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FEFECT OF BIO AND MINERAL FERTILIZERS ON THE PRODUCTION OF THYME (*Thymus vulgaris*, L.) PLANT.

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ABSTRACT

A nutritional study was carried out on thyme *Thymus vulgaris*, L. plants at the Farms of Agric. Research Center in EL-Baramon (Mansoura) and in the Laboratory of the Veg.& Flori. Dept., Fac. of Agric. Mansoura Univ. during the two seasons of (2001 and 2002). The investigation was planed to study the effect of nitrobein (2kg/fed.) and phosphorein (2kg/fed.), nitrobein (2kg/fed.) plus calcium super phosphate (150kg/fed.), phosphorein (2kg/fed.) plus ammonium sulphate (300kg/fed.) and calcium super phosphate(150kg/fed.) plus ammonium sulphate (300kg/fed.) on the vegetative growth, and yield of leaves and essential oil of thyme plant. Data were collected on plant height, number of branches/plant and fresh and dry weight of herb and leaves. In addition, essential oil of dry leaves was extracted and determined, and the thymol concentration in the essential oil was measured using G.L.C. separation. Chemical composition (nitrogen, phosphorus, potassium and total carbohydrate), percentages in dry leaves were also measured.

The results showed that in both seasons, all fertilizer treatments produced significantly better growth and yield than the untreated control. Treatments included phosphorein (phosphorein plus ammonium sulphate and nitrobein plus phosphorein) produced the tallest plants, the highest number of branches, highest fresh and dry weights of herb and leaves per plant, and the highest yield of dry leaves per feddan, followed by nitrobein plus calcium superphosphate treatment, while inorganic fertilizer only treatment was the least in all parameters.

The results also showed that the highest essential oil percentage in the dry leaves and thymol (main constituent) content along with the highest yield of the essential oil per feddan in both seasons were produced by nitrobein plus calcium superphosphate treatment, which also resulted the highest percentages of phosphorus, potassium and total carbohydrates in dry leaves of thyme plants. Although the treatments included biofertilizers did not significantly differ in the yield of the dry leaves or the essential oil among each other, they were significantly better than using mineral fertilizers alone.

INTRODUCTION

Thyme (*Thymus vulgaris* L.), Fam. Lamiaceae, is an important aromatic and me dicinal plant, which grows wild and well in Sinai, Egypt (Julia, 1977). The drug occurs in segments of the leaves and flowering tops, which contained essential oil, the most important constituents of essential oil is the phenolic thymol (Harold *et al.*,1984). Essential oil of thyme is extensively used in pharmaceutical industry, i.e antiseptic, antioxidative and as a natural food preservative (Deans *et al.*, 1993).

Nitrogen, phosphorus and potassium are very important elements for plant growth (Radwan, 1980). However, the intensive use of expensive

mineral fertilizers in recent years resulted in increasing the production costs of the crops in addition to the environmental pollution problems (Pokorna, 1984).

Biofertilizers are microbial inoculants used for application to either seeds or soil for increasing soil fertility (Subb Rao, 1981). Commercial biofertilizer (nitrobein) increased the vegetative growth and the essential oil percentage in the seeds of both fennel and dill plants (Abd El-Rahman,2001). Eid and El-Ghawwas (2002) mentioned that using nitrobein and microbein biofertilizers increased plant hight and dry weight of majoram plant. Abdel-Kader and Ghaly (2003) on coriander plants reported that phosphorein plus nitrobein treatment gave the heighest linalool (81.3%) compared with the control.

Therefore the use of biofertilizers could supply plants with their needs of nitrogen and phosphorus during their growth by cheap means, and would lead to significant decrease in production costs of different crops.

The aim of this research was to study the effects of biofertilizers alone or in combination with mineral fertilizers on vegetative growth, leaves and oil yield and chemical composition of leaves and oil of thyme plants in order to achieve high yield with minimum pollution to the environment.

MATERIALS AND METHODS

This work was carried out on thyme, *Thymus Vulgaris,* L., plant at the Farm of the Agricultural Research Center in EL-Baramon (Mansoura), and in the Laboratory of the Veg. & Flori. Dept. Fac. of Agric. Mansoura Univ. during two successive seasons of 2001 and 2002. The present investigation aimed to study the effect of biofertilizers alone or in combination with mineral fertilizers on vegetative growth, leaves and oil yield and chemical composition of leaves and essential oil of thyme plants.

Seeds of thyme (local variety), were sown in the nursery on February 15th on both seasons. The seedlings were transplanted to the experimental field after six weeks from sowing on 1st April, when they reached about 8-10 cm height

The field was divided to experimental plots, each one was 3.5 m^2 (2 × 1.75m) and each plot included 3 rows (each 1.50m long and rows were 50cm apart) each plot contained 15 plants. The seedlings were planted at 25 cm apart in hills. Irrigation was carried out whenever needed.

Biofertilizer application:

Biofertilizers (nitrobein and phosphorein) were added after mixing with sand each one at the rate of 1.75 g/plot (2 kg/fed.) divided into three equal portions. The seeds were inoculated with one portion before sowing, whereas the second portion was added to the plants during transplanting practice (after six weeks from sowing) and the last one was added after six weeks from transplanting the plants.

Mineral fertilizer application:

Potassium fertilizer was applied to all plots including the control ones in the form of potassium sulphate (48% K_2O) at 150kg/fed. The amount of potassium was added as one dressing during soil preparation before planting.

Nitrogen fertilizer was added in the form of ammonium sulphate (20.5%N) at the rate of 300 kg/fed. divided into three equal portions, as monthly side dressings, starting after one month from planting.

Phosphorus fertilizer in the form of calcium superphosphate (15.5% P_2O_5) was applied at 150kg/fed. as one dressing during soil preparation before planting.

The experiment was designed as randomized complete block design including five treatments, each of them was replicated four times as follows:

1- Control.
2- Nitrobein + Phosphorein.
3- Nitrobein + Calcium superphosphate.
4- Phosphorein + Ammonium sulphate.
5- Ammonium sulphate + Calcium superphosphate.

Thyme plants were cut at the soil surface at the end of July in both seasons (after 120 days from transplanting), leaves were separated by hand from the herb, and the following data were recorded:

1- Vegetative growth characters

Twelve plants were chosen randomly from each treatment and the following characters were recorded :

1-1 Plant height (cm) was measured from the soil surface to the end of the main stem.

1-2 Number of branches/plant.

1-3 Herb fresh and dry weights (g/plant).

1-4 Leaves fresh and dry weight (g/plant) and yield of dry leaves (kg/fed.)

N.B. Herb and leaves were dried in perforated paper bags at room temperature until constant weight.:

2- Essential oil determination:

The dried leaves were subjected to the following determinations:

2.1 Essential oil percentage: oil samples of dry leaves (25g) were extracted by hydro-distillation in Clevenger apparatus according to the **Egyptian Pharmacopoeia (1984)**.

2.2 Essential oil yield (Liter/fed.): was calculated by multiplying the essential oil percentage by leaves dry weight/fed.

2-3 Thymol percentage: gas Liquid Chromatography analysis was carried out at the Medicinal and Aromatic Plants Section, Agric. Res. Center to separate and identify the main component of thyme essential oil (thymol) according to (Guenther and Joseph, 1978).

3- Chemical analysis of dry leaves:

Leaves samples of both seasons were dried at 70°C for 48-72hrs. to constant weight. The dry materials were finally ground and used for determination of nitrogen and potassium percentage as described method by (Jackson, 1967) and phosphorus percentage using spectrophotometer method as described by (Humphries, 1956). The total carbohydrates percentage was determined according to (Doubois *et al.*, 1956).

Soil analysis

Before the start of the experiment, soil samples were taken at 30 cm deep from the soil surface and were analyzed for chemical and mechanical analysis at the laboratory of Soil Science Dept. Fac. of Agric. Mansoura Univ.

Table(A): Chemical and mechanical properties of the experimental soil.

Mechanical analysis %			Organic matter%	Chemical analysis PPM			PH	E.C mmhos / cm
Sand	Silt	Clay		N	P	K		
12.65	17.6	66.7	2.3	24.6	6.2	450	7.6	1.40

Statistical analysis

A randomized complete block design with 4 replicates was used according to Steel and Torrie (1980). Data were subjected to the statistical analysis according to the analysis of variance procedure (ANOVA) using SAS computer software (SAS Institute, 1985). The treatment means were compared using the least significant difference (L.S.D) procedure as mentioned by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

1. Effect of different fertilizer treatments on the vegetative growth:

a) Plant height and number of branches/plant:

All fertilizer treatments produced significantly taller plants and more branches than the control in both seasons (Table 1). In both seasons, thyme plants treated with nitrobein + phosphorein or phosphorein plus ammonimum sulphate produced taller plants with more branches than the other three treatments including the control. In general, plants received mineral fertilizers only were less in height and branching than the other three treatments containing biofertlizers alone or mixed with inorganic fertilizers. Similarly, nitrobein and phosphorein were found to increase height and branching of fennel and dill (Abd El-Rahman, 2001) and marjoram (Eid and El-Ghawwas, 2002).

Table(1):Effect of bio and mineral fertilizers on plant height and number of branches/plant of (*Thymus vulgaris*, L.) during two seasons (2001 and 2002).

Fertilizers treatments	Plant height (cm)		Number of branches /plant	
	1st season	2nd season	1st season	2nd season
Control	35.92	32.75	5.17	4.75
Nitrobein + Phosphorein	47.17	46.00	7.67	7.75
Nitrobein + Calcium superphosphate	45.19	45.54	7.42	7.17
Phosphorein + Ammonium sulphate	46.33	47.75	7.65	7.75
Ammonium sulphate + calcium superphosphate	42.25	38.88	7.08	6.33
L.S.D. at 5%	4.43	5.20	0.87	0.82

Our results showed that inoculation with nitrobein and/or phosphorein increased height and branching of thyme plants which coincided with the results of Abd EL-Latif and Salem (2002) who found that nitrobein and phosphorein increased plant growth of *Tagetes minuta*. The significant increases in plant height and branching might be a result of increasing metabolic activities of the plant under the effect of the combination between bio- and mineral fertilizers in the two seasons.

b) Herb and leaves fresh weight:

Table (2) showed that, in both seasons, all tretments produced more fresh weight of both herb and leaves than the control. The highest fresh weight of both herb and leaves fresh weights resulted from phosphorein plus ammonium sulphate and nitrobein + phosphorein treatments. The highest fresh weight produced by these two treatments was because they produced more branching compared with other treatments as shown in Table (1). It is worthy to note that treatments included phosphorein were very effective in increasing the fresh weight of both the herb and the leaves. These results were similar to those found by Abdel-Kader and Ghaly (2003), who showed that inoculation with phosphorein plus inorganic fertilizers or phosphorein plus nitrobein and inorganic fertilizers produced best growth of coriander plants. Using inorganic fertilizers only produced the least weight of leaves than the other fertilizer treatments in both seasons. One can suggest that biofertlizers take more time to make nitrogen and phosphorus available to be used by the plant than the inorganic ones, but they provided these elements to the plant over a longer period to meet with the needs of the progressive development of the plant.

Table(2): Effect of bio and mineral fertilizers on herb and leaves fresh weight of (*Thymus vulgaris*, L.) during (2001 and 2002) seasons.

Fertilizers treatments	Herb fresh weight (g/plant)		Leaves fresh weight (g/plant)	
	1st season	2nd season	1st season	2nd season
Control	77.51	104.26	42.88	57.38
Nitrobein + Phosphorein	171.91	200.93	100.96	124.28
Nitrobein + Calcium superphosphate	142.69	192.86	75.61	118.43
Phosphorein + Ammonium sulphate	190.24	217.06	105.15	125.50
Ammonium sulphate + calcium superphosphate	135.62	173.97	66.34	85.56
L.S.D. at 5%	34.98	34.19	20.71	19.23

c) Herb and leaves dry weight:

As shown from the data in Table (3), the dry weight of herb and leaves per plant followed a similar trend to that of the herb and leaves dry weight. In both seasons, phosphorein plus ammonium sulphate produced the highest herb and leaves dry weight followed by nitrobein plus phosphorein treatment, then nitrobein plus calcium superphosphate, while inorganic fertilizers only were the least. Attia (2000) and Badawi (2000) reported that biofertilizers were effective in increasing the total carbohydrates in henna and roselle leaves. As a consequence, it seems that treatments included

biofertilizers in this study increased the dry weight of the leaves by increasing their carbohydrate contents.

Table (3): Effect of bio, mineral fertilizers and their combination on herb and leaves dry weight of (*Thymus vulgaris*, L.) during two seasons (2001 and 2002).

Fertilizers treatments	Herb dry weight (g/plant)		Leaves dry weight (g/plant)	
	1st season	2nd season	1st season	2nd season
Control	23.19	31.09	12.12	16.24
Nitrobein + Phosphorein	47.90	58.25	28.17	33.90
Nitrobein + Calcium superphosphate	41.43	54.87	21.34	33.40
Phosphorein + Ammonium sulphate	51.17	62.59	28.99	35.27
Ammonium sulphate + calcium superphosphate	38.74	51.20	19.61	25.17
L.S.D. at 5%	9.86	11.304	4.32	4.90

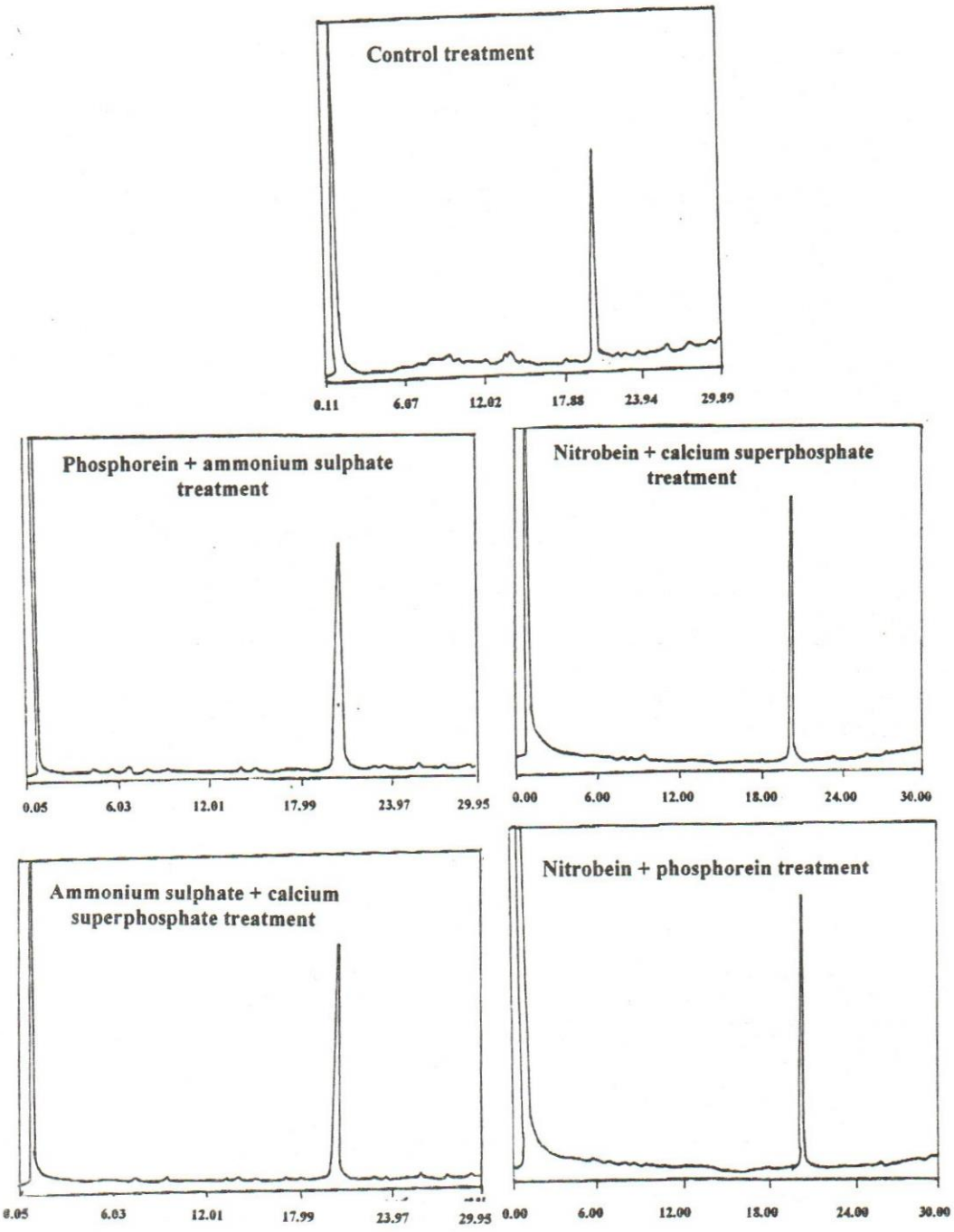
2. Effect of different fertilizer treatments on the essential oil and thymol percentages:

All fertilizer treatments produced more essential oil than the control in both seasons (Table 4).. Nitrobein + calcium superphosphate gave the highest significant oil percentage (2.24 and 2.25%) in both seasons, respectively, while ammonium sulphate plus calcium superphosphate (inorganic fertilizers only) produced the least concentration (1.42 and 1.15%) among all fertilizer treatments. Thus, the data clearly showed that the three treatments containing biofertilizer produced higher essential oil percentage than the treatment using inorganic fertilizers only. The increase in oil percentage might be due to the beneficial effect on these elements on the synthesis of carbohydrates in plant tissues, which was used for oil formation.

The data of Table (4) and Figures (1 to 5) also showed that although thymol concentration was not significantly affected, all fertilizer treatments relatively increased thymol percentage compared with the control. Also, the highest relative value of thymol concentration was that of nitrobein plus calcium superphosphate treatment (91.73 and 92.96% in the first and second seasons), followed by phosphorein plus ammonium sulphate, then nitrobein plus phosphorein, while plants received chemical fertilizers only had relatively the least value among the fertilizer treatments.

Table (4): Effect of bio and mineral fertilizers on essential oil % and thymol % of (*Thymus vulgaris*, L.) during two seasons (2001 and 2002).

Fertilizers treatments	Essential oil %		Thymol %	
	1st season	2nd season	1st season	2nd season
Control	0.83	1.00	54.18	71.01
Nitrobein + Phosphorein	1.53	1.75	89.27	89.52
Nitrobein + Calcium superphosphate	2.24	2.25	91.73	92.96
Phosphorein + Ammonium sulphate	1.44	1.75	91.29	91.58
Ammonium sulphate + calcium superphosphate	1.42	1.15	84.49	84.68
L.S.D. at 5%	0.37	0.53	N. S.	N. S.



Figs (1 - 5) :G.L.C. of thymol % in thyme essential oil as affected by bio and mineral fertilizers during (2001) season.

These results were in accordance with those reported by El-Sawy *et al.* (1998) on *Ammi visnaga*, Ibrahim (2000) on *Founiculum vulgare* and Abd El-Latif and Salem (2002) on *Tagetes minuta*, who mentioned that the combination between bio and NPK fertilizers caused increases in the main constituents of essential oil.

3. Effect of different fertilizer treatments on yields of the dry leaves and essential oil :

Concerning leaves dry weight and oil yield per faddan, Table (5) showed that all fertilizer treatments produced significantly more yields than the control. The highest yield of dry leaves in both seasons was produced by phosphorein plus ammonium sulphate (497.02 & 604.52 kg/fed.) and nitrobein plus phosphorein (482.86 & 581.13 Kg/fed.), followed by nirobein plus calcium superphosphate (365.81 & 572.54 Kg/fed), while the lowest was that of mineral fertilizers only which averaged 336.15 and 431.49 kg/fed. in the first and second seasons respectively. These results followed the same trend as other growth parameters mentioned earlier.

The data in Table (5) showed clearly that treatments included one or more biofertilizers produced significantly more oil yield than inorganic fertilizer alone and the control. However, the trend of the essential oil yield was a bit different from that of the dry leaves yield, since nirobein plus calcium superphosphate (which ranked third for dry leaves yield) had the highest oil yield of 8.19 and 12.88 litre/fed., in the first and second seasons respectively, followed by nitrobein plus phosphorein and phosphorein plus ammonium sulphate treatments. This was due to nitrobein plus calcium superphosphate treatment produced significantly more oil percentage than the other two treatments as shown in previous Table (4). The highest essential oil percentage compensated for the less dry leaves yield of this treatment and resulted in highest essential oil yield per feddan.

Table (5): Effect of bio and mineral fertilizers on yield of dry leaves and essential oil of (*Thymus vulgaris*, L.) during (2001 and 2002).

Fertilizers treatments	Dry leaves yield (kg/fed.)		E. oil yield (lt/fed.)	
	1st season	2nd season	1st season	2nd season
Control	207.76	278.39	1.72	2.78
Nitrobein + Phosphorein	482.86	581.13	7.39	10.17
Nitrobein + Calcium superphosphate	365.81	572.54	8.19	12.88
Phosphorein + Ammonium sulphate	497.02	604.52	7.16	10.58
Ammonium sulphate + calcium superphosphate	336.15	431.49	4.77	4.96
L.S.D. at 5%	95.82	84.16	1.36	2.56

Previous reports showed that inorganic fertilization increased both plant weight and oil yield of thyme such as Shalaby and Razin (1992), Younis (1998) and Morsy (1999). Also, biofertilizers were found to increase essential oil yield of *Thymus toesevii*, Hristova, *et al.*(1999). Our results showed that addition of biofertilizers alone or in combination with inorganic ones was more effective on yield than using inorganic fertilizers alone. These results coincide

with the researches, on palmarosa, Maheshwari *et al.* (1998), chamomile, Abd El-Latif *et al.* (2002) and coriander, Abdel-Kader and Ghaly (2003). The increase in essential oil yield per faddan seemed to be a result of the increase in leaves yield per faddan as previously mentioned, as well as the increment in the essential oil yield per plant as a result of treating thyme plants with nitrobein plus calcium super phosphate.

4- Effect of different fertilizer treatments on chemical contents in leaves:

Table (6) showed that the nitrogen percentage in leaves of thyme plants was slightly increased as a result of fertilization treatments in both seasons compared to control. Nitrobein plus phosphorein treated plants had relatively higher nitrogen than the other three treatments in both seasons. These results were in accordance with those obtained by Fayez *et al.* (1985) on wheat and Hassouna *et al.* (1994) on alfalfa who reported that total nitrogen content significantly increased after inoculation with Azotobacter combined with a moderate application of nitrogen fertilizer. Increasing the nitrogen content might be related to the effect of those fertilization elements on the absorption and translocation of nitrogen.

Table (6): Effect of bio and mineral fertilizers on nitrogen, phosphorus, potassium and total carbohydrate percentages in the dry leaves of (*Thymus vulgaris*, L.) during two seasons (2001 and 2002).

Fertilizers treatments	Nitrogen %		Phosphorus %		Potassium %		Total carbohydrate %	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
Control	1.83	2.97	0.25	0.21	2.00	2.45	37.80	21.00
Nitrobein + Phosphorein	3.20	3.89	0.49	0.38	2.80	3.10	47.40	52.20
Nitrobein + Calcium superphosphate	2.97	3.43	0.62	0.52	2.45	3.25	59.40	57.00
Phosphorein + Ammonium sulphate	2.97	3.66	0.43	0.48	2.30	3.15	58.80	53.70
Ammonium sulphate + Calcium superphosphate	2.74	3.19	0.36	0.26	2.10	2.95	46.20	40.20
L.S.D. at 5%	N. S.	N. S.	0.30	0.21	0.94	N. S.	9.66	13.38

It was also noticed that, the phosphorus percentage in leaves was higher in all fertilizer treatments than the control in both seasons. The highest phosphorus percentage was found in plants treated with nitrobein plus calcium superphosphate (0.62 and 0.52%) in both seasons. These findings in agreement with those obtained by Haggag and Azzazy (1996). The increase in the phosphorus content in leaves as a result of fertilization might be due to the increase of absorption of phosphorus and its role of production of nucleic acids.

Data recorded in Table (6) also showed that all fertilizer treatments resulted in significant increase in potassium percentage compared with the control in both seasons similar to the trend phosphorus percentage. These results were in accordance with those found by Nofal *et al.* (2001) on *Ammi visnaga*. The stimulating effect of fertilizer treatments on potassium accumulation may be due to their effect on enhancing the plant metabolism.

Regarding the total carbohydrates percentage in leaves, the data clearly showed that all fertilizer treatments significantly increased the total carbohydrates percentage in both seasons compared with the control. Plants treated with nitrobein plus calcium superphosphate contained more percentage of total carbohydrates (59.4 and 57.00% in the first and second seasons respectively) than the other three treatments.

The positive increase in percentage of total carbohydrates were in harmony with those obtained by (Morsy, 1999) on thyme plants and (Badawi, 2000) on roselle leaves. Thus, the application of bio and mineral fertilization increased the total carbohydrates percentage within the thyme plants, which might be due to the effect of direct or indirect of the anabolic process of carbohydrates metabolism. These nutrients participate in chlorophyll metabolism, leading to more chlorophyll content, which participates directly in the metabolism of carbohydrates (Devlin, 1975).

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تأثير التسميد الحيوى و المعدنى على إنتاج نبات الزعتر (*Thymus vulgaris*, L.)

حكمت يحيى مسعود* – هشام هاشم عبد القادر*– طه عبد اللطيف – منال محمد مليجى***

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***قسم النباتات الطبية والعطرية – معهد بحوث البساتين – القاهرة**

أجريت دراسة تسميدية على نبات الزعتر بمزرعة مركز بحوث البساتين بالبرامون (المنصورة) ومعمل قسم الخضروالزينة بكلية الزراعة – جامعة المنصورة خلال موسمين زراعيين متتاليين ٢٠٠١–٢٠٠٢. تمت دراسة تأثير النتروبين (٢كجم/فدان) + الفوسفورين (٢كجم/فدان) ، النتروبين (٢كجم/فدان) + سوبر فوسفات الكالسيوم (١٥٠كجم/فدان) ، الفوسفورين (٢كجم/فدان) + سلفات أمونيوم (٣٠٠ كجم/فدان) ، سوبر فوسفات الكالسيوم (١٥٠كجم/فدان) + سلفات أمونيوم (٣٠٠ كجم/فدان) على النمو الخضرى ومحصول الأوراق والزيت العطرى لنبات الزعتر. و لقد أخذت النتائج على صفات طول النبات و عدد الأفرع للنبات ووزن العشب و الأوراق الطازج و الجاف. بالإضافه الىإستخلاص وتقدير الزيت العطرى للأوراق الجافة وقياس تركيز مركب الثيمول (المركب الرئيسى) فى الزيت العطرى باستخدام طريقة الفصل الكروماتوجرافى كما تم تقدير النسبة المئوية للمكونات الكيماوية (النتروجين – الفوسفور – البوتاسيوم – الكربوهيدرات الكلية) فى الأوراق الجافة.

أوضحت النتائج ان جميع المعاملات السمادية أعطت نموا و محصولا أفضل من الكونترول بدون معاملة. ولقد أعطت المعاملات التى تحتوى على الفسفورين (الفوسفورين + سلفات الأمونيوم ومعاملة النتروبين + الفوسفورين) أطول النباتات وأكبر عدد من الافرع للنبات وأكبر وزن طازج و جاف للعشب والأوراق للنبات و أكبر محصول من الأولراق الجافة للفدان و تلاهما فى الترتيب معاملة النتروبين + سوبر فوسفات الكالسيوم فى حين أن معاملة التسميد المعدنى كانت الأقل.

أظهرت النتائج أيضا أن أعلى نسبة مئوية للزيت العطرى فى الأوراق الجافة والثيمول (المركب الرئيسى) ومحصول الزيت العطرى للفدان فى كلا الموسمين نتج عن معاملة النتروبين + سوبر فوسفات الكالسيوم و هى المعاملة التى أعطت أيضا أعلى نسبة من الفوسفور و البوتاسيوم لنباتات والكربوهيدرات الكلية فى الأوراق الجافة لنباتات الزعتر. و بالرغم من أن المعاملات التى تحتوى على الأسمدة الحيوية لم تختلف معنوبا فيما بينها فى محصولى الأوراق الجافة أو الزيت إلا أنها كانت أفضل بدرجة معنوية من إستخدام السماد المعدنى وحده.