

RESPONSE OF ROSEMARY PLANTS (*Rosmarinus officinalis* L.) TO ORGANIC AND BIOFERTILIZERS IN REPLACEMENT OF CHEMICAL FERTILIZATION

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ABSTRACT

Pot experiment was carried out during two successive seasons 2008 and 2009 to investigate the effect of organic fertilizer (compost) ,with or without biofertilizer inoculation (*Azotobacter chroococcum* and *Bacillus megaterium*) in comparison with the recommended dose of NPK as control on growth, yield and chemical composition of rosemary plants. Data obtained clearly revealed that plants received the compost in combination with a mixture of biofertilizers recorded a considerable increments with regard to growth characters, macronutrients, phytohormones (IAA, GA₃ and CK) and essential oil components (1,8-Cineol, Camphor, α-Pinene, β- Pienene, Bornyl acetate and Borneol). In addition, the significant increases were recorded on fresh weight (g/plant) and herb dry weight (g/plant) in both seasons, as well as essential oil percentage and oil yield/plant in the fresh herb in the combined of compost and mixture of biofertilizers as compared to the control treatment. These findings clearly indicated that organic fertilizer (compost) and biofertilizers mixture could replace the application of mineral fertilizers and consequently minimize the pollution of the agricultural environment.

Key words : biofertilizer, organic fertilizer, rosemary.

1. 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.

According to Anjara (1996), UNESCO (1996), Bhat (1997), and World Bank (1997) rosemary plants (*Rosmarinus officinalis* L.) are considered as an important aromatic and medicinal crop in Egypt with high production and great applications. It is used extremely as anti-spasmodic, analgesic, anti-cancer, anti-diarrhea, anti-gout, anti-influenza, whooping cough and neuralgia, bile stones, increase urine flow, diaphoretic and appetizer, urinary system stones and improves blood circulation as well as emmenagogue, diuretic and anti-congestion. In addition, it can be used for edema, knocks, bruises, sprains and pulls. It speeds up the healing of wounds and eases mouth infection when used as a gargle. It is also used in the treatment of

female genital disorders when used as vaginal douche. It can be used also as antimicrobial, antioxidant, antiseptic. aphrodisiac, astringent, carminative, parasiticide and insect repellent. Moreover, the oil is extensively used in detergents, cosmetics, household sprays and perfumes. The herb and its oil are largely employed in most major food categories, especially meat products as well as soft drinks.

In the recent years, 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 on 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, resulted in a final product with high ability to improve soils and enhanced plant growth. This was reported by Lampkin (1990), Mohamed and Matter (2001), Badran (2002) and Ghallab and El-Gahdban (2004). 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 rhizospheric biology and the discovery of the promotive function of special groups of microorganisms such as *Azospirillum*, *Azotobacter*, *Acetobacter*, *Bacillus*, *Serratia* and *Pseudomonas* which are known as plant growth promoting rhizobacteria (PGPR). They appear to be frequent colonizers of important medicinal crops including *Ammi visnaga*, Lemongrass, Palmarosa and Marjoram (Masheshwari *et al.*, 1995, El-Sawy *et al.*, 1998, Harridy *et al.*, 2001 and Ghalla' and El-Gahdban, 2004). Such beneficial effects of these plant growth promoting rhizobacteria (PGPR) could be attributed to the biological nitrogen fixation and production of phytohormones (gibberellins and cytokinins like substances as 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 rhizobiacteria on aromatic and medicinal crops are 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 indicate that application of bacterial inoculants as biofertilizers resulted in improvement of growth and productivity of aromatic and medicinal crops, in addition to the reduction of their N-requirements

to 50% of the recommended dose (Harridy *et al.* 1998).

The aim of the present study was to investigate the effects of compost solely or combined with inoculation mixture of biofertilizers, *i.e.*, *Azotobacter chroococcum* and *Bacillus megaterium* on the growth, oil yield and chemical composition of rosemary plants and to compare this effect with that of recommended NPK fertilization.

2. MATERIALS AND METHODS

The present work was conducted in pot experimental farmyard, Ramsis region, Cairo, Egypt during the two successive seasons; 2008 and 2009. The total area of the farmyard was 175 m². Plastic pots diameter was 40 cm and 30 cm in depth. Some physical and chemical analyses (Table 1) of the experimental soil used were carried out as described by Page *et al.* (1982) before planting.

Terminal cuttings (15 – 20 cm. in length) of rosemary (*Rosmarinus officinalis* L.) were transplanted on the 15th of March 2008, on 9th November 2008; the plants were cut 15 cm above the soil surface at the first season, and on the 25th of April 2009 at the second season. The seedlings of rosemary plant were supplied from the Experimental Farm of Medicinal and Aromatic Plants, Faculty of Pharmacy, Cairo Univ., Giza, Egypt. The seedlings were given the recommended agronomic managements except the fertilization.

Pot experiment during the two seasons included four treatments as follows:

- 1- The recommended quantities of NPK fertilizers which are 200 kg N/ fed.
as ammonium nitrate (33.5% N), 100 kg., P₂O₅/ fed. as calcium superphosphate (15.5% P₂O₅) and 100 kg K₂O/ fed. as potassium sulfate (48% K₂O) (control)
- 2- Mixture of Biofertilizers *Azotobacter chroococcum* + *Bacillus megaterium*.
- 3- Compost
- 4- Mixture of biofertilizers + Compost

Pots were laid out in a completely randomized design with five replications according to Gomez and Gomez (1984), with enough space between them to keep the plants from shading each other.

Biofertilizers are represented by Nitrobin and Phosphorin as commercial names containing 1x10⁸ CFU/ml from *Bacillus megaterium* and *Azotobacter chroococcum* individually in Biofertilizer unit, Soils Water and Environ. Res. Inst., Dept. of Microbiology (A. R. C.), Giza,

Response of rosemary plants (Rosmarinus officinalis L.) to

Egypt. Both biofertilizers were mixed well together in a liquid at equal portions. Roots of rosemary seedling were dipped into the mixture of biofertilizers immediately before planting. Also, this mixture was added to plant rhizosphere through a view holes (2 and 4) already made in

of the compost was conducted according to Page *et al.* (1982).

The compost (5 ton/fed.) and NPK fertilization treatments were incorporated with the potting medium, two weeks before transplanting.

In both seasons, plant height, number of

Table (1). Some mechanical and chemical properties of the used soil.

Property	Value	Property	Value
Clay %	34	Ca ⁺² (meq/l)	7.00
Silt %	21	Mg ⁺² (meq/l)	2.39
Coarse sand %	7	Na ⁺ (meq/l)	0.24
Fine sand %	38	K ⁺ (meq/l)	0.85
Soil texture class	Clay loam	CO ₃ ⁻ (meq/l)	0.00
Organic matter %	1.5	HCO ₃ ⁻ (meq/l)	2.00
pH	7.4	SO ₄ ⁻² (meq/l)	12.50
E _c (ds/m)	0.30	Cl ⁻ (meq/l)	0.61
		Total N %	0.35

soil surface for inoculated treatments at 30 and 60 days after transplanting.

The compost was obtained from the Soil Department, A.R.C. Various properties of the compost is shown in Table (2). Chemical analysis

branches/plant and herb fresh and dry weight/plant were recorded.

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

Table(2):Means value of various properties of compost in both seasons

Property	Value
pH (1:5)	7.5
Ec (1: 5 extract) ds/m	3.1
Organic-C %	33.11
Organic matter %	70
Total-N %	1.82
Total-K %	1.25
C/N ratio	18.19
Total-P %	1.29
Fe-ppm	1019
Mn-ppm	111
Cu-ppm	180
Zn-ppm	280
NH ₄ ⁺ - N (ppm)	274.7
NO ₃ ⁻ - N (ppm)	33.1
Ashes %	30
Total content of Bacteria	2.5 x 10 ⁷
Total content of Fungi	7 x 10 ⁵
Weed seeds	0
Nematode	0
Phosphate dissolving Bacteria	2.5 x 10 ⁶
Dehydrogenase activity (mg TPF/100g)	32.5
Nitrogenase activity (N mol C ₂ H ₄ /g/hr)	123.5
Humidity (%)	25

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 Kjeldahl apparatus of Parnas and Wanger as described by Jones *et al.*, (1991) was used. For total P and K determinations, the wet digestion of the plant material was carried out as recommended by Piper (1950). Phosphorus was determined colorimetrically using the stannous chloride reduced molybdophosphoric blue color method in sulphuric acid as described by Jackson (1973). Potassium was determined by the Atomic Absorption Spectrophotometer (GBC, 932 AA).

For determination of plant phytohormones freeze-dried rosemary leaves (equivalent 6 g F.W.) were ground to a fine powder with 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-tert-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 Palmer *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 column (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 temperatures 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 temperature 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).

3. RESULTS AND DISCUSSION

3.1. Growth characters

Growth characters of both seasons of rosemary plants as influenced by compost or biofertilizer treatment either alone or in combination are presented in Table (3).

In general, the results indicated that the compost associated with the inoculation of biofertilizer mixture significantly increased growth as compared to the control (plants receiving the recommended NPK dose) characters; *i.e.*, plant height, number of branches / plant and both of herb fresh and dry weight (g/plant) in both seasons. Concerning this, it is notable that compost treatment without inoculation also gave results similar to the results of the control plants in most morphological parameters. In addition, it was mentioned that the positive effects of the other treatments on all studied growth characters failed to reach the 0.05 level of significance.

The favorable effects of the combination between compost fertilizer and biofertilizers might be explained based on 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. This was reported by Bashan *et al.* (1989) who found that *Azospirillum* and *Pseudomonas* improved wheat growth through the significant increase in dry matter accumulated in

Table (3): Growth characters of rosemary plants as affected by compost, biofertilizers and their combination during 2008 and 2009 seasons.

Growth character	Plant height (cm)		No. of branches / plant		Herb fresh weight (g/plant)		Herb dry weight (g/plant)	
	1 st Season	2 nd Season	1 st Season	2 nd Season	1 st Season	2 nd Season	1 st Season	2 nd Season
Season	2008	2009	2008	2009	2008	2009	2008	2009
Treatment								
Control (NPK)	80.5	85.7	42	61	525.7	565.6	105.4	128.5
Biofertilizers	53.7	61.3	24	27	332.5	370.3	75.8	85.5
Compost	74.6	81.4	36	46	440.7	499.7	100.5	114.2
Compost + Biofertilizers	96.4	105.6	49	76	634.8	706.8	125.8	160.8
L. S. D 0.05	1.34	1.5	1.25	1.14	24.90	26.86	8.19	9.36

both roots and shoots of the treated plants.

Also, Bashan and Levanony (1990) proposed several possible modes of action of *Azospirillum* on plant growth and N₂ fixation, which contributes N to the plant. Hormonal effects, alter plant metabolism and growth. Moreover General growth improvement in the entire root system, result in enhanced mineral and water uptake. Moreover, Valssak and Reyndres (1980) found that *Azospirillum* produced Indole lactic acid (IAA) and gibberellins and the degree of sensitivity of plants to phytohormones are being suggested as the main reason for this phenomenon (Jlik, 1995). Furthermore, Ghallab and Salem (2001) found that wheat inoculated with *Azospirillum* spp. improved grain yield and total dry matter accumulation. Recently, Salem *et al.* (2006) found that flax cultivars inoculated with nitroben or phosphorben increased most of the studied growth and yield characters .

Moreover, the significant positive effect of compost fertilizer on vegetative growth characters might 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; Hussein, 2003 and Ghallab and El-Gahdban, 2004).

It is worth to mention that compost combined with biofertilizer mixture, lead to a high increase in dry weight of rosemary plants.

3.2. Chemical analysis

The effects of different treatments on the essential oil percentage (ml/100g.F. W. herb.); oil yield/plant; N, P, K, IAA, GA₃ and CK concentrations in the shoots of rosemary plants grown in both seasons (Table 4) were similar to those recorded for growth characters. The data clearly showed the significant increases in both essential oil percentage (22.6 and 26.5 % in both seasons, respectively) and oil yield/ plant (48.4 and 57.6 % in both seasons, respectively) in the fresh herb at the combination of compost and biofertilizer mixture as compared to the control. On the other hand, insignificant differences were detected among the other treatments (compost and biofertilizer mixture) and the control.

These results are in agreement with those obtained by Jacoub (1999) on *Ocimum basillicum* L. and *Thymus vulgaris* L. plants ; Mansour *et al.*,

Table (4): Essential oil %, oil yield, concentrations of N, P, K (mg/g D.W.), indole-3-acetic acid (IAA), gibberellic acid (GA₃) and cytokinins (CK) (µg/gF.W.) in shoots of rosemary plants as affected by biofertilizer, compost and their combination during 2008 and 2009 seasons.

Chemical composition	Essential oil % (ml./100 g.F.W.)	Oil yield (ml./plant)	N	P	K	IAA	GA ₃	CK
First cut (2008)								
Control	0.31	1.61	27.74	3.82	28.58	9.96	8.53	7.21
Biofertilizers	0.25	0.82	24.58	2.63	25.43	6.74	7.16	6.16
Compost	0.28	1.22	25.39	3.14	26.84	7.11	7.21	6.33
Compost+ Biofertilizers	0.38	2.39	36.65	4.52	31.68	11.85	9.89	8.98
L. S. D. 0.05	0.06	0.59						
Second cut (2009)								
Control	0.34	1.91	28.94	3.87	29.68	10.85	8.89	7.57
MB	0.28	1.02	26.88	2.92	27.59	9.76	7.57	6.34
Compost	0.31	1.54	27.38	3.56	28.97	9.96	7.86	6.52
Compost+ MB	0.43	3.01	38.94	4.96	34.21	12.37	10.57	9.34
L. S. D. 0.05	0.05	0.66						

(1999) on spearmint and marjoram plants ; Badran and Safwat (2004) on fennel plants and Ghallab and El-Gahdban (2004) on marjoram plants. The effect of compost on increasing the volatile oil percentage of shoots might be attributed to their enhancing effect on vegetative growth, in terms of the fresh yield and increasing the uptake of nutrient by roots. Similar results were recorded by Mohamed and Matter (2001) on marigold plants.

The effects of different treatments on the mean values of macro-nutrient concentrations in the shoots of rosemary plants in both growing seasons (Table 4) were similar to those recorded for growth characters (Table 3).

The data show the superiority of the combined treatment including compost and biofertilizers mixture which resulted in the highest promotive effects on the macronutrients accumulation which surpassed both other treatments. However, the positive effect of both other treatments on the values of macronutrient concentrations was insignificant.

The promotive effects of the biofertilization on macronutrients accumulation could be attributed to that the inoculation of rosemary 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 a mixture of *Azospirillum* and *Serratia* inoculation greatly enhanced the uptake of N, P, K, Fe, Mn , Zn and Cu as well as the accumulation of these minerals in 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 increase of mineral uptake by inoculated roots to the enhancement in proton efflux activity of wheat root inoculated with *Azospirillum*. Moreover, Omar *et al.* (1991) reported that inoculation with *Azospirillum* cultures stimulated the uptake and accumulation of the nutrients from the soil. Furthermore, 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 included 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 stimulated plant growth

(Gaskins *et al.*, 1985). The indirect effects included positive effects on symbiotic nitrogen fixation by enhancement of root nodules number or mass as reported by Yahalom and Okanad (1987).

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 contributed much to the increase in nutrients uptake. In this respect, Abd El-Moez *et al.*, (1999) on fennel and coriander plants and Ghallab and El-Gahdban (2004) on marjoram plants, found that the macro -nutrients uptake by plant roots increased significantly by the addition of organic composts to prepared soil. They attributed the results to the effect of organic fertilizer on improving not only soil physical and biological properties but also soil chemical characteristics resulting in more release of available nutrient elements to be absorbed by plant roots and the water efficiency by different plants.

Concerning the phytohormones , the results of the hormonal analysis [IAA, GA₃ and CK] in the leaves of the rosemary plants in both seasons 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 concentration.

The results obtained (Table 4) showed the positive response in the hormonal concentrations which reached their maximum when the compost was combined with biofertilizer mixture. However, the positive effects of the other treatments on hormonal concentrations failed to reach the 0.05 level of significant.

The promotive effects of the biofertilization mixture with the compost on phytohormones might be attributed to the production of growth promotive substances from rhizospheric microorganisms such as IAA and GA₃ (Gulam *et al.*, 1998). In this respect, *Azospirillum* produced 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 gibberellins (Bottini *et al.*, 1989), abscisic acid (ABA) (Kolp and Martin ,1989), and cytokinins (Horemans *et al.*, 1986).

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

3.3. Chemical composition of the essential oil

Table (5) showed that the highest content of 1,8-Cineol (the main component), as well as that of some other important components; *i.e.* Camphor, α -Pinene, β -Pinenene, Bornyl acetate and Borneol were obtained from plants fertilized with compost in combination with biofertilizers mixture compared to the control. This finding strongly confirmed the previous conclusion drawn about essential oil percentage, oil yield/plant and concentrations of macronutrients and phytohormones (Table 4). In this respect, Bajaj

studied morphological characters, different chemical constituents in the shoots of rosemary plants (essential oil percentage, oil yield/plant, percentage of oil components, macronutrients and hormones concentrations of rosemary leaves). This treatment resulted in considerable and even significant increases in all studied parameters, over the control (plants receiving only the recommended dose of NPK fertilizers).

Economic value taking into consideration the production of safe plants without using chemical fertilizers which in turn minimize the

Table (5): Effect of biofertilizer, compost and their combination on the essential oil components of rosemary plant during 2008 season.

Treatments	NPK	Biofertilizer	Compost	Compost+Biofertilizer
Oil components				
α -Pinene	4.22	2.54	3.32	6.79
Camphene	2.71	1.52	2.12	2.76
Camphor	11.92	7.32	9.96	14.76
β -Pinene	7.02	7.64	6.96	10.45
Limonene	2.09	1.52	1.06	1.95
P-cymene	5.46	3.43	4.35	5.54
1,8-Cineol	15.26	11.96	13.21	18.31
Linalool	9.97	3.1	8.22	8.88
Bornyl acetate	5.73	6.62	4.36	7.52
Borneol	7.68	8.55	6.74	10.89
γ -Terpinene	1.12	0.67	1.11	1.01
Thymol	0.18	0.02	0.08	0.11

(1999) and Nagata and Ebizuka (2002) indicated that all oil components of rosemary 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 indicated the fact that all oil components together could induce the medical effect. Therefore, the treatment of compost combined with biofertilizer mixture could help to increase the quality of rosemary essential oil. Also, these results in the increase of its use as stimulating in use as anti-spasmodic, anti-cancer, anti-diarrhea, anti-gout, edema, knocks, bruises, sprains, pulls, anti-microbial, detergents, household sprays and perfumes.

Conclusion

The obtained data emphasized the efficiency of treatment including combined compost and biofertilizers mixture in inducing increase in 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 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.

4. REFERENCES

Abd El-Moez M. R., Saleh A. L. and Wanas Sh. A. H. (1999). Influences of some organic composts on yield, nutrients uptake and consumptive use of fennel and coriander plants and some soil properties. *J. Agric., Mansoura Univ.*, 24 (10) : 6237-6253.

Amin G. A., Mostafa M. H., Amer A. A. and Refat I. R. (1999). Potential microorganisms for rapid and nutritionally enriched compost. *Proc. of 1st Cong. "Recent Technologies in Agriculture"*, Cairo Univ., Fac. Agric. Nov. 1999, Proceedings Vol. IV: 804-822.

- Anjara J. (1996). Ethnoveterinary pharmacology in India: past, present and future. In: Ethnoveterinary Research and Development. Eds. McCorkle, C.M., Mathias, E. and Schillhorn van veen, T.W., Intermediate Technology Publications, London, UK, p. 137 - 147.
- Badran F. S. and Safwat M. S. (2004). Response of fennel plants to organic and biofertilizers in replacment of chemical fertilization. Second International Conference of Organic Agriculture. Cairo International Conference Center, 25 -27 March, 2004. Nasr City, Cairo, Egypt. Absts. Book , 24 p.
- Badran M. S. S. (2002). Organic VS. mineral fertilization on yield and yield components of some barley varieties under sandy soil conditions. Proc. Minia 1st Conf. for Agric. & Environ. Sci., Minia, Egypt, March 25-28: 917-934.
- Bajaj Y. P. S. (1999). Biotechnology in Agriculture and Forestry 43 : Medicinal and Aromatic Plants XI . Springer, Berlin-Heidelberg, New York.
- Bashan Y. and Levanony H. (1990). Current status of *Azospirillum* inoculation technology *Azospirillum* as a challenge for Agriculture. Can. J. Microboil., 36: 591-608.
- Bashan Y., Ream Y., Levanony H. and Sade A. (1989). Nonspecific responses in plant growth, yield and root colonization or non cereal crop plant to inoculation with *Azospirillum brasilense*. Can. J. Bot., 67:1317-1324.
- Bhat K.K.S. (1997). Medicinal and plant information databases. In: Medicinal Plants for Forests Conservation and Health Care. eds. Bodeker, G. and Vantomne, P., FAO, Non-Wood Forest Products Series No. 11, FAO, Rome, 158 pp.
- Bottini R., Fulchieri Pearce D. and Pharis R. P. (1989). Identification of gibberellins GA₁, GA₃ and iso-GA₃ in cultures of *Azospirillum lipoferum*. Plant Physiol :45-47.
- Crozier A., Arruda P., Hasmin I. M., Ontero A. M. and Sandverg G. (1988). Analysis of indole - 3-acetic acid and related indoles in culture medium from *Azospirillum lipoferum* and *Azospirillum brasilense*. Appl. Environ. Microbiol., 54:2833-2837.
- Dashti N., Zhang F., Hynes R. and Smith D. L. (1997). Application of plant growth promoting rhizobacteria to soybean (*Glycine max* (L.) Merr) increases protein and dry matter yield under short season conditions. Plant and Soil, 188:33-41.
- Egyptian Phramacopoeia (1984). Egyptian Pharmacopoeia. General Organization for Governmental Printing Office, Ministry of Health, Cairo, Egypt. p. 31-33.
- El-Kouny H. M. (2002). Effect of organic fertilizer in different application rates under salinity stress Goudvion on soil and plant. International Symposium and Optimum Resources Utilization in Salt-affected Ecosystems in Arid and Semi-arid Regions, 8-11 April 2002, Cairo , Egypt, Le Meridian, Heliopolis Hotel, Abst. Book, 33 pp.
- El-Sawy M. Saleh, E. A., El-Borolloy M. A., Nokhal T. H., Fendriik I. and Sharaf M. S. (1998). Effectiveness of dual inoculation with diazotrophs and vesicular arbuscular mycorrhizae on the growth and Khellin content of *Ammi visnaga*. Arab Univ., J. Agric. Sci. Ain-Shams Univ., Cairo 6 (2):357-371.
- Fales H. M. and Jaouni T. M. (1973). Simple device for preparing ethercal diazomrthane without restoring to codisitillation. Anal. Chem, 45:2302-2303.
- Fallik E., Okin Y., Epstein E., Golduman A. and Fischer M. (1989). Identification and quantification of IAA and IBA in *Azospirillum brasilense* inoculated maize roots. Soil Biol. Biochem., 22:147-153.
- Faragl S. T. and Ali F. A. (2004) . Response of tomato plants to mineral , Bio- and organic fertilizers . Second International Conference of Organic Agriculture. Cairo International Conference Center, 25 -27 March, 2004. Nacr City , Cairo, Egypt. Absts. Book , 20 pp.
- Gaskins M. H., Albrect S. L. and Hubbel D. H. (1985). Rhizosphere bacteria and their use to increase productivity : A Review Agric. Ecosys. Environ. 12:99-116.
- Ghallab A. M. and El-Gahadban E.A.E. (2004). Physiological response of marjoram plants to biofertilizer and organic fertilization. J. Agric. Sci. Mansoura Univ., 29(4): 1743-1759.
- Ghallab A. M. and Salem S. M. (2001). Effect of some biofertilizer treatments on growth, chemical composition and productivity of wheat plants grown under different levels of NPK fetilization. Annals Agric. Sc., Ain Shams Univ., Cairo, 46 (2): 485-50.
- Gomez K. A and Gomez A. A. (1984). Statistical Procedures for Agricultural Research 2nd Ed. International Rice Res. Ins. J. Wiley and Sons New York, USA p. 377-434.

- Gulam R. M., Sajjaad M., Farooq L. and Kauser A. M. (1998). Identification of plant growth hormones produced by bacteria isolated from rice, wheat and kallar grass Malik K. A., M. Saijad Miliza; J. K. Ladna (eds). In : Nitrogen Fixation with Non-legumes Kluwer Academic Publishers. Dordrecht. Bostan. London p. 25-37.
- Gunther Z. and Joseph S. (1978). Handbook Series in Chromatography. CRC press, Inc., New York, USA. Pp. 325-469.
- Haahtela K., Knokko R., Laskso T., Williams P. H. and Korhonen T. K. (1990). Root associated Entero-bacter and Klebsiclla in *Poa pratensis* ; characterization of an iron-scavenging system and a substance stimulating root hair reduction. Mol. Plant Microb. Interact. 3:358-365.
- Harridy I. M. A. and Amara M. A. T. (1998). Effect of presowing inoculation of seeds by nitrogen fixed bacteria on growth, fruit production, sepals yield and chemical composition of roselle plants. Egyptian Journal of Applied Science 13 (6): 217-231.
- Harridy I. M. A., Soliman S. G. I. and Amara M. A. T. (2001). Physiological chemical and biological studies on lemongrass "*Cymbopogan citralus*" (DC) Staphf in Response to diazotrophic bacteria. J. Agric. Sci. Mansoura Univ., 26 (10): 6131-6154 (2001).
- Hartmann A. Stingh A. and Klingmuller W. (1983). Isolation and characterization of *Azospirillum* mutants excreting high amount of IAA. Can. J. Microbiol., 29:916-923.
- Horemans S. K. De Conink. J. Neuray; Hermans R. and Vlassak K (1986). Production of plant growth substances by *Azospirillum* sp. and other rihzosphere bacteria. Symbiosis, 2:341-346.
- Hussein M. M. M. (2003). Growth of *Senna occidentais* (L.) Link in sandy soil as affected by fertilization and soil amendments. Bull. Fac. Agric., Cairo Univ., 54:189-216.
- Jackson M. L. (1973). Soil Chemical Analysis Prentice-Hall of India Private. New Delhi, pp. 144-197.
- Jacoub R. W. (1999). Effect of Some Organic and Non Organic Fertilizers on Growth, Oil Yield and Chemical Composition of *Ocimum basilicum* L. and *Thymus vulgaris* L. Plants. Ph. D. Thesis, Fac. of Agric. Cairo Univ. Egypt.
- Jlik B. R. (1995). The enhancement of plant growth by free-living bacteria. Can. J. Microbial., 41: 109-117.
- Jones Jr., Benton J., Wolf B. and Mills H. A. (1991). Plant Analysis Hand book. Methods of Plant Analysis and Interretations, Micro-Macro Publishing. Inc., U. S. A. p. 30-34.
- Kolp W. and Martin P. (1989). Response of plant roots to inoculation with *Azospirillum brasilense* and to application of IAA. In *Azospirillum*-III. Genetics. Physiology and Ecology, Edited by Klingmiller. Springer-Verlag, Berlin, Hidelberg. Pp. 215-221.
- Kumar C. M. Agrawal M. and Gupta B. R. (1998). *Azospirillum* and its potential as biofertilizer. Fertilizer-News, 43 (11) : 49-50.
- Lampkin N. (1990). Organic Farming. Farming Press Book. United Kingdom. 63 pp.
- Iifshitz R. J., Kloepper W. and Kozlowski M. (1987). Growth promotion of canola seedlings by a strain of *Pseudomonas putida* under genotobiotics conditions Can. J. Microbiol (33) : 390-395.
- Mansour H. A., El-Maddawy E. I. and El-Ghadban E. A. (1999). Organic and inorganic fertilization of spearmint and marjoram. 1st Cong. "Recent Technologies in Agriculture", Cairo Univ., Fac. Agric. Nov. 1999, Proceedings Vol. I. 226-239 p.
- Masheshwari S. K., Gangrade S. K. and Sharma R. K. (1995). Differential responses of *Azotobacter* and nitrogen on biomass and oil yield of palamross. Crop Res. (Hisor) 1995, 10 (3) : 356-359. (C. F. Hort. Abst. 66 (12) 10759).
- Mohamed H. A. F. and Amara M. A. T. (2000): Response of tomato to biological and mineral fertilizers under calcareous soil conditions. Bull. Fac. Agric., Cairo Univ., 51:151-174.
- Mohamed S. A. and Matter F. M. A. (2001). Effect of ammonium nitrate and organic fertilizers on growth, volatile oil yield and chemical constituents of marigold (*Taget mimuta* L.) plant Fayoum. J. Agric., Res. & Dec. 15 (1) : 95-107.
- Nagata T. and Ebizuka Y. (2002). Biotechnology in Agriculture and Forestry 51 : Medicinal and Aromatic Plants XII. Springer, Berlin-Heidelberg, New York.
- Okon Y. and Labandera Gonzalez C. A. (1994). Agronomic application of *Azospirillum* in improving plant productivity with rhizosphere bacteria. Ryder, M. H. P. M. Stephens. and G. D. Bowen eds *Commonweultie Sciemifie* and

- Industrial Research Organlcation Adeliaide, 274-278.
- Omar M. N. A., Heagazy M. H., Abd El-Aziz R. A., Abo-Soliman M. S. M and Sobh M. M. (1991). Effect of inoculation with rhizobacteria on yield of wheat under graded level of nitrogen fertilization. *Annals. Agric. Sci. Ain Shams Univ., Cairo*, 36 (1): 99-104.
- Page A. I., Miller R. H. and Keeney T. R. (1982). *Methods of Soil Analysis part 2 Amer. Soc. Agr. Inc. Madison WI*:595.
- Piper C. S. (1950). *Soil and Plant Analysis. The Univ. of Adelaide* p.30.
- Palmer M. V., Hogan R. and Wareing P. F. (1981). Cytokinins metabolism *Phaseolus vulgaris* L. identification of endogenous cytokinins and metabolism of (8-C¹⁴) dihydrozeatin in stems of decapitated plants. *Planta*, 153-297-302.
- Salem M. S. A., Zidan S. Z. and Esmail M. M. (2006). Effect of some biological and mineral fertilizers on some growth and yield charactrs of two flax cultivars. *Bull. Fac. Agric., Cairo Univ.*, 57(2) : 261- 276.
- UNESCO (1996). *Culture and Health, Orientation Texts – World Decade for Cultural Development 1988 – 1997, Document CLT/DEC/PRO – 1996, Paris, France*, 129 pp.
- Valssak K. and Reyndres L. (1980). *Azospirillum rhizocoenoses in agricultural practice. IV. Int. Symp. On N-Fixation. Camberra*. p. 423-436.
- Vogel A. I. (1975): *A Text Book of Practical Organic Chemistry. Published by English Language Book Society and Longman Group Limited 3rd Ed.*, pp. 197-596.
- World Bank (1997). *Medicinal Plants – Rescuing a Global Heritage*. eds. Lambert, J., Srivastava, J. and Vietmeyer, N., Technical Paper No. 355, 61pp.
- Yahalom E. Y. A. and Okanad D. (1987). *Azospirillum* effects on sucepitability to *Rhizobium* nodulation and nitrogen fixation of several forage legumes. *Can. J., Microbiobiol.*, 33:510-514.

إستجابة نباتات حصابان للتسميد العضوى والحيوى كبديل لإستخدام التسميد الكيمايى

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ملخص

تم إجراء هذه التجربة خلال موسمين زراعيين متتالين: ٢٠٠٨ و ٢٠٠٩ بهدف دراسة تأثير كلا من السماد العضوى (الكمبوست) والاسمدة الحيوية (الأزوتوباكتر *Azotobacter chroococcum* والباسلس *Bacillus megaterium*) أو خليط منهما مقارنة بالتسميد الكيمايى الموصى به من NPK على النمو والمحصول والتركيب الكيمايى لنبات حصابان. أظهرت البيانات المتحصل عليها بوضوح أن إستخدام خليط من السماد العضوى والاسمدة الحيوية سجلت زيادة ملحوظة فى قياسات النمو للنبات، الهرمونات النباتية (الأوكسينات والجبريلينات والسيبتوكينينات)، مكونات الزيت الطيار (1,8-Cineol, Camphor, α -Pinene, β - Pinene Bornyl acetate and Borneol) وبالإضافة إلى ذلك سجلت زيادة كبيرة فى كلا من الوزن الغض والجاف فضلا عن زيادة فى محصول الزيت الطيار فى النبات والتي تم فيها الجمع بين الأسمدة العضوية والحيوية مقارنة بالتسميد الكيمايى الموصى به . تؤكد هذه النتائج بوضوح إلى أن خلط السماد العضوى (الكمبوست) بالاسمدة الحيوية يمكن أن تستخدم كبديل لإستخدام الأسمدة الكيمايية والتي تؤدي إلى زيادة جودة حصول نبات حصابان كما ونوعا وبالتالي تقليل تلوث البيئة الزراعية.