The future Journal of Biology
2019;3:26-33

USING SOME BIOFERTILIZERS TREATMENTS TO PROMOTE GROWTH AND OIL YIELD OF ROSEMARY PLANT (*Rosmarinus officinalis* L.) GROWN IN SANDY CALCAREOUS SOIL

Abeer M. Shehata[1], Walid, S. E. Nosir[2], and Adel F. Ahmed[1]

1- Medicinal & Aromatic Plants Res. Dept., Hort. Res. Institute, Agric. Res. Centre, Egypt.
2- Hort. Dept., Fac. Agric., Zagazig Univ., Egypt

RESEARCH PAPER *Accepted: 10 April 2019 published online: 25 July 2019*

ABSTRACT

In order to study the effect of phosphate dissolving bacteria "*Bacillus megatherium* L." (BM), *Azospirillum brasilense* (AZB) and Arbuscular Mycorrhiza fungi (AMF) and their mixture treatments on vegetative growth, herb mineral content and volatile oil of rosemary plants (*Rosmarinus officinalis*), a field experiment was conducted in two successive seasons (2016/2017 and 2017/2018) at private Farm located at Sanuor Village, BaniSuif Governorate, by using a randomized complete block design (RCBD) in simple design. The obtained results referred to that inoculation with Arbuscular Mycorrhiza Fungi (AMF), *Bacillus megatherium* strain (BM), *Azospirillum brasilense* strain (AZB) each alone or in mixture significantly increased rosemary growth (plant height, number of shoots/plant as well as herb fresh and dry weights/plant), chemical constituents (total carbohydrates, nitrogen, phosphorus and potassium percentages as well as iron, manganese and zinc as ppm) and volatile oil parameters (volatile oil percentage, volatile oil yield per plant) compared to the control (un inoculated plants). The maximum values in above mentioned parameters were observed in mixed treatment of (AMF+ BM+ AZB) compared to the other ones and control plants. Generally, results of the present work demonstrated that application of biological fertilizers plays a remarkable role in improving growth parameters, chemical constituents and volatile oil of rosemary plants under BeniSuif Governorate conditions.

Key words: *Rosemary - biofertilizers - chemical constituents - volatile oil.*

INTRODUCTION

Rosemary (*Rosmarinus officinalis*, L.) of the family Lamiaceae (Labiatae) is one of the most valuable spices, aromatic and medicinal plant that widely utilized around the world. Rosemary is native to the Mediterranean regions. Therefore, it well grows all throughout the year under Egyptian conditions. Implementation of naturally occurring antioxidants rather than synthetic antioxidants is a promising technology to increase the shelf life of foods.

Rosemary organic residues after the distillation of leaves after manufacturing of essential oils was used to prevent lipid oxidation and color changes in meat products (**Nieto *et al*., 2010**; **Banon *et al*., 2012**). Rosemary essential oil, phenolics and extract, stimulates circulation of blood and used as an antifungal, antibacterial, anticancer agents and antiviral (**Sharabani *et al*., 2006**; **Genena *et al*., 2008**; **Yi and Wetzstein, 2010**; **Tsai *et al*., 2011**).

It is also known that *Bacillus megatherium* induces the production of plant hormones such as cytokinins, IAA and GA (**Glick 1995**). Growth promotion by *Bacillus megatherium* may be mediated by brassinosteroid, IAA, gibberellins and salicylic acid signaling pathways **(Ryu *et al*., 2005)**. Bacteria of the genera *Azotobacter*, *Azospirillum* and *Klebsiella* are non-symbiotic nitrogen fixing microorganisms; that generate ammonia for their own utilize and provide the plant with nitrogen as an exchange for carbon and protected habitat (**Marschner, 1995**).

Correspondent Author: Walid Nosir
w.nosir@gmail.com

ISSN:2572-3006(Print)2572-3111(Online)
http://www.thefuturejournals.org

Also, *Azospirillum* species are nitrogen-fixing organisms (diazotrophs), can forming an associative with the roots of several important economically plant (**Vande Broek and Vanderleyden, 1995**). The previous studies indicated that *Azospirillum* improves plant growth (**Cohen *et al*., 2007**), like most organisms, *Azospirillum* uses ammonium salts as a preferred nitrogen source. In addition, Arbuscular mycorrhizal fungi (AMF) form is a symbiotic association with more than 80% of land plant families. AMF consists of an internal phase inside the root and an external phase, or extra radical mycelium (ERM) phase, which can form an extensive network within the soil (**Gosling *et al*., 2006**). AMF benefit their host principally by enhencing uptake of relatively immobile phosphate ions, due to the ability of the fungal to gridge beyond the phosphate depletion zone that quickly develops around the roots (**Smith and Read, 1997**).

Therefore this study was aimed to investigate the effect of biofertilizers as *Bacillus megatherium* L." (BM), *Azospirillum brasilense* (AZB) and Arbuscular Mycorrhiza fungi (AMF) and their mixture treatments on the plant growth, yield components, volatile oil content and some chemical contents in leaves determine the suitable treatments for the maximum production of rosemary (*Rosmarinus officinalis*) plant.

MATERIALS AND METHODS

This study was carried out during two successive seasons 2016/2017 and 2017/2018 at privet farm located at Sanour Village, BeniSuif Governorate, Egypt. Where, the plant was grown in sandy calcareous soil. The samples were analyzed at medicinal and aromatic plants Laboratory at the Agriculture Research Centre, BeniSuif Station. The physical and chemical properties of the used soil are shown in Table (1) according to (**Chapman and Pratt, 1978**).

Table 1. Physical and chemical properties of the used soil at 0-30 cm

Character	Value	Character	Value
Sand %	77.20	**Available P%**	15.12
Silt %	10.70	**Exch. K^+mg/100 g**	2.11
Clay %	12.10	**Exch. Ca^{++} mg/100 g**	31.74
Organic matter%	0.92	**Exch. Na^+mg/100 g**	2.40
$CaCO_3$ %	18.9	**Fe (ppm)**	8.54
pH (1 : 2.5)	8.82	**Cu (ppm)**	2.06
E.C. (m mhos/cm)	2.64	**Zn (ppm)**	2.75
Total N%	0.08	**Mn (ppm)**	8.26
Soil type		Sandy loam	

Plant material:

Soft cuttings of rosemary plants (*Rosmarinus officinalis*) were supplied from the Medicinal and aromatic Researches Department, Mallawy Research Station, Agriculture Research Centre, Egypt and were cultivated under greenhouse at October 15th until March 15th of both seasons and then the plants were transplanted in the experimental field on March 15th in both seasons.

The plot area was 3.00 ×3.60 m included six rows. Each row was 60 cm wide and three meters in length. The transplants were transplant in hills on one side of the row, and hills spaced 50 cm, a part.

Bacterial strains:

The Fungi and bacterial strains used in the experiment were: *Arbuscular mycorrhizal* fungi (AMF), *Bacillus megatherium* strain (BM) and *Azospirillum brasilence*strain (AZB) and their mixtures of the three used strains microorganisms.

Strains of *Azospirillum, Bacillus megatherium (var. phosphaticum)* and *Arbuscular* mycorrhiza fungi were kindly isolated and propagated at Laboratory of Microbiology, Minia University, Egypt. Strains of Azospirillum were grown on

The future Journal of Biology
2019;3:26-33

Doberiner medium but strains of *Bacillus megatherium* were grown on nutrient broth medium. Strains were grown in liquid medium on a rotary shaker at 30 °C and 120 rpm, then the culture were added to the plants, three times/year, at a rate of 10 ml per plant, each ml contain 10^8 cell of *Azospirillum* or *Bacillus megatherium*. However, *Arbuscular* mycorrhiza fungi were developed on onion plants roots, then the onion soil added to the rosemary plant in order to 10g/plant. However, each 1 gram contained 10^8 cells.

Bio-fertilizers were applied either separately or in a mixture three times to the soil around each plant at 10ml/plant for *Azospirillum* and 10g/plant for Mycrrohiza (1 ml or 1g contains 108 cells of bacteria or fungi). The first dose was added after two weeks from transplanting. Also, the second and third applications were applied after two weeks intervals.

Treatments:

Treatments included Control (un-treated plants), Arbuscular Mycorrhiza Fungi (AMF), *Bacillus megatherium* strain (BM), *Azospirillum brasilense* strain (AZB), Arbuscular Mycorrhiza + *Bacillus megatherium* (AMF + BM), Arbuscular Mycorrhiza + *Azospirillum* (AMF + AZB), *Bacillus megatherium* + *Azospirillum* (BM + AZB) and Arbuscular Mycorrhiza + *Bacillus megatherium* + *Azospirillum* (AMF+ BM+ AZB). The eight treatments were arranged in randomized complete block design with three replicates.

All regular agricultural practices that were commonly applied in ornamental and medical plants as usual in the region, included chemical "fertilization 200 kg ammonium nitrate, 150 kg superphosphate and 100 kg potassium sulfate / fed / year, irrigation and hoeing and pest management.

Data recorded:

The plants were harvested in two separated cuts (in Jun 15^{th} and 15^{th} October) in both seasons and the following data were recorded:

Plant growth parameters: Plant height (cm), mean of shoots length (cm) and number of shoots/plant as well as fresh and dry weights of herb /plant (g) were determined.

Chemical constituents: After every cut, 10 g of each dried herb was randomly taken from each treatment and oven dried at 70°C till constant weight and total carbohydrates, nitrogen, phosphorus and potassium percentages of rosemary dry herb according to the methods described by **AOAC (1990)**, **Hucker and Catroux (1980)**, **Chapman and Pratt (1978)** and **Jackson (1970)**, respectively. However, Fe, Zn and Mn (ppm) in the dry herb were determined according to **Chapman and Pratt (1978)**.

Volatile oil: At harvest time (at the end of every cut) about 10 g of each dried sample (herb) was separated triturated and steam-hydro distilled for 3 hours. The extraction of oils was carried out according to method of **European Pharmacopoeia (1983)**. Also, volatile oil yield per rosemary plant (ml) was calculated.

Statistical design:

The statistical analysis of the obtained data was carried out according to **Snedecor and Cochran (1990)** using New L.S.D. at 5% level.

RESULTS AND DISCCUSION

Rosemary growth parameters:

Data presented in Tables 2 and 3 show that inoculation with Arbuscular Mycorrhiza Fungi (AMF), *Bacillus megatherium* strain (BM), *Azospirillum brasilense* strain (AZB) each alone or in mixture significantly increased rosemary growth

ISSN:2572-3006(Print)2572-3111(Online)
http://www.thefuturejournals.org

The future Journal of Biology
2019;3:26-33

(plant height, number of shoots/plant as well as herb fresh and dry weights/plant) compared to the control (un inoculated one). The highest values in this connection were obtained from the treatment of AMF + BM + AZB as mixture compared to the other treatments under study in the two cuts during both seasons.

In this connection, the favourable effect of mycorrhizae on increasing the dry weight of onion plants, owes directly to their effect on releasing phosphorus in the soil and its absorption by the plant, consequently producing activation energy to utilization of metabolites and building the cells, as well as development of the plant growth (**Pacovsky and Fuller, 1986**). *Azosprillum*, in addition to N fixation ability, improve root growth by produce growth stimulants and subsequent increase in water and nutrient uptake rate, that raising yield (**Tilk *et al*., 2005**). Also, **Sokhangoy *et al*. (2005)** revealed that application of biological fertilizers plays a remarkable role in improving growth characteristics and yield compounds of *Anethum graveolens*.

The positive effect of biofertilization on enhancing plant growth was observed on **Abdullah *et al*. (2012)** and **Hassan *et al*. (2015)** on rosemary plants, also, **Hendawy *et al.* (2010)** on *Thymus vulgaris* plants and **Nassar *et al*. (2015)** on thorn apple (*Datura stramonium* L.) plant.

Rosemary chemical constituents:

Comparison of the means of different treatments revealed that Arbuscular Mycorrhiza Fungi (AMF), *Bacillus megatherium* strain (BM), *Azospirillum brasilense* strain (AZB) and the mixture of these biofertilizers resulted in significant differences in total carbohydrates % and total nitrogen % (Table 4), total phosphorus % and potassium % (Table 5) and Fe, Mn and Zn as ppm (Table 6) compared to control. Moreover, the mixture of AMF + BM + AZB which followed by the mixture of AMF with either of BM or AZB recorded the highest values of chemical constituents of rosemary herb compared to the other treatments under study in both cuts during the two seasons.

Nassar *et al*. (2015) found that chemical contents of thorn apple were the maximum with the plants infected with biofertilizers containing nitrogen fixers (namely, *Azotobacter chrococcum* and *Azospirillum brasilense*) and phosphate dissolving bacteria (namely, *Bacillus megaterium* var. *phosphoticum*) as compared to the uninoculated ones. Furthermore, **Al-Maghraby *et al*. (2018)** reported that the biofertilizer treatments among onion Giza 20 cultivar, inoculation with mycorrhizae at 4 kg/feddan, significantly increased N, P and K uptake by roots, bulb and leaves at 105 days after transplanting.

Rosemary volatile oil:

The top most increase in volatile oil percentage, volatile oil yield per plant were observed with biofertilizers inoculation with triple mixture of Arbuscular Mycorrhiza Fungi (AMF), *Bacillus megatherium* strain (BM) and *Azospirillum brasilense* strain (AZB) compared to the other treatments under study in the two cuts during both seasons (Table 7). In this research inoculation with three strains AMF, BM and AZB alone or with duple mixture of them succeeded in make significant increase in this regard compared to control in both cuts during the first and second seasons.

The application of phosphate solubilizing bacteria (*Pseudomonas putida* and *Bacillus lentus*) and nitrogen fixing bacteria (*Azotobacter* sp. and *Azospirillum* sp.) in pumpkin plants has been led to an increase in oil, seed and fruit yield, especially the fatty acid content (**Habibi *et al*., 2011**). In addition, **Abdullah *et al*. (2012)** on rosemary found that the highest oil percentage and yield in fresh herb were given by adding *Azotobacter Chroococcum* (AZ) +

Bacillus megaterium (B1) + *Bacillus circulanse* (B2) compared to other biofertilizers treatments. **Patel *et al*. (2016)** found that the bioactive component (plumbagin) was high with application of Azospirillum (0.026%w/w) using HPLC. The results of this study suggest that bio-fertilizers have the potential to increase the growth and bioactive component of *Plumbago zeylanica*.

CONCLUSION

In general, results of the present study showed that inoculation of biological fertilizers (Arbuscular Mycorrhiza Fungi, *Bacillus megatherium* strain and *Azospirillum brasilense* strain) plays a remarkable role in improving plant growth parameters, herb chemical constituents and volatile oil of rosemary (*Rosmarinus officinalis*) plant and they can be viewed as a suitable treatment for enhancing productivity under BeniSuif Governorate conditions.

REFERENCES

Abdullah, A. T.; Hanafy, M. S.; EL-Ghawwas, E. O. and Ali, Z.H. (2012). Effect of compost and some biofertilizers on growth, yield, essential oil productivity and chemical composition of *Rosmarinus officinalis*, L. plants. J. Hort. Sci. & Ornamen. Plants, 4 (2): 201-214.

Al-Maghraby, A. Y.; Bardisi, A. and Mohsen, A. A. M. (2018). Effect of phosphorus fertilization and inoculation with mycorrhizae on plant dry weight and chemical composition of two onion cultivars. Zagazig J. Agric. Res., 45 (6A): 1885-1899.

AOAC (1990). Official Methods of Analysis.15th Ed. Association of Official Analytical Chemists, Inc., Virginia, USA.

Banon, S.; Mendez, L. and Almela, E. (2012). Effects of dietary rosemary extract on lamb spoilage under retail display conditions. Meat Sci., 90 (3):579‑583.

Chapman, D. H. and Pratt, R. F. (1978). Methods of Analysis for Soils, Plants and Waters. Div. Agric. Sci. Univ. of California USA pp16-38.

Cohen, A. T.; Mariela, P.; Rubén, B. and Patricia, P. (2007). *Azospirillum brasilense* and ABA improve growth in arabidopsis. International Plant Growth substances Association 19th Annual Meeting PuertoVallarta, Mexico-July, 21-25.

European Pharmacopoeia (1983). Maissoneuve, SA: Sainte Ruffine. p. 1-8.

Genena, A. K.; Hense, H.; Smania, A. and Souza, S. M. de (2008). Rosemary (*Rosmarinus officinalis*)-A study of the composition, antioxidant and antimicrobial activities of extracts obtained with supercritical carbon dioxide. Ciencia e Tecnologia de Alimentos, 28 (2): 463-469.

Glick, B. R. (1995). The enhancement of plant growth by free-living bacteria. Can. J. Microbiol., 41:109–117

Gosling, P.; Hodge, A.; Goodlass, G. and Bending, G.D. (2006). Arbuscular mycorrhizal fungi and organic farming. Agric., Ecos. And Environ., 113: 17‑35.

Habibi, A.; Heidaril, G. and Sohrabi1, Y. (2011). Influence of bio, organic and chemical fertilizers on medicinal pumpkin traits. J. Med. Plants Res., 5: 5590–5597.

Hassan, E. A.; Ali, A. F. and El Gohary, A. E. (2015). Enhancement of growth, yield and chemical constituents of rosemary (*Rosmarinus officinalis*, L.) plants by application of compost and biofertilization treatments. Middle East J. Agric. Res., 4 (1): 99-111.

Hendawy S.F.; Ezz El-Din, Azza A.; Aziz, Eman E. and Omer, E.A. (2010). Productivity and oil quality of *Thymus vulgaris* L. under organic fertilization conditions. Zean J. Appl. Sci., 3(2): 203-216.

Hucker, T. W. G. and Catroux, G. (1980). Phosphorus in sewage ridge and animal waster slurries. Proceeding of the EEC Seminar, Haren (Gr); Groningen Netherlands 12, 13 June.

Jackson, M.L. (1970). Soil and Plant Chemical Analysis Prentice Hall. Englewood Gliffs, N. J.

Marschner, H. (1995). Mineral nutrition of higher plants (2nd ed). Academic Press, London.

Nassar, R. M. A.; Boghdady, M. S. and Selim, Dalia A. (2015). Effect of mineral and bio-fertilizers on vegetative growth, mineral status, seed yield, tropane alkaloids and leaf anatomy of thorn apple plant (*Datura stramonium* L.). Middle East J. Agric. Res., 4 (1): 99-111.

Nieto, G. Dıaz, P.; Banon, S. and Garrido, M. D. (2010). Dietary administration of ewe diets with a distillate from Rosemary leaves (*Rosmarinus officinalis* L.): influence on lamb meat quality. Meat Sci., 84:23‑29.

Pacovsky, R.S. and Fuller, G. (1986). Development of two endomycorrhizal symbioses on soybean and comparison with phosphorus fertilization. Plant and Soil, 195: 361-377.

Patel, H. D.; Krishnamurthy, R. and Azeez, M. A. (2016). Effect of biofertilizer on growth, yield and bioactive component of *Plumbago zeylanica* (Lead Wort). J. Agric. Sci., 8 (5): 141-151.

Ryu, C. M.; Hu, C. H.; Locy, R. D. and Kloepper, J. W. (2005). Study of mechanisms for plant growth promotion elicited by rhizobacteria in Arabidopsis thaliana. Plant Soil, 268:285–292.

Sharabani, H.; Izumchenko, E.; Wang, Q.; Kreinin, R.; Steiner, M.; Barvish, Z.; Kafka, M.; Sharoni, Y.; Levy, J.; Uskokovic, M.; Studzinski, G.P. and Danilenko, M. (2006). Cooperative antitumor effects of vitamin D_3 derivatives and rosemary preparations in a mouse model of myeloid leukemia. Int. J. Cancer, 118 (12): 3012‑3021.

Smith, S. E. and Read, D. J. (1997). Mycorrhizal Symbiosis. Academic Press, London.

Snedecor, G. W. and Cochran, W. G. (1990). Statistical Methods. pp:369-375. 11th. Ed. Iowa State College Press. Ames, Iowa, U.S.

Sokhangoy, S. H.; Ansari Kh. and Asli, D. E. (2012). 'Effect of bio-fertilizers on performance of dill (*Anethum graveolens*)'. Iranian Journal of Plant Physiology, 2 (4): 547-552.

Tilk, K. V. B. R.; Ranganayaki, N.; Pal, K. K.; De, R.; K.Saxena, A.; Nautiyal, C. S.; Tripathi, A. K. and Johri, B. N. (2005). Diversity of plant growth and soil health supporting bacteria. Current Sci., 98: 136-150.

Tsai, C.W.; Lin, C.Y.; Lin, H.H. and Chen, J.H. (2011). Carnosic acid, a rosemary phenolic compound, induces apoptosis through reactive oxygen species-mediated p38 activation in human neuroblastoma IMR-32 cells. Neurochem Res., 36 (12): 2442‑2451.

Vande Broek, A. and Vanderleyden, J. (1995). Review: genetics of the *Azospirillum*-plant root association. Crit. Rev. Plant Sci., 14: 445–66.

Yi, W. and Wetzstein, H. Y. (2010). Biochemical, biological and histological evaluation of some culinary and medicinal herbs grown under greenhouse and field conditions. J. Sci. Food Agric., 90 (6): 1063‑1070.

The future Journal of Biology
2019;3:26-33

Table 2. Effect of Mycrrohizal fungi (AMF), *Bacillus megatherium* var. *phosphaticum* (BM) and *Azospirillum brasilense* (AZB) and their mixtures on plant height (cm) and number of shoots /plant of rosemary in the two cuts during 2017 and 2018 seasons

Treatments	Plant height (cm)				Number of shoot /plant			
	2017 season		2018 season		2017 season		2018 season	
	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
Control	23.2	25.4	23.5	22.6	6.1	7.2	8.5	9.2
AMF	31.2	35.3	27.3	26.1	9.4	11.7	13.4	16.6
BM	44.1	43.6	42.5	44.4	7.3	8.4	11.5	13.3
AZB	36.5	38.4	37.3	36.7	8.8	9.8	10.3	11.4
AMF + BM	51.2	52.5	49.2	51.6	16.1	17.3	16.6	18.3
AMF + AZB	54.3	57.3	51.7	54.4	12.3	14.6	19.2	20.5
BM + AZB	48.1	49.6	46.5	49.6	10.8	15.5	15.6	17.7
AMF + BM + AZB	56.8	60.7	55.8	58.5	19.5	20.9	21.7	22.6
New L.S.D. at 5 %	**3.7**	**4.1**	**3.5**	**5.4**	**1.82**	**1.71**	**1.21**	**1.35**

Table 3. Effect of Mycrrohizal fungi (AMF), *Bacillus megatherium var. phosphaticum* (BM) and *Azospirillum brasilense* (AZB) and their mixtures on fresh and dry weights of herb/plant (g) of rosemary in the two cuts during 2017 and 2018 seasons

Treatments	Herb fresh weight / plant (g)				Herb dry weight / plant (g)			
	2017 season		2018 season		2017 season		2018 season	
	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
Control	59.2	67.3	55.8	61.4	18.9	22.1	17.2	19.9
AMF	64.4	71.9	68.7	69.8	23.5	25.9	22.1	23.8
BM	61.7	70.5	59.9	64.4	22.9	24.7	21.7	24.9
AZB	62.6	73.2	61.7	67.2	23.7	24.7	22.6	25.5
AMF + BM	76.9	79.3	71.8	76.9	25.9	29.3	25.1	28.6
AMF + AZB	78.3	84.2	77.1	76.9	27.5	30.2	24.9	25.8
BM + AZB	71.4	81.9	67.7	69.9	21.8	28.6	20.3	23.4
AMF + BM + AZB	121.7	135.5	117.8	121.8	32.8	35.4	28.5	29.9
New L.S.D. at 5 %	**7.9**	**6.5**	**6.9**	**7.2**	**3.2**	**3.3**	**2.9**	**3.7**

Table 4. Effect of Mycrrohizal fungi (AMF), *Bacillus megatherium var. phosphaticum* (BM) and *Azospirillum brasilense* (AZB) and their mixtures on total carbohydrates and nitrogen percentages of rosemary in the two cuts during 2017 and 2018 seasons

Treatments	Total carbohydrates (%)				Total nitrogen (%)			
	2017 season		2018 season		2017 season		2018 season	
	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
Control	13.1	12.9	12.9	12.8	1.89	1.81	1.78	1.77
AMF	15.7	16.9	15.3	15.1	2.02	2.03	2.11	2.14
BM	14.4	15.1	14.3	14.6	1.97	1.99	2.00	2.01
AZB	14.9	14.7	13.9	14.1	2.08	2.07	2.07	2.09
AMF + BM	18.8	18.3	17.7	17.9	2.01	2.08	1.99	2.03
AMF + AZB	18.1	18.7	18.9	17.9	2. 13	2.12	2.13	2.14
BM + AZB	15.5	16.1	16.6	16.7	2.11	2.10	2.01	2.11
AMF + BM + AZB	19.4	18.7	19.9	19.6	2.19	2.17	2.21	2.22
New L.S.D. at 5 %	**1.1**	**1.7**	**1.5**	**1.4**	**0.43**	**0.51**	**0.44**	**0.42**

Table 5. Effect of Mycrrohizal fungi (AMF), *Bacillus megatherium var. phosphaticum* (BM) and *Azospirillum brasilense* (AZB) and their mixtures on total phosphorus and potassium percentages of rosemary in the two cuts during 2017 and 2018 seasons

Treatments	Total phosphorus (%)				Potassium (%)			
	2017 season		2018 season		2017 season		2018 season	
	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
Control	0.19	0.19	0.17	0.17	1.81	1.83	1.71	1.82
AMF	0.25	0.23	0.21	0.22	1.84	1.90	1.92	1.93
BM	0.28	0.25	0.24	0.27	1.99	2.11	1.83	1.89
AZB	0.21	0.22	0.21	0.22	1.99	1.95	1.79	1.81
AMF + BM	0.28	0.29	0.28	0.27	2.09	2.11	2.19	2.19
AMF + AZB	0.26	0.26	0.22	0.25	2.01	2.09	2.07	2..08
BM + AZB	0.27	0.28	0.28	0.27	1.98	2.11	2.19	2.11
AMF + BM + AZB	0.30	0.31	0.3-	0.29	2.20	2.22	2.21	2.22
New L.S.D. at 5 %	**0.08**	**0.09**	**0.07**	**0.06**	**0.39**	**0.42**	**0.41**	**0.38**

Table 6. Effect of Mycrrohizal fungi (AMF), *Bacillus megatherium var. phosphaticum* (BM) and *Azospirillum brasilense* (AZB) and their mixtures on Fe, Mn and Zn (ppm) of rosemary in the two cuts during 2017 and 2018 seasons

Treatments	Fe (ppm)				Mn (ppm)				Zn (ppm)			
	2017 season		2018 season		2017 season		2018 season		2017 season		2018 season	
	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
Control	43	44	46	43	21	23	31	30	28	27	29	29
AMF	65	72	77	82	42	44	46	49	33	34	32	36
BM	61	65	60	71	39	44	47	44	32	32	30	32
AZB	51	52	59	57	31	33	33	43	31	33	33	32
AMF + BM	72	79	81	88	51	55	52	61	35	37	37	38
AMF + AZB	69	77	62	83	48	55	50	52	36	36	36	39
BM + AZB	59	61	67	68	46	49	44	49	32	36	33	35
AMF + BM + AZB	83	95	86	89	62	68	71	77	38	39	38	40
New L.S.D. at 5 %	**12.1**	**13.7**	**11.9**	**12.5**	**9.2**	**8.9**	**7.1**	**8.0**	**1.3**	**2.2**	**1.8**	**1.7**

Table 7. Effect of Mycrrohizal fungi (AMF), *Bacillus megatherium var. phosphaticum* (BM) and *Azospirillum brasilense* (AZB) and their mixtures on volatile oil (%) and volatile oil yield / plant (ml) of rosemary in the two cuts during 2017 and 2018 seasons

Treatments	Volatile oil (%)				Volatile oil yield / plant (ml)			
	2017 season		2018 season		2017 season		2018 season	
	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
Control	0.97	0.99	0.95	0.96	0.62	0.63	0.59	0.66
AMF	1.06	0.98	1.10	1.12	0.89	0.92	0.84	0.91
BM	0.99	0.99	1.01	1.03	0.77	0.79	0.80	0.82
AZB	0.98	1.04	1.08	1.09	0.79	0.83	0.82	0.83
AMF + BM	1.09	1.05	1.11	1.12	1.11	1.09	1.09	1.16
AMF + AZB	1.12	1.11	1.21	1.23	0.91	1.01	0.99	1.01
BM + AZB	1.05	1.09	1.13	1.15	0.97	0.98	0.99	1.03
AMF + BM + AZB	1.19	1.23	1.26	1.31	1.21	1.29	1.22	1.32
New L.S.D. at 5 %	**0.05**	**0.07**	**0.09**	**0.7**	**0.12**	**0.09**	**0.11**	**0.11**