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PHYSIOLOGICAL RESPONSE OF MARJORAM PLANTS TO BIOFERTILIZER AND ORGANIC FERTILIZATION

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ABSTRACT

Two pot experiments were carried out during two successive seasons 2000 and 2001 to investigate the effect of organic fertilizer (Biogreen) at three rates; 5, 10 and 15 m^3/fed. with or without biofertilizer inoculation (namely; *Azobacter crococcum; Azospirillum brasilense and Bacillus polymyxa*) in comparison with the recommended dose of NPK (control) on growth, yield and chemical composition of marjoram plants. Data obtained clearly revealed that plants treated with the highest level of compost (15 m^3 / fed.) either alone or inoculated with mixture of biofertilizers recorded a considerable increments with regard to growth characters, macronutrients, phytohormones; (IAA, GA_3, and CK) and essential oil components; (α-terpineol, α-terpenolene, Linalool, Geraniol, Cineole, Linalyl acetate and Citronellol). In addition, the significant increases were 15.8, 19.9 and 14.4% for herb fresh weight (g/plant) and 19.8, 16.9 and 14.7% for herb dry weight (g/plant) in first, second and third cuttings, respectively, as well as essential oil percentage (25 % ,26% and 28 % in the three cuttings, respectively) and oil yield/plant (44 %, 52% and 47 % in the three cuttings, respectively) in fresh herb at the highest compost level as compared to the control treatment. Also marjoram plants which received the highest level of compost in combination with biofertilizer mixture significantly surpassed the control plants in herb fresh weight (g/plant) by 22.1 29.1 and 32.1% and herb dry weight (g/plant) by 25, 32.6 and 40% in first, second and third cuts, respectively, as well as the essential oil percentage by 38%, 38 % and 40% in the three cuttings, respectively and oil yield per plant by 69 %, 79% and 91 % in the three cuttings, respectively. On the other hand, the present data disclosed that nearly the same marjoram fresh and dry weight (g/plant) as well as essential oil percentage and oil yield per plant of the control plant could be obtained if marjoram plants received the lowest and moderate rates of compost fertilizer alone or combined with biofertilizers mixture. These findings clearly indicate that organic fertilizers (Biogreen) and biofertilizers mixture could replace the application of mineral fertilizers and consequently minimize the pollution of the agricultural environment.

Key words: *biofertilizer, marjoram , organic fertilizer*

INTRODUCTION

Medicinal plants occupy a prominent position, because of the increasing demand of the local industry and export. In order to cover such increase, an increasing interest in the cultivation of medicinal and aromatic plants has been settled in Egypt. Recently, a considerable attention has been directed in the newly reclaimed lands to improve the growth and the yield of various aromatic and medicinal plants.

Marjoram plants (*Origanum majorana* H. or *Majourana hortensis* L.) is considered as an, important medicinal crop in Egypt with high production and great applications. The air dried leaves and flowering tops are used as condiment for seasoning soups dressing, stews, poultry and sausage. It is also used as a stimulating in tooth pastes, whooping cough and larynx infections.

In the recent years, the safe agriculture is one of the main attitudes in the world (El-Kouny, 2002). Also, 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 in the health of human and animal consumers. As a result of this awareness, strict regulations and restrictions have been imposed in several countries (especially in the European markets) prohibiting the impact of "Chemically–grown" products. This has led growers of medicinal and aromatic plants in many countries to adopt organic and biological agricultural methods (for fertilization, pest controls, ... etc).

Composting of agricultural residues by supplying the natural microbial flora present on them with their requirements of inorganic nutrients such as nitrogen and phosphorous and by applying a proper moistening and turning, results in a final product with high ability to improve soils and enhance plant growth reported by Lampkin (1990), Mohamed and Matter (2001) and Badran (2002). Moreover, compost with its content of humic substances and microbial materials, has been shown to improve soil physical, chemical and microbiological conditions, moisture content, and reduce leaching of nutrients, water run-off and soil erosion (Amin et al., 1999).

Biofertilization is used in order to compensate a part of the mineral fertilizer doses, taking in consideration the complementary or synergistic effects of such combination between bio-and mineral fertilization. This could be of economic value from the applied point of view of minimizing the used doses of the mineral fertilizers and consequently reduce agricultural costs as well as soil pollution.

Over the last two decades, the biofertilizers are increasingly used in modern agriculture due to the extensive knowledge in rizosphare biology and the discovery of the promotive function of special groups of microorganisms such as *Azospirillum, Azobacter, Acetobacter Bacillud, Serratia* and *Psdomonas* known as plant growth promoting rhizobacteria (PGPR). They appear to be frequent colonizers of important medicinal crops including *Ammi visnaga*, Lemongrass and Palmarorsa (Masheshwari *et al.*, 1995, El-Sawy *et al.*, 1998 and Harridy *et al.*, 2001). Such beneficial effects of these plant growths promoting rhizobacteria (PGPR) could be attributed to the biological nitrogen fixation and production of phytohormones (gibberellins and cytokinins like substances well as auxins) that promote root development and proliferation, resulting in efficient uptake of water and nutrients (Hartmann *et al.*, 1983 and Haahtela *et al.*, 1990).

The beneficial effects of rhizobicteria on medicinal crops recently increased due to their potential use as biofertilizers (Okon and Labandera – Gonzalez, 1994 and Kumar *et al.*, 1998). Thus, the extensive researches in this field strongly indicated that application of bacterial inoculants as biofertilizers resulted in improvement of growth and productivity for medicinal crops, in addition to the reduction of their N-requirements to 50% of the recommended dose (Harridy and Mervat, 1998). Therefore, the aim of the present study was to investigate the effects of compost (Biogreen) solely or combined with inoculation mixture of biofertilizers, i.e. *Azobacter chroococcum, Azospirillum brasilense and Bacillus polymexa* on the growth, oil yield and chemical composition of marjoram plants and to compare this effects with that of recommended NPK fertilization.

MATERIALS AND METHODS

Two pot experiments were conducted in the Medicinal and Aromatic plant Research Department, Horticulture Research Institute (HRI), Agricultural Research Center (ARC) during the two successive seasons 2000-2001. In both experiments, three marjoram seedling (12-15 cm in height, with 10-12 leaves) were planted on the 20^{th} of March, 2000 in the first season and on the 14^{th} of March, 2001 in the second one in pots of 30 cm, in diameter filled with sandy soil (Obtained from the shabrament area, Giza). Two weeks later, only one vigorous seedling was left to grow in each pot. The marjoram plants were irrigated with tap water when needed . Some physical and chemical analysis (Table 1) of the soil under study was carried out as described by page *et al.*, (1982) in both seasons before planting

Marjoram seedling (50-60 days after germination) were obtained from the Experimental station of the Horticulture Research Institute in South Al-Tahrir.

Both two pot experiments during the two successive seasons included seven treatments with four replicates as follows:

1) * R. Q. F. (control)
2) Compost at rate 84 cm^3 / pot / season (equivalent to 5 m^3 / fed./ season) (C_1).
3) Two rates of C_1 [168 cm^3 / pot season (10m^3 / fed./ season)] (C_2)
4) Three rates of C_1 [252 cm^3 / pot season (15 m^3 / fed./ season)] (C_3)
5) C_1 +** Mixture of biofertilizers (C_1 + Bio).
6) C_2 + Mixture of biofertilizers (C_2+Bio).
7) C_3 + Mixture of biofertilizers (C_3 + Bio).

Where :

* R.Q.F. = The recommended quantities of NPK fertilizer which are 2.8 g. Ammonium sulphate (20.5% N); 2.1 g. calcium superphoshate (15.5% P_2O_5)and 1.4 g. Potassium sulfate (48% K_2O).

** Mixture of biofertilizers = *Azotobacter chroococcum*; *Azospirillum brasilense* and *Bacillus polymexa*.

These treatments were distributed in complete randomized design according to Gomez and Gomez (1984).

Three bacterial cultures containing 1x1$10^8$ CFU/ml from *Bacillus polymexa; Azospirillum brasilense and Azotobacter chroococcum* were prepared individually in Biofertilizer unit, Soils water and Environ. Res. Inst., Dept. of microbiology (A. R. C.), Giza, Egypt, Then, they were mixed well together in liquid at equal portions. Roots of marjoram seedling were dipped into the mixture of biofertilizers immediately before planting . Also, this mixture was added to plant rhizospher at rate of 10 ml for each pot for inoculated treatments (5, 6 and 7) at 30 and 60 days after treasplanting.

Table (1): Some physical and chemical analysis of Experimental soil

Parameter	Value
Sand (%)	86.4
Silt (%)	7.2
Clay (%)	6.1
Texture grade	Sandy
PH (soil paste)	7.6
EC (ds/m at 25° C)	0.37
Cations (m eq/1) Ca^{++}	0.94
Mg^{++}	0.53
Na^{+}	0.59
K^{+}	0.28
Anions (m eq/1) $CO^{=}_{3}$	0.00
HCO^{-}_{3}	1.41
Cl^{-}	0.29
$SO^{=}_{4}$	0.65
Total – N (%)	0.030
Organic – C (%)	0.37

The compost (Biogreen) was obtained from the Soil Department. ARC. The chemical composition of the compost is shown in Table (2). Chemical analysis of the compost was conducted according to Page et al., (1982).

The compost and NPK fertilization treatment were divided into two doses. The first dose from compost was incorporated with the potting medium, two weeks before transplanting, and the second dose from NPK or compost were applied two weeks before the first cut (75 days from transplanting). On the other hand, the first dose of NPK was added two weeks after transplanting .

Three cuttings plants from each treatment were taken after 90, 150 and 210 days from transplanting by cutting the vegetative parts of all sampled plants 5 cm above the soil surface. In each sample, plant height, number of branches/plant; and herb fresh and dry weight/plant were recorded.

The mean values of growth determined in the two seasons were statistically analyzed and the means were compared using L. S. D. values at 5% level (Gomez and Gomez, 1984).

2.1. Chemical analysis:

The shoot was chemically analyzed in order to determine their chemical constituents. For the determination of total nitrogen, the modified " Micro Kjeldhl" apparatus of Parnas and Wanger as described by Jones et al., (1991) were used. For total P and K, determination, the wet digestion of plant material was carried out as recommended by Piper (1947). Phosphorus was estimated colorimetrically using the stannous chloride reduced molybdophosphric blue color method in sulphoric acid system as described by Jackson (1973). K was determined by the Atomic Absorption Spectrophotometer (GBC, 932 AA).

Table (2): Chemical analysis of compost (Biogreen) in both seasons

Property	Value
PH (1:5)	8.11
EC (1:5 extract) ds/m	8.21
Organic – C%	12.54
Organic matter %	21.57
Total – N%	1.41
C/N ratio	8.89
Total – P%	1.19
N-NH_4^+ (ppm)	274.7
N-NO_3^- (ppm)	33.1
Total content of bacteria	2.5 x 10^7
Total content of Fungi	7 x 10^5
Total content of Actinomycetes	3.5 x 10^5
Phosphate dissoluing bacteria	2 x 10^6
Dehydrogenase activity (mg TPF/100g)	32.5
Nitrogen activity (N mol C_2H_4 / g/ hr)	123.6
E4/E6 (aquenous extract 1:10)	3.64
Seed germination test for cress seeds after 48 hr.%	79.5

Freeze-dried marjoram leaves (equivalent 6 g F.W.) were ground to a fine powder within a mortar and pestle. The powdered material was extracted three times (1x3 h. 2x1 h) with methanol (80% v/v, 15 ml./g F. W.), supplemented with butylated hydroxy toluene (2. (6) – Di-tret-Butyl-P-crosol) as an antioxidant, at 4°C in darkness. The extract was centrifuged at 4000 rpm. The supernatant was transferred into flasks wrapped with aluminum foil and the residue was again twice extracted. The supernatants were combined and the volume was reduced to 10 ml at 35° C under vacuum. The aqueous extract was adjusted to pH 8.6 and extracted three times with an equal volume of pure ethyl acetate. The combined alkaline ethyl acetate extract was dehydrated over anhydrous sodium sulphate then filtered. The filtrate was evaporated to dryness under vacuum at 35°C and redissolved in 1 ml absolute methanol . The methanol extract was used after methylation according to Plamer et al., (1981) for the determination of cytokinins. The remaining aqueous extract was acidified to pH 2.6 and extracted as previously described by ethyl acetate. The methanol extract was used after methylation according to Fales and Jaouni (1973) for determination of gibberlic acid (GA) and indole-acetic acid (IAA). The quantification of the endogenous phytohormones was carried out with Ati-Unicum gas-liquid chromatography, 610 Series, equipped with flame ionization detector according to the method described by Vogel (1975). The fractionation of phytohormones was conducted using a coiled glass coulumn (1.5 m x 4 mm.) packed with 1% OV-17. Gases flow rates were 30, 30, 330 ml/min., for nitrogen, hydrogen and air, respectively. For cytokinins fractionation, the temperature were for injector 260°C, detector 300 °C and column initially for 3 min. at 220 °C then programmed at 20 °C/min. for 220 °C to 240 °C. then isothermally at 240 °C for 8 min. For IAA and GA, the initial column temperture was 200 °C for 3 min. then programmed at 20 °C/min. for 200 °C to 220 °C, then isothermally at 220 °C for 4 min, then programmed at 20 °C/min. for 220 °C to 240 °C, then isothermally at 240 °C for 6 min. The peaks identification and quantification of phytohormones were performed by using external authentic hormones and a Microsoft program to calculate the concentrations of the identified peaks.

The oil percentage was determined in the fresh herb using the method described by the Egyptian Pharmacopoeia (1984), and the essential oil yield per plant was calculated in proportion to the herb fresh weight (Oil yield/plant = plant fresh weight x oil percentage).

Chemical analysis for essential oil was conducted by using Ati-Unicum gas liquid chromatography (GLC), 610 series, to determine their main constituents as described by Gunther and Joseph (1978).

It is to be mentioned here that only the second season was taken for chemical analysis. Moreover, the average values of the various characters determined in the two successive seasons were only tabulated and discussed in the present investigation.

RESULTS AND DISCUSSION

3.1. Growth characters:

Growth characters in all cuttings of marjoram plants were influenced by compost treatments either alone or in combination with biofertilizer mixture (Table 3).

In general, results indicate that all growth characters in all cuttings; the highest compost level (15 m^3/fed.) significantly increased as compared to the control treatment (plants receiving the recommended NPK dose). These increments reached 15.8, 19.9 and 14.4% for herb fresh weight (g/plant) and 19.8, 16.9 and 14.7% for herb dry weight (g/plant) in the three cuttings, respectively; as compared to the control. No significant differences were detected in all growth characters between marjoram plants receiving the compost at lowest and moderate doses (5 and 10 m^3/fed.) and plants fertilized by NPK (control).

These results coincide with those obtained by Abd El-Moez *et al.*, (1999) on fennel and coriander plants; Abd El-Moez and Saleh (1999) on Roselle plants; Mansour *et al.*, (1999) on spearmint and marjoram plants; Mohamed and Matter (2001) on marigold plants ; Harridy *et al.*, (2001) on lemongrass and Badran and Safwat (2004) on fennel plants.

The significant positive effect of compost fertilizer on vegetable growth characters may be due to the improvement in soil physical and biological properties and also, the chemical characteristics resulting in more release of available nutrient elements to be absorbed by plant root and its effect on the physiological processes such as photosynthesis activity as well as the utilization of carbohydrates, in addition to water use efficiency by different plants (Abd El-Moez *et al.*, 1999) and Hussein (2003).

When the different doses of compost fertilizer were associated with the inoculation with biofertilizer mixture (Table 3), positive responses in all growth characters; *i.e.* plant height, number of branches / plant and both of herb fresh and dry weight (g/plant) in all cuttings were recorded against control. The highest combination level of compost, and biofertilizer mixture (plants receiving 15 m^3/fed. compost combined with inoculated biofertilizer mixture), such positive responses recorded significant increments over all other treatments in all cuttings. These increments were 22.1, 29.1 and 32.1% for herb fresh weight, and 25, 32.6 and 70% for herb dry weight in first, second and third cuts, respectively, as compared to the control. Also, it is be mentioned here that, the positive effects of the other combination treatments on all studied growth characters failed to reach the 0.05 level of significant.

The favorable effects of the combination between compost fertilizer and biofertilizers may be explained on the basis of the beneficial effects of bacteria on the nutrient availability, vital enzymes, hormonal stimulating effects on plant growth or the increasing of photosynthetic activity. Supportive evidence for this view was reported by Bashan *et al.*, (1989) who found that *Azopirillum* and *Pesdomonas* improved wheat growth through the significant increase in dry matter which accumulates in both roots and shoots of treated plants.

Also, Bashan and Levanony (1990) proposed several possible modes of action of *Azospirillum* on plant growth and N_2 fixation, which contributes N to the plant. Hormonal effects, which alter plant metabolism and growth. General growth improvement in the entire root system, resulted in enhanced mineral and water uptake. Moreover, Valssak and Reyndres (1980) found that Azopirillum produces IAA, Indol lactic acid, gibberllins by bacteria and degree of sensitivity of plants to phytohormones are being suggested as the main reason for this phenomenon (Jlik, 1995). Recently, Ghallab and Salem (2001) found that wheat inoculating with *Azopirillum spp*. improved grain yield and total dry matter accumulation. Also, Hanafy Ahmed et al., (2002) found that sweet pepper inoculation with rhizobacterien or microbien increased most of the studied growth characters and total fruit yield.

It is worth to be mention her from the results that the two treatments, i.e. the highest level of compost either alone or combined with biofertilizer mixture , lead to high increase in dry weight of marjoram plants. This results in the increase of its use as condiment for seasoning soups dressing, stews, poultry and sausage

3.2. Chemical analysis:

The effect of different treatment on the essential oil percentage (mL/100g. F. W. herb.); oil yield/plant; concentrations of N, P, K, IAA, GA_3 and CK in the shoots of the three cuttings of marjoram plants grown in the second season (Table 4) were similar to those recorded for growth characters. The data clearly show the significant increases in both essential oil percentage (25 %, 26% and 28 % in the three cuttings, respectively) and oil yield/ plant (44 %, 52% and 47 % in the three cuttings , respectively) in fresh herb at the highest compost level as compared to the control treatment. On the other hand insignificant differences were detected between the other levels of compost (5 and 10 m^3/ fed.) and the control.

Majoram plants which received the highest level of compost in combination with biofertilizer mixture significantly surpased the control plants in the essential oil percentage by (38%, 38 % and 40 % in the three cuttings, respectively) and oil yield per plant by (69 %, 79% and 91 % in the three cuttings, respectively); meanwhile the positive effect of the other combination treatments on oil yield per plant and the essential oil percentage was insignificant.

These results are in agreement with those obtained by Jacoub (1999) on *Ocimum basillicum L.* and *Thymus vulgaris L.* plants ; Mansour et al., (1999) on spearmints and majoram plants and Badran and safwat (2004) on fennel plants. The effect of compost on increasing the voltile oil percentage of shoots might be attributed to their enhancing effect on vegetative growth, in terms of the fresh yield and increasing the uptake on nutrient by roots . Similar results were recorded by Mohamed and Matter (2001) on marigold plants.

Concerning the effect of different treatments on the mean valus of macro-nutrients concentrations in the shoots of marjoram plants in the all cuttings of the second growing season [Table 4] were similar to those in recorded for growth characters [Table 3].

Table (3): Growth characters of marjoram plants as affected by different levels of compost and their combinations with biofertilizer mixture (combined analysis for two seasons).

Growth characters	Plant height (cm)			No. of branches / plan			Herb fresh weight (g/plant)			Herb dry weight (g/plant)		
Cuts / Treatments	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
Control (NPK)	15.58	20.17	27.23	13.72	18.43	21.51	11.61	14.14	19.5	5.35	7.31	11.90
C_1 (5 m^3 / fed.)	14.89	18.99	25.96	12.85	17.29	20.25	10.51	12.81	17.89	4.55	6.71	10.73
C_2 (10 m^3 / fed.)	15.15	19.48	26.35	13.11	17.95	21.11	11.21	13.24	18.18	4.89	7.01	11.24
C_3 (15 m^3 / fed.)	16.95	21.55	28.88	14.89	19.89	23.42	13.45	16.95	22.31	6.41	8.55	13.65
C_1 + MB	16.21	20.98	27.91	14.39	19.23	22.17	12.89	15.43	20.85	5.99	7.91	12.64
C_2 + MB	16.22	21.31	28.45	14.72	19.56	22.85	13.01	16.05	21.11	6.18	8.11	13.01
C_3 + MB	18.85	26.25	37.58	17.56	24.69	30.33	14.18	18.26	25.75	6.69	9.69	16.66
L. S. D0.05	0.7	1.25	1.37	1.13	1.37	1.46	1.56	1.98	2.61	1.01	1.19	1.27

C = Compost; MB = Mixture of biofertilizer

The data clearly show the superiority of the highest compost level either alone or combined with biofertilizer mixture which resulted in the highest promotive effects on the macro-nutrients accumulation which surpassed all other treatments However, slight increases were obtained by the plants treated with the lowest and moderate compost levels combined with bofertilizer mixture.

Increasing N, P and K concentrations by compost fertilization might be due to the increase in root surface per unit of soil volume as well as the high capacity of the plants supplied with compost fertilizer in building metabolites, which in turn contribute much to the increase of nutrient uptake. In this respect, Abd El-Moez *et al.*, (1999) on fennel and coriander plants, found that the macro and micro nutrients uptake by roots plant increased significantly by addition organic composts to prepared soil. They attributed results to the effect of organic fertilizer in improving not only soil physical and biological properties but also chemical characteristics resulting in more release of available nutrient elements to be absorbed by plant roots and the water efficiency by different plants.

Table (4): Essential oil percentage; oil yield; concentrations of N, P, K (mg/g. D.W), Indole-3-acetic acid (IAA), gibberelic acid (GA_3) and cytokinins (CK) (µg/g. F.W.) in shoots of marjoram plants as affected by different levels of compost and combination between compost and biofertilizers during 2001 season.

Growth characters	Oil % (ml./100 g.F.W.)	Oil yield (ml./plant)	N	P	K	IAA	GA_3	CK
	1st cutting							
Control	0.31	0.036	26.73	3.71	27.79	9.91	8.32	7.15
C_1 (5 m^3/Fed.)	0.26	0.027	24.69	2.61	25.42	8.86	7.22	6.07
C_2 (10 m^3/fed.)	0.28	0.031	25.28	3.11	26.71	9.16	7.39	6.24
C_3 (15 m^3/fed.)	0.39	0.052	35.54	4.42	30.79	11.34	9.76	8.39
C_1 + MB	0.33	0.043	27.26	3.88	28.29	10.28	8.86	7.67
C_2 + MB	0.34	0.044	28.23	3.99	28.93	10.85	9.17	8.06
C_3 + MB	0.43	0.061	37.97	4.91	32.01	12.82	10.92	8.79
L. S. D. 0.05	0.05	0.015						
	2nd cutting							
Control	0.34	0.048	28.75	3.99	29.89	10.66	8.95	7.69
C_1 (5 m^3/Fed.)	0.29	0.037	26.55	2.81	27.33	9.53	7.76	6.53
C_2 (10 m^3/fed.)	0.31	0.041	27.19	3.34	28.72	9.85	7.95	6.71
C_3 (15 m^3/fed.)	0.43	0.073	38.21	4.75	33.11	12.19	10.49	9.02
C_1 + MB	0.36	0.056	29.31	4.18	30.42	11.05	9.53	8.25
C_2 + MB	0.39	0.063	30.36	4.29	31.11	11.67	9.86	8.67
C_3 + MB	0.47	0.086	40.83	5.28	34.42	13.79	11.74	9.45
L. S. D. 0.05	0.06	0.017						
	3rd cutting							
Control	0.35	0.068	29.91	4.15	31.19	11.19	9.31	7.99
C_1 (5 m^3/Fed.)	0.30	0.054	27.61	2.92	28.42	9.91	8.07	6.79
C_2 (10 m^3/fed.)	0.32	0.058	28.28	3.98	29.87	10.24	8.27	6.98
C_3 (15 m^3/fed.)	0.45	0.089	39.74	4.94	34.43	12.68	10.91	9.39
C_1 + MB	0.37	0.077	30.48	4.35	31.64	11.49	9.91	8.58
C_2 + MB	0.40	0.084	31.57	4.48	32.35	12.14	10.25	9.02
C_3 + MB	0.49	0.092	42.46	5.49	35.79	14.34	12.21	9.83
L. S. D. 0.05	0.07	0.018						

C = Compost; MB = Mixture of biofertilizer

The promotive effects of the bioferilization on macro-nutrients accumulation could be attributed to the inoculation of majoram plants resulted in a promotive effect on root development and consequently their function in the uptake of both water and nutrients. Similar results were reported by Harridy *et al.*, (2001) on lemongrass. More supportive evidences for the obtained results were found in the work of Ghallab and Salem (2001) on wheat who found that mixture of *Azospirllium* and *Serratia* inoculation greatly enhanced the uptake of N, P, K, Fe., Mn, Zn and Cu as well as accumulation of these minerals in the wheat plants. Moreover, Faragl and Ali (2004) found that using 75 % NPK + Biofertilizers [HALEX2] + organic fertilizers caused increasing N, P and K percentage in the leaves of tomato plants. Also, Bashan *et al.*, (1989) attributed the increased mineral uptake by inoculated roots to the enhancement in proton efflux activity of wheat root inoculated with *Azopirllium*. Moreover, Omar *et al.*, (1991) reported that inoculation with *Azopirllium* cultures stimulated the uptake and accumulation of the nutrients from the soil. Recently, Dashti *et al.*, (1997) suggested that the promotive mechanism of growth and nitrogen fixation induced by plant growth promoting rhizobacteria (PGPR) included both direct and indirect effects. The direct effects include an increase in the mobilization of insoluble nutrients followed by enhancement uptake by the plants (Lifshitz *et al.*, 1987) and production of plant growth regulators that stimulate plant growth (Gaskins *et al.*, 1985). The indirect effects include positive effects on symbiotic nitrogen fixation by enhancement of root nodule number or mass are reported by Yahalom *et al.*, (1987).

Concerning the phytohormones , the results of the hormonal analysis [IAA, GA_3 , and CK] in the leaves of the marjoram plants in the all cuttings of the second season as affected by different treatments are presented in Table (4) confirmed the previous results obtained in Table (3) as well as essential oil percentage, oil yield [ml. / plant] and macro-nutrients accumulation [Table 4]. Obtained results clearly show the positive response in the hormonal concentrations which their maximum when the highest level of compost either alone or combined with biofertilizer mixture. However, slight increases were obtained by the plants treated with the lowest and moderate compost levels combined with Biofertilizers mixture.

The promotive effects of the biofertilization specially the biofertilizer mixture with the compost at 15 m^3/Fed. on phytohormons may be attributed to the production of growth promotive substance from rihzospheric microorganisms such as IAA and GA_3 (Gulam *et al.*, 1998). In this respect. *Azopirllium* produce several plant hormones in liquid culture, mainly IAA. Other hormones were detected at much lower concentration, indole-3 butyric acid (IBA) (Fallik *et al.*, 1989), indole –3 ethanol and indole – 3 – methanol (Crozier *et al.*, 1988). Several gibberllins (Bottini *et al.*, 1989), abscisic acid (ABA) Kolp and Martin (1989), and Cytokininid [Horemans *et al.*, 1986].

It is well established that biofertilizers can lower the amount of the added inorganic nitrogen fertilizer to the soil and consequently reduces soil pollution.

3.3. Chemical composition of oil:

Table (5) shows that the highest contents of terpinen-4-ol (the main component), as well as that of some other important components; I.e. α-teripineol , α – terpenolene, linalool, geraniol, cineole, linalyl acetate and citronellol were obtained from plants fertilized with the highest level of compost in combination with biofertilizer mixture followed with compost at 15 m^3/Fed. and then the lowest and moderate doses of compost in combination with biofertilizer mixture, compared to the control.

Table (5): **Effect of different levels of compost and combination between compost and biofertilizer on the essential oil components in *Origanum marjoram* H. plant during 2001 season.**

Components / Treatments	α-pinene	β-pinene	Limonene	Cineole	Linalool	α- Teripineol	Terpinen-4-ol	Citronellol	Linalylacetate	α—Terpinyl acetale	Geraniol	α–Terpenolene	Unidentified (U)
							1st cutting						
Control (NPK)	5.21	11.53	11,37	0.94	1.25	1.43	5o.72	0.44	0.38	3.24	1.27	0.59	0.72
C_1 (5 m^3/Fed.)	0.32	4.29	1.81	0.81	1.18	0.89	47.32	0.41	0.34	1.05	1.18	0.54	3.12
C_2 (10m^3/Fed.)	1.08	5.21	2.18	0.88	1.24	1.02	49.71	0.44	0.36	1.21	1.25	0.57	4.44
C_3 (15 m^3/Fed.)	1.26	5.69	3.7	1.34	1.68	1.33	58.39	0.81	0.84	2.02	1.61	1.73	9.36
C_1 + MB	1.23	4.83	2.65	1.03	1.43	1.27	52.38	0.53	0.66	1.79	1.43	0.99	12.67
C_2 + MB	1.29	5.97	2.79	1.09	1.59	1.29	53.56	0.66	0.76	1.93	1.58	1.11	13.66
C_3 + MB	1.98	6.71	4.24	1.69	1.81	1.38	65.41	1.08	1.48	2.19	1.76	2.95	14.87
							2nd cutting						
Control (NPK)	5.72	12.95	12.77	1.06	1.41	1.61	56.99	0.50	0.43	3.65	1.43	0.67	0.81
C_1 (5 m^3/Fed.)	0.35	4.81	2.03	0.91	1.33	1.01	53.17	0.46	0.39	1.18	1.33	0.61	3.51
C_2 (10m^3/Fed.)	1.19	5.85	2.45	0.99	1.39	1.15	55.86	0.49	0.41	1.36	1.41	0.65	4.99
C_3 (15 m^3/Fed.)	1.42	6.40	4.16	1.51	1.91	1.65	65.61	0.91	0.95	2.29	1.84	1.94	10.51
C_1 + MB	1.39	5.43	2.98	1.16	1.61	1.43	58.85	0.59	0.74	2.02	1.61	1.11	14.24
C_2 + MB	1.46	6.71	3.14	1.23	1.79	1.46	60.18	0.75	0.85	2.17	1.78	1.25	15.35
C_3 + MB	2.23	7.54	4.76	1.90	2.03	1.69	73.49	1.21	1.67	2.47	1.98	3.31	16.71
							3rd cutting						
Control (NPK)	5.95	13.47	13.29	1.11	1.47	1.67	59.27	0.53	0.45	3.79	1.48	0.69	0.48
C_1 (5 m^3/Fed.)	0.36	5.01	2.11	0.95	1.38	1.05	55.29	0.48	0.41	1.23	1.38	0.63	3.65
C_2 (10m^3/Fed.)	1.24	6.08	2.55	1.03	1.45	1.19	58.19	0.51	0.43	1.41	1.47	0.67	5.19
C_3 (15 m^3/Fed.)	1.48	6.66	4.33	1.57	1.99	1.55	68.23	0.95	0.99	2.38	1.92	2.02	10.91
C_1 + MB	1.45	5.65	3.09	1.21	1.67	1.48	61.21	0.62	0.77	2.11	1.67	1.15	14.81
C_2 + MB	1.51	6.98	3.27	1.27	1.86	1.52	62.59	0.78	0.88	2.27	1.85	1.31	15.96
C_3 + MB	2.32	7.84	4.95	1.98	2.11	1.61	76.43	1.26	1.74	2.57	2.15	3.44	17.38

C = Compost; MB=Mixtutre of biofertilizer

This finding strongly confirm the previous conclusion drawn about essential oil percentage, oil yield and concentrations of macronutrients and phytohormones (Table 4). Also, almost no differences between the moderate compost level (10 m^3/Fed.) and the control. In contrast, the different fertilizer treatments generally decreased the contents of α-pinene, β-pinene, Limonene and α-terpineol acetale, compared to the control. The previous obtained results are in agreement with those obtained by Mansour *et al.*, (1999) and Harridy *et al.*, (2001). In this respect, Bajaj (1999) and Nagata and Ebizuka (2002) indicated that all oil components of marjoram plants have medical useful effect ; in the sense that any components having high concentration in oil and high medical effect if well purified and used alone , does not give the required medical effect unless it is found together with other oil components even with less percentage. This indicates the fact that all oil components rally togther to induce the useful medical effect. Therefore, the two treatments. i.e. the highest level of compost either alone or combined with biofertilizer mixture, help increase the quality of marjoram essential oil. Also, this results in the increase of its use as a stimulating in tooth pastes, whooping cough and larynx infections.

CONCLUSION

The obtained data clearly emphasize the efficiency of increasing compost fertilizer level in inducing gradual increase in most of the studied morphological characters, different chemical constituents in the shoots of marjoram plants [essential oil percentage, oil yield, percentage of oil components, macronutrients and the hormonal concentrations of marjoram leaves]. Plants received the highest dose (15 m^3/Fed.) of compost either alone or combined with inoculcation of biofertilizers mixture recorded more promotive effects. These treatments resulted in considerable and even significant increases in all studied parameters, over the control treatment (plants receiving only the recommended dose of NPK fertilizer).

Also, no significant differences were detected in all studied parameters between marjoram plants receiving the lowest and moderate of compost fertilizer solely or combined with inoculation of biofertilizers mixture and plants fertilized by the complete recommended dose of NPK (control).

Economic value taking in consideration the production of safe plants without using chemical fertilizers which in turn minimize the agricultural costs as well as the pollution of the Egyptian environment. Therefore, such effects will surely minimize the costs in terms of potential benefits to be derived from using compost either alone or combined with inoculation with biofertilizers mixture for the various plant species to approach its optimal productivity, taking into consideration the economic point of view to attain the minimum level of agricultural costs.

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الاستجابة الفسيولوجية لنباتات البردقوش للتسميد الحيوى والعضوى

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تم اجراء تجربتى أصص خلال موسمي ٢٠٠٠، ٢٠٠١ لدراسة تأثير ثلاث معدلات من التسميد العضوى (البيوجرين) ٥، ١٠، ١٥ م^{٣} / فدان مع تلقيح أو بدون تلقيح بخليط من التسميد الحيوى ***(Azobacter crococcum; Azospirillum brasilense and Bacillus polymyxa)*** مقارنة مع الموصى به من التسميد النيتروجيني والفوسفاتي والبوتاسي (كنترول) على النمو والمحصول والتركيب الكيماوى لنباتات البردقوش. المنزرعة فى التربة الرملية (الاراضى المستصلحة حديثا).

* أظهرت النتائج أن المعاملة بالمستوى العالى من الكمبوست (١٥ م^{٣} / فدان) إما منفردا أو بالتلقيح مع خليط التسميد الحيوى لنباتات البردقوش سجلت زيادة معنوية كبيرة فى قياسات صفات النمو النباتية والهرمونات (الاوكسينات و الجبرلليات والسيتوكينينات) وعناصر النيتروجين و الفوسفور والبوتاسيوم والمكونات الاساسية للزيت الطيار (ألفا تربينول وألفا تربينولين ولينالول جيرانول وسيانول وليانيل اسيتات وسيترونيلول).

وقد سجلت النباتات المعاملة بالمستوى العالى من الكمبوست زيادة معنوية فى الوزن الطازج للنبات متفوقة على نباتات المقارنة (المسمدة بالكميات الموصى بها من السماد الكيماوى فقط) بنسبة بلغت (١٥,٨، ١٩,٩، ١٤,٤%) فى الحشة الاولى والثانية والثالثة على التوالى وكذلك الوزن الجاف للنبات بنسبة (١٩,٨، ١٦,٩، ١٤,٧%) فى الحشة الاولى والثانية والثالثة وأيضا النسبة المئوية للزيت الطيار للنبات الطازج (٢٥% و ٢٦% و ٢٨ % فى الحشات الثلاثة على التوالى) ومحصول الزيت للنبات الطازج (٤٤ % و ٥٢% و ٤٧% فى الحشات الثلاثة على التوالى) وأظهرت النتائج أيضا أن المعاملة بالمستوى العالى للكمبوست مختلطا بالتسميد الحيوى قد أدت إلى زيادة معنوية بلغت (٢٢,١، ٢٩,١، ٣٢,١%) للوزن الطازج للنبات و (٢٥، ٣٢,٦، ٤٠%) للوزن الجاف للنبات فى الحشة الاولى والثانية والثالثة على التوالى وبلغت نسبة الزيادة فى الزيت الطيار للنبات الطازج (٣٨ % و ٣٨% و ٤٠ % فى الحشات الثلاثة على التوالى) ونسبة الزيادة فى محصول الزيت للنبات الطازج (٦٩ % و ٧٩% و ٩١ % فى الحشات الثلاثة على التوالى) بالمقارنة بالكنترول.

أدت معاملة النباتات بالمستوى المنخفض والمتوسط من الكمبوست [٥، ١٠ م^{٣}/ فدان] منفردا أو مختلطا بالسماد الحيوى الى محصول من العشب الطازج والجاف و كذلك النسبة المئوية للزيت الطيار ومحصول الزيت فى النبات الطازج بما يعادل الناتج من نباتات المقارنة. وتشير النتائج إلى إمكانية الاستخدام الناجح للأسمدة الحيوية والعضوية (الكمبوست) كبديل فعال وآمن بدلا من الاسمدة الكيماوية دون أن تتأثر الصفات الكمية والنوعية للمحصول وهذا يؤدى الى تقليل التكلفة والحد من التلوث البيئى.