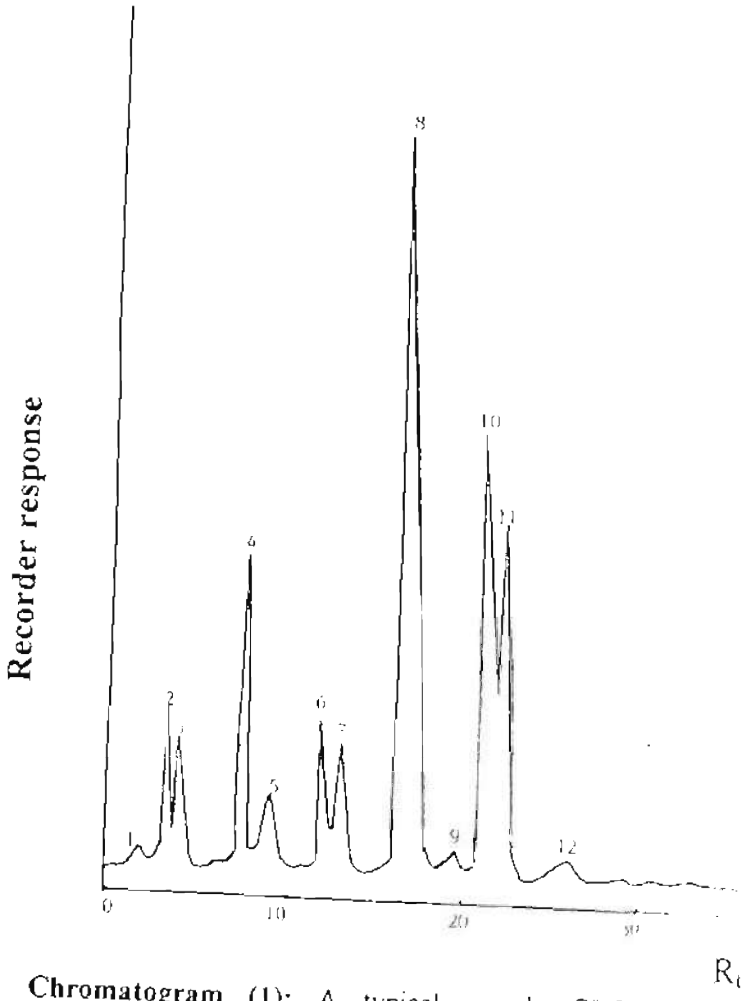
Table (9): Effect of inoculation with *A.chroococcum*, *B.circulans*, VAM or their mixture in the presence of full or half dose of NPK fertilizers on percentages and yearly volatile oil yield (fed $^{-1}$)of marjoram herb.

Treatments		Volatile Oil %							
		First 2000/2001 season				Second 2000/2001 season			
Inoculation	NPK rate	1st cut	2nd cut	3rd cut	EYO[5]	1st cut	2nd cut	3rd cut	EYO
Uninoculated	fd[1]	1.51	1.43	1.37	30.24	1.48	1.41	1.39	31.38
A.chroococcum	hd[2]	1.57	1.43	1.40	29.82	1.56	1.40	1.39	30.60
	fd	1.60	1.51	1.44	33.60	1.59	1.49	1.45	37.20
B.circulans	hd	1.51	1.45	1.40	29.70	1.52	1.39	1.40	29.28
	fd	1.53	1.49	1.41	32.82	1.57	1.45	1.43	33.48
VAM[3]	hd	1.65	1.55	1.44	36.12	1.65	1.56	1.49	36.42
	fd	1.72	1.63	1.51	41.94	1.75	1.65	1.52	43.38
Mixture[4]	hd	1.75	1.63	1.53	46.14	1.80	1.60	1.55	46.32
	fd	1.79	1.63	1.51	38.22	1.67	1.56	1.55	36.90
L.S.D. at 5 %		0.07	0.03	0.06	1.73	0.05	0.04	0.03	1.65
L.S.D. at 1 %		0.09	0.04	0.09	2.38	0.07	0.05	0.05	2.28

1 fd: full dose
2.hd: half dose
3. VAM: Vesicular arbuscular mycorrhizae
4. Mixture: *A.chroococcum* + *B.circulans* + VAM
5.EYO: Estimated Yearly oil yield fed^{-1} (liter)

2.2.2. Volatile oil constituents

GLC analysis of marjoram volatile oil mixture showed that oil samples obtained from uninoculated control plants showed the presence of 12 components (see chromatogram 1). Ten of them were identified, while the other two components (peaks No.9 and 12) were not identified due to the lack of the authentic samples . Data presented in Table (10) indicate that the major oil components in marjoram oil sample from plants of the control treatment were : α-terpineol, geranyl acetate, cineole and citronellol, while the other components were found in small quantities . Mixed inoculation in the presence of half or full dose of NPK, resulted in increased concentrations of geranyl acetate, cineole, linalool, limonene and α-terpenolene while decreased concentration of α-terpineol was detected in comparison to the uninoculated control plants. Geraniol concentration tended to increase due to inoculation with VAM or *A.chroococcum* plus application of full or half dose of NPK compared with the control . Citronellol concentration on the other hand, was not affected due to different treatments . Similar results were recorded by El-Gadban *et al.* (2002) on marjoram plant .



Chromatogram (1): A typical sample GLC trace shows the separation of constituents of marjoram volatile oil for the control treatment (given full dose of inorganic NPK-fertilization)

Table (10): The obtained constituents of marjoram volatile oil and their percentage as affected by single or mixed inoculation with *A.chroococcum*, *B.circulans* and VAM combined with inorganic NPK fertilization during 2000/2001 season.

Peak No.	Components	Treatments								
		Inoculation								
		Uninoculated	*A.chroococcum*		*B.circulans*		VAM[3]		Mixture[4]	
		NPK rates								
		fd[1]	hd[2]	fd	hd	Fd	hd	Fd	hd	fd
1	α-Pinene	1.89	1.82	1.71	2.92	2.13	1.85	1.81	0.89	0.72
2	β-Pinene	6.81	7.63	6.53	7.85	5.91	6.93	6.64	4.31	4.81
3	Limonene	5.80	5.77	5.31	5.38	5.43	6.03	6.21	7.79	7.73
4	Cineole	10.72	10.11	10.22	10.29	10.44	10.89	11.43	12.40	14.25
5	Linalool	3.23	3.11	3.83	3.42	3.62	3.32	3.00	5.51	5.70
6	Citronellol	6.24	6.31	6.34	6.71	5.71	6.31	6.33	6.11	5.96
7	Geraniol	4.53	6.87	6.90	5.09	4.11	7.12	7.65	5.30	4.63
8	α.-Terpineol	33.32	27.50	29.33	23.58	26.55	28.93	27.39	23.71	21.66
9	Unidentified	1.95	2.59	1.79	3.37	2.31	1.51	2.05	0.97	1.12
10	Geranylacetate	18.61	20.74	21.32	22.91	26.01	19.87	20.72	26.21	26.44
11	α-Terpendene	5.87	5.61	5.65	9.13	6.00	6.00	6.02	6.62	5.91
12	Unidentified	1.03	1.94	1.06	2.34	1.78	1.24	0.75	0.17	1.07

1 fd: full dose
2.hd: half dose
3 , VAM: Vesicular arbuscular mycorrhizae
4. Mixture: *A.chroococcum* + *B.circulans* + VAM

2.3. N , P and K percentages in plant herb

Data in Table (11) indicated that N, P and K percentages in marjoram plant herb were considerably influenced by the inoculation with *A.chroococcum*, *B.circulans*, VAM or their mixture combined with NPK fertilizers .The highest N,P and K percentages were recorded for mixed inoculation of marjoram supplementation of half dose of NPK . Plants inoculated with VAM given full dose of NPK, and half dose of NPK represented the second and third order of descending magnitude where N, P and K % were higher than other treatments . On the other hand, plants inoculated with *A.chroococcum* or *B.circulans* in the presence of half dose of NPK, showed decreased N, P and K % in their tissues compared with the uninoculated control plants. Treating plants with *A.chroococcum*, *B.circulans* or their mixture plus VAM with full dose of NPK amendment increased N,P and K % in marjoram plant herb over control plants . Similar results were obtained by Azcon and Barea (1998) on lavender, El-Gadban *et al.* (2002) on marjoram and Kandeel *et al.* (2002)on basil plants.

Table (11): Effect of inoculation with *A.chroococcum*, *B.circulans*, VAM or their mixture in the presence of full or half dose of NPK fertilizers on N, P and K% in marjoram plant herb at 2000/2001 season.

Treatments		N %			P %			K %		
Inoculation	NPK rate	1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut	1st cut	2nd cut	3rd cut
Uninoculated	hd[1]	2.41	2.67	2.74	0.47	0.52	0.57	2.73	2.81	2.79
A.chroococcum	hd[2]	2.30	2.49	2.67	0.42	0.53	0.57	2.69	2.80	2.83
A.chroococcum	fd	2.49	2.72	2.75	0.48	0.54	0.59	2.72	2.93	2.97
B.circulans	hd	2.29	2.43	2.59	0.37	0.47	0.54	2.65	2.79	2.81
B.circulans	fd	2.35	2.57	2.75	0.47	0.54	0.59	2.84	2.94	2.95
VAM[3]	hd	2.68	2.83	2.98	0.57	0.61	0.73	2.79	2.89	2.99
VAM	fd	2.97	3.01	3.11	0.63	0.69	0.77	2.81	2.97	3.08
Mixture[4]	hd	3.12	3.21	3.25	0.65	0.69	0.86	2.87	2.98	3.12
Mixture	sfd	2.42	2.73	2.89	0.59	0.66	0.71	2.77	2.88	2.93

1 fd: full dose
2.hd: half dose
3 . VAM: Vesicular arbuscular mycorrhizae
4. Mixture: *A.chroococcum* + *B.circulans* + VAM

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إنتاجيه نبات البردقوش تحت تأثير التداخل بين التسميد المعدني و الحيوي

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تم إجراء تجربه حقليه لدراسة تأثير تلقيح نبات البردقوش ببكتريا الأزوتوباكتر كروكوكم ، والباسيلس سيركيولانس ، وفطريات الميكوريزا المكونة للحويصلات والتفرعات الشجيرية أو خليط منهم فى وجود المعدل الحقلي الموصى به كاملاً أو نصف هذا المعدل من التسميد المعدني من النتروجين والفسفور والبوتاسيوم على نمو النبات ، والأنشطة الميكروبية فى الريزوسفير (متمثلة فى معدل انطلاق ك ا[2] واختزال الأستيلين وإصابة الجذر بفطريات الميكوريزا) ، ومحصوليه الزيت الطيار (مع استخدام الكروماتوجرافي لتحديد مكوناته)،ومحتوى المجموع الخضري من ن، فو ، بو وقد تم تسجيل هذه القياسات فى ثلاث حشات خلال موسم النمو ، وقد أظهرت نتائج هذه الدراسة أن التلقيح المختلط من مخصبات حيوية مجتمعه مع إضافة نصف المعدل السمادي المعدني (ن ، فو ، بو) أعطى أعلى زيادات معنوية في القياسات الخاصة بالنمو ، والنسبة المئوية للزيت الطيار وكذا المحصول السنوي من الزيت للفدان وكذلك محتوى المجموع الخضري من، ن ، فو ، بو كما أدى لزيادة نسبه بعض الزيوت الطيارة مثل geranyl acetate , α-terpineol بينما انخفضت نسبه ماده cineole, linalool, limonene and α-terpenolene مقارنه بالكنترول كما أدى التلقيح بالخليط الى زيادة كثافه أعداد الميكروبات الكليه وكذلك الأزوتوباكتر وبكتريا السليكات فى ريزوسفير البردقوش ، كما زاد معدل انطلاق ك ا[2] ونشاط إنزيم النتروجينيز ومعدل الإصابة وإنتاج حراثيم الميكوريزا . من جهه أخرى أدى التلقيح بأي من الأزوتوباكتر أو الباسيلس في وجود نصف معدل التسميد المعدني (ن ، فو ، بو) إلى انخفاض القياسات السابقة الذكر مقارنة بالنباتات غير الملقحة مع التسميد بالمعدل الكامل من التسميد المعدني ولكن أدى استخدام الميكوريزا بمفردها أو بالاشتراك مع خليط البكتريا الى زيادة معدلات الصفات المختبرة