

EFFECT OF PLANTING SPACE AND SOME BIOFERTILIZATION ON PLANT GROWTH, YIELD AND CHEMICAL COMPOSITION OF LEMON BALM (*Melissa officinalis* L.) PLANTS.

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ABSTRACT

Therefore, an experiment was undertaken to determine the suitable planting spaces (40, 50 and 60 cm) of *Melissa officinalis* L., plant and the best treatment of nitrogen, phosphate and potassium biofertilizers treatments plus half dosage of the full recommended dose of NPK mineral fertilizer.

Results showed that planting spaces recorded various different effects on growth characters. The narrow space (40 cm) resulted in the tallest plants, the highest Leaves and essential oil yield per fed, while the wider space (60 cm) produced the heaviest leaves dry weight per plant, the highest number of branches, essential oil percentage, total chlorophyll content as well as N, P, K and total carbohydrates percentages.

The most growth characters and yield as well as chemical composition improved and increased when soil was inoculated by a different inoculums of nitrogen-fixing bacteria (nitrobein), *Bacillus polymyxa* as a phosphate dissolving bacteria (phosphorein), and foliar spray of potassium as a (potassein-p) were used in combination with half of the full recommended dose of NPK mineral fertilizer compared with the control treatment received only the full recommended dose of NPK mineral fertilizer.

Moreover, the interaction treatments indicated that the highest means values for most growth characters, active constituents and chemical composition were recorded at 40 cm plant space with biofertilizers treatment (nitrobein + phosphorein + potasei-p) as a mixture plus half of the recommended dose of NPK mineral fertilizer. Gas chromatography analysis of essential oil indicated that the main components were citral and geranial. The components were positively affected by different combined treatments between planting space and biofertilization.

So, cultivated lemon balm plants at 40cm planting space and applying 50 % of the recommended dose of NPK plus mixture of biofertilizers can save half of the quantity of mineral fertilizer decreases the production cost and environmental pollution as well as gives high quality product.

INTRODUCTION

Melissa officinalis L. is a member of Lamiaceae family known as Lemon balm plant native to the Mediterranean region. The plant is an important medicinal and aromatic plant characterized by fragrant. The leaves which be collected before the plant flowers contain high quality essential oil of lemon scent and its constituents are camphene, β -pinene, myrecene, linalool, nerol, geraniol, citral, geranial and geranyl acetate. It calms a nervous stomach, colic, or heart spasms. The leaves are reputed to also lower blood pressure, (keville, 1999).

Plant density is very important factor affecting growth and yield of medicinal and aromatic crops, (Delaluz *et al.*, 2002).

However, Saglam (2004) on *Melissa officinalis*; Aflatonini (2005) on mint (*Mentha* sp.) and El-Sherbeny *et al.* (2007) on *Ruta graveolens* they found that the highest dry herb yield, essential oil amount and composition of plants was obtained from the closer plant distance compared with wider spacing.

Recently, under Egyptian conditions a great attention is being devoted to reduce the high rates of mineral fertilizers, the cost of producing and environmental pollution caused by repeated application of mineral fertilizers. Biofertilizers play a very important role in modern agriculture, in achieving higher productions at lower input costs, (Bagde *et al.*, 2010).

Kandeel *et al.* (2002) on *Ocimum basilicum*; Abo-Baker and Mostafa (2011) on roselle plant and Sakr *et al.* (2012) on marjoram plant they revealed that the inoculation with the mixture of biofertilizers combined with 50% or 100% mineral fertilizers improved most of vegetative characterizes, increased yield and the content of main active constituents compared to control as well as reducing the requirements of NPK mineral fertilizers.

This research was aimed to evaluate the effect of suitable planting space with N₂ fixing bacteria (nitrobein), phosphate dissolving bacteria (phosphorein), and foliar spray of potassium as (potasein-p) singly or as mixtures of biofertilizers combined with half dose of the full recommended NPK mineral fertilizer on plant growth, yield and essential oil productivity of lemon balm (*Melissa officinalis* L.) plants. The goal was to minimize inorganic fertilize (NPK) usage.

MATERIALS AND METHODS

This study was carried out at the Research Farm of Medicinal and Aromatic Plants, Fac. Agric., Mansoura Univ., in the two consecutive seasons of 2009 / 2010 and 2010 / 2011 to study the effect of planting spaces and biofertilization in combination with 50% of NPK mineral fertilizer on growth, yield and chemical composition of lemon balm plants. Transplants planted on February 6th of both seasons. The plot area was 4.80 m² (3.20 x 1.5m), the total number of plants in every plot was 24, 18 and 15 plant regarding to the planting space. Some physical and chemical properties of soil during the two seasons were carried out at Laboratory of Soil, Agric. Res. Center, (Table, A).

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

Physical properties	1st season	2nd season	Chemical properties	1st season	2nd season
Coarse sand (%)	1.66	1.96	CaCO ₃ (%)	2.53	3.85
Fine sand (%)	19.99	20.99	Organic matter (%)	1.96	2.12
Silt (%)	26.52	28.72	Available N (ppm)	42	48
Clay (%)	49.63	51.0	Available P (ppm)	3.22	3.88
pH	7.17	8.25	Available K (ppm)	315	330

The plants of control treatment received only the full recommended dose (RD) of NPK mineral fertilizer, according to the Ministry of Agriculture, Egypt, which was including ammonium nitrate (33.5 % N) at the rate of 100 kg / fed, calcium super phosphate (15.5 % P₂O₅) at the rate of 100 kg / fed, and potassium sulfate (48 % K₂O) at the rate of 48 kg / fed. Calcium super phosphate (P) was added during soil preparation. Ammonium nitrate and potassium sulfate (N and K) added at two equal batches; the first one after 45 days after transplanting, the second one was added after two weeks of the first cut.

Biofertilizer inoculations were *Azospirillum brasilense* as N₂-fixing bacteria (nitrobein) and phosphate dissolving bacteria *Bacillus polymyxa* as a (phosphorein). For the biofertilization treatments, roots of transplants were inoculated at planting and then added to the soil around each plant once every month until the end of the experiment at the rate of 4.80g / plot / season then plants were irrigated immediately. As a source of potassium we used foliar spray of potassein-p at rate of 1.15 ml / plot / season after a month from transplanting and once every month and after the first cut. The fertilizers were obtained from the General Organization for Agriculture Equalization Fund (GOAEF), Ministry of Agriculture, Egypt.

Detail of experiment treatments:

- T1- Control (100 % RD of NPK) at 40, 50 and 60 cm plant space
- T2- Nitrobein + ½ RD of NPK at 40, 50 and 60 cm plant space
- T3- Phosphorein + ½ RD of NPK at 40, 50 and 60 cm plant space
- T4- Potassein + ½ RD of NPK at 40, 50 and 60 cm plant space
- T5- Nitrobein+ Phosphorein+ ½ NPK at 40, 50 and 60 cm space
- T6- Nitrobein + Potassein+ ½ NPK at 40, 50 and 60 cm plant space
- T7- Phosphorein+ Potassein+ ½ NPK at 40, 50 and 60 cm plant space
- T8- Nitrobein+ Phosphorein+ Potassein+ ½ NPK at 40, 50 and 60 plant space

Plants were harvested twice during each season, the first cut at commencement of flowering on the last week of July and the second one on the first week of October. At harvesting the studied characteristics were fate for determining and recording plant height (cm), number of branches / plant, dry weight of leaves (g / plant) and leaves yield (ton / fed). For estimation of essential oil percent (%) according to Egyptian Pharmacopeia (1984), and essential oil yield (liter / fed) were determined and calculated for each cut and for each season. The essential oil constituents were analyzed and determined at the second cut in the second season samples using Gas liquid chromatography (GLC) analysis according to Guenther and Joseph (1978). Total chlorophyll (mg/g-F.W.) was determined in fresh leaf samples according to Moran (1982). Dried herb (oven dried at 70 °C) was analyzed to determine percentages of total carbohydrate according to Herbert (1971), nitrogen as described by Wilde *et al.* (1985), phosphorus according to Chapman and Pratt (1975) and potassium according to Cottenie *et al.* (1982).

The eight treatments were arranged in three replication using a split plot in randomized complete block design, the main plots, having the different plant spaces (40, 50 and 60cm), while the sub plots; NPK 100% of the

mineral fertilizer and some biofertilizers plus 50% of NPK. Data of the present study was subjected to analysis of variance (ANOVA) by the general linear models (GLM) procedure using (SAS) statistical analysis system. Mean comparisons were performed using the least significant differences (L.S.D.) method at significance level of 5% according to Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Growth and yield:

Effect of planting space:

The results in Table (1 and 2) indicated that different plant spacing markedly effect on vegetative growth characters. The narrow space (40cm) resulted in the tallest plants (42.08 and 43.89 cm) and the heaviest leaves yield (1.81 and 1.21 ton /fed) compared to the wider planting space (60cm), which resulted (37.50 and 39.46 cm) and (1.39 and 1.11 ton /fed) in the both cuts respectively, in the first season. The wider plant spacing was more effective to produce significant increment for branches number per plant and leaves dry weight per plant. It was noticed that increasing planting space gradually from 40cm to 60cm increased number of branches as (33.92 to 36.04 and 28.33 to 33.08 / plant), the leaves dry weight was (86.29 to 106.57 and 57.66 to 85.67 g / plant) in the both cuts respectively, in the first season. The results of second season gave the same trend. This increment in plant height and yield by decreasing the plant distance might be due to the competition between plants for obtaining more light and then the plant is bushy when narrow spaces tends to increase in plant height rather than branching. These results are in the same line with many researchers such as Saglam *et al.* (2004) and Harshavardhan *et al.* (2007) on *Melissa officinalis*; Massoud *et al.* on rosemary plant and Ibrahim (2010) on *Aloysia triphylla*.

Effect of biofertilizers treatments :

It was clear from data in (Table, 1 and 2) that fertilizing lemon balm plants using different biofertilizers and amended with a half dose of NPK had significant effects on plant growth characters.

The significantly increasing tallest plants (43.59 and 50.54 cm), number of branches (40.78 and 37.56 / plant), leaves dry weight (117.66 and 82.07 g / plant) and leaves yield (1.93 and 1.33 ton / fed) were resulted from the treatment (nitrobein, phosphorein and potassein-p) plus 50% recommended dose of NPK in the two cuts, respectively. Mentioned results were recorded in the first season and the same behavior was recorded in the second season for the same treatment compared to the control treatment (full recommended dose of NPK). These results may be due to the role of *Azotobacter* and *Azospirillum* in nitrogen fixation (Fayez *et al.*, 1985). The phosphate dissolving bacteria (PDB) also may have increased the availability of phosphorus, and it may have increasing the uptake of trace elements (El-Haddad *et al.*, 1993). Similar results are in agreement with those of Mahfouz (2003) on *Majorana hortensis*; Al-Qadasi (2004) on *Ocimum basilicum*; Hamed (2004) on *Salvia officinalis*; Massoud *et al.* (2004) on thyme plants; Yousef, 2005 on *Melissa officinalis* and Sakr *et al.* (2012) on marjoram plants.

Table (1): Effect of plant space, biofertilizers and their interaction on plant height (cm) and number of branches / plant of lemon balm plants during the two seasons of 2009/2010 and 2010/2011.

Fertilizers (B)	Plant height (cm)								Number of branches / plant							
	Planting space (cm) (A)															
	1 st season (2009 / 2010)															
	1 st cut				2 nd cut				1 st cut				2 nd cut			
	40 cm	50 cm	60 cm	Mean (B)	40 cm	50 cm	60 cm	Mean (B)	40 cm	50 cm	60 cm	Mean (B)	40 cm	50 cm	60 cm	Mean (B)
T1	45.00	42.20	39.58	42.26	49.17	48.70	44.33	47.40	36.33	36.50	38.00	36.94	33.33	35.33	36.33	35.00
T2	40.33	39.67	37.13	39.04	39.40	37.57	36.40	37.79	30.00	31.33	33.00	31.44	24.67	29.33	30.33	28.11
T3	38.67	37.53	35.67	37.29	38.90	35.53	34.57	36.33	29.67	30.33	31.67	30.56	24.33	26.67	27.67	26.22
T4	36.90	34.83	31.27	34.33	35.67	32.50	29.33	32.50	28.00	29.33	31.00	29.44	23.00	25.33	26.33	24.89
T5	44.33	43.00	39.33	42.22	50.03	48.53	45.53	48.03	36.67	37.00	38.33	37.33	33.00	36.67	37.33	35.67
T6	43.07	41.13	38.33	40.84	42.67	41.47	39.07	41.07	36.00	36.33	37.67	36.67	28.00	34.33	34.67	32.33
T7	41.27	41.03	38.00	40.10	42.03	40.53	38.97	40.51	35.33	35.67	37.00	36.00	25.33	31.67	33.00	30.00
T8	47.10	43.00	40.67	43.59	53.23	50.90	47.50	50.54	39.33	41.33	41.67	40.78	35.00	38.67	39.00	37.56
Mean (A)	42.08	40.30	37.50		43.89	41.97	39.46		33.92	34.73	36.04		28.33	32.25	33.08	
L.S.D at 5 %	A 1.68	B1.83	AB 3.20		A 1.78	B 2.09	AB 3.62		A 2.30	B2.61	AB4.53		A 0.99	B 1.57	AB 2.64	
2 nd season (2010 / 2011)																
T1	50.93	48.13	47.67	48.91	45.27	42.57	41.00	42.94	36.00	39.33	41.33	38.89	32.33	32.67	35.00	33.33
T2	46.33	44.33	43.93	44.87	41.33	39.00	36.00	38.78	32.33	33.00	36.67	34.00	28.33	26.33	30.00	28.22
T3	45.00	42.60	41.33	42.98	37.80	36.60	34.67	36.36	29.00	32.67	35.00	32.22	24.67	26.67	28.00	26.44
T4	42.33	40.17	39.43	40.64	37.73	34.97	32.50	35.07	26.67	31.67	33.67	30.67	23.00	23.33	26.33	24.22
T5	51.13	50.87	48.33	50.11	48.07	45.27	41.33	44.89	36.00	40.00	41.33	39.11	34.33	34.67	37.33	35.44
T6	47.73	46.67	45.33	46.58	45.10	40.77	39.33	41.73	35.33	36.67	39.33	37.11	31.33	32.05	33.67	32.33
T7	47.53	45.67	44.40	45.87	40.20	39.37	38.67	39.41	34.00	34.67	38.00	35.56	29.33	31.00	32.33	30.34
T8	55.10	52.00	49.27	52.12	52.50	48.43	45.33	49.09	38.00	41.67	42.67	40.78	34.33	36.67	40.67	37.22
Mean (A)	48.26	46.30	43.96		43.50	40.87	38.60		33.42	36.83	39.66		29.71	30.42	32.92	
L.S.D at 5 %	A 1.16	B1.72	AB 2.91		A 1.31	B 1.78	AB 3.04		A 2.66	B 1.91	AB 3.69		A 2.35	B 2.67	AB 4.64	

T1=control (100%NPK) T2=nitrobein+50%NPK T3=phosphorien+50%NPK T4=potassien+50%NPK T5= nitrobein+ phosphorien+50%NPK
T6= nitrobein+ potassien+50%NPK T7= phosphorien+ potassien+50%NPK T8== nitrobein+ phosphorien+ potassien +50%NPK

Table (2): Effect of plant space, biofertilizers and their interaction on leaves dry weight (g / plant) and leaves yield (ton / fed) of lemon balm plants during the two seasons of 2009/2010 and 2010/2011.

fertilizers (B)	Leaves dry weight (g / plant)								Leaves yield (ton / fed)							
	Planting space (cm) (A)															
	1 st season (2009 / 2010)															
	1 st cut				2 nd cut				1 st cut				2 nd cut			
	40 cm	50 cm	60 cm	Mean (B)	40 cm	50 cm	60 cm	Mean (B)	40 cm	50 cm	60 cm	Mean (B)	40 cm	50 cm	60 cm	Mean (B)
T1	96.14	109.71	115.87	107.24	61.67	76.53	90.32	76.17	2.02	1.75	1.51	1.76	1.30	1.22	1.17	1.23
T2	80.90	86.77	101.47	89.71	56.09	69.65	81.98	69.24	1.70	1.39	1.32	1.47	1.18	1.11	1.06	1.12
T3	71.78	78.74	88.09	79.54	51.55	64.59	77.10	64.41	1.51	1.26	1.14	1.31	1.08	1.03	1.00	1.04
T4	60.96	67.23	82.90	70.36	46.66	60.16	73.94	60.25	1.28	1.08	1.07	1.14	0.98	0.96	0.95	0.96
T5	99.54	109.65	120.48	109.89	63.85	81.34	95.22	80.14	2.09	1.75	1.57	1.80	1.34	1.30	1.23	1.29
T6	90.08	95.95	111.08	99.04	59.34	73.86	87.24	73.48	1.89	1.54	1.44	1.62	1.25	1.18	1.13	1.19
T7	85.59	87.99	103.00	92.19	55.54	71.06	83.74	70.11	1.80	1.41	1.34	1.52	1.17	1.13	1.08	1.13
T8	105.34	118.00	129.63	117.66	66.57	81.79	97.84	82.07	2.21	1.89	1.68	1.93	1.40	1.31	1.27	1.33
Mean (A)	86.29	94.26	106.57		57.66	72.37	85.92		1.81	1.51	1.39		1.21	1.15	1.11	
L.S.D at 5 %	A 2.40 B 4.01 AB 6.71				A 2.25 B 2.84 AB 4.88				A 0.036 B 0.065 AB 0.11				A 0.042 B 0.052 AB			
2 nd season (2010 / 2011)																
T1	90.27	101.25	116.09	102.54	71.69	85.43	95.26	84.13	1.92	1.58	1.48	1.66	1.51	1.37	1.23	1.37
T2	78.96	86.33	98.53	87.94	64.74	76.53	83.19	74.82	1.66	1.38	1.28	1.44	1.36	1.22	1.08	1.22
T3	75.04	84.66	93.92	84.54	59.41	67.45	74.60	67.15	1.58	1.35	1.22	1.38	1.25	1.07	0.97	1.10
T4	69.57	80.15	90.71	80.14	55.92	62.22	69.85	62.66	1.46	1.28	1.17	1.30	1.17	1.00	0.91	1.03
T5	91.28	98.97	114.39	101.55	73.28	84.31	94.44	84.01	1.90	1.62	1.50	1.67	1.54	1.35	1.24	1.38
T6	84.65	94.17	106.06	94.96	69.74	80.73	85.81	78.76	1.78	1.51	1.37	1.55	1.46	1.29	1.12	1.29
T7	81.32	89.38	101.20	90.63	67.34	78.59	84.51	76.81	1.71	1.43	1.31	1.48	1.41	1.26	1.09	1.25
T8	97.96	108.88	120.79	109.21	79.12	88.17	95.45	87.58	2.05	1.74	1.57	1.79	1.66	1.41	1.24	1.44
Mean (A)	83.63	92.97	105.21		67.66	77.92	85.39		1.76	1.49	1.36		1.42	1.25	1.11	
L.S.D at 5 %	A 2.14 B 3.88 AB 6.45				A 1.97 B 2.58 AB 4.41				A 0.038 B 0.074 AB 0.12				A 0.036 B 0.040 AB 0.070			

T1=control (100%NPK) T2=nitrobein+50%NPK T3=phosphorien+50%NPK T4=potassien+50%NPK T5= nitrobein+ phosphorien+50%NPK
T6= nitrobein+ potassien+50%NPK T7= phosphorien+ potassien+50%NPK T8= nitrobein+ phosphorien+ potassien +50%NPK

Effect of the interaction treatments :

Regarding to combined effect of different plant spacing and various bio-fertilizers (Table, 1 and 2), the obtained data revealed that the plant supplied with a mixture of biofertilizers treatment plus 50% RD of mineral fertilizer and grown under closer planting distance condition (40cm) gave tallest plant (47.10 and 53.23 cm) and heaviest leaves yield (2.21 and 1.40 ton / fed) in both cuts, respectively in the first season. Thus the maximum number of branches per plant was (41.67 and 39.00 / plant) and the heaviest leaves dry weight (129.63 and 97.84 g / plant) in the both cuts respectively, during the first season were noticed as a result of wider distance (60cm between plants) which gave an increment values (comparing with narrow distance 40cm) compared to the control treatment. The obtained results showed that using bacteria inoculation combined with 50% mineral fertilizers at closer planting space improved, in most cases, the growth characters and increased leaves yield or at least did not differ significantly from the control (full recommended dose of NPK fertilizers alone). The stimulatory effect of the treatments on plant growth may be due to the role of NPK fertilization on the plant physiological processes as well as the micro-organisms which increase soil available nitrogen and consequently increase formation of metabolites which encourage the plant vegetative growth, (El-Merich *et al.*, 1997).

Essential oil productivity :

Effect of planting space:

Results in Table (3) indicated that essential oil percentage was not affected by planting distances between plants. In the first season the essential oil percentage was (0.36, 0.36 and 0.37) and (0.31, 0.32 and 0.32) at the three planting space (40, 50 and 60cm) in the two cuts, respectively. While, the essential oil yield increased by decreasing plant density from (7.98 and 5.14 L / fed) at 40cm planting space to (6.82 and 4.44 L / fed) at 60cm planting space at the both cuts respectively. The results of the second season gave the same trend. The increment in oil yield as a result of wider spacing may be attributed to the heavier fresh weight of the plants (Table, 2). The same results were obtained by Saglam *et al.* (2004) and Harshavardhan *et al.* (2007) on *Melissa officinalis*, Massoud *et al.* (2007) on *Rosmarinus officinalis* proved that the content and quality of essential oil were not influenced by plant spacing.

Effect of bio-fertilizers:

Data in Table (3) showed that the highest mean values of essential oil percentage and yield of the two cuts in both seasons increased significantly. The highest percentage and yield of essential oil were (0.45 and 0.41 %) and (10.56 and 6.93 L / fed) of plants treated with biofertilizers (nitrobein, phosphorein and potassein-p) plus 50 % NPK, followed by (0.40 and 0.39 %) and (8.90 and 6.15 L / fed) of plants treated with biofertilizer treatment (notrobein + phosphorein) plus 50 % NPK mineral fertilizer in the two cuts respectively, during the first season.

Table (3): Effect of plant space, biofertilizers and their interaction on essential oil percentage (%) and yield (liter/fed) of lemon balm plants during the two seasons of 2009/2010 and 2010/2011.

Fertilizers (B)	Essential oil percentage (%)								Essential oil yield (liter /fed)							
	Planting space (cm) (A)															
	1 st season (2009 / 2010)															
	1 st cut				2 nd cut				1 st cut				2 nd cut			
	40 cm	50 cm	60 cm	Mean (B)	40 cm	50 cm	60 cm	Mean (B)	40 cm	50 cm	60 cm	Mean (B)	40 cm	50 cm	60 cm	Mean (B)
T1	0.40	0.40	0.40	0.40	0.38	0.38	0.38	0.38	9.45	8.32	8.19	8.65	6.30	5.76	5.59	5.88
T2	0.32	0.32	0.31	0.32	0.25	0.26	0.25	0.25	6.30	5.92	5.59	5.94	3.78	3.52	3.25	3.52
T3	0.30	0.31	0.30	0.32	0.23	0.23	0.24	0.23	5.88	5.44	5.20	5.51	3.15	2.88	2.73	2.92
T4	0.28	0.27	0.27	0.27	0.20	0.22	0.22	0.21	5.04	4.48	4.42	4.65	2.73	2.56	2.47	2.59
T5	0.40	0.41	0.43	0.40	0.39	0.38	0.39	0.39	9.87	8.64	8.19	8.90	6.51	6.08	5.85	6.15
T6	0.38	0.38	0.39	0.38	0.37	0.35	0.36	0.36	8.40	7.84	7.54	7.93	6.09	4.96	4.90	5.32
T7	0.34	0.36	0.35	0.35	0.31	0.33	0.34	0.33	7.35	6.88	6.36	6.86	5.04	4.48	4.45	4.66
T8	0.44	0.48	0.43	0.45	0.41	0.42	0.40	0.41	11.55	11.04	9.10	10.56	7.35	7.20	6.24	6.93
Mean (A)	0.36	0.36	0.37		0.31	0.32	0.32		7.98	7.32	6.82		5.14	4.68	4.44	
L.S.D at 5%	A 0.081 B 0.13 AB 0.22				A 0.035 B 0.044 AB 0.076				A 1.16 B 1.98 AB 3.32				A 0.57 B 0.72 AB 1.23			
2 nd season (2010 / 2011)																
T1	0.40	0.39	0.40	0.40	0.37	0.38	0.38	0.38	9.45	8.32	8.19	8.65	7.35	6.72	6.24	6.77
T2	0.34	0.32	0.32	0.33	0.28	0.30	0.31	0.30	7.35	5.92	5.46	6.24	5.04	4.64	4.42	4.70
T3	0.31	0.31	0.32	0.31	0.26	0.28	0.30	0.28	6.30	5.60	5.20	5.70	4.20	4.00	3.90	4.03
T4	0.28	0.30	0.31	0.29	0.27	0.27	0.28	0.27	5.25	5.12	4.94	5.10	3.99	3.36	2.99	3.44
T5	0.38	0.40	0.40	0.39	0.37	0.37	0.39	0.38	9.87	8.48	8.45	8.93	7.14	7.04	7.02	7.06
T6	0.36	0.37	0.37	0.36	0.34	0.35	0.36	0.35	8.40	7.36	6.76	7.51	6.51	5.76	5.33	5.87
T7	0.35	0.36	0.35	0.35	0.31	0.32	0.33	0.32	7.77	7.04	6.11	6.97	5.67	5.12	4.81	5.20
T8	0.42	0.42	0.43	0.43	0.39	0.40	0.38	0.39	11.13	9.76	8.97	9.95	8.40	8.32	7.02	7.91
Mean (A)	0.36	0.36	0.37		0.32	0.33	0.34		8.19	7.20	6.68		6.04	5.62	5.22	
L.S.D at 5%	A 0.020 B 0.050 AB 0.082				A 0.010 B 0.019 AB 0.032				A 0.30 B 0.95 AB 1.56				A 0.13 B 0.34 AB 0.55			

T1=control (100%NPK) T2=nitrobein+50%NPK T3=phosphorien+50%NPK T4=potassien+50%NPK T5= nitrobein+ phosphorien+50%NPK
T6= nitrobein+potassien+50%NPK T7= phosphorien+potassien+50%NPK T8=nitrobein+phosphorien+potassien+50%NPK

Essential oil content was strongly affected by aqueous extract of biofertilizer application for two cuts compared with their corresponding control (NPK mineral fertilizers).

The effect of biofertilizer on increasing the essential oil synthesis in the herb might be attributed to their enhancing effect on increasing the uptake of nutrients by plant roots especially phosphorus element as phosphate group one linked by (ATP). Hence, the biosynthesis of essential oil is dependent on inorganic P content in the plant, (Kapoor *et al.*, 2004). These results agree with those of Kandeel *et al.* (2001) found that the highest percentage of fennel volatile oil resulted from inoculating the plants with *Azotobacter* + *Azospirillum* in the presence of a full dose of N, P and K. Omer *et al.* (2008) who found a positive correlation between nitrogen fertilizer and essential oil content in herbage of *Ocimum syriacum* and *Ocimum americanum*, respectively.

Effect of the interaction treatments :

Data in the same Table (3) indicated that the interaction between the three planting spaces (40, 50 and 60cm) and the combination of biofertilizers treatments plus 50% RD of recommended NPK mineral fertilizer had significantly effect on essential oil (%) and yield (L / fed) in the two cuts during both seasons. The same results were agreement with Ibrahim (2010) on *Aloysia triphylla* plants.

Essential oil components (%):

The G.L.C. analysis of the essential oil samples for the second season, at planting space (40cm) was obtained in (Table, 4) and illustrated in Figure (1) revealed a total of 10 compounds represented from 34.46 to 93.19 % in lemon balm essential oil. It is noted that the planting spaces or the biofertilizers treatments had different effects on total identified and major constituents of lemon balm essential oil.

Table (4): Essential oil components (%) affected by biofertilizers at plant space (40cm) of lemon balm plants in the second season 2010/2011.

Components	Essential oil components (%) at planting space 40 cm							
	T1	T2	T3	T4	T5	T6	T7	T8
Camphene	1.75	2.26	2.65	1.13	1.77	1.14	1.65	2.03
β-pinene	7.15	6.63	-	-	4.71	-	-	1.30
Myrcene	-	-	0.98	0.93	0.49	0.68	-	-
Linalool	4.76	6.48	1.13	0.41	2.29	1.09	2.60	1.79
Nerol	8.77	4.93	3.79	0.52	1.30	10.35	3.49	7.51
Geraniol	4.85	2.62	1.29	0.23	4.60	-	1.90	4.61
Citral	18.96	13.82	24.14	8.56	27.35	25.74	18.54	30.06
Geranial	28.61	28.09	22.69	12.48	35.22	34.22	34.11	35.65
Geranyl acetate	2.25	5.09	4.02	3.28	2.50	4.53	3.16	2.03
β-caryophellen	4.61	4.53	10.31	6.92	4.11	5.57	9.68	8.20
Known	81.72	74.45	71.00	34.46	91.34	83.32	75.13	93.19

Fig1

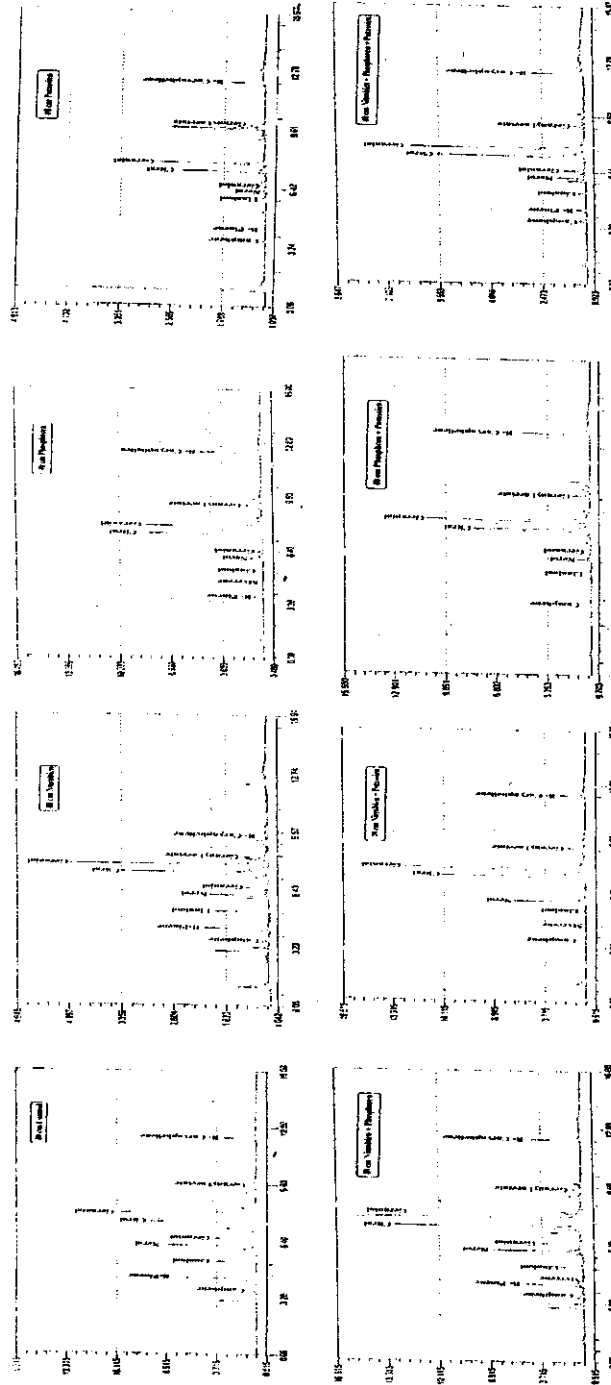


Fig. (1): G.L.C. Chromatographic analysis of lemon balm oil components (%) from different biofertilizers treatments at planting space 40cm.

The highest value of main components (citral and geranial) was obtained from plants grown in 40cm planting space plus the combination of mixture tribal biofertilizers treatments (nitrobein + phosphorein + potasein-p) plus 50 % recommended dose of NPK mineral fertilizer as recorded 30.06 and 35.65 % respectively followed by the mixture of nitrobein + phosphorein plus 50 % RD of NPK which gave 27.35 and 35.22 %, while the least values 8.56 and 12.48 % resulted from the treatment potasein-p plus 50 % NPK. From these results it may be concluded that all treated plants with the mixture of biofertilizers plus 50% of recommended dosage NPK caused an increase in the main compounds of lemon balm oil in comparison with full dose of NPK. Generally, treating the plants with effective microorganisms increased the known compounds, which are responsible for the medicinal activity of these plants.

Very similar results were mentioned by El-Sawy *et al.* (1998) reported that inoculation of *Ammi visnaga* seeds with a mixture of *Azotobacter* and *Azospirillum* along with a full dose of rock phosphate and inorganic nitrogen fertilizers increased the content of khellin. Mahfouz (2003) reported that the treatment of microorganisms increased the main compounds of *Marjorana hortensis* essential oil more than the untreated plants. Mahfouz and Sharaf-Eldin (2007) found that the highest anethol of fennel plant occurred with the half dose of NPK and inoculation with *Bacillus megatherium*.

Chemical composition:

Effect of plant space:

Data in Table (5) indicated that in both seasons the planting space significantly led to enhance the percentages of the three elements of N, P and K in the dry herb of plants. The highest values of N, P and K were (2.07, 0.47 and 2.80 %) obtained from the wider planting space 60cm, while the closer planting space 40cm gave (1.92, 0.45 and 2.62 %), respectively in the first season. The results of the second season gave the same trend. It may be concluded from the previous results, increasing the planting distance led to an increase in the uptake of NPK by the plants and this may be due to the increased area surrounding each plant; resulting in less competition between the plants and more spread of roots, consequently more availability of these elements to the plants and increased growth.

The highest value of total carbohydrate 19.85 % and of total chlorophyll 0.94 mg / g -F.W. was obtained from the wider planting space 60 cm, while the closer planting space 40cm gave 18.96 % and 0.90 mg / g-F.W., of total carbohydrate and total chlorophyll, respectively in the first season. The results of the second season gave the same trend, (Table, 6).

The increase in chlorophylls content is attributed to the existence of nitrogen (either from chemical or bio source) in the structure of the porphyrin which is found in chlorophyll pigments. The favorable effect of the different fertilization treatments on the synthesis and accumulation of carbohydrates may be attributed to the increase in the contents of chlorophylls and cytochrome enzymes results in an increment in the photosynthetic rate and a promotion in carbohydrate synthesis and accumulation.

Table (5): Effect of plant space, biofertilizers and their interaction on N, P and K (%) of lemon balm plants during the two seasons of 2009/2010 and 2010/2011.

Fertilizers (B)	N %				P %				K %			
	Planting space (cm) (A)											
	1 st season (2009 / 2010)											
	40 cm	50 cm	60 cm	Mean (B)	40 cm	50 cm	60 cm	Mean (B)	40 cm	50 cm	60 cm	Mean (B)
T1	2.24	2.34	2.41	2.33	0.50	0.51	0.54	0.53	2.84	2.94	3.00	2.93
T2	1.70	1.79	1.84	1.77	0.41	0.43	0.43	0.42	2.51	2.61	2.69	2.61
T3	1.51	1.57	1.62	1.57	0.39	0.40	0.41	0.40	2.40	2.47	2.55	2.47
T4	1.34	1.42	1.48	1.42	0.36	0.37	0.39	0.37	2.22	2.33	2.43	2.32
T5	2.21	2.29	2.36	2.29	0.51	0.52	0.53	0.52	2.80	2.84	3.00	2.90
T6	2.11	2.20	2.29	2.20	0.48	0.49	0.51	0.50	2.72	2.76	2.88	2.78
T7	1.94	2.04	2.09	2.02	0.44	0.45	0.46	0.45	2.63	2.75	2.83	2.74
T8	2.30	2.39	2.48	2.39	0.52	0.54	0.55	0.54	2.93	3.06	3.12	3.04
Mean (A)	1.92	2.01	2.07		0.45	0.47	0.47		2.62	2.72	2.80	
L.S.D at 5 %	A 0.059	B 0.079	AB 0.014		A 0.028	B 0.029	AB 0.051		A 0.003	B 0.004	AB 0.007	
2 nd season (2010 / 2011)												
T1	2.35	1.93	2.01	2.43	0.51	0.52	0.53	0.52	2.95	2.99	3.06	3.00
T2	1.80	1.98	2.07	1.95	0.42	0.43	0.44	0.43	2.57	2.67	2.76	2.67
T3	1.66	1.76	1.79	1.73	0.39	0.41	0.41	0.41	2.44	2.53	2.60	2.53
T4	1.49	1.55	1.61	1.55	0.36	0.37	0.38	0.37	2.31	2.35	2.46	2.37
T5	2.28	2.46	2.53	2.42	0.51	0.52	0.54	0.52	2.88	2.94	3.06	2.96
T6	2.14	2.34	2.41	2.30	0.48	0.49	0.50	0.49	2.80	2.82	2.96	2.86
T7	1.94	2.14	2.18	2.08	0.44	0.45	0.46	0.45	2.70	2.81	2.90	2.80
T8	2.39	2.60	2.69	2.56	0.52	0.52	0.55	0.53	2.98	3.06	3.16	3.07
Mean (A)	2.00	2.15	2.22		0.46	0.47	0.48		2.69	2.76	2.86	
L.S.D at 5 %	A 0.023	B 0.036	AB 0.06		A 0.003	B 0.004	AB 0.007		A 0.023	B 0.036	AB 0.06	

The promoting effect of planting space was found by many authors such as, Abd EL-Salam (1994) on *Pimpinella anisum* plants; Kassem (2002) on rosemary plants and Ibrahim (2010) on *Aloysia triphylla* plants, mentioned that the NPK contents and total chlorophylls, as well as total carbohydrates content increased by increasing the spacing between plants.

Effect of Biofertilizers:

Tables (5 and 6) also showed that inoculating lemon balm plants with a tribal mixture of biofertilizers treatment + 50% NPK resulted in the highest nitrogen percentage. This is due to the effect of all strains. In addition, phosphate-dissolving bacteria secrete organic acids, which lead to a transfer of fixed phosphate to available phosphate. This may increase growth of roots in the soil that can take up phosphorus.

Table (6): Effect of plant space, biofertilizers and their interaction on total carbohydrate (%) and total chlorophyll (mg / g - F.W.) of lemon balm plants during (2009/10) and (2010/11) seasons.

Fertilizers (B)	Total carbohydrate (%)				Total chlorophyll (mg/g-F.W.)			
	Planting space (cm) (A)							
	1 st season (2009 / 2010)							
	40 cm	50 cm	60 cm	Mean (B)	40 cm	50 cm	60 cm	Mean (B)
T1	19.60	20.20	21.15	20.22	0.97	0.98	1.00	0.98
T2	18.05	18.65	19.10	18.60	0.85	0.87	0.89	0.87
T3	17.32	17.95	18.50	17.92	0.83	0.84	0.86	0.89
T4	16.85	17.45	17.80	17.37	0.80	0.83	0.83	0.82
T5	19.30	19.75	20.35	19.80	0.95	0.97	1.00	0.97
T6	18.65	19.20	19.85	19.24	0.93	0.95	0.97	0.95
T7	18.24	18.85	19.40	18.83	0.89	0.92	0.93	0.92
T8	19.84	20.30	21.30	20.43	0.97	0.99	1.02	0.99
Mean (A)	18.96	19.61	19.85		0.90	0.92	0.94	
L.S.D at 5 %	A 0.659	B 0.648	AB 1.247		A 0.009	B 0.011	AB 0.019	
2 nd season (2010 / 2011)								
T1	19.96	20.61	21.23	20.60	0.97	0.99	1.01	0.99
T2	18.74	19.04	19.55	19.11	0.86	1.39	0.91	0.89
T3	18.09	18.50	18.95	18.51	0.83	0.85	0.87	0.85
T4	17.58	18.25	18.45	18.09	0.81	0.82	0.86	0.83
T5	19.54	20.32	20.75	20.20	0.97	0.99	1.00	0.99
T6	19.10	19.70	20.35	19.72	0.94	0.95	0.97	0.95
T7	18.75	19.25	20.05	19.35	0.90	0.91	0.92	0.91
T8	20.15	20.72	21.60	20.82	0.98	1.01	1.02	0.99
Mean (A)	18.90	19.45	20.01		0.91	0.93	0.94	
L.S.D at 5 %	A 0.667	B 0.776	AB 1.357		A 0.006	B 0.007	AB 0.013	

Therefore, the percentages of N, P and K elements in the leaves were increased and this increment led to promote the growth and yield of lemon balm plants. Moreover, phosphate solubilizing bacteria release organic and inorganic acids which reduce soil pH leading to change of phosphorus and other nutrients to available forms ready for uptake by plants, (Singh and Kapoor, 1999).

Effect of the interaction treatments :

Lemon balm nutrient contents N, P and K (%), total carbohydrates (%) and Chlorophyll (mg-g /F.W.) were affected by plant space and biofertilizers use (Table, 5 and 6). Regarding to combined effect of various biofertilizers and different plant spacing the obtained data revealed that the maximum plant height and the heaviest fresh weight of leaves were recorded from plant supplied with nitrobein, and grown under closer distance condition (40cm). The obtained data in Table (6) revealed that the maximum total carbohydrate and total Chlorophyll as well as nutrient content N, P and K were recorded from plants supplied with a mixture of (nitrobein, phosphorein

and potassein-p) plus 50% of recommended dose of NPK mineral fertilizer and grown under wider distance effect (60cm).

The increase in the contents of nutrients in the dry matter of *Melissa officinalis* as a result of the fertilization treatments is reasonable, since raising NPK levels as a result of fertilization treatments in the root medium led to more root growth. This may be accompanied by converting the unavailable forms of nutrient elements to available forms by the microorganisms in biofertilizer, more absorption of essential elements from the soil and their accumulation in plant tissues, (Cocking, 2003). In this regard, Belimov *et al.* (1995) reported that the inoculation with bacterial mixtures provide a more nutrition for the plants and improvement in root uptake of both nitrogen and phosphorus as a balance result of mechanism of interaction between nitrogen fixing and phosphate solubilizing bacteria.

CONCLUSION

According to the previous results, it appears that the two factors of various planting distances and Biofertilizers have an important role in growth and yield characters as well as essential oil quality and quantity accumulation for *Melissa officinalis* plants.

Finally, using biofertilizer (combined strains) plus the half dose of recommended NPK at 40cm planting space gives the greatest plant growth, leaves yield and essential oil content, saves half of the quantity of chemical fertilizers, decreases the production cost and the environmental pollution.

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تأثير مسافة الزراعة وبعض الأسمدة الحيوية على النمو و المحصول في نبات الترنجان

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تم تنفيذ تجربتين حقليتين بمزرعة النباتات الطبية والعطرية - كلية الزراعة - جامعة
المنصورة خلال موسمين زراعيين ناجحين ٢٠٠٩/٢٠١٠ و ٢٠١٠/٢٠١١ لتحديد أنسب مسافة
زراعة من بين ثلاث مسافات هي ٤٠، ٥٠، ٦٠ سم وأفضل معاملة سماد حيوي من النتروبيين
والفوسفورين والرش الورقي بالبوتاسين_فو للحصول على أفضل نمو ومحصول ورقى ومحتوى
زيت عطرى لنبات الترنجان.

وكانت أهم النتائج كالتالى:

١- أثرت مسافات الزراعة بين النباتات تأثيرا معنويا على كل الصفات المدروسة، وأعطت
المسافات الضيقة ٤٠ سم أعلى هذه القيم من حيث أطول النباتات وأعلى محصول ورقى وكذا
أعلى محصول من الزيت العطرى. بينما أعطت المسافات الواسعة ٦٠ سم أكبر عدد من الأفرع
الجانبية وأكبر وزن للأوراق الجافة للنبات وكذا أعلى نسبة مئوية للزيت العطرى فى كلا
الموسمين.

٢- على الجانب الآخر فإن إضافة الأسمدة الحيوية قد أظهر تحسن فى معظم صفات النمو
ومحصول الزيت. ولقد تم للحصول على أطول النباتات و أكبر عدد من الأفرع الجانبية وأكبر
وزن للأوراق الجافة للنبات ومكونات الزيت العطرى وكذلك محتوى الكلوروفيل الكلى وأعلى
نسبة مئوية لكل من النتروجين والفوسفور والبوتاسيوم وكذلك الكربوهيدرات الكلية بخلط كل من
النتروبيين والفوسفورين والبوتاسين مع نصف الجرعة الموصى بها من السماد الكيماوى
واضافتهم للتربة والى النبات.

٣- كان للتفاعل بين عاملى الدراسة تأثيرا معنويا على جميع الصفات تحت الدراسة فى كلا
موسمى الزراعة. فقد أوضحت النتائج أن أعلى القيم لمتوسطات معظم صفات النمو ومحصول
الأوراق ومحتوى الزيت العطرى كان من الزراعة على مسافة ٤٠ سم بين النباتات وإضافة
الأسمدة الحيوية الثلاثة (النتروبيين والفوسفورين والبوتاسين-فو) مع نصف الجرعة الموصى بها
من السماد الكيماوى. وهذه النتائج قريبة من النتائج المتحصل عليها من استخدام المعدلات
الموصى بها من السماد الكيماوى.

التوصية :

من النتائج السابقة يمكن التوصية بالزراعة على مسافة ٤٠ سم بين النباتات واستخدام
مخلوط من النتروبيين والفوسفورين والبوتاسين_فو مع نصف المعدلات الموصى بها من السماد
الكيماوى لتقليل تكاليف الانتاج والحد من تلوث البيئة والحصول على أفضل نمو ومحصول ورقى
ومحتوى من الزيت العطرى لنباتات الترنجان.

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