Effect of Potassium Fertilizer and Biofertilizers Inoculation on Vegetative Growth and Volatile Oil Content of Rosemary

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ABSTRACT: The field experiments was carried out at the Horticulture Researches Sabhia Station , Horticulture Research Institute, Agriculture Research Center, Alexandria, Egypt during 2014 to 2016 in order to study the effect of potassium and biofertilization on growth, chemical composition of essential oil and major compounds of rosemary (*Rosmarinus officinalis* L.). The experimental design was randomize complete block design with three replicates. The main results could be summarized as follows: (1) the K+ fertilization treatments, differently, affected the mean values of all studied characters, whereas the application treatment of (150 kg K2SO4/fed + NP mineral significantly, increased plant fresh and dry weights (g/plant), (2) the application of 300 kg K2SO4/fed + phosphorein + Ceraline treatment; gave the highest chemical composition (P and K%) and carbohydrate (%) during both seasons, whereas, the highest mean value of N% was recorded by 450 kg K2SO4/fed + Cerealin + phosphorein, and (3) the application of 20 L/fed Potassmage + phosphorein + Ceraline; gave rise to the highest essential oil (%). Also, the various fertilizers; brought about the highest major's compounds (α-Pinene, β- Pinene, Cineol, Camphor, Borneol, Borneol acetate and Eugenol) percentages during 2015 season.

Key words: Mineral potassium, Biofertilization,Rosemary, Major active compounds.

INTRODUCTION

Medicinal plants occupy a prominent position, because of the increasing demands of both the local industry and exportation. 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 and to improve the growth and the yield of various aromatic and medicinal plants (Hassan *et al*., 2006).

Rosemary (*Rosmarinus officinalis* L.*)* Fam. Lamiaceae (Labiatae) is a shrubby evergreen bush (grows in the Mediterranean countries) up to 2 meters high with silver green needless, shaped leaves and pote blue flowers. The whole plant is strongly aromatic and is one of the important medicinal aromatic and spices plants. It is analgesic, antioxidant, antiseptic carminative, fungicidal, nerving stomachic and toxic. It is, extensively, used in soap manufacture, perfumes, especially meat products, as well as serves as a source of material antioxidants (Lawiess, 1992).

Potassium is an essential macro-element for plant growth and it is important in agriculture practices. It plays an important role in several physiological processes in plant such as energy transfer, formation of sugars, starch, and protein in plant (Kassem, 1997). Furthermore, biofertilization is an important factor being used to produce without some mineral fertilizers that cause environmental pollution problems and high rates of it; leads to decrease the potential activity of microflora and the mobility of organic matters. Hence, the attention has been focused on the researches of biofertilizers to provide as alternative to specific chemical fertilizer. In this context biofertilizers play vital role for increasing the number of microorganisms and accelerate certain microbial process in the rhizosphere of inoculated soil of plants which change unavailable forms to the available forms of some nutrients to the plants (Kandeel *et al.*, 2001; Mohamed and Abdu, 2004; Hassan *et al.*,2006).

This research, hence, is an attempt to find out the best fertilizer combination of chemical fertilizer and biofertilizer that enhance the vegetative growth and chemical composition of rosemary (*Rosmarinus officinalis* L.).

MATERIALS AND METHODS

Two field experiments were carried out at the Horticulture Researches Sabhia Station , Horticulture Research Institute, Agriculture Research Center, Alexandria, Egypt, during two growing seasons of 2014 - 2016, in order to study the effect of potassium and biofertilization on growth and chemical composition of rosemary (*Rosmarinus officinalis* L.).The experimental design of the present study was a randomized complete block design with three replicates. Experimental unit for all treatments contains 9 plants.

The experimental soil analyses during growing season of the present study were determined according to Page *et al*. (1982)as presented in Table (1).

Table (1).The physical and chemical properties of the experimental soil in 2015.

Soil properties	2015
A- Particle size distribution (%)	
Sand%	30.50
Silt%	33.50
Clay%	36.00
Soil texture	Clayey loam soil
B- Chemical analysis	
pH (1:1)	7.40
EC (1:1) dS/m	2.30
1- Soluble cations (1:2) (cmol/kg soil)	
K^+	1.12
Ca^{++}	4.20
Mg^{++}	3.20
Na^+	8.10
2- Soluble anions (1:2) (cmol/kg soil)	
$CO^=_3 + HCO^-_3$	2.90
Cl^-	12.10
$SO^=_4$	0.55
Calcium carbonate (%)	7.80
Total nitrogen (%)	1.20
Available Phosphorus (mg/kg)	3.90
Available K (mg/kg)	170.90

Plant material:

The rooted cuttings were obtained from MAPRD farm in AL- Quanater Alkheria and planted in the field on February 8th 2014 in pots 50 cm diameter , and the treatments were conducted as follows:

1- 10 L Potassmage/fed + biofertilizer (Cerealine + Phosphorein).
2- 15 L Potassmage/fed + biofertilizer (Cerealine + Phosphorein).
3- 20 L Potassmage/fed + biofertilizer (Cerealine + Phosphorein).
4- 10 L Potassmage/fed + NP mineral fertilization.
5- 15 L Potassmage/fed + NP mineral fertilization.
6- 20 L Potassmage/fed + NP mineral fertilization.
7- 150 kg/fed K_2SO_4 + biofertilizer (Cerealine + Phosphorein).
8- 300 kg/fed K_2SO_4 + biofertilizer (Cerealine + Phosphorein).
9- 450 kg/fed K_2SO_4 + biofertilizer (Cerealine + Phosphorein).
10- 150 kg/fed K_2SO_4 + NP mineral fertilization.
11- 300 kg/fed K_2SO_4 + NP mineral fertilization.
12- 450 kg/fed K_2SO_4 + NP mineral fertilization.
13- Control (Recommended dose of NPK)

The plants were taking cut on November 15th 2014 and June 11th 2015 for both growing seasons 2015 and 2016. The plants were left in the field to the second season 2016 and the treatments were reported on them during the second season. The main average of two cut were calculate for all determine characters of two seasons.

Applied mineral fertilizers:

Potassium sulphate (K_2SO_4) (48% K_2O) treatments were used at rates of 150,300 and 450 kg/fedan. The used chemical fertilizers were mixed (NP) as recommended dose as 300 kg ammonium sulfate (20.5% N) per feddan and phosphorus at the rate of 300 kg calcium super phosphate (15.5% P_2O_5) per feddan.NP mineral fertilizers added as constant level for different levels of k_2so_4 and Potassmage (Treatments No.4,5,6,10,11 and 12).The recommended dose of NPK (2:1:1) as control treatment was applied as ammonium sulphate (20.5%N), calcium superphosphate (15.5% P_2O_5) and potassium sulphate (48% K_2O) at (150 kg/fed, respectively) which are the recommended dose.

All mineral fertilizers treatments were divided into three equal parts, the first one was applied one month after sowing time, the second one was applied after the first cut and the third one was applied after the second cut.

Applied bio- fertilizers:

The used bio- fertilizers of bacteria *Bacillus megatherum* and phosphorus dissolving bacteria (P.D.B). Bacillus circulanis (silicate potassium dissolving Bacterea) [K. D. B]. Potassmage contained *Azospirillum Lipoferum* and *Azotobacterea Chroococcum* , and Cerealine provided from National Research Center. Inoculations with biofertilzers were done with irrigation water on May 21 each growing season.

At harvest dates in the two growing seasons, guarded plants were randomly taken from each plot and the following characteristics were recorded:

Vegetative characteristics:

The following data were recorded at harvesting time of each cut season:

1. Plant height (cm).
2. Number of branches per plant.
3. Leaf area index (LAI).
4. Plant fresh weight (g).
5. Plant dry weight (g).

NPK and total carbohydrate % analyses:

Element percentage and total carbohydrate (%) was conducted in dry herb at 70ºC for 72 hours, at constant weight then ground to a fine powder for the determination of both nutrient elements and carbohydrates (Herbert and Philips 1971).

Plant digestion was made on eight of the dried samples (0.2 g) by using a mixture of hydrogen peroxide and sulfuric acid at a ratio (4:10).

1- Nitrogen % was determined using the micro Kieldahl method according to Black (1983)
2- Phosphorus% was determined colorimetrically according to Jackson (1967).
3- Potassium % was estimated using flame photometer method according to Richards (1954).
4- Total carbohydrates percentages in the herb were determined according to Herbert *et al*. (1971).

Rosemary essential oil percentage:

The essential oil percentage was determined in the air dried herb according to British Plarmacopoeia (1963) by solvent method in order to extract the essential oil.

Essential oil analysis and its major's component:

The essential oils (for second growing season) were diluted in diethyl ether (20 ml in 1 ml) and analyzed with GC (HP 8644) with flame ionization detector (FID) on a fused silica 132 capillary column DB-5, 25 m in length, 0.32 mm i.d., and 0.5 mm film thickness.133 Helium was used as the carrier gas with a flow rate of 1.6 ml/min; the detector 134 temperature was 260 °C, the oven temperature was programmed to increase from 130 to 260 °C at a rate of 4 °C/min. The split injector was heated at 250 °C, the split 136 ratio was 15:1. Data were processed on a DP 800 integrator. The percentage of majors constituents (α- Pinene, β- Pinene, Cineol, Camphor, Borneol, acetate and Eugenol) were estimated by measuring the peak area of the different compounds of the chromatogram according to Heftman (1967) and Gunther and Joseph (1978). Sources of the principal components of rosemary which used as reference for determined essential oil of Rosemary by GC were Ciba Gigi, NY, USA. For α- Pinene, β- Pinene, Cineol, Camphor, Borneol, acetate and Eugenol.

Statistical Analysis:

The obtained data were, statistically, analyzed according to Gomez and Gomez (1984). The L.S.D. at 5% level of probability was used to declare the significant for differences among means.

RESULTS AND DISCUSSION

A- Growth parameters:

The obtained results, given in Table (2) cleared that fertilizer's treatments exhibited a significant effect on all estimated traits during both seasons. Application the treatment 150 kg/fed K_2SO_4 + NP mineral, significantly, increased plant height, fresh and dry weights (g/plant), except number of branches/plant which was increased with application of 150 kg/fed K_2SO_4 + biofertilizer NP (Cerealine + Phosphorein)during both seasons. Its could be concluded that the positive effect on growth characters in response to K_2SO_4 fertilizer levels may be attributed to increasing maentration in plant tissues. The previous results agree, more or less, with the finding of Rashed (2002) on parsley, Abdel- Wahab (2000) on rosemary; Kandeel *et al*. (2001) and Mohamed and Abdu (2004) on *Foeniculum vulgare*.

B- Chemical composition and carbohydrate (%):

The data in Table (3) showed that all treatment of fertilization affected, significantly, chemical composition (N, P and K %) and carbohydrate (%) during both seasons. It is clear from data that the highest mean values of chemical composition (P and K%) and carbohydrate (%) resulted from the treatments of 300 kg/fed K_2SO_4 + biofertilizer NP (Cerealine + Phosphorein) in both seasons, and the highest mean value of N% recorded by 450 kg K_2SO_4 + biofertilizer NP (Cerealine + Phosphorein) in both seasons.

The increment of chemical composition (N, P and K%) and carbohydrate content of plant leaves using the treatments of potassium sulfate and (NP) biofertilization may be attributed to increase in the occupancy root zone of plant as a results of adding fertilization treatments which reflected on nutrients uptake by plants and confirm the enhancement of vegetative growth. Similar results, more or less, were obtained by Kassam (1997) on rosemary, Kandeel *et al*. (2001) and Abou- El- Maged *et al.* (2008) on fennel; Rashed (2002) on *Petrselinium sativum*. Likwise, the results showed significant differences for potassium sulfate + NP biofertilization in both seasons , which gave the greatest values for all chemical composition.

Table (2). Growth characters of rosemary plants as affected by potassium and bio- fertilizer during 2015 and 2016 seasons.

Treatments	Plant height (cm)		No. of branches/plant		Fresh weight/plant (g)		Dry weight/plant (g)	
	2015	2016	2015	2016	2015	2016	2015	2016
10 L/fed Potassmage+(Cerealine + Phosphorein)	39.80	45.00	26.07	29.30	200.3	191.4	90.8	91.60
15 L/fed Potassmage + (Cerealine + Phosphorein)	35.50	45.40	24.70	27.30	154.2	154.8	82.70	75.40
20 L/fed Potassmage + (Cerealine + Phosphorein)	36.30	45.40	20.80	25.30	115.4	148.3	60.20	75.8
10 L/fed Potassmage + NP mineral fertilization	40.00	49.60	33.20	31.50	112.2	127.2	58.7	64.7
15 L/fed Potassmage + NP mineral fertilization	42.60	44.97	22.70	33.30	93.5	93.87	46.5	50.90
20 L/fed Potassmage + NP mineral fertilization	35.80	47.60	27.80	33.10	211.3	223.00	97.7	122.30
150 kg K_2SO_4/fed + Cerealine + Phosphorein	33.39	46.00	38.07	33.10	225.4	243.30	98.9	123.6
300 kg K_2SO_4/fed + Cerealine + Phosphorein	31.16	31.87	39.70	43.90	309.5a	310.60c	100.3	172.3
450 kg K_2SO_4/fed + Cerealine + Phosphorein	30.90	43.20	26.00	24.60	171.5	153.10	97.5	80.00
150 kg K_2SO_4/fed + NP mineral fertilization	31.10	46.80	22.80	22.47	178.4	199.80	88.6	142.8
300 kg K_2SO_4/fed + NP mineral fertilization	44.26a	58.90a	31.70	43.70	28.40a	439.40a	201.6a	223.00a
450 kg K_2SO_4/fed + NP mineral fertilization	35.03	52.97	36.50	39.60	207.50	228.60	157.0	185.5
Control	40.07	54.70	35.70	39.20	292.10	267.50	150.4	190.6
L.S. D. $_{0.05}$	**0.90**	**0.95**	**0.30**	**0.50**	**4.7**	**5.60**	**3.7**	**4.50**

Table (3). Chemical composition of rosemary as affected by potassium and biofertilizer during 2015 and 2016 seasons.

Treatments	Nitrogen (%)		Phosphorus (%)		Potassium (%)		Carbohydrate (%)	
	2015	2016	2015	2016	2015	2016	2015	2016
10 L/fed Potassmage + (Cerealine + Phosphorein)	3.10	3.30	0.43	0.52	1.62	1.65	43.60	44.20
15 L/fed Potassmage + (Cerealine + Phosphorein)	1.72	1.63	0.51	0.55	1.45	1.47	43.10	41.00
20 L/fed Potassmage + (Cerealine + Phosphorein)	2.30	2.41	0.59	0.63	1.48	1.52	44.50	38.00
10 L/fed Potassmage + NP mineral fertilization	2.61	2.70	0.60	0.67	1.50	1.53	41.60	40.70
15 L/fed Potassmage + NP mineral fertilization	1.90	1.98	0.46	0.52	1.56	1.60	45.70	41.30
20 L/fed Potassmage + NP mineral fertilization	1.75	1.88	0.45	0.68	1.59	1.64	50.70a	51.50a
150 kg K_2SO_4/fed + Cerealine + Phosphorein	2.44	2.66	0.71	0.79	1.63	1.67	46.40	47.30
300 kg K_2SO_4/fed + Cerealine + Phosphorein	2.85	2.72	0.65	0.73	1.69	1.71	45.30	46.20
450 kg K_2SO_4/fed + Cerealine + Phosphorein	3.98	3.50	0.54	0.63	1.65	1.70	44.70	48.60
150 kg K_2SO_4/fed + NP mineral fertilization	2.70	2.60	0.52	0.55	1.73	1.84	49.60	49.80
300 kg K_2SO_4/fed + NP mineral fertilization	2.65	2.75	0.50	0.58	1.57	1.66	40.80	42.70
450 kg K_2SO_4/fed + NP mineral fertilization	3.04	3.20	0.46	0.53	1.67	1.70	42.90	43.10
Control	1.90	1.88	0.43	0.47	1.20	1.38	40.50	33.00
L.S. D. $_{0.05}$	**0.40**	**0.30**	**0.04**	**0.06**	**0.05**	**0.16**	**0.51**	**0.48**

C- Essential oil (%):

The essential oil percentage was affected by using potassium fertilization during both seasons; whereas, the highest essential oil % was found to be recordes due to the application of 10 L Potassmage/fed + (NP mineral fertilization) during both seasons as shown in Table (4). This may finding be taken place due to the synergiatic effect of biofertilizers (Potassmage + Phosphorein + Cerealine) to increase the availability of NPK to be uptake by plants at a considerable rate to build up more metabolites necessary for including the volatile oil synthesis. These results were in agreement with those of Abdel Wahab (2000) on rosemary who used 100% NPK + organic manure, which gave the highest values of volatile oil percentage in both seasons.

Table (4). Volatile oil (%) as affected by potassium and biofertilizer during 2015 and 2016 seasons.

Treatments	2015	2016
10 L/fed Potassmage + (Cerealine + Phosphorein)	0.42	0.50
15 L/fed Potassmage + (Cerealine + Phosphorein)	0.44	0.70
20 L/fed Potassmage + (Cerealine + Phosphorein)	0.51	0.75
10 L/fed Potassmage + NP mineral fertilization	0.57a	0.80a
15 L/fed Potassmage + NP mineral fertilization	0.38	0.45
20 L/fed Potassmage + NP mineral fertilization	0.54	0.76
150 kg K_2SO_4/fed + Cerealine + Phosphorein	0.53	0.74
300 kg K_2SO_4/fed + Cerealine + Phosphorein	0.51	0.73
450 kg K_2SO_4/fed + Cerealine + Phosphorein	0.48	0.55
150 kg K_2SO_4/fed + NP mineral fertilization	0.45	0.54
300 kg K_2SO_4/fed + NP mineral fertilization	0.48	0.57
450 kg K_2SO_4/fed + NP mineral fertilization	0.55	0.77
Control	0.53	0.66
L.S. D. $_{0.05}$	**0.03**	**0.04**

D- Major components percentage of essential oil:

The effect of fertilization treatments on essential oil's major compound (α-Pinene, β- Pinene, Cineol, Camphor, Borneol, acetate and Eugenol) percentages are shown in Table (5). The results indicated that using potassium's fertilization which had significant effect on the studied major compound percentages of rosemary oil contents. The application of different fertilization; gave rise to the highest percentages of major compounds in 2014/2015 season. Similar results were reported by Darzi *et al*. (2006) on fennel, and Ismal *et al.* (2009) on Majoram plant.

Table (5). Chemical composition of rosemary oils major components (%) during 2016 season.

T/comp.	α- Pinene	β- Pinene	Cineol	Camphor	Borneol	Borneol acetate	Eugenol
1	12.07	3.01	13.92	17.52	11.49	6.03	2.44
2	14.60	3.75	18.97	22.63	12.84	6.89	2.67
3	15.20	5.13	19.13	21.72	9.14	7.27	1.24
4	16.82	4.91	19.44	23.51	10.06	7.09	1.84
5	12.22	3.46	17.63	21.11	15.80	9.00	1.32
6	10.90	3.12	20.52	27.52	11.88	9.98	2.28
7	14.87	3.34	21.76	27.01	11.01	9.83	2.01
8	13.99	3.37	21.88	27.25	10.26	9.31	2.00
9	14.1	4.02	21.91	27.58	10.06	9.02	1.91
10	13.71	4.39	21.12	28.53	9.26	8.10	1.32
11	16.89	4.32	21.53	23.45	7.76	8.43	1.28
12	15.71	3.33	19.72	23.72	7.48	7.76	1.29
13	11.99	3.50	19.25	24.66	13.75	10.76	1.31
L.S.D.$_{0.05}$	**0.05**	**0.03**	**0.50**	**0.40**	**0.30**	**0.45**	**0.12**

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الملخص العربي

تأثير التسميد البوتاسي والتلقيح الحيوي على النمو الخضري ومكونات الزيت العطري لنباتات حصالبان

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أقيمت التجربة الحقلية بمحطة بحوث البساتين بالصبحية ،معهد بحوث البساتين، مركز البحوث الزراعية ، إسكندرية، مصر، أثناء موسمي نمو ممتد من ٢٠١٥ الى ٢٠١٦ لدراسة تأثير التسميد المعدني والحيوي البوتاسي على النمو والمحتوى الكيماوي والنسبة المئوية للزيت لنباتات الحصالبان– صممت التجربة بتصميم القطاعات العشوائية الكاملة مع ثلاث مكررات ويمكن تلخيص أهم النتائج فيما يلي:

معاملات التسميد أثرت بقيم مختلفة على كل الصفات عند إضافة المعاملة ١٥٠كجم كبريتات البوتاسيوم/فدان + التسميد المعدني للنتروجين والفوسفور زادت معنوية طول النبات، الوزن الطازج والجفاف (جم/نبات). مع إضافة المعاملة ٣٠٠ كجم كبريتات بوتاسيوم/فدان + الفوسفورين + السيريالين أعطت أعلى محتوى كيماوي (النسبة المئوية الفوسفور والبوتاسيوم) والنسبة المئوية للكربوهيدرات في كلا الموسمين.أيضاً إضافة ٤٥٠ كجم كبريتات بوتاسيوم/فدان + سيريالين + فوسفورين أدت إلى زيادة النسبة المئوية للنتروجين في كلا الموسمين. أدى إضافة ٢٠ لتر بوتاسيوماج /فدان + الفوسفورين + السيريالين أعلى نسبة مئوية للزيت. أيضاً اختلافات التسميد أدى إلى ارتفاع المكونات الفعالة للزيت (– Pinene, –Pinene, Cineol, Camphor, – Borneol, acetate and Eugenol) كنسبة مئوية في الموسم ٢٠١٥.