

EFFECT OF SOME ORGANIC AND BIO-FERTILIZERS ON QUALITY AND QUANTILY OF *Rosmarinus officinalis* L. PLANTS

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ABSTRACT

Two field experiments were carried out on rosemary plants (*Rosmarinus officinalis* L.) at the experimental Baramoon Research Farm, Dakahlya Governorate, Egypt, during two successive seasons, 2010-2011 and 2011-2012, to study the effect of different sources of organic manure (FYM) mixed of poultry and cattle manures, humic acid, biofertilization nitrogen fixing bacteria (microbein) and phosphate dissolving bacteria (phosphorein), as well as active dry yeast on vegetative growth, herb yield, essential oil production and chemical composition of plants.

The results clear that in both seasons, all fertilizer treatments achieved significantly better growth and yield than the untreated plants (control) which received recommend dose of NPK. Treatments included that plant biomass responded linearly to (FYM) application and most values of all measurements were realized at the rate of 30 m³/fed. than obtained at both 20, 10 m³/fed. in comparison with those of control plants in both seasons, respectively. Also, it is evident that the application of (FYM) at rates of 10, 20 and 30 m³/fed., interacted with combinations of both (phosphorein + microbein), (phosphorein + yeast) and (phosphorein + humic acid) improved significant increments in the all growth characters compared with control plants, and the highest one was realized with combination of (FYM) at rate of 30 m³/fed. + phosphorein + humic acid.

These findings clearly indicate that phosphorein, humic acid and 30 m³/fed. of (FYM) could be used as traditional fertilizers instead of chemical ones and may consequently minimized pollution of agricultural environments.

INTRODUCTION

Rosmary(*Rosmarinus officinalis* L.) Fam. Lamiaceae is one of the important medicinal and aromatic perennial plants. It is used externally as parasiticide, cicatrisant, for muscular pains and rheumatism, dermatitis, dandruff and exzema. It promotes hair growth and stimulates scalp. Internally it is used for asthma, bronchitis, whooping cough, to stimulate poor circulation, it is employed for palpitation, debility, headache, neuralgia, renal fatigue, nervous exhaustion, and stress-related disorders, dyspepsia, flatulence, hepatic disorders, hyper cholesterolaemia, and jaundice. Its oil is extensively used in soap, detergents, cosmetics, house-hold sprays and perfumes especially colognes. Also extensively used in most major food categories, especially meet products, and drinks. Serves as a natural antioxidant (Lawless, 1992).

Chemical nutrients especially nitrogen, phosphorus and potassium are very important for plants, because N, P and K partake in structure of several components of the whole plants (protein, hormones, amino acids, enzymes, nucleic acids, fats and regulation of water conditions). However, using intense chemical fertilization cause serious problems on human health by pollution of the whole environmental conditions (soil, air and drainage water).

Farmyard manure (FYM) added to soils to improve their physical and chemical properties. It increases the soil fertility owing to its composition from macro and micro elements, amino acids, organic acids, sugars and organic matter. Also, it is a considerable useful habitat for several beneficial microorganisms. In the presence of organic materials, the number of N_2 fixes bacteria, mycorrhizae and phosphate solubilizing microorganisms increases in the soil as well as plant production, EL-Mahrouk (2000).

Moreover (FYM) are utilized for the change of soil texture, supplying nutrients, and they are considered safe for human health. Organic matter improves the aeration and drainage of compacted soils, the water holding capacity and also increases the soil exchange capacity i.e. its ability to absorb nutrients, Bryan and Lance (1991).

Humic substances are organic compounds that result from the decomposition of plant and animals materials. Humic acid and their salts which derived from coal and other sources may provide a viable alternative to liming, to ameliorate soil acidity and improve soil structural stability. It is the humic fractions (humic acid, fulvic acid and humin) of the soil organic matter that are responsible for the generic improvement of soil fertility and improved productivity, Kononova (1966) and Fortun *et al.*, (1989). On other hand, Russo & Beriyn (1990), Sanders *et al.*, (1990) and Pioncelot (1993) stated that increasing the permeability of plant membranes due to humate application resulted in improving growth of various groups beneficial microorganisms, accelerate cell division, increased root growth and all plant organs for a number of horticultural crops and turf grasses, as well as, the growth of some trees.

Bio-fertilizers are the most reliable tools to reduce the rate of chemical fertilizers applied for medicinal plants production in all types of soil and hence decreasing environmental pollution, EL-Mahrouk (2000). The significant effect of bio-fertilizers may be due to the effect of different strain groups and nutrients mobilizing microorganisms which help in availability of metals and their forms in the composted material and increased levels of extractable minerals, EL-Kramany *et al.*, (2000). Eisa (2004) reported that microbein and nitrobein bio-fertilizers increased the essential oil content per plant and oil yield per fed., of *Salvia officinalis* plants. Massoud *et al.*, (2004) showed that nitrobein plus phosphorein gave significant increase in plant height, number of branches and plant fresh and dry weight of thyme plants.

Yeast as a natural biostimulator is very safe to human, animals and environments, EL-Araby (2004). It is natural source of many growth substances (thiamine, riboflavin, cholin, niacin, pyridoxine, folic acid and vitamin B₁₂) and most of nutrient elements (Na, Ca, Fe, K, P, S, Zn and Si), as well as, organic compounds i.e. protein, carbohydrate, nucleic acids and

lipids, Nagodawithana, (1991). The various positive effects of applying dry yeast were attributed to its content of different nutrients, higher percentage of proteins, large amount of vitamin B and natural growth hormones, namely, cytokinins, in addition, application of yeast is very effective in releasing CO₂ which improved photosynthesis, Idso *et al.*, (1995). The vegetative growth, essential oil content and composition were increased in the sprayed plants with dry yeast, EL-Hindi and EL-Boraie, (2004) on marigold and Heikal (2005) on thyme.

Interaction between phosphorus fertilizer and active dry yeast as a bio-fertilizer increased greatly the vegetative growth parameters and carbohydrates contents as well as N, P and K percentages of *Vigna radiate*, L. Fouda (2005).

The aim of this respect, was to investigate the effect of organic manures (FYM manures+humic acid) and biofertilizers (phosphorein, microbein and yeast) and their interactions on growth, essential oil productivity and chemical components of peppermint plants.

MATERIALS AND METHODS

This study was conducted at the experimental Baramoon Research Farm, Dakahlya Governorate, Egypt during the two successive seasons 2010/2011 and 2011/2012, aiming to investigate the effect of (FYM), humic acid, microbein, phosphorein and yeast as well as their interactions on vegetative growth, herb yield, essential oil production and chemical composition of rosemary plants.

Uniform cuttings of *Rosmarinus officinalis* L. (rosemary) plants were taken from symmetry mother plants and planted in the nursery for rooting on Oct. 15th 2010 and 2011 seasons and transplanted on Mar.15th as rooted cuttings. . The soil of the experimental location was clay texture.

Randomized soil samples were obtained from the field to determine the physical and chemical contents according to the standard method described by Wild *et al.* (1985). Soil properties are presented in Table (A).

Table (A): Some physical and chemical characteristics of the experimental soil in the two seasons.

Season	Sand (%)	Silt mm (%)	Clay mm (%)	CaCO ₃ mm (%)	pH mm	Available nutrients (ppm)		
						N	P	K
2010	19.80	27.52	44.62	2.89	7.35	15.7	12.60	33.70
2011	20.99	29.47	47.12	3.21	7.65	17.10	12.90	36.60

The plot of control received full doses of (NPK) at the rate of recommended rate of Agriculture Ministry of Egypt, whears remain treatments received half dose of NPK.

Organic fertilizer was prepared from two types of organic manure (Poultry and cattle manures) named farmyard manure (FYM). Both of two types were mixed together at equal quantities before addition, as shown in table (B) then they were addressed at rates of (47 , 95 and 142 cm³/plot) as

(10, 20 and 30 m³/ fed) FYM, before planting during soil preparation as for the treatments were conducted for the experimental design in the two seasons.

Table (B): Chemical analysis of the added (FYM) in both seasons (2010/2011 & 2011/2012).

Properties	2010/2011	2011/2012
Organic matter	63.35	63.35
Total carbon	36.06	36.06
Total nitrogen	1.61	1.61
C/N ratio	1:20.25	1:20.25
Total phosphate	1.03	1.03
Total potassium	0.96	0.96
pH	8.15	8.15

Humic acid is an organic material was added 6 L/fed., drench with water irrigation divided four equal doses. The first dose was applied after one week from planting of seedlings followed with other doses at intervals two weeks between them.

Bio-fertilizers were provided by the General Organization for Agricultural Equalization Fund (G.O.A.E.F.), Ministry of Agriculture Egypt. The examined biofertilizers were:

Microbein: It contains live cells of efficient bacteria strains for N-fixing bacteria (*Azotobacter* sp., *Azospirillum* sp., *Pseudomonas* sp., *Rhizobium* sp., and *Bacillus megatherium*).

Phosphorein: It contains live cells of efficient bacteria strains as phosphate dissolving bacteria (*Bacillus megatherium*). (El-Zeiny *et al.*, 2001).

All the bacterial growth media were used at a rate of 400 g/fed. (0.2 gm/plot/season). The application of active dry yeast (*Saccharomyces cerevisiae*) was done as foliar spray with concentration of 3 mg/L., three times during growing of each seasons. The first foliar spraying after one week planting, the second one month after planting and the third two months after planting .

The experimental design :

The experimental design was split-plot design with 3 replicates. (FYM) treatments were main plots at rates 10, 20 and 30 m³/fed., while microbein, yeast and humic acid interacted with phosphorein were sub-main plots.

The treatments :

- 1- Control which received full dose of NPK.
- 2- (FYM) 10m³/Fed + half (NPK).
- 3- (FYM) 20m³/Fed + half (NPK).
- 4- (FYM) 30m³/Fed + half (NPK).
- 5- Microbein + phosphorein 4kg/Fed + half (NPK).
- 6- Microbein + phosphorein 4Kg/Fed + (FYM) 10m³/Fed + half (NPK).
- 7- Microbein + phosphorein 4Kg/Fed + (FYM) 20m³/Fed + half (NPK).
- 8- Microbein + phosphorein 4Kg/Fed + (FYM) 30m³/Fed + half (NPK).
- 9- Yeast 3Gm/L + phosphorein 2Kg/Fed + half (NPK).

- 10- Yeast 3Gm/L + phosphorein 2Kg/Fed +(FYM)10m³/Fed+half (NPK).
- 11- Yeast 3Gm/L + phosphorein 2Kg/Fed +(FYM)20m³/Fed+half (NPK).
- 12- Yeast 3Gm/L + phosphorein 2Kg/Fed +(FYM)30m³/Fed+half (NPK).
- 13- Humic acid 6L/Fed + phosphorein 2Kg/Fed+ half (NPK).
- 14- Humic acid 6L/Fed + phosphorein 2Kg/Fed + (FYM) 10m³/Fed + half (NPK).
- 15- Humic acid 6L/Fed + phosphorein 2Kg/Fed + (FYM) 20m³/Fed + half (NPK).
- 16- Humic acid 6L/Fed + phosphorein 2Kg/Fed + (FYM) 30m³/Fed + half (NPK).

Plants were harvested twice yearly by cutting the aerial parts of each plant (10 cm) above the soil surface. The first cut was carried out in the second week of June and the second cut was in the second week of September.

Five plants were randomly chosen from each treatment at two cuts, in both seasons, respectively. The vegetative growth parameters (plant height, number of branches, plant fresh and dry weight as well as herb yield) were recorded.

Essential oil percentages was determined from dry leaves according to the method described the Egyptian Pharmacopoeia (1984). The essential oil obtained from the second cut of the second season was analyzed using Gas Liquid Chromatography technique (GLC), which carried out at the Laboratory of Aromatic and Medicinal plants research, Cairo, Egypt, on department.

The use of GLC in the quantitative determination was performed using the methods described by Bunzen *et al.*, (1969) and Hoftman (1967).

Tissue analysis of microelements were carried out in the Chemistry Dept., Fac. Agric., Mansoura Univ. Plant samples were dried in an electric oven at 70 °C for 48 according to A.O.A.C. (1970). Nitrogen percentage was determined according to method of Kjeldahl as described by Jackson (1973). Phosphorus percentage was determined according to Murphy and Reily (1962). Potassium percentage was determined according to Wilde *et al.*, (1985).

The split-plot design in a completely randomized block with 3 replicates was used in both growing seasons. Obtained data was subjected to the statistical analysis of variance (ANOVA) in split-plot design as mentioned by Gomez and Gomez (1984)

RESULTS AND DISCUSSION

1- Plant growth characters:

Data presented in Tables (1, 2, 3 and 4) indicated that providing rosemary plants with different levels of (FYM), (phosphorein + microbein), (phosphorein + yeast), (phosphorein + humic acid) and their interactions, exerted significant differences concerning vegetative growth characters expressed as plant height, number of branches, herb fresh and dry weights compared with control. The highest vegetative growth characters resulted from fertilized plants with the interaction between (FYM) 30 m³/fed., and (phosphorein + humic acid) on other hand.

Table (1): Plant height (cm) of rosemary as affected by (FYM), phosphorein, microbein, yeast and their interactions during 2011 and 2012 seasons for two cuts.

Characters	First season 2010/2011									
	Plant height									
	First cut					Second cut				
Treatments	control	FYM 10 m ³ /fed.	FYM 20 m ³ /fed.	FYM 30 m ³ /fed.	Mean (A)	control	FYM 10 m ³ /fed.	FYM 20 m ³ /fed.	FYM 30 m ³ /fed.	Mean (A)
control	40.30	50.90	55.00	58.90	51.20	47.00	53.30	57.70	62.50	55.00
Phosphorein+Microbein	59.70	62.40	66.00	69.30	64.40	60.40	61.40	66.30	70.20	54.58
Phosphorein+Yeast	56.40	60.00	62.40	65.70	61.00	57.20	59.40	64.40	68.30	62.40
Phosphorein+Humic acid	62.80	65.90	69.70	71.90	67.60	67.00	68.80	70.40	73.40	69.90
Mean (B)	54.80	59.80	63.30	66.50	-----	57.90	60.80	64.70	68.60	-----
L.S.D at 0.05	A		B		A x B	A		B		A x B
L.S.D at 0.01	2.59		2.09		3.17	3.76		3.64		6.62
	4.43		3.15		5.16	5.36		4.35		10.47
Characters	Second season 2011/2012									
	First cut					Second cut				
	Treatments	control	FYM 10 m ³ /fed.	FYM 20 m ³ /fed.	FYM 30 m ³ /fed.	Mean (A)	control	FYM 10 m ³ /fed.	FYM 20 m ³ /fed.	FYM 30 m ³ /fed.
control	45.00	49.30	55.40	59.70	52.35	51.80	56.70	59.50	65.50	58.37
Phosphorein+Microbein	60.70	63.20	66.80	69.80	55.12	62.90	62.80	67.70	70.30	55.92
Phosphorein+Yeast	55.50	60.70	64.50	67.40	52.02	58.90	61.20	64.30	68.70	53.27
Phosphorein+Humic acid	66.80	69.90	72.30	75.90	71.22	67.80	69.30	70.90	73.70	70.42
Mean (B)	57.00	50.77	64.75	68.20	-----	60.35	62.50	65.60	69.55	-----
L.S.D at 0.05	A		B		A x B	A		B		A x B
L.S.D at 0.01	1.69		2.53		3.64	2.42		2.90		5.69
	2.27		3.45		6.26	4.15		4.17		8.13

- All treatments received half dose of (NPK) except control received full dose of(NPK).

Table (2): Number of branches/plant of rosemary as affected by (FYM), phosphorein, microbein, yeast and their interactions during 2011 and 2012 seasons for two cuts.

Characters	First season 2010/2011									
	Number of branches / plant									
	First cut					Second cut				
Treatments	control	FYM 10 m ³ /fed.	FYM 20 m ³ /fed.	FYM 30 m ³ /fed.	Mean (A)	control	FYM 10 m ³ /fed.	FYM 20 m ³ /fed.	FYM 30 m ³ /fed.	Mean (A)
control	31.30	33.70	35.82	39.21	34.96	32.81	34.61	36.91	39.26	39.26
Phosphorein + Microbein	34.61	36.22	39.31	42.61	38.19	34.82	36.92	39.62	43.41	43.41
Phosphorein + Yeast	33.87	35.90	38.32	41.72	37.45	33.10	35.42	38.31	41.22	41.22
Phosphorein + Humic acid	36.82	38.82	32.22	43.41	37.82	37.22	39.62	41.91	43.81	43.81
Mean (B)	34.10	36.16	36.42	41.73	-----	34.48	36.64	39.19	41.93	-----
L.S.D at 0.05	A		B		A x B	A		B		A x B
L.S.D at 0.01	2.09		1.16		2.87	2.09		1.16		2.14
	3.08		2.88		4.69	3.11		2.67		4.09
Characters	Second season 2011/2012									
	First cut					Second cut				
	Treatments	control	FYM 10 m ³ /fed.	FYM 20 m ³ /fed.	FYM 30 m ³ /fed.	Mean (A)	control	FYM 10 m ³ /fed.	FYM 20 m ³ /fed.	FYM 30 m ³ /fed.
control	12.50	13.90	15.60	18.90	15.20	33.70	35.21	37.31	39.71	36.48
Phosphorein + Microbein	14.90	16.30	18.90	21.80	17.98	35.50	36.71	38.62	40.91	37.93
Phosphorein + Yeast	13.20	15.10	17.20	20.60	16.53	34.30	35.00	37.31	39.11	31.43
Phosphorein + Humic acid	16.4	18.90	20.9	23.60	19.95	38.20	39.91	42.31	44.61	36.21
Mean (B)	14.25	16.050	18.15	21.23	-----	35.42	36.71	39.00	41.08	-----
L.S.D at 0.05	A		B		A x B	A		B		A x B
L.S.D at 0.01	1.14		2.09		2.68	1.17		2.07		2.28
	2.17		3.08		4.39	2.36		3.56		4.36

- All treatments received half dose of (NPK) except control received full dose of(NPK).

The pre-mentioned increases refers to the essential and vital role of (FYM), biofertilizers phosphorein, microbein and yeast as well as humic acid, and their combinations. The stimulatory effect of (FYM) as a result of improving physical, chemical, biological, texture and drainage of the soil which in turn, positively influence the growth, Salem (1986). The increases in vegetative growth characters owing to yeast may be due to its content of tryptophan, Abd EL-Latif (1987) and precursor of IAA, Moor (1979). Increments resulted from biofertilized treatments due to the supplements with nitrogen which is considered a precursor of protein synthesis and a vascular osmoticum. The osmotic compounds in the cell sap are important in order to allow cell enlargement. These results are supported by Hamza *et al.*, (2007) on *Plantago ovata* Forsk plants and Massoud (2007) on marjoram plants. On other hand, Kononova (1966) and Fortun *et al.*, (1989) performed that humic acids are known to possess many beneficial agricultural properties, they participate actively in the decomposition of organic matter, improve soil texture, enhancement of photosynthesis which resulted in greater plant growth. The results of this study are in harmony of those obtained with Massoud *et al.*, (2010) on marjoram plants.

Table (3): Herb fresh weight/plant (g) of rosemary as affected by (FYM), phosphorein, microbein, yeast and their interactions during 2011 and 2012 seasons for two cuts.

	First season 2010/2011											
	Herb fresh weight / plant											
	First cut					Second cut						
	control	FYM 10 m ³ /fed.	FYM 20 m ³ /fed.	FYM 30 m ³ /fed.	Mean (A)	control	FYM 10 m ³ /fed.	FYM 20 m ³ /fed.	FYM 30 m ³ /fed.	Mean (A)		
control	210.30	306.70	344.70	391.40	313.27	235.80	315.20	352.90	403.10	326.75		
Phosphorein + Microbein	395.40	418.20	434.80	459.80	427.05	402.10	427.80	443.90	466.20	435.00		
Phosphorein + Yeast	369.80	401.50	410.90	450.10	408.10	381.10	407.50	414.70	454.30	414.40		
Phosphorein + Humic acid	432.50	459.70	470.80	490.20	463.30	450.30	464.90	481.80	507.20	476.05		
Mean (B)	352.00	396.52	415.30	447.90	-----	357.32	403.85	423.32	457.70	-----		
L.S.D at 0.05	A		B		A x B		A		B		A x B	
L.S.D at 0.01	26.80		31.50		76.50		30.20		37.50		85.60	
	43.50		55.10		91.40		45.20		57.10		97.10	
	Second season 2011/2012											
	First cut					Second cut						
	control	FYM 10 m ³ /fed.	FYM 20 m ³ /fed.	FYM 30 m ³ /fed.	Mean (A)	control	FYM 10 m ³ /fed.	FYM 20 m ³ /fed.	FYM 30 m ³ /fed.	Mean (A)		
	control	215.60	320.20	352.70	397.60	321.52	241.30	327.10	360.70	410.20	334.82	
Phosphorein + Microbein	397.50	423.50	439.30	365.90	406.55	409.50	436.20	449.80	471.90	441.85		
Phosphorein + Yeast	371.70	410.50	411.90	456.90	396.85	391.10	414.70	418.60	459.20	420.90		
Phosphorein + Humic acid	445.90	465.10	478.80	497.60	471.85	459.80	472.50	483.10	410.60	456.50		
Mean (B)	357.67	404.83	420.67	429.50	-----	375.92	412.62	428.05	437.97	-----		
L.S.D at 0.05	A		B		A x B		A		B		A x B	
L.S.D at 0.01	34.10		40.50		86.10		36.50		45.60		93.10	
	50.20		64.30		96.20		55.40		67.50		101.20	

- All treatments received half dose of (NPK) except control received full dose of(NPK).

The interactions showed that there were significant differences as plant growth characters were concerned. These results were similar in the two seasons.

Table (4): Herb dry weight/plant (g) of rosemary as affected by (FYM), phosphorein, microbein, yeast and their interactions during 2011 and 2012 seasons for two cuts.

	First season 2010/2011									
	Herb dry weight / plant									
	First cut					Second cut				
	control	FYM 10 m ³ /fed.	FYM 20 m ³ /fed.	FYM 30 m ³ /fed.	Mean (A)	control	FYM 10 m ³ /fed.	FYM 20 m ³ /fed.	FYM 30 m ³ /fed.	Mean (A)
control	126.09	137.68	140.07	146.21	137.51	128.11	137.71	141.81	147.16	138.70
Phosphorein + Microbein	147.43	149.16	150.97	154.57	150.53	148.10	149.98	151.80	154.08	151.00
Phosphorein + Yeast	144.65	147.31	148.20	153.45	148.40	144.70	147.81	148.65	152.81	148.50
Phosphorein + Humic acid	150.75	154.10	158.69	156.12	154.91	152.44	155.06	155.61	158.61	155.43
Mean (B)	142.23	147.06	149.48	152.59	-----	143.33	147.64	149.43	153.16	-----
L.S.D at 0.05	A		B		A x B	A		B		A x B
L.S.D at 0.01	19.10		24.80		49.80	23.60		31.20		61.70
	25.70		35.40		77.30	31.80		44.20		73.80
	Second season 2011/2012									
	First cut					Second cut				
		control	FYM 10 m ³ /fed.	FYM 20 m ³ /fed.	FYM 30 m ³ /fed.	Mean (A)	control	FYM 10 m ³ /fed.	FYM 20 m ³ /fed.	FYM 30 m ³ /fed.
control	126.76	138.15	141.72	146.87	138.37	129.41	137.91	142.63	148.09	139.51
Phosphorein + Microbein	146.75	149.70	151.35	153.91	150.43	147.97	151.09	152.41	154.92	151.80
Phosphorein + Yeast	144.03	147.10	148.33	153.21	148.16	145.74	148.59	148.10	152.38	148.70
Phosphorein + Humic acid	152.09	155.20	155.72	162.21	156.30	153.57	154.09	156.02	158.09	155.44
Mean (B)	142.41	147.54	149.28	145.05	-----	143.42	147.92	149.79	153.37	-----
L.S.D at 0.05	A		B		A x B	A		B		A x B
L.S.D at 0.01	23.40		26.80		54.80	24.70		33.80		67.80
	34.80		44.70		83.90	34.50		49.80		84.90

- All treatments received half dose of (NPK) except control received full dose of(NPK).

2- Essential oil productivity :

The essential oil percentage and content in the dried leaves of rosemary plants varied from one treatment to other (Tables 5 and 6). The highest increasing in oil percentage were obtained from plants fertilized with (FYM) 30 m³/fed. combined with interaction of (phosphorein + microbein) which were (1.69, 1.70 and 1.76, 1.82 %) for two cuts in both two seasons, respectively, whereas same treatment achieved the most increments of essential oil content cc/plant in the two cuts for both two seasons and the differences were significant in comparison with those obtained by control plants, (2.29, 2.33 and 2.37, 2.49 cc/plant).

Similar results of positive effect of both (FYM) or biofertilizers (phosphorein and microbein) on the essential oil productivity were obtained by Shalan *et al.*, (2001) on chamomile, Sakr (2001) on peppermint, Abd EL-Latif *et al.*, (2002) on *Matricaria chamomilla*, Hamed (2004) on *Salvia*

officinalis, Massoud (2007) on marjoram and EL-Sanafawy (2007) on *Ocimum basilicum* and marjoram plants

Table (5): Essential oil percentage of rosemary as affected by (FYM), phosphorein, microbein, yeast and their interactions during 2011 and 2012 seasons for two cuts.

Characters	First season 2010/2011									
	Essential oil percentage									
	First cut					Second cut				
Treatments	control	FYM 10 m ³ /fed.	FYM 20 m ³ /fed.	FYM 30 m ³ /fed.	Mean (A)	control	FYM 10 m ³ /fed.	FYM 20 m ³ /fed.	FYM 30 m ³ /fed.	Mean (A)
control	0.46	1.11	0.98	0.84	0.85	0.48	1.16	1.01	0.90	0.89
Phosphorein + Microbein	0.69	1.18	1.35	1.48	1.17	0.53	1.19	1.34	1.51	1.14
Phosphorein + Yeast	0.88	1.10	1.05	1.02	1.01	0.91	1.11	1.08	0.99	1.02
Phosphorein + Humic acid	1.41	1.15	1.02	0.97	1.14	1.30	1.17	1.04	0.96	1.12
Mean (B)	0.86	1.13	1.10	1.08	-----	0.80	1.16	1.12	1.09	-----
L.S.D at 0.05	A		B		A x B	A		B		A x B
L.S.D at 0.01	0.07		0.03		0.09	0.07		0.04		0.10
	0.12		0.05		0.12	0.13		0.06		0.15
Characters	Second season 2011/2012									
	First cut					Second cut				
	Treatments	control	FYM 10 m ³ /fed.	FYM 20 m ³ /fed.	FYM 30 m ³ /fed.	Mean (A)	control	FYM 10 m ³ /fed.	FYM 20 m ³ /fed.	FYM 30 m ³ /fed.
control	0.46	1.16	1.04	0.86	0.88	0.52	1.19	1.07	0.88	0.91
Phosphorein + Microbein	0.50	1.21	1.43	1.54	1.17	0.54	1.24	1.44	1.61	1.21
Phosphorein + Yeast	0.95	1.17	1.12	1.03	1.07	0.99	1.18	1.13	0.97	1.07
Phosphorein + Humic acid	1.46	1.22	1.08	1.01	1.19	1.35	1.24	1.09	0.96	1.16
Mean (B)	0.84	1.19	1.17	1.11	-----	0.85	1.21	1.18	1.10	-----
L.S.D at 0.05	A		B		A x B	A		B		A x B
L.S.D at 0.01	0.02		0.03		0.09	0.03		0.04		0.10
	0.04		0.05		0.09	0.06		0.06		0.14

- All treatments received half dose of (NPK) except control received full dose of(NPK).

These increases might be attributed to the enhancing effect of organic and biofertilizers on vegetative growth, in terms of fresh yield besides increasing uptake of nutrients especially phosphorus element which linked by phosphate bounds which is adenosine triphosphate (ATP). In this form, the energy can be undergoing processes such activation uptake and the synthesis of various organic compounds such as essential oil, EL-Ghadban *et al.*, (2003) and Heikal (2005).

Table (6): Oil content of rosemary cc/plant as affected by (FYM), phosphorein, microbein, yeast and their interactions during 2011 and 2012 seasons for two cuts.

Characters	First season 2010/2011									
	Oil content									
	First cut					Second cut				
	control	FYM 10 m ³ /fed.	FYM 20 m ³ /fed.	FYM 30 m ³ /fed.	Mean (A)	control	FYM 10 m ³ /fed.	FYM 20 m ³ /fed.	FYM 30 m ³ /fed.	Mean (A)
control full dose (NPK)	0.58	1.52	1.37	1.22	1.17	0.61	1.60	1.43	1.32	1.24
Phosphorein + Microbein	1.01	1.76	2.03	2.29	1.77	0.78	1.78	2.03	2.33	1.73
Phosphorein + Yeast	1.27	1.62	1.55	1.56	1.50	1.32	1.64	1.60	1.51	1.52
Phosphorein + Humic acid	2.12	1.77	1.61	1.51	1.75	1.98	1.81	1.62	1.52	1.73
Mean (B)	1.25	1.67	1.64	1.64	-----	4.24	1.71	1.67	1.67	-----
L.S.D at 0.05	A B		A x B			A B		A x B		
L.S.D at 0.01	0.31	0.43	0.63			0.38	0.41	0.61		
	0.45	0.51	0.77			0.48	0.53	0.75		
Characters	Second season 2011/2012									
	First cut					Second cut				
	control	FYM 10 m ³ /fed.	FYM 20 m ³ /fed.	FYM 30 m ³ /fed.	Mean (A)	control	FYM 10 m ³ /fed.	FYM 20 m ³ /fed.	FYM 30 m ³ /fed.	Mean (A)
	control	0.58	1.60	1.47	1.26	1.23	0.67	1.64	1.53	1.30
Phosphorein + Microbein	0.73	1.81	2.16	2.37	1.77	0.80	1.87	2.19	2.49	1.84
Phosphorein + Yeast	1.37	1.72	1.66	1.58	1.58	1.44	1.75	1.67	1.47	1.58
Phosphorein + Humic acid	2.22	1.89	1.68	1.64	1.86	2.07	1.91	1.70	1.52	1.80
Mean (B)	1.22	1.75	1.74	1.71	-----	0.99	1.79	1.77	1.69	-----
L.S.D at 0.05	A B		A x B			A B		A x B		
L.S.D at 0.01	0.31	0.30	0.42			0.31	0.30	0.41		
	0.38	0.34	0.57			0.38	0.34	0.52		

- All treatments received half dose of (NPK) except control received full dose of(NPK).

3- Herb and oil yield :

Data reported in Table (7) revealed significant differences in dried herb yield of rosemary plants due to different levels of FYM fertilizer (10, 20 and 30 m³/fed.) solely or combined with interactions of both (phosphorein + microbein), (phosphorein + yeast) and (phosphorein + humic acid).

Fertilization with the highest level of (FYM) 30 m³/fed., combined with interaction (phosphorein + humic acid) produced the heaviest dried herb yield (2.935 and 2.971 ton/fed.) as a total for two cuts in both two seasons, respectively, compared with control plants.

Concerning the effect of different levels of fertilizers in this respect, on essential oil yield, data was tabulated in Table (7) and it showed that the highest yearly essential oil production (Liter/fed.) have achieved when plants fertilized with (FYM) 30 m³/fed. combined with interaction of (phosphorein + microbein) such as data recorded (77.61 and 81.65 Liter/fed.) as a total essential oil yield in both cuts for two seasons, respectively, when compared with control.

Table (7): Herb (ton/fed.) and essential oil yield (Liter/fed.) of rosemary plants as affected by (FYM), phosphorein, microbein, yeast and their interactions during 2011 and 2012 seasons for two cuts.

Characters Treatments	Yearly dry weight (ton/fed.)						Yearly essential oil (L/fed.)					
	First season 2010/2011			Second season 2011/2012			First season 2010/2011			Second season 2011/2012		
	1 st cut	2 nd cut	Total	1 st cut	2 nd cut	Total	1 st cut	2 nd cut	Total	1 st cut	2 nd cut	Total
control	2.118	2.119	4.237	2.130	2.171	4.301	9.71	10.25	19.99	9.79	11.26	21.00
(FYM) 10 m ³ /fed.	2.313	2.313	4.626	2.321	2.317	4.630	25.54	26.88	52.42	26.88	27.55	54.43
(FYM) 20 m ³ /fed.	2.353	2.382	4.735	2.381	2.396	4.777	23.02	24.02	47.04	24.70	25.70	50.40
(FYM) 30 m ³ /fed.	2.456	2.472	4.928	2.467	2.488	4.955	20.50	22.18	42.68	21.17	21.84	43.01
Microbein Phosphorein	2.477	2.489	4.966	2.465	2.485	4.950	16.97	13.10	30.07	12.26	13.44	25.70
Mic. + Phos. (FYM) 10 m ³ /fed.	2.506	2.520	5.026	2.515	2.538	5.053	29.57	29.90	59.47	30.41	31.41	61.83
Mic. + Phos. (FYM) 20 m ³ /fed.	2.536	2.550	5.086	2.543	2.560	5.103	34.10	34.10	68.20	36.29	36.79	73.08
Mic. + Phos. (FYM) 30 m ³ /fed.	2.597	2.588	5.185	2.586	2.603	5.189	38.47	39.14	77.61	39.82	41.83	81.65
Yeast Phosphorein	2.430	2.431	4.861	2.420	2.448	4.868	21.34	22.18	43.52	23.02	24.19	47.21
Yeast + Phos. (FYM) 10 m ³ /fed.	2.475	2.483	4.958	2.471	2.496	4.967	27.22	27.55	54.77	28.90	29.40	58.30
Yeast + Phos. (FYM) 20 m ³ /fed.	2.490	2.450	4.940	2.492	2.488	4.980	26.04	26.88	52.92	27.89	28.06	55.95
Yeast + Phos. (FYM) 30 m ³ /fed.	2.578	2.567	5.145	2.571	2.560	5.131	26.21	25.37	51.58	26.54	24.70	51.24
Humic acid Phosphorein	2.533	2.561	5.094	2.555	2.580	5.135	35.62	33.26	68.88	37.30	34.78	72.08
Hum. acid + Phos. (FYM) 10 m ³ /fed.	2.589	2.605	5.194	2.607	2.589	5.196	21.34	30.41	51.75	31.75	32.01	63.76
Hum. acid + Phos. (FYM) 20 m ³ /fed.	2.607	2.614	5.221	2.616	2.621	5.237	27.05	27.22	54.27	28.22	28.56	56.78
Hum. acid + Phos. (FYM) 30 m ³ /fed.	2.623	2.665	5.288	2.725	2.655	5.380	25.37	25.54	50.91	27.55	25.54	53.05

- All treatments received half dose of (NPK) except control received full dose of(NPK).

Phos. = Phosphorein. Mic. = Microbein. Hum. = Humic

The highest increase in oil yield/fed. was obtained from combinations between both of organic and biofertilizers, may be due to the increase in herb yield as well as the increment in the essential oil percentage. On other hand, this increment may be due to that mineral nutrition exerted some effects either directly on the enzyme system dealing with this conversion or indirectly by its effects on photosynthetic process.

4- Essential oil components :

Data presented in Table (8) and illustrated in Figure (1) identified (10) compounds separated from peppermint herb oil samples produced from plants fertilized with (FYM) or inoculated with phosphorein, microbein, yeast, humic acid and their interactions. The obtained chromatograms revealed the presence of (22) components from which (10) components were identified by the retention times obtained from pure authentic substances. The (10) main components are illustrated in Table (8).

It is evident from the results that the compound of menthrole recorded the highest values (29.39, 28.05, 22.97 and 22.67 %) when plants treated with (microbein + phosphorein + FYM 20 m³/fed.), (microbein + phosphorein + FYM 10 m³/fed.), (humic acid + phosphorein + FYM 10 m³/fed.) and (yeast + phosphorein + FYM 20 m³/fed.) interaction, respectively- followed by menthone compound which recorded (28.38%) which was the best when plants treated with (microbein + phosphorein + FYM 20 m³/fed.). It is clear that co-operation between both of biofertilizers and organic manure activated biosynthesis reactions of rosemary plants resulting the most values of main components.

Table (8): GLC of rosemary plants oil as affected by (FYM), phosphorein, microbein, yeast and their interactions during 2011 and 2012 seasons for two cuts.

Main components	α -pinene	camphene	β -pinene	Linalool	1,8 cineole	camphor	α -terpineol	borneol	borny acetate	β -caryophyllene
Control	10.87	10.12	4.17	13.45	15.77	9.56	5.30	3.84	3.55	3.45
(FYM) 10 m ³ /fed.	11.06	9.42	5.47	16.28	17.59	7.48	7.07	5.37	4.99	2.84
(FYM) 20 m ³ /fed.	11.27	10.92	5.94	14.40	18.01	9.79	8.08	3.83	3.21	2.56
(FYM) 30 m ³ /fed.	10.46	9.72	5.37	14.48	19.04	9.30	6.89	4.68	3.73	3.69
Microbein + Phosphorein	11.00	10.01	5.72	13.01	16.72	9.97	6.34	5.05	4.11	2.36
Mi. + Ph. + (FYM) 10 m ³ /fed.	11.40	9.82	4.05	16.57	28.05	8.58	6.04	2.27	1.62	0.78
Mi. + Ph. + (FYM) 20 m ³ /fed.	11.27	11.17	3.39	28.38	29.39	4.11	3.62	1.93	1.74	1.15
Mi. + Ph. + (FYM) 30 m ³ /fed.	14.22	12.44	5.14	16.99	17.72	9.35	6.06	3.89	3.39	2.65
Yeast + Phosphorein	13.23	11.79	5.44	17.22	19.12	8.46	5.83	3.32	2.88	1.42
Yeast + Ph. + (FYM) 10 m ³ /fed.	11.56	10.96	3.08	14.06	20.85	6.60	5.64	2.80	1.80	0.50
Yeast + Ph. + (FYM) 20 m ³ /fed.	14.30	10.78	3.92	14.94	22.67	6.36	4.95	3.77	2.20	1.01
Yeast + Ph. + (FYM) 30 m ³ /fed.	10.68	9.48	4.70	14.42	18.89	8.80	5.53	3.87	2.43	1.09
Humic acid + Phosphorein	11.05	9.41	4.41	14.61	17.72	8.60	5.44	3.76	2.23	1.11
Hu. acid + Ph. + (FYM) 10 m ³ /fed.	10.65	9.66	3.37	19.62	22.97	8.63	5.68	2.51	1.13	0.42
Hu. acid + Ph. + (FYM) 20 m ³ /fed.	11.76	11.27	5.19	17.97	18.51	8.98	5.74	3.99	2.94	1.50
Hu. acid + Ph. + (FYM) 30 m ³ /fed.	13.28	12.05	4.30	15.16	21.31	6.12	5.53	4.28	2.66	2.07

- All treatments received half dose of (NPK) except control received full dose of(NPK).

Farmyard

manure = Phosphorein =
 FYM, Ph. , Microbein = Mi. , Humic acid = Hu. , Feddan = Fed.

Figure (1): Effect of FYM, Microbein, phosphorein, yeast, humic acid and their interactions on the essential oil components (%) of rosemary plants during 2012 season.

(1) α -pinene / (2) camphene / (3) β -pinene / (4) Linalool / (5) 1,8 cineole / (6) camphor / (7) α -terpineol (8) borneol / (9) borny acetate / (10) β -caryophyllene.

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تأثير بعض الأسمدة العضوية والحيوية على إنتاج وجودة الزيت على نبات الحصابان

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أجريت هذه الدراسة خلال موسمين ٢٠١١ ، ٢٠١٢ في مزرعة البرامون - محطة بحوث البساتين - المنصورة - محافظة الدقهلية لدراسة تأثير سماد عضوى الفارم يارد وحمض الهيوميك وبعض الأسمدة الحيوية مثل الخميرة الجافة والفسفورين والميكروبيين والتفاعلات بين هذه العناصر على نمو وإنتاج الزيت الطيار ومكوناته في نبات الحصابان.

تم معاملة عقل الحصابان بسماد الفارم يارد بتركيزات ١٠ ، ٢٠ ، ٣٠ م^٣/الفدان في معاملات منفصلة ثم سمدت نباتات أخرى بتفاعلات من الفوسفورين + الميكروبيين مع كل من التركيزات الثلاثة للسماد العضوى ومعاملات أخرى سمدت بالفوسفورين + الخميرة مع كل من معدلات السماد العضوى الثلاثة ومعاملات ثالثة سمدت بالفوسفورين + حمض الهيوميك مع كل من معدلات السماد العضوى الثلاثة أيضاً في موسمي الزراعة المتتابعين كما سمدت نباتات الكنترول بالجرعات الموصى بها من السماد الكيماوى لوزارة الزراعة بجمهورية مصر العربية.

وأظهرت النتائج تحسن وتفوق الصفات الخضرية المختلفة لنباتات الحصابان عن نباتات الكنترول مثل ارتفاع النبات وعدد الأفرع الجانبية والوزن الطازج والجاف للنبات وللقدان والنسبة المئوية للزيت في النبات وإنتاج المحصول للفدان ومكوناته نتيجة لكل المعاملات وكانت أفضل المعاملات هي الفوسفورين + الخميرة + فارم يارد ٣٠ م^٣/الفدان بالتبادل مع الفوسفورين + حمض الهيوميك + فارم يارد ٣٠ م^٣/الفدان على الصفات المختلفة تحت الدراسة وكانت الزيادات جميعها معنوية.

أظهر التحليل الكروماتوجرافى للزيت أنه يحتوى على عدد (٢٧) من المكونات الرئيسية تم التعرف على عدد (١٠) مركبات منها وكان مركب ١.٨ سينيل هو الرئيسى على الإطلاق وقد أدت المعاملات المذكورة كلها إلى زيادات واضحة عن الكنترول.

وبذلك يمكن التوصية للحصول على محصول وافر من عشب وزيت الحصابان ذو مواصفات أفضل بمعاملة النباتات بالفوسفورين + الميكروبيين + فارم يارد ٣٠ م^٣/الفدان ذات التأثير الكلى على إنتاج الزيت لنبات الحصابان.

قام بتحكيم البحث

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