

Effect of Nitrogen and Potassium Biofertilization on Growth, Yield and Essential Oil Production of White Horehound, *Marrubium vulgare* L. Plant

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Abstract: *Marrubium vulgare* L., family Lamiaceae, is a new important pharmaceutical plant cultivated recently under Egyptian conditions. Two field experiments were conducted to study the effect of nitrogen (N0, N1, N2 and N3) (Biofert) and potassium (K0, K1, K2 and K3) (Available K) biofertilizers and interaction between them on growth, herb yield, essential oil yield and oil constituents of white horehound plant during two consecutive seasons of 2011 and 2012. The results in both seasons pointed out that 20 L/ feddan (feddan = 4200 m²) of nitrogen or potassium biofertilizers significantly increased the plant height, fresh and air dry herbs weights and essential oil yield per plant and per feddan compared to control plants (without inoculation). Similar effects were noticed in combination treatments between nitrogen and potassium biofertilizers at high concentration for each at 20 L/ feddan (N3 K3) treatment compared with unfertilized control plants (N0 K0 treatment). C-MS analysis was performed to detect the essential oil compounds. Eleven components have been identified in the white horehound essential oil representing 88.36 and 87.35% of the oil in both seasons. The major components were β -caryophyllene (27.62 and 28.11%) and Germacrene-D (25.87 and 23.35%) in the first and second seasons, respectively. The other components of the oil were delta-Cadinene, α -Copaene, Humulene, β -Bourbonene, Nerolidol, Caryophyllene oxide, γ -Terpinene, D-Limonene and Sabinene. Generally β -Caryophyllene, Germacrene-D% and some other components unaffected by biofertilization treatments.

Keywords: *Marrubium vulgare* • Nitrogen • Potassium • Biofertilizers • Essential oil • β -Caryophyllene • Germacrene-D

INTRODUCTION

White horehound (*Marrubium vulgare* L.) plant belongs to the family Lamiaceae, native to Europe; northern Africa and Asia. It is a gray-leaved herbaceous perennial plant, which grows to 25-45 cm tall. The leaves are 2-5 cm long with a densely crinkled surface and are covered in downy hairs. The flowers are white, borne in clusters on the upper part of the main stem.

Egyptian Priests called this plant the Seed of Horus, or the Bull's Blood and the Eye of the Star. It was a principal ingredient in the Caesar's antidote for vegetable poisons. Gerard recommends it, in addition to its uses in coughs and colds, to those that have drunk poison or have been bitten of serpents and it was also administered for mad dog's biting. It was once regarded as an

anti-magical herb. Horehound is a serviceable remedy against Cankerworm in trees and it is stated that if it be put into new milk and set in a place pestered with flies, it will speedily kill them all. Aqueous extracts of *Marrubium vulgare* L. provides a source of natural antioxidants, which inhibit LDL oxidation and enhance reverse cholesterol transport and thus can prevent cardiovascular diseases development. These antioxidant properties increase the anti-atherogenic potential of HDL [1].

Many investigators studied the effect of organic and biofertilizers on the growth and essential oil yield and constituents of medicinal and aromatic plant [2-9].

The composition of the essential oil obtained from the dried flowering aerial parts of *Marrubium vulgare* L. (Labiatae) was determined by GC and GC/MS. Morteza-Semnani *et al.* [10] identified twenty components

in the essential oil of *M. vulgare*. The major constituents of the essential oil were beta -bisabolene (20.4%), delta -cadinene (19.1%) and isocaryophyllene (14.1%). Mahnaz-Khanavi *et al.* [11] identified 34 components in the essential oil of *M. vulgare* that by GC-MS representing 95.1% of the total oil β -bisabolene (25.4%), β -Caryophyllene (11.6%), Germacrene-D (9.7%) and β -farnesene (8.3%) as the major components of white horehound.

Nitrogen is most recognized in plants for its presence in the structure of protein molecule, it plays an important role in synthesis of plant constituents through the action of different enzymes. Potassium is a key essential plant nutrient although it is not a constituent of any plant part and it acts as catalyst for many of the enzymatic processes which are necessary for plant growth. It also regulates the opening and closing of stomata which affect carbon dioxide uptake for photosynthesis.

The objective of this study was to investigate the effect of nitrogen and/or potassium as biofertilizers on growth and essential oil yield and constituents of *Marrubium vulgare* L. plants.

MATERIALS AND METHODS

The field experiment in this study was carried out in Sekem Company Experimental Farm in Bilbes, Sharqia Governorate, Egypt (50 km North Cairo) and laboratories of the National Research Center, Medicinal and Aromatic Plants Department, Dokki, Giza, Egypt during the two successive seasons of 2011 and 2012.

Layout of the Experiment: This experiment was designed using a factorial split plot design, with 16 treatments with three replicates (4 nitrogen treatments x 4 potassium

treatments, including the control). The nitrogen treatments were assigned to the main plots, while potassium treatments were assigned to the sub-plots.

Experimental Procedures

Plant Material: White horehound (*Marrubium vulgare* L.) seeds were imported from Jellitto Standensamen GmbH, Schwarmstedt, Germany by Sekem Company, Egypt through Dr. S. Hendawy, National Research Center, Dokki, Giza, Egypt. The seeds were sown on 15th February 2011 and 2012 (in the two seasons) in nursery beds inside greenhouse in peat moss medium. One month and half (45 days) after sowing, the seeds on 1st April 2011 and 2012, seedlings of 10-15 cm height were transplanted to prepared plots in the experimental field (certified field for organic production). The physical and chemical characteristics of the soil of the experimental field were determined according to Jackson [12] and are shown in Table 1.

The soil was prepared on 15th January in the both seasons, EL-Nile compost was added at rate of 10 m³/ feddan during the soil preparation. The experimental field was divided into 48 plots; each plot was 1×2.8 m (2.8 m²) and divided into 2 rows with 50 cm apart and 35 cm between plants. The seedlings were planted in each plot on 1st April in both seasons, at 35 cm between each two plants adjacent to drippers line (2-line), which were 50 cm apart and each line contains 8 seedlings (each plot contains 16 seedlings).

Biofertilization Treatments: Nitrogen biofertilizer (Biofert) contains nitrogen fixing bacteria of *Azotobacter* spp., *Azospirillum* spp., *Klebsiella* spp., *Closteridium* spp., *Streptomyces* spp., *Thermoactinomyces* spp. and *Pseudomonas* spp, the total count of bacteria was

Table 1: Mechanical and chemical analysis of the experimental soil

Mechanical analysis										
	Very coarse sand (2-1 mm)	Coarse sand (1-0.5mm)	Medium sand (0.5-0.25mm)	Fine sand (0.25-0.1mm)	Veryfine sand (0.1-0.05mm)	Silt+Clay (0.05>mm)	Texture			
Year										
2011	41.42	42.99	0.12	9.38	4.85	1.24	Sandy			
2012	35.72	49.86	0.13	8.94	4.18	1.17	Sandy			
Chemical analysis										
Millie equivalent/Liter										
		E.C. (dSm ⁻¹)	Cations				Anions			
	pH (2.5:1)	(5:1)	Ca ⁺⁺	Mg+ +	Na ⁺	K ⁺	CO ₃ ⁻	HCO ₃ ⁻	Cl ⁻	SO ₄ ⁻
2011	8.26	2.5	7.0	3.0	10.5	0.9	Nil	2.6	10.8	7.9
2012	8.15	2.1	9.0	4.5	10.9	0.8	Nil	2.2	10.9	12.1

9.1×10^8 cell/ml. Potassium biofertilizer (Available K) contains potassium solubilizing bacteria such as: *Clostridium* spp., *Bacillus* spp. and *Azospirillum* spp., the total count of bacteria was 1.4×10^7 cell/ml. The liquid biofertilizers (Biofert and Available K) were obtained from laboratories of Sekem Co., Cairo, Egypt.

The different treatments of nitrogen and potassium biofertilizers were added as soil drench adjacent to the seedling on 1st May in both seasons. Nitrogen biofertilizer treatments were applied at rate of 0, 10, 15 and 20 L/ feddan (N0, N1, N2 and N3, respectively), while potassium biofertilizer treatments applied at rate of 0, 10, 15 and 20 L/feddan (K0, K1, K2 and K3, respectively). Both biofertilizers were added separately or in combination with the other treatments. All plants received common agricultural practices including regular irrigation and manual weed control.

Treatments:

The biofertilization treatments were as follows:

1-N0 K0 (control)	9- N2 K0
2-N0 K1	10-N2 K1
3-N0 K2	11-N2 K2
4-N0 K3	12-N2 K3
5-N1 K0	13-N3 K0
6-N1 K1	14-N3 K1
7-N1 K2	15-N3 K2
8-N1 K3	16-N3 K3

These treatments were carried out for each two successive seasons.

Recorded Data

Vegetative Growth: The plants were harvested, at the early bloom stage (on 15th July, 2011 and 2012 in the two seasons, respectively). The plants were harvested by cutting vegetative parts 10 cm above the soil surface. Data were recorded in the two seasons as the following:

- Plant height (cm)
- Fresh weight g/plant: Fresh weight g/plant was measured immediately after harvest.
- Fresh weight of herb (Kg/ fed).
- Air dry weight of herb (g/plant).
- Air dry weight of herb (Kg/ fed).

Essential Oil Production:

- Essential oil % in fresh herb.

The oil percentage was determined in fresh herb in both seasons using the hydro-distillation method described by Guenther [13]. The extracted volatile oil was dehydrated by anhydrous sodium sulphate and stored in refrigerator until GC-MS analysis.

Essential Oil Yield/Plant (ml):

Essential oil yield per plant = oil % \times herb fresh weight/plant.

- Essential oil yield/ feddan (L)

Oil yield per feddan = [oil yield/ plant (ml) \times number of plants/fed*]/ 1000

*Number of plants/fed for (50 \times 35) =22857 plants/fed.

- Essential oil components

Samples taken from the oil obtained in the two seasons were analyzed using GC-MS analysis, to determine their main constituents. The use of GC-MS in the quantitative determinations was performed using the methods described by Adams [14].

Statistical Analysis of Data: Recorded data on growth yield, oil content and oil yield were statistically analyzed and separation of means was performed using the Least Significant Difference (L.S.D.) test at the 5% level, as described by Little and Hills [15].

RESULTS AND DISCUSSION

Vegetative Growth and Herb Yield

Plant Height: Data presented in Table 2 show the effect of N and K biofertilizers and their interaction on height of horehound plants in the two seasons.

Nitrogen biofertilization treatments (N2 and N3) significantly increased plant height compared to untreated plants (N0) in both seasons, while N1 treatment insignificantly increased plant height compared to N0 treatment in the first season and insignificantly decreased in the second season, the values were 41.71 and 38.92 cm. for N1 and N0, respectively in the first season and 43.86 and 44.91 cm for N1 and N0, respectively in the second season.

Potassium biofertilization treatments (K1, K2 and K3) significantly increased plant height compared to K0 in the first season, similar results were obtained with K2 and K3 treatments in the second season when compared to K1 and K0 treatments.

Table 2: Effect of nitrogen and potassium biofertilizers as well as their interactions on plant height (cm) of *Marrubium vulgare* L. plant in the two seasons 2011 and 2012

First season,2011					
Treatments	N0	N1	N2	N3	Mean
K0	33.10	40.22	41.16	42.60	39.15
K1	40.66	42.63	45.00	45.30	43.39
K2	41.30	42.23	43.30	44.50	42.83
K3	40.63	41.76	42.50	45.10	42.49
Mean	38.92	41.71	42.99	44.25	
LSD at 0.05 N			3.41		
K			3.41		
N x K			6.82		
Second season,2012					
Treatments	N0	N1	N2	N3	Mean
K0	35.96	42.20	46.30	48.63	43.27
K1	44.43	43.43	45.50	48.63	45.50
K2	47.96	45.63	51.06	53.96	48.15
K3	51.30	44.20	54.30	54.16	50.99
Mean	44.91	43.86	49.29	51.35	
LSD at 0.05 N			4.03		
K			4.03		
N x K			8.05		

N0,N1,N2 and N3 = N biofertilizer at 0,10,15 and 20 L Biofert/fed., respectively

K0,K1,K2 and K3 = K biofertilizer at 0,10,15 and 20 L Available K/fed., respectively

Regarding the interaction between the effects of nitrogen and potassium biofertilizers on plant height, data presented in Table 2 show significant differences between the heights of plants receiving the various combinations of these two elements in both seasons. In the first season, all combination treatments significantly increased the plant height when compared to N0K0 treatment (control). In the second season, N2K3, N3K3 and N3K2 treatments produced significantly higher plants compared to the other combination treatments and N0K0 un-inoculated plants (control).

The favorable effect of nitrogen fertilization may be attributed to the fact that nitrogen is the most essential element for the plants, leads to the production of extra protein and allows the leaves of the plants to grow larger and hence to have larger surface area available for photosynthesis. Potassium is essential for many physiological processes such as photosynthesis, activation of enzymes, protein synthesis and stomatal movements.

Similar increases in the height of plants receiving organic and biofertilizers have been reported by other studies [16- 19].

Fresh Herb Weight (gm / plant and kg / feddan):

Data presented in Tables 3 and 4 show the effect of N and K biofertilizers and their interaction on fresh weight gm/plant and kg / feddan of *Marrubium vulgare* L. plants in the two growing seasons.

Nitrogen biofertilizer at higher concentration N3 had a significant effect on herb fresh weight per plant and per feddan, the values were 180.15 gm and 4117.69 kg, respectively compared with other N levels N2, N1 and N0 (unfertilized plants). The averages were 168.43, 146.05 and 128.31g/plant, respectively and 3849.88, 3338.26 and 2944.55 Kg/feddan respectively in the first season.

The results in the same Tables 3 and 4 show that fresh weight gm/plant or kg/feddan were significantly affected by potassium biofertilization treatments K3 and K2 compared with control plants K0 (un-inoculated plants). Application of K2 (15L/feddan) treatment increased both fresh weight gm/plant and Kg/feddan than K1 treatment, but these increases were insignificant between them in the first season.

Regarding the interaction effect between nitrogen and potassium biofertilization on the fresh herb weight of horehound plants g per plant and Kg per feddan,

Table 3: Effect of nitrogen and potassium biofertilizers as well as their interactions on fresh weight g / plant of *Marrubium vulgare* L. plant in the two seasons 2011 and 2012

First season,2011					
Treatments	N0	N1	N2	N3	Mean
K0	105.36	136.33	158.00	168.30	142.00
K1	130.33	143.66	170.53	175.12	154.91
K2	138.66	148.86	171.50	181.76	160.20
K3	140.90	155.33	173.70	195.40	166.33
Mean	128.31	146.05	168.43	180.15	
LSD at 0.05 N			9.41		
K			9.41		
N x K			18.81		
Second season,2012					
Treatments	N0	N1	N2	N3	Mean
K0	153.50	160.78	172.93	189.77	169.25
K1	173.82	179.24	196.30	214.84	191.05
K2	175.83	199.13	200.22	215.44	197.66
K3	182.04	202.85	204.90	215.76	201.39
Mean	171.30	185.50	204.90	208.95	
LSD at 0.05 N			15.05		
K			15.05		
N x K			30.10		

N0,N1,N2 and N3 = N biofertilizer at 0,10,15 and 20 L Biofert/fed., respectively

K0,K1,K2 and K3 = K biofertilizer at 0,10,15 and 20 L Available K/fed., respectively

Table 4: Effect of nitrogen and potassium biofertilizers as well as their interactions on fresh weight (kg/ feddan)of *Marrubium vulgare* L. plant in the two seasons 2011 and 2012

First season,2011					
Treatments	N0	N1	N2	N3	Mean
K0	2408.36	3116.17	3611.40	3846.83	3245.69
K1	2979.02	3283.78	3897.87	4002.72	3540.95
K2	3169.50	3402.64	3919.97	4154.64	3661.69
K3	3221.31	3550.45	3970.26	4466.25	3802.07
Mean	2944.55	3338.26	3849.88	4117.69	
LSD at 0.05 N			215.00		
K			215.00		
N x K			429.90		
Second season,2012					
Treatments	N0	N1	N2	N3	Mean
K0	3508.70	3674.94	3952.73	4337.68	3868.51
K1	3973.08	4096.88	4486.83	4910.77	4366.89
K2	4019.10	4551.51	4576.43	4924.31	4517.84
K3	4160.88	4629.07	4683.47	4931.70	4601.28
Mean	3915.44	4238.10	4424.87	4776.12	
LSD at 0.05 N			392.70		
K			392.70		
N x K			785.30		

N0,N1,N2 and N3 = N biofertilizer at 0,10,15 and 20 L Biofert/fed., respectively

K0,K1,K2 and K3 = K biofertilizer at 0,10,15 and 20 L Available K/fed., respectively

Table 5: Effect of nitrogen and potassium biofertilizers as well as their interactions on air dry weight (gm /plant) of *Marrubium vulgare* L. plant in the two seasons 2011 and 2012

First season,2011					
Treatments	N0	N1	N2	N3	Mean
K0	33.05	47.68	43.55	49.94	43.56
K1	44.32	45.89	55.09	50.97	49.07
K2	39.25	47.87	56.62	62.65	51.60
K3	45.82	49.46	54.96	54.36	51.15
Mean	40.61	47.73	52.56	54.48	
LSD at 0.05	N		4.33		
	K		4.33		
	N x K		8.65		
Second season,2012					
Treatments	N0	N1	N2	N3	Mean
K0	48.16	56.23	47.67	56.32	52.10
K1	59.11	57.25	63.42	62.53	60.58
K2	49.75	64.03	66.11	74.26	63.54
K3	59.19	65.43	70.11	66.54	65.32
Mean	54.05	60.74	61.83	64.91	
LSD at 0.05	N		6.33		
	K		6.33		
	N x K		12.67		

N0,N1,N2 and N3 = N biofertilizer at 0,10,15 and 20 L Biofert /fed., respectively

K0,K1,K2 and K3 = K biofertilizer at 0,10,15 and 20 L Available K/fed., respectively

data recorded in Tables 3 and 4 showed significant differences in the fresh herb weight of plant and per feddan. In both seasons, N3K3 treatment (high level of N and K) significantly increased the fresh weight per plant and per feddan, the averages were 195.40 gm and 4466.25 Kg, respectively compared with control plants (N0K0), with mean values of 105.36 gm/plant and 2408.36 Kg/feddan in first season.

In the second season, similar results were recorded as in the first one, there were steady increase in fresh weight g/plant and Kg/ feddan with increasing the concentration of the both biofertilizers (N and K) compared with control plants.

Also, the same trend was noticed with the interaction treatments as showed in the first season, i.e. N3K3 treatment significantly increased fresh weight per plant and per feddan compared to N0K0 treatment (control plants).

Biofertilizers exert a positive effect on plant growth. The mechanism can be through the synthesis of phytohormones, i.e. nitrogen fixing bacteria such as Azotobacter and Azospirillum have the ability to not only fix nitrogen but also release certain phytohormones of GA₃ and IAA nature which could stimulate plant growth and absorption of nutrients.

Similar increase in fresh weight as a result of biofertilization treatments have been reported [19- 23].

Air Dry Herb Weight (g/plant and kg/feddan): Data presented in Tables 5 and 6 show the effect of N and K biofertilizers and interaction between them on air dry herb weight g/plant and Kg / feddan of *Marrubium vulgare* L. plants in the two growing seasons.

Nitrogen biofertilization treatments N2 and N3 (15 and 20 L / feddan) applied to *Marrubium vulgare* L. plant significantly increased dry herb weight than K0 control in both seasons (with mean values of 52.56, 54.48, 61.83 and 64.91 g/plant in the first and second seasons, respectively) in comparison with N0 and N1 treatments in the first season and N0 treatment only in the second one. Furthermore N1 treatment in the second season also significantly increased herb dry weight. Similar results recorded in the air dry herb weight kg/feddan in both seasons.

Application of potassium biofertilizer to horehound plants significantly increased air dry weight in both seasons (with mean values of 49.07, 51.60 and 51.15 gm/plant in the first season, respectively, while the mean values were 60.58, 63.54 and 65.32 gm/plant in the second season, respectively) in comparison with control (K0) which resulted the values of 43.56 and 52.10 gm/plant in

Table 6: Effect of nitrogen and potassium biofertilizers as well as their interactions on air dry weight (kg/ feddan) of *Marrubium vulgare* L. plant in the two seasons 2011 and 2012

First season, 2011					
Treatments	N0	N1	N2	N3	Mean
K0	755.57	1089.89	995.49	1141.55	995.63
K1	1013.09	1049.05	1259.26	1165.09	1121.62
K2	896.90	1094.23	1294.16	1431.99	1179.32
K3	1047.38	1130.50	1256.37	1242.65	1169.23
Mean	928.24	1090.92	1201.32	1245.32	
LSD at 0.05	N		98.89		
	K		98.89		
	N x K		197.80		
Second season, 2012					
Treatments	N0	N1	N2	N3	Mean
K0	1100.94	1285.32	1089.59	1420.57	1224.11
K1	1351.07	1308.71	1449.66	1429.40	1384.71
K2	1137.21	1463.68	1511.07	1697.35	1452.33
K3	1352.98	1495.68	1602.65	1519.61	1492.73
Mean	1235.55	1388.35	1413.24	1516.73	
LSD at 0.05	N		144.80		
	K		144.80		
	N x K		289.60		

N0, N1, N2 and N3 = N biofertilizer at 0, 10, 15 and 20 L Biofert/fed., respectively

K0, K1, K2 and K3 = K biofertilizer at 0, 10, 15 and 20 L Available K/fed., respectively

the first and second seasons, respectively. Similar results recorded in the air dry herb weight kg/feddan in both seasons.

Regarding the interaction between nitrogen and potassium biofertilization treatments on air dry herb weight g/plant of *Marrubium vulgare* L. in both seasons, data presented in Table 5 show significant differences between air dry weights (g/plant) resulted from application of various combinations of nitrogen and potassium treatments. Maximum values of air dry weight (g/plant) were observed with N3K2 treatment (with mean values of 62.65 and 74.26 g/plant in the first and second seasons, respectively) in comparison with other different combination treatments. The minimum values of air dry herb weight were recorded with N0K0 (control) and N0K2 treatments, the values were 33.05 and 39.25 g/plant, respectively in the first season and N2K0 treatment (47.67 g/plant) in the second season. Similar results recorded in air dry yield (kg /feddan) in both seasons (Table 6).

The favorable effect of nitrogen fertilization may be due to the fact that N is the most essential element for plant growth [24]. Potassium is essential for many physiological processes, such as photosynthesis, activation of enzymes, protein synthesis, osmotic regulator and stomatal movements [25]. These results agreed with results of other researches [8, 19, 26].

Essential Oil Production

Essential Oil % in Fresh Herb: Data recorded on the essential oil percentage in the fresh herb of *Marrubium vulgare* L. plants in the two seasons (Table 7) show no significant differences in the essential oil percentage in the plants fertilized by nitrogen biofertilization at rates of N0, N1, N2 and N3 (the mean values were 0.046, 0.043, 0.041 and 0.046% in the first season, respectively and 0.041, 0.043, 0.043 and 0.043% in the second season, respectively). N1 and N2 treatments slightly decreased essential oil % compared with N0 (control) and high N level (N3) treatments in the first season. All N levels (N1, N2 and N3) slightly increased essential oil percentage in the second season.

Potassium biofertilizer at rates of K1, K2 and K3 significantly increased volatile oil % compared to control plants (K0) in the first season. On the other hand, all K levels had insignificant effect on the essential oil % in the second season.

Regarding the interaction between the effects of nitrogen and potassium biofertilizer on the essential oil percentage, the data presented in Table 7 show significant differences between in essential oil percentages in plants fertilized with various combinations in the first season. Maximum essential oil percentages were recorded in plants fertilized by N0 K3 and N1 K3 treatments, with averages of 0.060 and 0.051 %, respectively.

Table 7: Effect of nitrogen and potassium biofertilizers as well as their interactions on essential oil % of *Marrubium vulgare* L. plant in the two seasons 2011 and 2012

First season, 2011					
Treatments	N0	N1	N2	N3	Mean
K0	0.030	0.029	0.030	0.046	0.034
K1	0.045	0.045	0.042	0.046	0.045
K2	0.048	0.046	0.046	0.045	0.046
K3	0.060	0.051	0.045	0.045	0.050
Mean	0.046	0.043	0.041	0.046	
LSD at 0.05	N		n.s		
	K		0.009		
	N x K		0.018		
Second season, 2012					
Treatments	N0	N1	N2	N3	Mean
K0	0.035	0.044	0.045	0.043	0.042
K1	0.040	0.044	0.041	0.043	0.042
K2	0.046	0.042	0.043	0.045	0.044
K3	0.043	0.042	0.041	0.039	0.041
Mean	0.041	0.043	0.043	0.043	
LSD at 0.05	N		n.s		
	K		n.s		
	N x K		n.s		

N0, N1, N2 and N3 = N biofertilizer at 0, 10, 15 and 20 L Biofert/fed., respectively

K0, K1, K2 and K3 = K biofertilizer at 0, 10, 15 and 20 L Available K/fed., respectively

On the contrary, the minimum oil percentage (0.029%) was recorded in N1K0 treatment.

In the second season, all combination treatments of N and K biofertilizers had insignificant effect on the essential oil percentages, this mean that N and K biofertilizer treatments had no effect on synthesis and accumulation of the essential oil in *Marrubium vulgare* L. plants in this season which may be due to some environmental factors, such as temperature or relative humidity.

The increase in fresh herb essential oil % with the increase in potassium at high concentration (K3) might be due to an important role of K in the metabolic processes like photosynthesis, respiration and carbohydrate synthesis, this leads to increase of the essential oil synthesis.

Similar increase in the essential oil % of plants fertilized with organic and biofertilizers were reported by other works [2, 5, 9]. They reported that biofertilization treatments increased the percent of essential oil.

Essential Oil Yield (ml/plant and L/feddan): The results presented in the two seasons (Tables 8 and 9) show that the essential oil yield/plant and per feddan of *Marrubium*

vulgare L. plants were significantly affected by nitrogen biofertilizer. In both seasons, plants fertilized with the highest level (N3) gave the highest essential oil yield with mean values of 0.082 and 0.089 ml/plant in the first and second seasons, respectively. These values were significantly higher than those obtained from non-inoculated plants (N0) which gave essential oil yields of 0.061 and 0.073 ml/plant in the two seasons, respectively. Plants treated by N1 gave 0.063 ml/plant in the first season. Similar results were recorded on essential oil yield L/feddan in both seasons.

Data recorded in Tables 8 and 9 also show that the essential oil yield (ml/ plant) was increased significantly in both seasons as a result of applying the K2 and K3 treatments compared to the un-inoculated control plants. The mean values were 0.074 and 0.083 compared to 0.049 ml/plant, respectively in the first season, while the mean values were 0.087 and 0.083 ml/plant compared to 0.071 ml/plant, respectively in the second season. Generally, similar results were obtained on the essential oil yield L/feddan in the first season, while in the second season, all K biofertilizer levels gave essential oil yield ranging from 1.614 to 1.988 L/feddan with insignificant differences between treatments.

Table 8: Effect of nitrogen and potassium biofertilizers as well as their interactions on oil yield (ml/plant) of *Marrubium vulgare* L. plant in the two seasons 2011 and 2012

First season, 2011					
Treatments	N0	N1	N2	N3	Mean
K0	0.032	0.039	0.047	0.077	0.049
K1	0.059	0.064	0.072	0.081	0.069
K2	0.066	0.068	0.079	0.082	0.074
K3	0.085	0.079	0.078	0.088	0.083
Mean	0.061	0.063	0.069	0.082	
LSD at 0.05 N			0.012		
K			0.012		
N x K			0.024		
Second season, 2012					
Treatments	N0	N1	N2	N3	Mean
K0	0.053	0.070	0.078	0.081	0.071
K1	0.079	0.079	0.081	0.093	0.083
K2	0.081	0.084	0.086	0.097	0.087
K3	0.079	0.085	0.084	0.084	0.083
Mean	0.073	0.080	0.082	0.089	
LSD at 0.05 N			0.012		
K			0.012		
N x K			0.024		

N0, N1, N2 and N3 = N biofertilizer at 0, 10, 15 and 20 L Biofert/fed., respectively

K0, K1, K2 and K3 = K biofertilizer at 0, 10, 15 and 20 L Available K/fed., respectively

Table 9: Effect of nitrogen and potassium biofertilizers as well as their interactions on oil yield (L / feddan) of *Marrubium vulgare* L. plant in the two seasons 2011 and 2012

Season,2011 and 2012					
First season,2011					
Treatments	N0	N1	N2	N3	Mean
K0	0.723	0.899	1.082	1.752	1.114
K1	1.342	1.455	1.638	1.844	1.570
K2	1.516	1.562	1.806	1.874	1.690
K3	1.935	1.813	1.790	2.012	1.888
Mean	1.379	1.432	1.579	1.871	
LSD at 0.05 N	K	N x K	0.1320.1320.264		
Second season,2012					
Treatments	N0	N1	N2	N3	Mean
K0	1.204	1.608	1.783	1.859	1.614
K1	1.585	1.798	1.851	2.118	1.838
K2	1.851	1.912	1.973	2.217	1.988
K3	1.798	1.943	1.928	1.920	1.897
Mean	1.610	1.815	1.884	2.029	
LSD at 0.05	N		0.295		
	K		0.295		
	N x K		0.590		

N0, N1, N2 and N3 = N biofertilizer at 0, 10, 15 and 20 L Biofert/fed., respectively

K0, K1, K2 and K3 = K biofertilizer at 0, 10, 15 and 20 L Available K/fed., respectively

Regarding the interaction between the effects of nitrogen and potassium biofertilizers treatments on the essential oil yield (ml/plant) and L per feddan, data presented in Tables 8 and 9 show significant differences between the essential oil yield ml/plant and L/feddan in plants receiving the various combinations of the two elements. In both seasons, the highest essential oil yield were 0.088

and 0.097 ml/plant and 2.012 and 2.217 L/feddan were resulted from plants fertilized with N3K3 and N3K2 treatments in the first and second seasons, respectively. On the other hand, the lowest essential oil yields were 0.032 and 0.053 ml/plant and 0.723 and 1.204 L/feddan were recorded with unfertilized plants with (N0K0 treatment) in the first and second seasons, respectively.

Table 10: Effect of nitrogen and potassium biofertilizers as well as their interactions on the components (%) in the essential oil of *Marrubium vulgare* L. plant in the two seasons 2011 and 2012

Treatments	The components (%) of the essential oil									
	First season, 2011					Second season, 2012				
	N0	N1	N2	N3	Mean	N0	N1	N2	N3	Mean
Sabinene										
K0	0.30	0.36	0.23	---	0.30	---	---	---	---	---
K1	2.24	1.52	---	0.95	1.57	---	---	---	---	---
K2	1.66	0.51	1.18	1.61	1.24	---	---	---	---	---
K3	1.21	0.64	1.51	1.79	1.29	---	---	---	---	---
Mean	1.35	0.76	0.97	1.45	1.10	0.49	---	---	---	---
D-Limonene										
K0	1.35	2.33	0.99	0.32	1.25	2.03	0.31	2.42	1.08	1.46
K1	1.72	2.07	0.79	1.41	1.50	---	0.34	---	0.48	0.41
K2	3.64	1.89	3.23	1.92	2.67	3.28	---	1.63	0.58	1.83
K3	2.81	2.14	3.10	2.64	2.67	0.21	0.55	0.85	---	0.54
Mean	2.38	2.11	2.03	1.57	2.02	1.84	0.40	1.63	0.71	1.15
γ -Terpinene										
K0	1.33	1.16	1.12	0.31	0.98	0.83	---	1.13	0.34	0.77
K1	2.48	4.73	0.49	1.52	2.31	---	---	---	---	---
K2	5.62	1.84	3.45	2.24	3.29	1.91	---	0.68	---	1.30
K3	3.19	2.41	3.58	2.50	2.92	---	0.30	---	---	0.30
Mean	3.16	2.54	2.16	1.64	2.37	1.37	0.30	0.91	0.34	0.73
α -Copaene										
K0	6.96	7.57	5.41	6.69	6.66	8.23	7.96	7.81	6.95	7.74
K1	6.60	5.72	7.23	6.67	6.56	9.07	8.55	7.19	8.67	8.37
K2	5.36	6.90	6.58	4.64	5.87	7.54	6.64	6.72	6.15	6.76
K3	6.80	6.82	7.15	6.46	6.81	8.22	8.34	5.46	6.21	7.06
Mean	6.43	6.75	6.59	6.12	6.48	8.27	7.87	6.80	7.00	7.48
β -Bourbonene										
K0	5.13	5.54	3.25	5.10	4.76	4.55	4.50	6.32	4.00	4.84
K1	5.20	4.03	5.17	4.05	4.61	4.18	2.71	5.27	6.63	4.70
K2	5.56	5.26	4.07	3.24	4.53	5.35	3.26	4.54	3.17	4.08
K3	5.40	6.26	5.27	3.45	5.10	5.11	5.34	4.28	2.78	4.38
Mean	5.32	5.27	4.44	3.96	4.75	4.80	3.95	5.10	4.15	4.50
β -Caryophyllene										
K0	34.94	28.99	30.31	30.27	31.13	32.29	27.10	30.31	27.59	29.32
K1	27.85	23.24	30.86	26.49	27.11	30.91	30.99	32.43	29.87	31.05
K2	25.76	27.76	26.99	25.29	26.45	30.32	24.27	28.37	24.97	26.98
K3	25.27	26.18	23.10	28.55	25.78	30.18	23.48	20.73	26.02	25.10
Mean	28.46	26.54	27.82	27.65	27.62	30.93	26.46	27.96	27.11	28.11
Humulene										
K0	5.42	4.96	5.65	4.72	5.19	5.75	5.12	5.35	5.38	5.40
K1	5.33	4.51	5.58	4.46	4.97	5.40	6.29	5.94	4.94	5.64
K2	3.20	4.89	4.94	4.62	4.41	5.47	4.30	5.52	5.90	5.30
K3	4.69	5.07	4.57	4.76	4.77	5.47	4.31	4.14	5.26	4.80
Mean	4.66	4.86	5.19	4.64	4.59	5.52	5.01	5.24	5.37	5.29
Germacrene-D										
K0	22.63	26.79	29.98	28.23	26.91	22.91	27.79	22.16	23.51	24.09
K1	22.86	28.59	26.12	27.23	26.20	26.91	27.02	19.87	21.57	23.84
K2	25.17	27.01	27.08	25.27	26.13	21.14	28.24	22.13	23.58	23.77
K3	23.45	23.95	23.42	26.12	24.24	25.14	20.71	17.80	23.07	21.68
Mean	23.53	26.59	26.65	26.71	25.87	24.03	25.94	20.49	22.93	23.35

Table 10: Continued

The components (%) of the essential oil										
First season, 2011						Second season, 2012				
Treatments	N0	N1	N2	N3	Mean	N0	N1	N2	N3	Mean
δ-Cadinene										
K0	5.13	7.05	6.69	6.79	6.42	7.96	9.99	6.97	9.18	8.53
K1	7.07	6.58	7.48	7.18	7.08	8.25	8.03	6.81	7.27	7.59
K2	6.44	6.72	6.82	5.77	6.44	6.85	10.03	7.70	8.14	8.18
K3	7.03	6.58	7.86	5.52	6.75	8.37	15.34	7.27	8.96	9.99
Mean	6.42	6.73	7.21	6.32	6.67	7.86	10.85	7.19	8.39	8.57
Caryophyllene oxide										
K0	1.48	2.53	2.07	2.45	2.13	2.45	2.92	3.49	3.59	3.11
K1	3.24	2.51	3.26	1.86	2.72	2.17	2.42	4.40	4.56	3.39
K2	4.43	2.35	1.61	2.17	2.64	2.69	2.23	3.72	2.32	2.74
K3	2.72	2.65	2.54	1.75	2.42	2.94	3.15	3.59	4.14	3.46
Mean	2.97	2.51	2.37	2.06	2.48	2.56	2.68	3.80	3.65	3.18
Nerolidol										
K0	3.72	2.99	6.08	5.70	4.62	3.39	6.22	3.95	5.93	4.87
K1	4.54	2.61	3.63	4.25	3.76	4.27	2.43	7.62	3.78	4.53
K2	---	5.17	2.90	5.85	4.64	3.30	4.75	6.00	3.43	4.37
K3	4.54	4.63	5.04	3.62	4.46	5.34	8.35	4.65	6.28	6.16
Mean	4.27	3.85	4.41	4.86	4.35	4.08	5.44	5.56	4.86	4.99
F.S						S.S				
Eleven components representing =						87.35%				
Other components representing =						12.65%				

N0,N1,N2 and N3 = N biofertilizer at 0,10,15 and 20 L Biofert/fed., respectively

K0,K1,K2 and K3 = K biofertilizer at 0,10,15 and 20 L Available K/fed., respectively

The role of N and K in the synthesis and accumulation of essential oil in aromatic plants may be due to the fact that both elements are essential for many physiological processes such as photosynthesis, translocation of photosynthetics into sink organs, activation of enzymes and stomatal movements lead to increasing the synthesis and accumulation of essential oils in different plant organs. These results agree with other studies [8, 9, 19, 21, 27].

Essential Oil Components by GC-MS: The mean constituents of the essential oil of all treatments as identified by GC-MS. Eleven components have been identified in the essential oil (Table 10 and Figs.1-4). The major components were β -Caryophyllene (27.62 and 28.11 %) and Germacrene -D (25.87 and 23.35 %) in the first and second seasons, respectively, followed by d-Cadinene (6.67 and 8.57 %), α -Copaene (6.48 and 7.48%), Humulene (4.59 and 5.29%), β -Bourbonene (4.75 and 4.50%), Nerolidol (4.35 and 4.99 %), Caryophyllene oxide

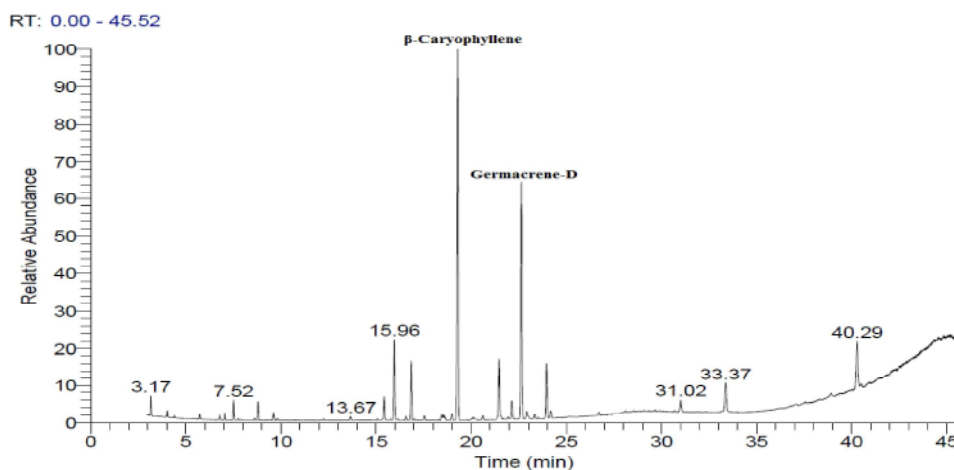


Fig. 1: GC-MS chromatogram of *Marrubium vulgare* L. essential oil distilled from N0K0 non-inoculated plants in the first season, 2011

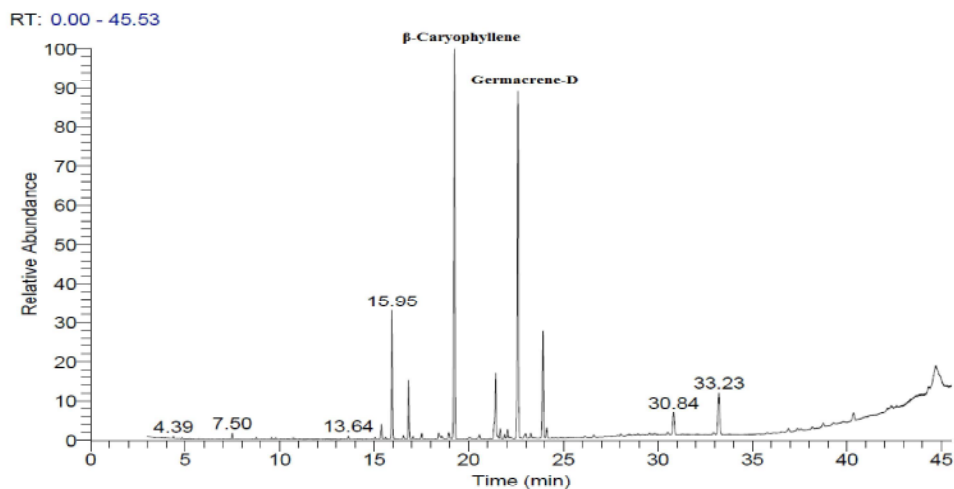


Fig. 2: GC-MS chromatogram of *Marrubium vulgare* L. essential oil distilled from N0K0 non-inoculated plants in the second season, 2012

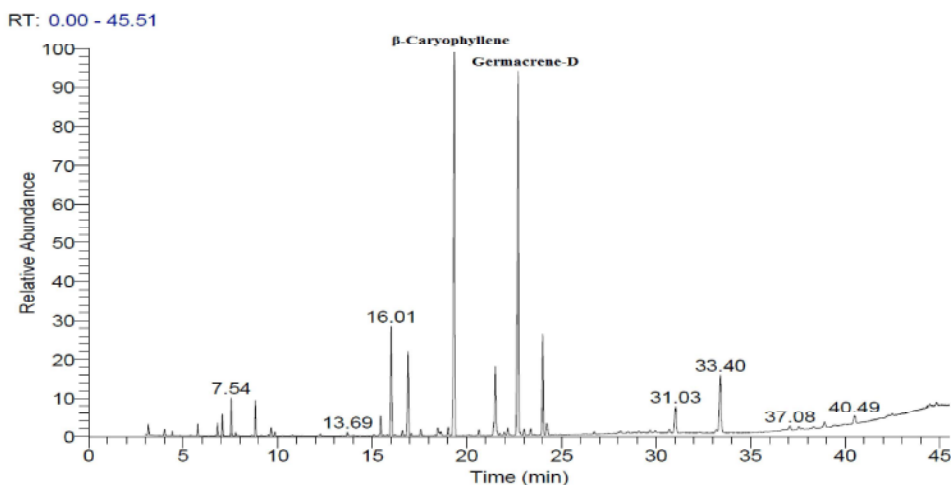


Fig. 3: GC-MS chromatogram of *Marrubium vulgare* L. essential oil distilled from N3K3 treated plants (20 L Biofert + 20 L Available K / feddan) in the first season, 2011

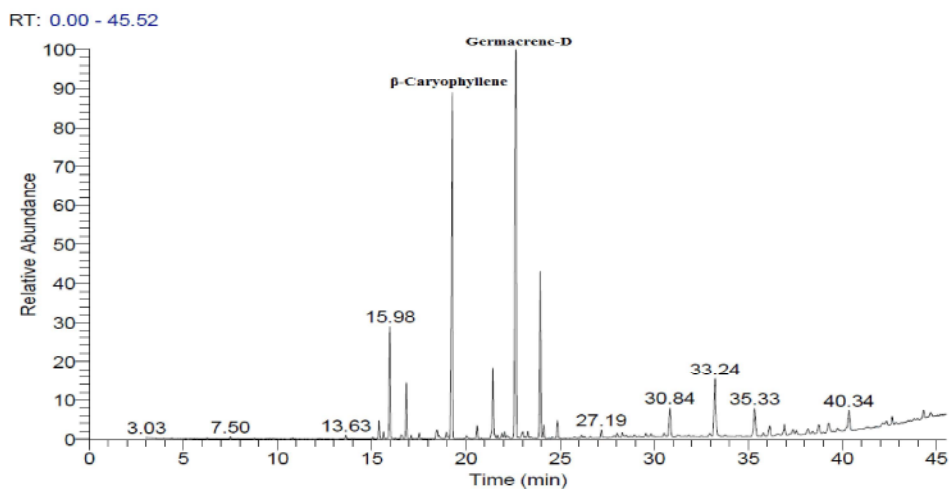


Fig. 4: GC-MS chromatogram of *Marrubium vulgare* L. essential oil distilled from N3K3 treated plants (20 L Biofert + 20 L Available K / feddan) in the second season, 2012

(2.48 and 3.18 %), γ -Terpinene (2.37 and 0.73 %), D-Limonene (2.02 and 1.15 %) and Sabinene (1.10 and 0.00 %) in the first and second seasons, respectively. These components in the first season, representing 88.36% of the total essential oil while in the second season representing 87.35 %.

The highest mean contents of β -Caryophyllene (28.46 and 30.93%) were recorded in the essential oil of unfertilized plants in the first and second seasons, respectively. On the other hand, the highest mean contents of Germacrene-D % were recorded in the oil of plants fertilized with N3 and N1 (26.71 and 25.94%) in the first and second seasons, respectively.

Results recorded in the two seasons also show that unfertilized plants with K resulted in the highest β -Caryophyllene % and Germacrene-D % in comparison with K2 and K3 treatments. The mean values of β -Caryophyllene were 31.13, 26.45 and 25.78 % in the first season and 29.32, 26.98 and 25.10 % in the second season, respectively. While the Germacrene-D was 26.91, 26.13 and 24.24 % in the first season and 24.09, 23.77 and 21.68 % in the second season, respectively.

Regarding the interaction effect between N and K biofertilizers on the β -Caryophyllene and Germacrene-D % in the essential oil, data presented in Table (10) and Figs (1-4) show that the highest β -Caryophyllene (34.94 and 32.43 %) were recorded in the essential oil plants unfertilized by N0K0 and fertilized by N2K1 treatments in both seasons, respectively. The highest Germacrene-D values (29.98 and 28.24 %) were recorded in the essential oil extracted from plants fertilized by N2K0 and N1K2 treatments in both seasons, respectively.

Generally, other mean components in the essential oil of *Marrubium vulgare* L., such as d-Cadinene, a-Copaene, Humulene, β -Bourbonene, Nerolidol, Caryophyllene oxide, γ -Terpinene, D-Limonene and Sabinene percentages had no detectable trend as affected by N and K biofertilizer treatments as well as interaction between them in the first and second seasons. These results agreed with Nagy and Svajdlenska [28] on *Marrubium vulgare* L., they found that β -Caryophyllene (45.80%) and Germacrene-D (14.40%) were the major constituents. The same results were found by Mahnaz-Khanavi *et al.* [11] and Dobrescu *et al.* [29] on *Marrubium vulgare* L. plants.

Recommendation: The biofertilization of white horehound (*Marrubium vulgare* L.) plants with nitrogen biofertilizer (Biofert) or potassium biofertilizer (Available K) and their combination at high level for each (20 L/feddan) gave the maximum values of plants height, fresh and dry herb weights and essential oil production.

The biofertilizers can be an alternative to chemical fertilizers for increasing white horehound production, minimizing environmental pollution and sustaining agriculture.

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