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STUDIES ONPRODUCTION OFTHYMUS PLANT *(Thymus vulgaris* L. *)* PART ONE. EFFECT OF FOLIAR SPRAY WITH NPK, MICRO NUTRIENTS AND AMINO ACIDS ON THE VEGETATIVE GROWTH AND VOLATILE OIL PERCENTAGE IN THE DRY HERB

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ABSTRACT

This research was carried out at the Experimental Farm of Agricultural and in the Laboratory of the Vegetable and Floriculture Department, Faculty of Agriculture, Mansoura University during the two successive seasons 2014 and 2015 in order to investigate the effect of foliar spray with NPK, micro nutrients and amino acids on the vegetative growth and volatile oil percentage of thyme (*Thymus vulgaris* L.) plants.Treatments included three levels of each of NPK (20-20-20) alone, NPK+ micronutrients andNPK + micronutrients + amino acids, and untreated control. One year old plants in 30 cm plastic pots were cut to 5 cm from the soil surface and sprayed 5 times (at 15 days intervals) with NPK (20-20-20) solution at low, medium, and high levels(175, 350, 700 mg/L). In addition other plants were sprayed at three levels with Low, medium and high (50, 100, and 200 mg/L)concentrations with each of Disper complex fertilizer (micronutrients)andAmino mineral fertilizer (NPK + micronutrient + amino acids) solution. NPK (20-20-20) fertilizer concentrations were added separately to Disper complex treatments, so that each 50 mg contained ١٧٥ mg/L NPK, each 100 mg/L contained 350mg/L NPK, and the 200mg/L contained 700 mg/L NPK (20-20-20). The control (non-fertilized) plants were sprayed with water only. Data were studied on vegetative growth parameters and volatile oil percentage in the dry herb after the last spray by 15 days. The results showed that all fertilizer treatments improved vegetative growth and oil percentage compared with the control. The high level of fertilizers resulted in taller plants, more herb fresh and dry weight, more number of branches per plant, and more oil percentage compared with the other two levels. The high level of Amino mineral (200 mg/L) and Disper complex (200mg/L + 700 mg/L NPK) produced taller plants than the high level of NPK treatment (700 mg/L). The high level of Amino mineral treatment (200 mg/L) produced the tallest plants, highest herb fresh and dry weight, and number of branches per plant in addition to volatile oil percentage in the dry herb. In most cases, the high level of Disper complex treatment ranked second after the high level of Amino mineral Treatment. These results indicated the importance of using microelements plus amino acids to increase growth and essential oil percentage of Thyme plants.

INTRODUCTION

Thyme, common thyme, or garden thyme *(Thymus vulgaris L.)* is an a member of the *Lamiaceae* family, and one of the most popular medicinal and aromatic plants (Nickavar et al., 2005).The plant is perennial dwarf shrub native to the Mediterranean region (Letchamo and Gosselin, 1995 and Stahl-Biskup and Saez, 2002). The plant has gained important position in world trading expansion resulted from increased tendency toward cultivating

of this plant and also consumption of natural by products (Bagaliyan and Nagdi, 2000).

The green part of thyme plant constitutes the most popular herbal medicine and spice, used in all developing countries. The plant has an agreeable aromatic smell and a warm pungent taste, and fragrance of its leaves is due to an volatile oil, which gives it value for culinary purposes, and its medicinal properties(Masada, 1976;Bremness, 1997; Prakash, 1990;Jeno, 1996; Philips, 1998 and Chandler-Ezell, 2004).

Thyme also is used in medicine as carminative, antifungal, antioxidant, antiviral,antispasmodic,tonic, diaphoretic, diuretic, rheumatism and also used in the treatment of cancer and activities(Herrmann and Kucera 1967 ; Simon *et al.,* 1984; Deans and Waterman, 1993; Skwarek and Lutostanska, 1994; Zambonelli *et al.,* 1996; Chevallier, 1996; Barry-Ryan, 2009;Sokoviæ *et al.,* 2009;Vuuren *et al.,* 2009)

Nutritional factorsare very important for the production of plants and their chemical constituents (Abadi *et al.,* 2011). Some fertilizers contain only the macronutrients or micronutrients, and others include both categories. Relationship between micronutrients and macronutrients in plant growth environment would increase their productivity. The macro elements nitrogen, phosphorus and potassium play a key role in plant metabolism, also have an effect on the synthesis and accumulation of nutrients and secondary metabolites (Somida, 2002 and Omidbeigi, 2005and Koeduka *et al.,* 2006).Other nutrients, such as sulphur, calcium, magnesium and microelements, are also capable of changing essential oil yield and composition in medicinal plants (Wierdak, 2011).

Amino acids and enzymes play a key role in the biosynthesis of numerous compounds which are essential oil constituents (Koeduka et al. 2006). Amino acids foliar application was reported to improve growth of many medicinal and aromatic plants (Gamal El-Din *et al.,* 1997; Youssef *et al.,* 2004 andHadi *et al.,* 2011) as well as to affect their essential oil contents and composition (Gamal El-Din *et al.,* 1997;Talaat and Youssef, 2002; Yussef *et al.,* 2004;AbouDahab and Abd El-Aziz, 2006 and Abd El-Aziz and Balbaa, 2007).

The present investigation was planned to study the effect of foliar spray with NPK (20-20-20)alone, NPK +micronutrients andNPK + micronutrients +amino acids on the vegetative growth and volatile oil percentage of thyme (*Thymus vulgaris* L.) plants.

MATERIALS AND METHODS

This work was carried out at the Experimental Farm of Agricultural and in the Laboratory of the Vegetable and Floriculture Department,Faculty of Agriculture, Mansoura University during the spring of 2014 and 2015.

Plant material:

One year old thyme (*Thymus vulgaris* L.) transplants, about 10 cm in heightwere planted individually in 30 cm pots, were used in this experiment. Soilanalysis is shown in Table (1).

Table (1).Chemical soil analysis.

pH	Total dissolved salts		meq/L in 100 g soil							
	mmhos /cm	%	K^+	Na^+	Mg^{++}	Ca^{++}	SO_4^-	Cl^-	HCO_3^-	CO_3^{--}
8.86	0.36	0.12	0.12	0.28	0.56	0.88	0.50	0.85	0.50	0.00
ppm										
N			P				K			
89.78			21.00				240.00			

Treatments:

Pots were divided to 10 groups with 3 replicates each. Every replicate consisted of tree plants (pots). Nine groups consisted of three fertilizer types (NPK (20-20-20), NPK + Disper complex and Amino mineral fertilizers) with three levels (low, medium, and high), while the tenth group represented the untreated control.

Treatments were as follows:

1- **Control**(Plants were sprayed with tap water only, and its analysis is shown in Table (2).
2- LowNPK (NPK (20-20-20) 175 mg/ L).
3- MediumNPK (NPK (20-20-20) 350 mg/ L).
4- HighNPK (NPK (20-20-20) 700 mg/ L).
5- LowDisper complex (50 mg/ L Disper complex + NPK 175 mg/ L).
6- MediumDisper complex (100 mg/ L Disper complex + NPK 350 mg/ L).
7- HighDisper complex (200 mg/ L Disper complex + NPK 700 mg/ L).
8- Low Amino mineral (50 mg/L).
9- MediumAmino mineral (100 mg/L).
10- HighAmino mineral (200 mg/L).

Composition of fertilizer is shown in table (A). NPK fertilizer was used to equalize Nitrogen, Phosphorus, and Potassium in Disper complex treatments, since Disper complex contains micro elements only. Amino mineral fertilizer already contains 7% N, 7%P, and 7% K (each 50 mg/L contained 175 mg/ L, 100 mg/L contained 350 mg/L and 200 mg/L contained 700 mg/L NPK).

Analyses of the three fertilizers used are shown in Table(3).

All plants were cut to a height of 5 cm above soil level and then subjected to foliar sprays. Plants were sprayed five times with the previously mentioned concentrations at 15 days intervals. Plants were sprayed with the designated solutions with equal amount (300 ml).

Table (2).Chemical analysis of tap water.

E.C.	**0.88 (mmoh/cm)**
Total dissolved solids (T.DS)	563.2 ppm
pH	7.7
Anions (meq / l)	
CO3--	0
HCO3-	0.1
CL-	1.4
SO4--	2.9
Cations (meq / l)	
Ca++	1.2
Mg++	1.8
Na+	1.25
K+	0.15

Table (3).Composition of fertilizers.

Element	**Floral NPK (20-20-20)**	**Disper complex GS**	**Amino mineral**
N	20	-	7%
P	20	-	7%
K	20	-	7%
Zn	-	5%	2.5% .
Fe	-	5%	3.5%
Mn	-	4 %	2%
Mg	-	2%	1.5%
Cu	-	%٤	0.2%
Ni	-	-	0.01%
B	-	6%	0.1%
Mo	-	.2%	0.1%
Co	-	-	0.01%
S	-	-	7.37%
Amino acids	-	-	5.21%

Watering:

Plants werewateredwith tap water once every 3 days, since thyme plants are non-loving waterplants.

Experiment Design:

Experiment was designed with 3 main treatments fertilizer types and four fertilizer levels (control, low, medium and high) each treatment consisted of 3 replicates, and each replicate contained 3 plants.

Data recorded:

Fifteen days after the last spray, the heights of thyme plants were recorded and plants were cut at a 5 cm from the soil level and the following data were recorded:

1- Vegetative growthcharacters :

- Plant height (cm) was measured from the soil surface to the end of the main stem of each plant before harvest.
- Number of branches/plant.

- Herb fresh weight (g/plant).
- Herb dry weight (g/plant): plants were left to dry on bench inside the laboratory and their dry weights were recorded daily until constant weight.

2- volatile oil determination :

Thyme plants were collected at the harvest date in both seasons. Samples were air dried at room temperature and used for determined the essential oil percentage.

Essential oil percentage:

Herb samples (25g) were separately subjected to hydro-distillation in Clevenger apparatus for more than three hours according to the Egyptian Pharmacopoeia (1984), and oil was calculated as % of the dry herb.

RESULTS AND DISCUSSION

1-Effects on vegetative growth

Effect on plant height

a)Effect of fertilizer types

Data of table (4) show that there were no significant differences among the three fertilizers plant height in both seasons. However, in the first season, Amino mineral andDisper fertilizers produced slightly taller plants (34.31 and 34.06 cm respectively)than NPK plants (31.96 cm).

b) Effect of fertilizers levels

Data of table (4) also show that, in both seasons, all fertilizer levels produced significantly taller plants than the control. However, there were no significant differences in plant height among the three levels of fertilizers, but in the first season the high level produced slightly taller plants than other two levels.

c)Effects on the interaction

It is clear from table (4) that, in the first season, all fertilizer treatments produced significantly taller plants compared with the control. In the first season, The high level of Amino mineral produced tallest plants followed by high level of Disper complex (40.83 and 38.67 cm respectively). In the second season, fertilizer treatments produced slightly taller plants than the control.

Data showed that NPK fertilization improved plant height of thyme plant. In this concern, Younis (1998) found that NPK fertilizers increased height of thyme plants.

Amino mineral and Disper contain micro elements which were shown by Morsy (1999) that foliar application of trace-elements improved height of Thymusplant. Similar results also were reported by Nooh (1991) on sweet basil and Khater et al. (1996) on *Menthapiperita* L.

Amino mineral fertilizer contained Amino acids which were reported by Hadi et al. (2011) to increase plant height of chamomile.

Effect on number of branches / plant

a) Effect of fertilizer types

Data of table (4) show that, in both seasons, there were no significant differences between the three fertilizers in the number of branches of thymus plants. However, Amino mineral and Disper complex produced more number of branches than NPK.

b)Effect of fertilizers levels

Data of the same table (4) show that the high fertilizer level produced more number of branches per plant in the first and second seasons.

C) Effects on the interaction

It is clear from table (4) that, in both seasons, the high levels of Amino mineral and Disper complex produced highest number of branches per plant inthe first season (315.7 and 312,7 respectively) and did not significantly differ with the high level of NPK (271.2). In the second season the trend was very similar, but the medium level of Amino mineral produced more number of branches than the high level of either Disper complex or NPK. It worth noting that the high and medium levels of each fertilizer did not significantly differ in number of branches produced by thymus plant. .

Similarly, NPK fertilization wasreported to incease number of branches in *Thymus vulgaris* L. (EL-Ghadban, 1998 and Younis, 1998) and sweet basil (Mahgoub, 1995).

It was also reported that foliar spray with microelements increased number of branches of *Thymus vulgaris* L. (Morsy, 1999) and sweet basil(Nooh, 1991).

Foliar spray with amino acids also increased number of branches oflemongrass (Gamal El-Din *et al.,* 1997),basil (Talaat and Youssef, 2002),Datura (Youssef *et al.,* 2004), and chamomile(Hadi et al, 2011).

Table (4) Effect of fertilizer types and levels on plant height and number of branches / plant.

Treatments		Plant height (cm)		Number of branches	
		First season	Second season	First season	Second season
Fertilizer type	Amino mineral	34.31	29.98	286.0	126.915
	Disper complex	34.06	28.5	286.7	101.66
	NPK	31.96	30.4	286.0	126.915
LSD (5%)		NS*	NS	NS	NS
Fertilizer level	Control	28.17	27.6	278.7	89
	Low	34.64	31	267.2	103.8867
	Medium	34.31	30	276.2	114.33
	High	36.67	29.7	303.1	131.44
LSD (5%)		2.73	NS	36.36	20.3
Amino mineral	control	28.17	27.6	278.7	89
	Low	35.08	31.3	284.3	127
	Medium	33.17	31	265.3	138.3
	High	40.83	30	315.7	153.3
Disper complex	Control	28.17	27.6	278.7	89
	Low	34.67	27.6	245.7	91.66
	Medium	34.75	29.6	309.7	96.66
	High	38.67	29.1	312.7	129.3
NPK	control	28.17	27.6	253.7	89
	Low	34.17	34.33	271.7	93
	Medium	35.00	29.6	253.7	108
	High	30.50	30	271.2	111.7
LSD (5%)		4.73	NS	62.98	57.53

NS* Non significant at LSD=5%

Effect on herb fresh weight (g)

a) Effect of fertilizer types

Data of table (5) show that in both seasons, there were no significant differences in herb fresh weight among the three fertilizers types. However, Amino mineral fertilizer produced slightly more fresh weight per plant (37.13 and 29.28 g) in the first and second seasons respectively than other fertilizer types. In the second season, Disper complex produced slightly more fresh weight than NPK.

B) Effect of fertilizers levels

Data of the same table (5) show that, in both seasons, the highest fresh weight of the herb (37.67 and 33.52 g/plant) respectively was achieved using the high level of fertilizers.

C) Effects of the interaction

It is clear from table (6) that, in the first season, medium and high fertilizer levels produced significantly taller plants compared with the control. In both seasons, the high level of Amino mineral fertilizer produced the highest fresh weight of thyme plants (50.1 and 45.6 g/plant respectively). However, in both seasons there were no significant differences in plant fresh weight between the high or medium level of Amino mineral fertilizer.

Similarly, NPK fertilization was reported to increase fresh weight of *Thymus vulgaris* (EL-Ghadban, 1998 and Younis, 1998) and (EL-Ghadban,1994)plants.

In addition, previous reports showed that foliar spray with microelements increased fresh weight of *Thymus vulgaris* L.(Morsy, 1999) and (Khater *et al.,* 1996) plants.

Foliar spray with amino acids was reported to significantly increase vegetative growth and fresh weight of lemongrass (Gamal El-Din *et al.,* 1997),Datura,(Youssef et al.,(2004), basil(Talaat and Youssef, 2002) and chamomile flowers (Haadi *et al.,* 2011).

Effect on herb dry weight (g)

a) Effect of fertilizer types

Data of table (5) show that in the first season there were no significant differences in herb dry weight among the three fertilizers types. However, in the first season, Amino mineral produced slightly higher herb dry weight (14.64g) followed by Disper complex (14.68 g), but in the second season, NPK and Amino mineral fertilizers produced slightly more dry weights (15.43 and 15.98 g respectively) than Disper complex.

B) Effect of fertilizers levels

Data of the same table (5) show the high level of fertilizers produced higher dry weight of thymus plant (14.76 and 16.96 g) in the first and second seasons respectively. However, there were no significant differences between high or medium levels in both seasons.

C) Effects of the interaction

It is clear from table (5) that, in the first season, higher level of Amino mineral fertilizer produced highest dry weight of thymus plant (17.22 g) followed by its medium level (15.5 g), and high level of Disper complex

(15.17 g). However, there were no significant differences between the three mentioned treatments.

Table (5) Effect of fertilizer types and levels on herb fresh and dry weight / plant.

Treatments		Herb fresh weight /plant (g)		Herb dry weight / plant (g)	
		First season	Second season	First season	Second season
Fertilizer type	Amino mineral	37.13	29.28	14.64	15.98
	Disper complex	34.91	24.18	14.68	14.05
	NPK	35.8	32.6	13.92	15.34
LSD (5%)		NS*	NS	NS*	NS
Fertilizer level	Control	32.32	18.28	14.70	12.03
	Low	34.93	26.24	13.85	15.3
	Medium	36.36	30.02	14.23	14.79
	High	37.67	33.52	14.76	16.96
LSD (5%)		5.49	5.49	13.98	1.57
Amino mineral	control	32.32	18.28	14.70	12.03
	Low	39.94	30.67	14.15	8.5
	Medium	44.47	44.28	15.50	13.03
	High	50.01	45.6	17.22	16.66
Disper complex	Control	32.32	18.28	14.70	12.03
	Low	35.41	23.58	14.32	14.3
	Medium	36.13	25.96	14.53	14.43
	High	35.80	28.93	15.17	15.43
NPK	control	32.32	18.28	14.70	12.03
	Low	35.41	28.46	13.12	14.4
	Medium	36.13	30.53	13.62	14.6
	High	35.80	32.6	13.92	15.34
LSD (5%)		9.503	9.503	20.3	2.72

NS* Non significant at LSD=5%

Effect on oil percentage in dry herb

a) Effect of fertilizer type

Data of table (6) show that although no significant differences between the three fertilizers in their effects on oil percentage, Amino mineral produced highest oil percentage in both seasons (1.34 and 1.08 % in the first and second seasons respectively). Disper complex ranked second in the first season, but was third in the second season.

b) Effect of fertilizers levels

Data of table (6) show that the high level of fertilizers produced highest oil percentage in the first (1.43%) and second (1.21%) seasons.

c) Effects of the interaction

It is clear from table (6) that, in the first season, highest oil percentage was a result of medium and high levels of Amino mineral fertilizer (1.52 and 1.47% respectively. This held true for the high level of Amino mineral in the second season (1.56%). This supports that the high level of Amino mineral produced the highest oil percentage in both seasons. On the other hand,Disper complex and NPK produced almost similar oil percentages in both seasons.

In this concern, previous reports by Omidbaigi and Arjmandi (2002), Ali (2010), and Mehrnoush and Shahram (2014) on Thymus vulgaris L., Omer (1999) on Egyptian oregano,Daneshian et al. (2009) on basil confirmed a positive relation between fertilization (especially nitrogen) rate and oil content. On the contrary, Arabaci and Bayram (2004) reported that Thymus plants which were not fertilized by nitrogen fertilizer had more oil percentage.

Previous research showed that foliar spray with amino acids was reported to improve oil content in lemon grass (Gamal, El–Din, et al (1997), and chamomile (Hadiet at., 2011).

Table (6) Effect of fertilizer types and levels on the oil percentage of the dry herb.

Treatments		Essential oil (%)	
		First season	Second season
Fertilizer type	Amino mineral	1.34	1.08
	Disper complex	1.22	0.87
	NPK	1.19	0.92
	LSD (5%)	NS	NS
Fertilizer level	Control	1	0.57
	Low	1.22	1.04
	Medium	1.36	1.0
	High	1.43	1.21
	LSD (5%)	0.08	0.25
Amino mineral	control	1.0	0.57
	Low	1.38	1.21
	Medium	1.52	0.99
	High	1.47	1.56
Disper complex	Control	1.0	0.57
	Low	1.14	0.85
	Medium	1.33	1
	High	1.42	1.07
NPK	control	1.0	0.57
	Low	1.14	1.07
	Medium	1.23	1.02
	High	1.42	1.02
	LSD (5%)	0.15	0.44

NS* Non significant at LSD=5%

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دراسات على انتاج نبات الزعتر *(Thymus vulgaris L.)* الجزء الأول تأثير الرش بالنتروجين والفسفور والبوتاسيم والعناصر الصغرى والأحماض الأمينية على النمو الخضري والنسبة المئوية للزيت الطيار في العشب الجاف

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تم إجراء هذا البحث في محطة التجارب الزراعية ومعمل قسم الخضر والزينة بجامعة المنصورة خلال العامين المتتاليين ٢٠١٤ و ٢٠١٥ بهدف دراسة تأثير الرش بالنتروجين والفسفور والبوتاسيوم والعناصر الصغري والأحماض الأمينية على النمو الخضري والنسبة المئوية للزيت الطيار لنبات الزعتر (Thymus vulgaris L.) ، ولقد شملت المعاملات ثلاث مستويات لكل من ن-فو-بو (٢٠-٢٠-٢٠) ، ن-فو-بو مع العناصر الصغرى ، ن-فو-بو مع العناصر الصغرى والأحماض الأمينية ، الكنترول (بدون معاملة). ولقد تم قص نباتات الزعتر عمر سنة النامية في أصص ٣٠ سم لإرتفاع ٥ سم من سطح التربة وتم رش النباتات ٥ مرات (على فترات كل ١٥ يوما) بـ ن-فو-بو (٢٠-٢٠-٢٠) بتركيزات ١٧٥، ٣٥٠، ٧٠٠ جم/ لتر. بالاضافة إلى الرش بتركيزات ٥٠، ١٠٠، ٢٠٠ مل/ لتر لكل من سماد Disper complex (عناصر صغرى) وسماد Amino mineral (عناصر صغرى + أحماض أمينية). ولقد تم إضافة تركيزات من ن-فو-بو (٢٠-٢٠-٢٠) على حدة لسماد Disper complex بحيث أن كل ٥٠ جم/ لتر متشمل على ١٧٥ جم/ لتر ن-فو-بو ، كل ١٠٠ جم/ لتر تشمل ٣٥٠ جم/ لتر ن-فو-بو ، وكل ٢٠٠ جم/ لتر تشمل ٧٠٠ جم/ لتر ن-فو-بو (٢٠-٢٠-٢٠) ، وتم رش النباتات الكونترول (غير المعاملة) بالماء فقط. تم أخذ القياسات على النمو الخضري والنسبة المئوية في العشب الجاف عقب أخر رشة ب ١٥ يوما.

أظهرت النتائج أن كل المعاملات السمادية قد أدت إلى تحسين كل من النمو الخضري ونسبة الزيت بالمقارنة بالكونترول. وأدى التركيز المرتفع من الأسمدة للحصول على نباتات أكثر إرتفاعا ، وأكثر وزنا طازجا وجافا ، وعددا أكبر من الأفرع ، وأعلى في نسبة الزيت من كل من المستويين المتوسط والمنخفض. ولقد أدى الرش بـ المستوى المرتفع من ال Amino mineral (٢٠٠ مل/ لتر) إلى الحصول على أكثر النباتات إرتفاعا ، وأكثرها وزنا طازجا وجافا ، وأكبر عدد من الأفرع ، وأعلى نسبة مئوية للزيت الطيار في العشب الجاف. وفي معظم الحالات كان الرش بالمستوى المرتفع من ال Disper complex في المركز التاني بعد التركيز المرتفع من معاملة ال Amino mineral .

من هذه النتائج يمكن ان نوصي بأهمية الرش بالعناصر الصغرى والأحماض الأمينية لزيادة النمو الخضري و النسبة المئوية للزيت الأساسي في نباتات الزعتر.