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Effect of Compost and Bio-fertilizers on Growth, Yield and Essential Oil of Sweet Marjoram (*Majorana hortensis*) Plant

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ABSTRACT

A pot experiment was conducted to determine the effect of compost and bio-fertilizers on the growth, yield and oil constituents of marjoram (*Majorana hortensis* L.). Forty five days old seedling were transplanted in soil treated with 15 and 30% aqueous extracts of compost and/or biofertilizers (mixture of *Azospirillum brasilienses*, *Azotobacter chroococcum*, *Bacillus polymyxa* & *B. circulans*) in addition to the recommended nitrogen, phosphorus and potassium (NPK) doses as control. Use of combined treatment of bio-fertilizers gave better results for all studied traits than those obtained from either nitrogen fixers (*Azos. brasiliense*, *Azot. chroococcum* & *B. polymyxa*) or (*B. circulans*) alone. The oil percentage and yield per plant for three cuttings was almost twofold higher on fresh weight basis as a result of aqueous extracts of compost at low level + bio-fertilizers compared with control, indicating that combinations of low input system of integrated nutrient management could be beneficial to obtain relatively good yields of essential oil. Oil composition using GC/MS revealed that marjoram belongs to the *cis*-sabinene hydrate/terpinene-4-ol chemotype. The chemical composition of marjoram essential oil did not change due to the fertilization type or level; rather the relative percentages of certain constituents were affected. The highest level of *cis*-sabinene hydrate (18.47%) and terpinene-4-ol (24.24%) was obtained with aqueous extracts of compost at 30% + *B. circulans* and aqueous extracts of compost at 30% + (*A. brasiliense* + *A. chroococcum* + *B. polymyxa*), respectively. Other components showed different concentrations depending on treatment and level of compost.

Key Words: Bio-fertilizer; Compost; Aqueous extracts; Growth; Essential oil composition

INTRODUCTION

Composting is a biological process in which organic biodegradable wastes are converted into hygienic, hums rich product (compost) for use as a soil conditioner and an organic fertilizer (Popkin, 1995). These are also used to provide biological control against various plant pathogens (Hoitink & Grebus, 1994). Aqueous extracts of compost have also been suggested to replace synthetic fungicides (Zhang *et al*., 1998). The addition of municipal solid waste compost to agricultural soils has beneficial effects on crop development and yields by improving soil physical and biological properties (Zheljazkov & Warman, 2004). Also, poultry manure application significantly increased the herbage, essential oil content and dry matter yield in *Java citronella* plants (Adholeya & Prakash, 2004).

Biological activities are markedly enhanced by microbial interactions in the rhizosphere of plants (Tilak & Reddy, 2006). Such syntrophic associations are of ecological importance with implied agricultural significance. The plant growth promoting rhizobacteria (PGPRs) can influence plant growth directly through the production of phytohormones and indirectly through nitrogen fixation and production of bio-control agents against soil-borne phytopathogens (Glick, 2003). *Azospirillum* species are nitrogen-fixing organisms (diazotrophs), capable of forming an associative relationship with the roots of several economically important cereals (Vande Broek & Vanderleyden, 1995). Studies indicate that *Azospirillum* promotes plant growth (Cohen *et al*., 2007), but the exact mechanism of growth promotion has not been fully characterized (Dommelen *et al*., 1998). Like most organisms, *Azospirillum* uses ammonium salts as a preferred nitrogen source. In the absence of combined nitrogen and under microaerobiosis, the nitrogenase enzyme complex is synthesized and converts atmospheric N_2 to NH_4 (Dommelen *et al*., 1998).

B. cereus and *B. circulans* are reported as plant growth promoting rhizobacteria. Seed inoculation of maize, pigeonpea and wheat with *B. cereus*, *B. circulans*, *Azotobacter chroococcum*, *Azospirillum brasilense* and *Pseudomonas fluorescens* although produces beneficial effects on growth and yield, maize recorded maximum grain yield (Tilak & Reddy, 2006). The increase in grain yield of maize due to seed bacterization with *Bacillus cereus* and *B. circulans* was 43.8 and 50.8%, respectively over uninoculated control (Tilak & Reddy, 2006).

Marjoram (*Majorana hortensis* L.) is used world wide as a spice and a medicinal source in the form of the essential oil in aromatherapy due to its stimulant and antispasmodic properties. The chemical composition of marjoram oils revealed differences between plant species. Several workers

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reported that essential oil components of marjoram were terpinen-4-ol, gamma-terpinene, trans-sabinene hydrate, linalool, thujanol, terpinolene and thymol (Edris *et al*., 2003; El-Ghorab *et al*., 2004). Marjoram cultivation has economic impact due to its ability to produce and store essential oil used in perfumes and cosmetics industries. The percentage of essential oil, fresh and dry matter of marjoram plants positively responded to increased levels of composted manure compared with chemical fertilizer (Edris *et al*., 2003). Jung *et al*. (2004) found that pH, EC, total nitrogen and organic matter of food waste composted treated with miraculous soil microorganisms significantly increased the fresh weight of lettuce compared to mineral fertilizers. In addition, oil production in mint plants increased when plants were grown with biosolid (Scavroni *et al*., 2005).

Application of compost and bio-fertilizers to improve soil structure, fertility and consequently development and productivity of marjoram plants has received little attention. Objectives of this study were to evaluate growth, chemical composition, yield and quality of essential oil of marjoram plants and populations of bacteria, infection percentage of AM-mycorrhiza and nitrogenase enzyme activities in the rhizosphere under different treatments of compost and/or bio-fertilizers compared with mineral fertilizers.

MATERIALS AND METHODS

Mixed cultures of three nitrogen-fixers (*Azospirillum brasiliense*, *Azotobacter chroococcum* & *Bacillus polymyxa*) were locally grown on nitrogen deficient media (Abd El-Malek & Ishac, 1968; Dobereiner *et al*., 1976) and used in combination as bio-fertilizers. Cell suspension of *B. circulans* was grown on nutrient agar medium (Difco Manual, 1985) and applied single or mixed with N_2-fixing strains according to treatment used. Mixed cultures of bacterial species, containing $I \times 10^6$ colony forming units mL^{-1}, were used for plant inoculation. In addition, the aqueous extract from the raw materials (rice, broad bean, maize, wheat & white clover straws treated with cellulose decompoers) were made and analyzed chemically before application to the soil at 15 and 30%. Chemical and microbiolgical composition of aqueous extract of compost used to grow marjoram plants are presented in (Table I).

Table I. Chemical and microbiolgical composition of aqueous extract of compost used to fertilize *M. hortensis* plants

Parameter	Value
pH	7.10
E.C (dS m^{-1})	3.39
Organic carbon (%)	0.29
Total nitrogen (%)	0.01
C/N ratio	29: 1
Total phosphorous (%)	0.02
NH_4-N (mg kg^{-1})	35.50
NO_3-N (mg kg^{-1})	5.30
Total count of bacteria (c.f.u./mL)	0.01x 10^7
Total count of fungi (c.f.u./mL)	0.01x 10^5
Total actinomycetes (c.f.u./mL)	0.01x 10^6

A pot experiment was conducted at the experimental farm of Helwan University, Cairo, Egypt during 2006/2007. Marjoram seeds (obtained from Horticultural Research Institute, Agricultural Research Center, Ministry of Agriculture, Giza, Egypt) were sown in earthenware pots (40 cm in diameter) containing 15 kg of sandy loam soil. After emergence the number of seedlings was thinned to three per pot. Aqueous extract of compost was prepared and applied with the irrigation system at 15 and 30% by volume. For biofertilizer treatments, each pot was inoculated with 10 mL bacterial suspension of three nitrogen-fixer strains (*Azos. brasiliense*, *Azot. chroococcum* & *B. polymyxa*) and/or *B. circulans*. Control plants received the recommended nitrogen, phosphorus and potassium (NPK) doses as ammonium nitrate, calcium superphosphate and potassium sulphate. Aqueous extract of compost, bio-fertilizer and NPK fertilizers were applied in two doses at 45 and 60 days from planting and repeated after the first and second cuts. Physico-chemical properties of the soil used in the experiment were evaluated according to Jackson (1973). The soil type was sandy loam in texture with water holding capacity 29.0%, pH 7.8, O.M 0.35% and E.C. 1.15 dS m^{-1}. The soil analysis, 0.55% containing $CaCO_3$, available 4.46, 23.46, 169 and 32.2 mg 100 g^{-1} soil of P, K, Mg and Na, respectively and also available 7.2, 9.4, 2.80 and 4.82 ppm of Fe, Mn, Cu and Zn, respectively. Irrigation was regularly carried out at intervals according to weather conditions to keep the moisture content of the soil to field capacity.

At full blooming stage, the plant herbage was harvested by cutting 5 cm above the soil surface and plant growth parameter for three cuts were recorded as plant height, stem diameter, number of branches per plant, fresh and dry weight of herb. Plant samples were dried in an electric oven with drift fan at 70°C till constant dry weight. Representative fresh samples were taken from each treatment for determination of essential oil content and constituents. Plant roots with their adhering soil of each sample was prepared by adding 5 g to a sampling bottle containing 45 mL of sterilized water. Bottles were shaken for 10 min and further dilutions were prepared. The most probable number (MPN) technique was applied for the enumeration of N_2-fixers spp. in the rhizosphere. Aliquots (0.5 mL) of suitable dilution were transferred to each of 5 tubes containing 4.5 mL of selective liquid culture media for N_2-fixers. All tubes were incubated at 30ºC for 2-4 days. Estimation of root infection rate of native AM mycorrhizae was done following the method of Phillips and Hayman (1970), while nitrogenase activity was determined according to the method described by Somasegaran (1985).

Quantitative determination of marjoram essential oil obtained from different treatments were achieved by hydro-distillation during first, second and third cuts. Distillation of 100 g fresh herb was continued for 2.5-3.0 h after water boiling till no further increase in the oil was observed. The oil was permitted to stand undisturbed and the amount of oil obtained from plant material was calculated as:

Oil (%) = observed volume of oil (mL)/weight of sample (g) × 100

Essential oil yield per plant was calculated by multiplying the average fresh weight of plant by the average oil percentage.

Oil yield/plant = plant fresh weigh (g) × oil %

Essential oils from the first cut were separated and analyzed qualitatively by GC/MS in National Research Centre, Dokki, Cairo, Egypt. The GC analysis was carried out using Varian 3400 GC, equipped with a DB-5 fused silica capillary column. Mass spectrometer was a Varian–Finnigan SSQ 7000.

Total N was determined in the dried samples of the first cut, using the modified Micro-Kjeldahl method according to AOAC (1980). Macroelements were determined after wet digestion. Total P was determined by using vanadate-molybdate method by Jackson (1973). Potassium was measured by flame photomtere. Fresh herb of marjoram plants developed from the first cut was used for extraction of plant growth regulators as described by Shindy and Smith (1975). Gibberillic acid was determined using 1 mL ethyl acetate extract, 1 mL HCl followed by 1 mL Folin Denis's reagent, 3 mL water and mixed. The test tubes were put in boiling water bath for 5 min, left to cool and the absorbance was measured at 750 nm according to (Udagwa & Kinoshita, 1961).

The experiments were laid out in completely randomized design with four replicates per treatment. Three cuts were taken by about 4, 6 and 8 month from transplanting marjoram plants. Data obtained were analyzed by one-way analysis of variance. Comparison of means were made by the Duncan's multiple range test (P = 0.05).

RESULTS AND DISCUSSION

Growth. Application of aqueous extract of compost at 15, 30%, nitrogen fixer strains (*Azos. brasiliense*+*Azot. chroococcum* +*B. polymyxa*) and *B. circulans* alone or in combination increased the growth parameters of marjoram in terms of plant height, stem diameter, number of branches per plant, as well as fresh and dry weight of herb during three cutting (Table II). Such promoting effect was maximal in response to interaction between aqueous extract of compost at 15% with mixed nitrogen fixer strains + *B. circulans* for all growth parameters compared to their control (NPK fertilizers). Thus inoculation of marjoram plants with PGPRs and compost at early stage of development results in positive impact on biomass production by improving soil physical and biological properties, directly affecting root growth, production of phytohormones by bacteria, enhancement mineral uptake and transfer of nitrogen to the plant. On the other hand, Hameeda *et al*. (2007) found that the application of microbial inoculants along with higher concentrations of composts may not be synergistic for sorghum plant growth.

Use of combined treatment of N-fixers and *B. circulans* gave better plant height, stem diameter, number of branches per plant, as well as fresh and dry weight of marjoram than those obtained from either bio-fertilizer alone during three cutting as shown in (Table II). Improved plant growth by *A. brasilense* has been attributed both to production of plant hormones, especially growth promoters and by supplying combined nitrogen (Cohen *et al*., 2007; Pedraza *et al*., 2007). Aqueous extract of compost at 15 and 30% treatments promoted growth in marjoram, especially at 15% treatment compared to corresponding control (Table II). Hameeda *et al*. (2007) found that rice straw compost applied at 2.5 t ha^{-1} showed significant improvement in shoot length, leaf area, plant biomass, root volume and mycorrhizal colonization in sorghum plant (Table II).

Essential oil content. Essential oil content of marjoram was strongly affected by aqueous extract of compost and/or bio-fertilizer application for three cuts compared with their corresponding controls (NPK fertilizers). The highest mean values of essential oil percentage of the three cuts when pooled together increased significantly about 39.0 and 52.0%, while on a per plant basis oil yield increased by 122.91 and 171.08% relative to control plants by the interaction of nitrogen-fixers + *B. circulans* + aqueous extract of compost at 30% and 15%, respectively (Table III). The increase in oil yield might be due to either increase in vegetative growth or changes in leaf oil gland population and monoterpenes biosynthesis. Similarly, compost with mixed *Azotobacter – B. megaterium* gave the highest essential oil production/feddan in cumin plants (Safwat & Badran, 2002). In addition, aqueous extract of compost especially at 15% increased essential oil percentage and yield in marjoram plants compared with their control during three cuttings. Similar increases were obtained on marjoram (Edris *et al*., 2003) and *J. citronella* plants (Adholeya & Prakash, 2004).

Essential oil composition. Thirty compounds, accounting for more than 98% of the total volatiles in most marjoram samples were detected and identified (Table IV). There were only small, differences in oil composition as affected by compost and/or bio-fertilizer application. The predominant compounds present under all treatments were the monoterpenes *cis*-sabinene hydrate (18.47-6.74%), terpinen-4-ol (24.24-17.82%), p-cymene (13.90-6.64%), sabinene (8.26-6.20%), γ-terpinene (16.83-9.06%), α-terpinene (13.11-0.24%), trans-sabinene hydrate (6.59-1.62%) and α-terpinolene (5.25-2.05%). β-caryophyllene was the only sesquiterpene present in significant amounts (3.35-2.40%).

Aqueous extract of compost and/or bio-fertilizer treatments increased the level of terpinen-4-ol (the major compound in marjoram oil), γ-, α-terpinene, trans-sabinene hydrate, phellandrene, p-menth-1-en-8-ol accompanied by a decrease in the proportions of *cis* sabinene hydrat, p-cymene, α-terpinolene, linalyl acetate, β-caryophyllene and spathulene, relative to control (NPK fertilizer). Contents of other oil constituents varied without a clear trend. In

Table II. Effect of aqueous extracts of compost and bio-fertilizers on the vegetative growth of *Origanum majorana* plants

Treatments	Plant height (cm)			No. of branches plant^{-1}			Stem diameter (mm)		
	1st	2nd	3rdcut	1st	2nd	3rdcut	1st	2nd	3rdcut
Control (NPK)	40.5 ef	23.7 e	20.7 f	13.67 g	28.25 i	29.94 i	1.60 k	2.00 j	1.13 i
Cmpost I (15%)	45.7 cd	28.3 c	26.4 bcd	16.33 ef	38.58 g	34.22 h	1.70 j	3.03 f	2.17 g
Cmpost II (30%)	43.3 de	26.1 d	25.3 cde	14.17 fg	36.17 h	33.67 h	1.65 jk	2.68 h	1.30 h
Nitrogen fixer (N_2)	42.7 de	25.0 de	24.6 e	18.50 e	39.00 fg	39.11 g	2.40 h	2.43 i	1.30 h
Bacillus circulans (*B.C.*)	39.7 f	23.5 e	20.7 f	21.50 d	41.17 ef	42.67 f	2.70 f	3.00 f	1.37 h
Nitrogen fixer+*B.C.*	48.8 b	30.0 bc	26.7 bcd	28.67 b	46.33 c	54.44 c	4.80 c	4.00 d	4.20 c
Cmpost I+ N_2	45.7 cd	29.0 c	27.5 b	25.00 c	43.50 de	52.00 cd	3.00 e	4.27 c	4.03 d
Cmpost II+ N_2	43.3 de	28.5 c	25.6 cde	22.67 cd	43.33 de	45.94 e	2.60 g	2.80 g	2.37 f
Cmpost I + *B.C.*	48.0 cb	26.3 d	27.2 bc	28.67 b	44.73 cd	54.17 c	4.00 d	4.00 d	4.07 d
Cmpost II + *B.C.*	43.0 de	25.3 de	25.0 de	24.67 c	43.33 de	50.78 d	2.11 i	3.37 e	3.00 e
Cmpost I+ N_2 + *B.C.*	53.0 a	34.7 a	30.1 a	34.00 a	52.92 a	67.00 a	5.23 a	6.00 a	5.00 a
Cmpost II+ N_2 + *B.C.*	52.5 a	30.8 b	27.7 b	30.33 b	49.00 b	59.78 b	5.03 b	5.33 b	4.67 b
L.S.D at 5%	3.7	2.5	2.6	3.27	3.35	3.62	0.08	0.14	0.15

Treatments	Herb fresh weight (g)			Herb dry weight (g)		
	1st	2nd	3rdcut	1st	2nd	3rdcut
Control (NPK)	41.19 f	23.80 g	17.39 f	13.97 f	11.14 e	8.06 h
Cmpost I (15%)	47.13 e	26.58 f	21.46 ed	17.94 d	11.58 de	9.83 de
Cmpost II (30%)	41.30 f	25.01 fg	18.99 e	15.25 ef	11.41 de	8.90 g
Nitrogen fixer (N_2)	57.52 d	32.74 d	25.50 b	20.30 c	17.83 c	11.23 c
Bacillus circulans (*B.C.*)	61.56 c	33.06 d	25.58 b	21.10 bc	18.42 c	11.46 bc
Nitrogen fixer+*B.C.*	64.38 b	35.63 c	26.01 b	22.25 b	19.76 b	11.73 b
Cmpost I+ N_2	48.61 e	29.63 e	22.98 c	18.18 d	12.46 d	10.25 d
Cmpost II+ N_2	43.90 f	25.39 fg	19.96 de	16.66 de	11.52 de	9.57 ef
Cmpost I + *B.C.*	47.93 e	27.16 f	22.94 c	18.11 d	12.18 de	10.15 d
Cmpost II + *B.C.*	43.61 f	25.04 fg	19.27 e	16.54 de	11.44 de	9.30 fg
Cmpost I+ N_2 + *B.C.*	73.49 a	43.75 a	29.10 a	26.66 a	22.65 a	12.32 a
Cmpost II+ N_2 + *B.C.*	65.24 b	39.67 b	27.12 b	22.65 b	22.16 a	11.88 b
L.S.D at 5%	3.65	3.17	2.19	2.34	1.50	0.59

Means followed by the different letters in the same column are statistically different according to Duncan's multiple range tests P = 0.05

Table III. Effect of aqueous extracts of compost and biofertilizers on essential oil percent and yield of *Origanum majorana* plants

Treatments	Oil %			Oil yield (ml plant^{-1})		
	1st	2nd	3rdcut	1st	2nd	3rdcut
Control (NPK)	0.374 i	0.582 h	0.679 i	0.154 i	0.139 h	0.118 h
Cmpost I (15%)	0.554 e	0.644 ef	0.795 f	0.261 f	0.171 fg	0.171 e
Cmpost II (30%)	0.496 g	0.614 g	0.775 g	0.205 h	0.154 g	0.147 g
Nitrogen fixer (N_2)	0.399 h	0.586 h	0.720 h	0.230 g	0.192 e	0.184 d
Bacillus circulans (*B.C.*)	0.369 i	0.521 i	0.609 j	0.227 g	0.172 fg	0.156 fg
Nitrogen fixer+*B.C.*	0.630 b	0.741 b	0.826 c	0.406 c	0.264 c	0.215 c
Cmpost I+ N_2	0.619 c	0.705 c	0.809 d	0.301 d	0.209 d	0.186 d
Cmpost II+ N_2	0.611 c	0.668 d	0.805 e	0.268 f	0.170 fg	0.160 ef
Cmpost I+*B.C.*	0.597 d	0.647 e	0.802 e	0.286 e	0.176 ef	0.184 d
Cmpost II+*B.C.*	0.528 f	0.635 f	0.776 g	0.230 g	0.159 fg	0.150 fg
Cmpost I+ N_2+*B.C.*	0.727 a	0.849 a	0.919 a	0.534 a	0.371 a	0.267 a
Cmpost II+ N_2+*B.C.*	0.644 b	0.743 b	0.887 b	0.420 b	0.295 b	0.241 b
L.S.D. at 5%	0.016	0.014	0.006	0.021	0.022	0.016

Means followed by the different letters in the same column are statistically different according to Duncan's multiple range tests P = 0.05

addition, the interaction treatment of nitrogen-fixer strains + *Bacillus circulans* recorded the highest percent of γ-terpinene (16.83%), α-terpinene (13.11%), phellandrene (2.41%), α-myrcene (2.01%) but the lowest value of *cis*-sabinene hydrate (6.74%), relative to control. Moreover, aqueous extract of compost at 15% markedly increased the proportion of α-, γ- terpinenes, trans-sabinene hydrate and terpinen-4-ol. Edris *et al.* (2003) found that the relative percentage of certain constituents of marjoram essential oil were affected by fertilization type and level and that the highest concentration of *cis*-sabinene hydrate, under mature compost was reported for a crop fertilized at level I, while the highest concentration of terpinene-4-ol was detected at level II fertilization.

Nitrogen-fixers + *Bacillus circulans* + aqueous extract of compost at 15 and 30% increased terpinen-4-ol (21.74, 21.51), which was accompanied by a decrease in the proportion of *cis*-sabinene hydrate (12.67, 13.40%), relative to their controls (19.81, 17.81%), respectively (Table IV). Also, the level of numerous other monoterpenes including p-cymene, α-terpinolene as well as of the sesquiterpenes caryophyllene and α-humulene were concomitantly decreased relative to controls. In this connection, although cultivation of *Mentha piperita* with 28 t ha^{-1} biosolid is within the limits allowed such a rate, which increased oil yield but did not improve oil quality (Scavroni *et al*., 2005).

Protein content. Maximum value of crude protein (11.50%) was obtained by application of aqueous extract of

Table IV. Effect of aqueous extracts of compost and biofertilizers on composition of essential oil of *Origanum majorana* plants

Treatments Oil components (%)	Control (NPK)	Compost I (15%)	Compost II (30%)	Nitrogen fixer (N_2)	Bacillus circulans (B.C.)	N_2 + B.C.	Compost I + N_2	Compost II + N_2	Compost I + B.C.	Compost II + B.C.	Compost I + N_2+ B.C.	Compost II + N_2+ B.C.
α-pinene	3.98	4.29	4.75	4.35	3.97	3.65	2.47	2.29	2.57	2.11	2.63	2.21
Camphene	0.00	0.00	0.00	0.03	0.00	0.04	0.00	0.00	0.00	0.00	0.00	0.00
Sabinene	7.73	6.56	7.00	6.52	6.36	6.21	7.20	8.26	6.79	6.20	7.10	6.67
α- myrcene	1.32	1.85	1.79	1.66	1.58	2.01	1.86	2.04	1.12	1.53	1.82	1.68
Phellandrene	0.12	0.70	0.36	1.78	0.82	2.41	0.45	0.12	0.14	0.32	0.40	0.29
α-terpinene	0.24	11.30	11.25	10.51	8.89	13.11	8.60	7.87	12.95	7.54	11.89	11.18
p-cymene	13.90	6.88	6.64	6.69	11.66	8.81	8.40	6.89	7.29	7.25	7.48	7.95
γ-terpinene	9.06	16.44	15.49	15.54	12.18	16.83	12.37	11.37	14.75	10.83	15.08	13.48
trans-sabinene hydrate	1.62	2.60	2.02	2.75	3.47	1.98	4.45	5.37	3.58	6.59	2.56	4.33
α- trpinolene	5.25	5.07	4.53	4.34	4.00	5.21	3.49	2.08	3.47	2.05	4.40	3.29
cis-sabinene hydrate	17.81	9.32	13.15	11.14	12.70	6.74	15.59	16.56	13.48	18.47	12.67	13.40
α-terpineol	0.41	0.80	0.56	0.94	1.07	0.47	0.99	0.10	0.37	0.58	0.74	0.87
Terpinen-4-ol	19.81	21.75	17.82	20.58	20.48	20.39	20.10	24.24	19.49	21.08	21.74	21.51
P-menth-1-en-8-ol	2.82	3.31	3.17	4.33	3.82	3.73	4.21	2.98	4.04	3.69	4.18	3.53
Nerol	0.33	0.07	0.11	0.11	0.15	0.07	0.11	0.12	0.10	0.14	0.08	0.11
Linalyl acetate	5.25	2.70	3.42	2.20	2.87	2.03	3.15	2.99	3.05	4.42	2.00	3.14
Endobornyl actate	0.67	0.14	0.19	0.15	0.19	0.16	0.18	0.14	0.59	0.12	0.13	0.16
α-terpinyl propionate	0.55	0.43	0.34	0.18	0.43	0.42	0.40	0.00	0.00	0.38	0.28	0.00
Bicycloelemene	0.42	0.11	0.15	0.12	0.07	0.07	0.13	0.23	0.14	0.25	0.08	0.12
Neryl acetate	0.55	0.13	0.19	0.18	0.26	0.14	0.17	0.16	0.16	0.22	0.12	0.16
Geranyl acetate	0.50	0.23	0.35	0.33	0.28	0.23	0.18	0.17	0.22	0.29	0.20	0.20
β-caryophyllene	3.18	2.61	2.95	2.51	2.40	2.70	3.02	2.81	3.14	3.35	2.55	2.69
Aromadendrene	0.33	0.53	0.82	0.56	0.22	0.40	0.20	0.14	0.33	0.18	0.25	0.21
α-humulene	0.33	0.19	0.23	0.18	0.20	0.26	0.18	0.17	0.26	0.18	0.16	0.17
Ledene	0.00	0.44	0.00	0.42	0.23	0.55	0.00	0.00	0.00	0.00	0.38	0.00
Bicyclogermacrene	1.36	0.79	1.70	1.11	0.48	0.64	1.24	1.30	1.53	1.37	0.69	1.20
α-cadinene	0.09	0.07	0.10	0.07	0.04	0.07	0.04	0.03	0.06	0.04	0.04	0.04
Spathulenol	1.11	0.10	0.25	0.19	0.45	0.09	0.13	0.12	0.24	0.13	0.07	0.27
caryophyllene oxide	0.06	0.07	0.12	0.05	0.00	0.06	0.14	0.15	0.04	0.12	0.06	0.07
Unidentified compounds	1.20	0.52	0.55	0.48	0.73	0.52	0.55	1.30	0.10	0.57	0.22	1.07

Table V. Effect of aqueous extracts of compost and biofertilizers on the chemical constituents of *Origanum majorana* plants ± standard deviation of means

Treatments	Macronutrients %			Crude proteins	Gibberellic acid (GA_3)
	N	P	K	%	mg g^{-1} fresh wt
Control (NPK)	1.29±0.01 j	0.16±0.01 i	1.00±0.20 g	8.06±0.06 j	0.05±0.00 g
Cmpost I (15%)	1.35±0.02 i	0.21±0.04 g	1.58±0.02 de	8.42±0.10 i	1.19±0.01 d
Cmpost II (30%)	1.31±0.00 j	0.17±0.01 h	1.46±0.20 f	8.19±0.00 j	1.00±0.09 f
Nitrogen fixer (N_2)	1.49±0.01 f	0.27±0.02 f	1.57±0.03 de	9.33±0.07 f	1.24±0.09 d
Bacillus circulans (*B.C.*)	1.45±0.02 g	0.21±0.02 g	1.62±0.01 cde	9.06±0.13 g	0.05±0.00 g
Nitrogen fixer+*B.C.*	1.65±0.00 c	0.33±0.02 c	1.85±0.02 b	10.29±0.19 c	1.61±0.09 b
Cmpost I+N_2	1.56±0.02 d	0.29±0.01 d	1.63±0.02 cd	9.75±0.13 d	1.49±0.02 c
Cmpost II+N_2	1.52±0.02 e	0.29±0.04 d	1.54±0.02 e	9.50±0.13 e	1.48±0.06 c
Cmpost I+*B.C.*	1.52±0.02 e	0.29±0.02 d	1.68±0.02 c	9.50±0.13 e	1.25±0.05 d
Cmpost II+*B.C*	1.42±0.02 h	0.28±0.01 e	1.62±0.02 cde	8.90±0.14 h	1.08±0.06 e
Cmpost I+N_2+*B.C.*	1.84±0.02 a	0.35±0.03 a	2.02±0.03 a	11.50±0.13 a	1.85±0.03 a
Cmpost II+N_2+*B.C.*	1.78±0.02 b	0.34±0.01 b	1.88±0.02 b	11.13±0.13 b	1.64±0.03 b
L.S.D. at 5%	0.03	0.01	0.10	0.21	0.08

Means followed by the different letters in the same column are statistically different according to Duncan's multiple range tests P = 0.05.

compost at 15% + inoculation with both nitrogen-fixer strains and *B. circulans*, compared to 8.06% for controls plants (Table V). This can be assigned to direct effects of bacteria on root growth, phytohormones production, greater mineral uptake and transfer of nitrogen to the plant.

Macroelements. The highest values of nitrogen, phosphorus and potassium were obtained with bio-fertilizer treatments (*Azos. brasiliense* + *Azot. chroococcum* + *B. polymyxa* + *B. circulans*) + 15% aqueous extract of compost recording (1.84, 0.35 & 2.02%.) compared with (1.29, 0.16 & 1.00%) for their control plants, respectively (Table V). Similarly, nitrogenous fertilizer was better absorbed by inoculated maize plants with *Azos. lipoferum* strain CRT1 than by controls (Fages, 1994). Cleyet-Marel *et al.* (2001) found that inoculation of plant with PGPRs at early stage of development resulted in positive impact on biomass production through direct effects on root growth, production of phytohormones by bacteria, mineral enhancement uptake and transfer of nitrogen to the plant. Moreover, soil microorganisms that colonize the rhizosphere assist plants in the uptake of several vital nutrients, such as P, K and N, from soil (Cocking, 2003).

Table VI. Effect of aqueous extracts of compost and biofertilizers on the MPN of *Azottobacter, Azospirillum* and *Bacillus spp* (log. cfu g^{-1} soil) in *Origanum majorana* plants ± standard deviation of means

Treatments	*Azottobacter* sp.			*Azospirillum* sp.			*Bacillus* sp.		
	1st	2nd	3rdcut	1st	2nd	3rdcut	1st	2nd	3rdcut
Control (NPK)	5.05±0.05 f	5.49±0.01 e	5.75±0.31 c	5.42±0.09 h	5.66±0.01g	6.20±0.05 h	6.00±0.05 ef	6.25±0.09 f	6.63±0.08 de
Cmpost I (15%)	5.85±0.05 d	6.08±0.04 d	6.39±0.13 b	6.03±0.06 f	6.28±0.03 f	6.90±0.06 f	5.89±0.02 fg	6.13±0.08 g	6.70±0.18 cde
Cmpost II (30%)	5.73±0.20 e	6.09±0.05 d	6.46±0.06 b	6.31±0.06 e	6.47±0.04 e	6.74±0.14 g	5.54±0.06 h	5.93±0.08 h	6.19±0.07 f
Nitrogen fixer (N_2)	6.11±0.10 c	6.42±0.04 b	6.57±0.03 b	6.58±0.03 c	6.72±0.03 d	7.03±0.06 e	6.34±0.08 d	6.68±0.10 c	7.22±0.08 b
Bacillus circulans (*B.C.*)	5.95±0.04 d	6.18±0.03 c	6.40±0.02 b	6.42±0.04 d	6.46±0.05 e	6.86±0.07 f	5.85±0.25 g	6.24±0.08 fg	6.70±0.06 cde
Nitrogen fixer + *B.C.*	6.37±0.06 b	6.44±0.05 b	6.66±0.57 b	6.54±0.11 c	6.74±0.02 d	7.22±0.11 d	6.46±0.12 c	6.70±0.05 c	7.32±0.08 b
Cmpost I+ N_2	6.38±0.04 b	6.48±0.03 b	6.60±0.32 b	6.58±0.05 c	6.94±0.06 c	7.59±0.10 c	6.04±0.08 e	6.49±0.03 de	6.49±0.68 b
Cmpost II+ N_2	6.09±0.06 c	6.24±0.03 c	6.37±0.05 b	6.37±0.04 de	6.56±0.05 e	6.91±0.05 f	6.09±0.06 e	6.39±0.14 e	6.80±0.09 cd
Cmpost I+*B.C.*	6.29±0.08 b	6.46±0.05 b	6.73±0.53 b	6.12±0.17 f	6.54±0.03 e	7.04±0.10 e	6.32±0.02 d	6.52±0.03 d	6.93±0.03 c
Cmpost II+*B.C.*	5.91±0.08 d	6.10±0.06 d	6.38±0.03 b	5.90±0.10 g	6.25±0.33 f	6.72±0.15 g	6.22±0.06 d	6.88±0.10 b	7.49±0.02 b
Cmpost I+ N_2 +*B.C.*	6.67±0.12 a	6.83±0.10 a	7.35±0.15 a	7.27±0.08 a	7.64±0.06 a	8.36±0.13 a	6.76±0.03 a	7.40±0.18 a	8.41±0.18 a
Cmpost II+ N_2 + *B.C.*	5.72±0.15 e	6.11±0.08 d	6.45±0.20 b	7.02±0.07 b	7.29±0.02 b	7.86±0.10 b	6.60±0.07 b	6.95±0.05 b	7.48±0.10 e

Means followed by the different letters in the same column are statistically different according to Duncan's multiple range tests P = 0.05.

Table VII. Effect of aqueous extracts of compost and bio-fertilizers on nitrogenase activity and % of infection of AM mycorrhizae of *Origanum majorana* plants

Treatments	Nitrogenase activity (µ mol C_2H_4 g^{-1} soil h^{-1})			Infection of AM mycorrhiza (%)		
	1st	2nd	3rdcut	1st	2nd	3rdcut
Control (NPK)	0.44 j	1.17 j	1.41 g	46.33 g	47.66 g	41.66 f
Cmpost I (15%)	2.52 h	2.40 i	2.80 f	82.00 e	85.33 d	85.33 d
Cmpost II (30%)	1.60 i	1.30 j	1.61 g	80.70 e	81.10 e	81.33 e
Nitrogen fixer (N_2)	2.54 h	2.92 h	3.37 e	93.00 cd	95.00 b	92.33 c
Bacillus circulans (*B.C.*)	3.03 g	3.17 g	4.33 d	78.33 f	78.33 f	79.70 e
Nitrogen fixer+*B.C.*	5.20 c	5.40 c	5.67 c	94.33 bc	96.00 b	96.00 b
Cmpost I+ N_2	4.50 d	4.83 d	5.40 c	95.00 bc	96.33 b	95.66 b
Cmpost II+ N_2	3.40 f	3.87 f	4.16 d	96.00 b	96.66 b	91.33 c
Cmpost I+*B.C.*	3.69 e	4.43 e	5.40 c	91.33 d	93.00 c	93.00 c
Cmpost II+*B.C.*	3.30 f	3.77 f	4.33 d	76.66 f	82.33 e	81.33 e
Cmpost I+ N_2+*B.C.*	9.00 a	10.5 a	12.27 a	100.00 a	99.66 a	99.00 a
Cmpost II+ N_2+*B.C.*	8.77 b	9.33 b	10.63 b	95.33 b	95.33 b	97.00 b
L.S.D at 5%	0.21	0.29	0.43	3.04	2.44	2.68

Means followed by the different letters in the same column are statistically different according to Duncan's multiple range tests P = 0.05.

Gibberellins. Marked increase in the activity levels of gibberellin was observed in the marjoram plants treated with nitrogen-fixer strains + *B. circulans* recording 1.61 compared with 0.051 mg g^{-1} fresh weight for control plants (Table V). Moreover, the highest gibberellin content was obtained in the extract of marjoram plants grown in soil treated with aqueous extract of compost at 15 and 30% + nitrogen–fixers + *B. circulans* recording (1.85 & 1.64 mg g^{-1} fresh weight), respectively. According to Shukry *et al*. (1999), soil treatment with spent straw and raw straw mostly increased the endogenous level of auxin, gibberellins and cytokinins of the extracts of the terminal buds and roots of the treated cucumber plants at 0.2 and 1.0%, respectively but these activities were decreased below those of the corresponding control with concomitant increase in the growth inhibitors at 2.0% level of the two types of straw.

Nitrogen-fixers population. Supplementation of the soil with aqueous extract of compost and/or bio-fertilizers significantly increased the total number of bacteria during three cuttings (Table VI). Moreover, aqueous extract of compost at 15% + inoculation with nitrogen-fixers + *B. circulans* highly increased the populations of nitrogen-fixers in the rhizospere of marjoram plants, especially at the third cut. It is likely that application of compost can provide nutrients to soil and microflora including nitrogen-fixers, which were colonized in the rhizospere. Similarly, Belete *et al*. (2001) found that the addition of compost and inoculation with nitrogen-fixers can affect the biological characteristics of soil by enhancing N-fixing rhizobacteria, which are sensitive indicators of soil health ecosystem.

Infection of native AM-mycorrhizae. A highest infection percentage of AM-mycorrhiza (100%) during three cuttings was obtained with aqueous extract of compost at 15% + inoculation with both nitrogen-fixer strains and *B. circulans* (Table VII). This support the evidence that nitrogen-fixers in the presence of aqueous extract of compost enhanced mycorrhizal root colonization. Similarly, Organic matter present in compost water extract contains substrates, which stimulate the growth of mycorrhizal fungi (Thomas *et al*., 1999). Compost at 15% significantly influenced the indigenous AMF infectious propagules in soil. Similar increases were recorded in poultry manure amended plots in *J. citronella* plants (Adholeya & Prakash, 2004).

Nitrogenase enzyme activity. Nitrogenase enzyme activity in the soil of marjoram plants increased as a result of treatments with aqueous extract of compost at 15 and 30%, nitrogen-fixers strains, *B. circulans* alone or in combination compared with control (NPK fertilizers) during three cuttings (Table VII). Highest nitrogenase enzyme activity was obtained with aqueous extract of compost at 15% +

inoculation with both nitrogen-fixers and *B. circulans* recording 9.0, 10.5 and 12.27 μ mole $C_2 H_4$ g^{-1} dry root h^{-1} compared with 0.44, 1.17, 1.41 μ mole $C_2 H_4$ g^{-1} dry root h^{-1} for control plants at first, second and third cuts, respectively. Similarly, in maize and sorghum grown in the field, acetylene reduction was higher during the reproductive stage. This can be explained by an increase in the number of mature roots associated with *Azospirillum* at the beginning of reproductive growth. At this stage, there was an increase in uptake of available nitrogen by the plant that, together with leaching and denitrification processes, may deplete the soil of combined nitrogen, thus derepressing the nitrogenase of *Azospirillum* (Neyra & Dobereiner, 1977).

CONCLUSION

Inoculation of marjoram with 15% and 30% aqueous extracts of compost and/or biofertilizers have beneficial effects on plant growth, essential oil content and dry matter yield due to hormonal stimulation of root development and by supplying combined nitrogen. The increase was more pronounced mainly at 15% aqueous extracts of compost + nitrogen fixers + *B. circulans,* whereas the effect was less marked with nitrogen fixer strains, *B. circulans* or at high level of aqueous extracts of compost applied singly compared with mineral fertilizers. At low level of aqueous extracts of compost in the soil, simultaneous utilization of biologically fixed nitrogen may be possible but at higher levels, the potential for the nitrogen fixation may be decreased.

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