INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
1560–8530/2005/07–5–735–739
http://www.ijab.org

Response of Essential Oils, Phenolic Components and Polyphenol Oxidase Activity of Thyme (*Thymus vulgaris*, L.) to Some Bioregulators and Vitamins

FATMA REDA[1], E.A. ABDEL-RAHIM†, G.S.A. EL-BAROTY† AND HASNAA S. AYAD
Botany Department, National Research Center, Dokki, Giza, Egypt
†*Biochemistry Department, Faculty of Agriculture, Cairo University, Giza, Egypt*
[1]Corresponding author's e-mail: fatmareda2005@yahoo.com

ABSTRACT

This study was conducted to determine the response of essential oils content and constituents, phenolic components and polyphenol oxidase (PPO) activity of thyme plants (*Thymus vulgaris* L.) to different bioregulators (gibberellic acid, GA_3, indole-3-butyric acid, IBA, benzyladenine, BA) and vitamins (ascorbic acid, thiamine and nicotinamide). Three cuttings were periodically sampled of the treated and untreated plants. Essential oil percent and its constituents, total phenolic compounds and their components as well as polyphenol oxidase activity were significantly affected by the foliar application of bioregulators and vitamins used. The results revealed the effectiveness of certain bioregulators or vitamins at each cutting for increasing essential oil content (in particular thymol and carvacrol) as well as phenolic compounds (in particular rosmarinic acid).

Key Words: Thyme; Essential oils; Phenolic components; Polyphenol oxidase activity; Bioregulators; Vitamins

INTRODUCTION

Essential oils and phenolic compounds of thyme had several medicinal values, as they have strong antibacterial activity of common respiratory tract (Inouye *et al*., 2001) and *In vitro* antifungal activity (Plaza *et al*., 2004). In addition, essential oils obtained form thyme had antioxidant activities, which were the most potential natural antioxidant as compared with those extracted from basil, rosemary, chamomile, lavender and cinnamon (Lee & Shibamoto, 2002). Moreover, Angelini *et al.* (2003) had recently proved that essential oils of thyme inhibited the germination of different annual weeds. Schwarz *et al*. (1996) reported that the greatest amount of the main essential oil components of *Thymus vulgaris* L. were thymol, carvacrol and p-cymene as compared with other four thyme species. Yanishlieva *et al*. (1999) indicated that thymol was more effective antioxidant than carvacrol.

Bioregulators (growth regulating substances) and vitamins are known to regulate primary and secondary metabolism through regulation of enzymatic activities (Normanly *et al*., 1995; Mohr & Schopfer, 1995; Heldt, 1997). Several studies had demonstrated that essential oil of several plants could strongly be affected when treated exogenously with bioregulators and vitamins. For example, essential oil content and their constituents of *Mentha piperita* were changed in response to IAA and/or GA_3 (Ibrahim *et al*., 1992). Similarly, oil content of chamomile was also changed in response to BA treatments (Reda *et al*., 1999). Ascorbic acid or nicotinamide treatment of lemongrass (*Cymbopogen citratus*) caused pronounced increments in their essential oil content as well as the main oil components (Tarraf *et al*., 1999). Thiamine application increased essential oils of *Nigella sativa* L. (Naguib & Khalil, 2002) and rosemary (Youssef & Talaat, 2003). Little could be traced in the literature on the response of essential oils and phenolic compounds of thyme to bioregulators and vitamins. For example, GA_3 affected thyme oil production (Gastaldo *et al*., 1979). The objective of the present study was, therefore, to determine the response of essential oils content, oil components, phenolic compounds and the activity of polyphenol oxidase of thyme (*Thymus vulgaris*, L.) to foliar application of bioregulators (gibberellic acid, GA_3; indole-3-butyric acid, IBA; benzyladenine, BA) and vitamins (ascorbic acid, thiamine and nicotinamide) in three successive cuttings of the herb.

MATERIALS AND METHODS

Plant materials and growth conditions. Seedlings of thyme (*Thymus vulgaris* L.) family Lamiaceae were secured from Medicinal and Aromatic Plants Dept., Agricultural Research Centre, Ministry of Agriculture, Giza, Egypt.

The design of the experiment was complete randomized blocks with three replicates per each treatment. The experiment was conducted at the Experimental Farm of National Research Centre, Bolak El-Dakroor, Giza, Egypt. Uniform seedlings were transplanted 26th Feb. 2000, at the field plots of loamy soil. The seedlings were fertilized using the following fertilizers (given per feddan): 153.6 kg ammonium nitrate (33.5% N), 115.2 kg calcium super-phosphate (16% P_2O_5), 38.4 kg potassium sulphate (48% K_2O). The fertilizers were added in three doses; the first dose one month before the first cutting of plants, the second and third doses were added after ten days of the first and second cuttings, respectively. Water requirements and other agricultural practices were regularly fulfilled according to

the weather conditions and local recommendations during the plant growth. The experiments started Feb. 2000 and terminated April 2001. The day/night temperature ranged 24-40 and 8-25°C, respectively.

Bioregulator and vitamin treatments. Freshly prepared aqueous solutions of the bioregulators; Indole-3-butryic acid (IBA, Aldrich), benzyladenine (BA, Fluka) and gibberellic (GA_3, Merck) as well as vitamins; ascorbic acid (Merk), thiamine-HCl and nicotinamide (Sigma) were foliarly applied afternoon to the plants after 70 days form transplanting. Control plants were sprayed with distilled water. Foliar spraying of different treatments was repeated after 10 days from the first spray. Exogenous application of the solutions was carried out until running (20 mL/plant) using plastic automizer. Two concentrations of each treatment were used: 30 and 60 mg L^{-1}. Tepol was added (1 mL L^{-1}) as a wetting agent to the prepared solutions of bioregulators and vitamins before spraying.

Sampling. Three samples of the plants were drawn from all treatments representing the three successive cuttings. The overground aerial vegetative parts were cut 5 cm from the ground and one branch was left for further growth. The three cuttings were taken when the plants reached the stage before flowering which coincided with the following dates: The first cutting was on July 8, 2000; the second cutting was on November 22, 2000 and the third cutting was on April 1, 2001.

Determination of essential oils. Essential oil percent of thyme was determined in the air-dried aerial vegetative parts of plants (100 g) of each treatment according to British Pharmacopoeia (1980) using GLC (HP 6890 series GC-system, USA).

Determination of total phenols and fractions. Total phenols content of shoot fresh mass was determined using folin-Ciocalteu assay as detailed by Singleton and Rossi (1965) using pyrogallol as a standard. The method of Zheng and Wang (2001) was used to obtain the different fractions of phenolic compounds in the fresh thyme samples using HPLC (Shimadzu, Japan).

Determination of Polyphenol oxidase (PPO) activity. Enzyme extract and assay was carried out according to the modified method of Taneja and Sachar (1974). The oxidizing capacity of the enzyme extract was determined (spectrophotometrically) against pyrogallol and catechol**.**

Statistical analysis. Analysis of variance of the obtained data and L.S.D. at 0.05 level for significant F-test were calculated according to Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Essential oil percent. The greatest oil percent was obtained at treatment 30 mg L^{-1} of BA or nicotinamide in the herb sampled at cutting I and the increase in oil percent was 36 and 27%, respectively, over the control (Table I). At cuttings II and III, the essential oil percents were significantly decreased at most treatments, more so with 30 and 60 mg L^{-1} GA_3. Gastaldo *et al.* (1979) reported that GA_3 treatment did not influence the essential oil% of *Thymus vulgaris* L. On the other hand, essential oil content positively responded to certain level of GA_3 application, e.g. rosmary (El-Khateeb, 1989), *Mentha piperita* L. and *M. spicata* L. (Ghosh *et al*., 1993). It seems that BA or nicotinamide stimulated the biosynthesis of essential oil of thyme at cutting I where the prevailing temperature was favorable for this relative to cuttings II and III. This enhancement effect might be attributed to increased capacity of meristemic cells to build active substrate necessary for biosynthesis of essential oils (Tarraf *et al*., 1999; Mok & Mok, 2001)

Essential oil constituents. At all treatments, thymol was a predominant constituent (53.96-34.21%) followed by p-cymene (44.62–27.53%) and then that of carvacrol (5.22–3.03%) in the oil of thyme herbage (Table II). Thymol is the major component in thyme herb and antifungal potential against *Asperigillus parasiticus* (Farag *et al*., 1989a), antioxidant effect (Farag *et al*., 1989b) and antimicrobial activity (Farag *et al*., 1989c). Abou-Taleb *et al*. (1997) reported that the main compounds in thyme herb were p-cymene (34–39%), thymol (20.7–24.3%) and carvacrol (11.6–13.2%). Other constituents identified were α-pinene (0.81–3.31%), β-pinene (0.95–4.33%), camphor (4.17–2.15%), borneol (2.13–1.13%), and linalool (2.64–1.13%), which their relative percents decreased at different treatments, especially at 60 mg L^{-1} BA (Table II). El-Keltawi and Croteau (1987) concluded that the increase in oil yield of cytokinin–treated plants of several members of Labiatae as *Salvia officinalis* was the result of increased monoterpene biosynthesis. Other treatments increased the essential oil constituents, especially p-cymene and decreased thymol and carvacrol (Table II). This might be explained by the fact that p-cymene is one of the biogentic precursor of thymol and carvacrol (Omer, 1998). It appears that benzyladenine at 60 mg L^{-1} was the more effective bioregulator, which would enhance the enzymatic systems responsible for oxygenation of p-cymene to thymol.

At cutting II, thymol and carvacrol were increased at the treatments nicotinamide 30 and 60 mg L^{-1} thiamine whereas P-cymene content was increased at treatments GA_3 (60 mg L^{-1}), IBA (60 mg L^{-1}) and BA (30 mg L^{-1}) (Table III). Linalool was increased at most of the applied concentrations of bioregulators, 30 mg L^{-1} GA_3 followed by 30 mg L^{-1} IBA were more effective. Relatively high temperature prevailed during the growth period of plants until cutting might favored the biosynthesis of linalool and thymol in plants treated with thiamine or nicotinamide.

At cutting III, thymol content was increased while p-cymene was decreased in the herb at treatment 60 mg L^{-1} thiamine (Table IV). However, the opposite response was obtained at treatment 30 mg L^{-1} BA for thymol and p-cymene. In addition, treatment 60 mg L^{-1} BA caused pronounced increase in p-cymene content. Variations in the constituents of the essential oil of different plants reported to be due to the effect of environmental conditions and harvest

Table I. Oil percent of *Thymus vulgaris* L as affected by foliar application of bioregulators and vitamins at three cuttings. Each value is the mean of 3 replicates

Bioregulators and vitamins	Cutting I		Cutting II		Cutting III	
Treatments (mg L^{-1})	Oil%	as% of the control	Oil%	as% of the control	Oil%	as% of the control
Control (H2O)	1.10	100.0	1.30	100.0	1.30	100.0
Gibberellic acid (30)	1.20	109.1	0.65	50.0	0.75	57.7
Gibberellic acid (60)	1.15	104.5	0.72	55.4	0.70	53.9
Indole -3- butyric acid (30)	1.20	109.1	1.10	84.3	1.15	88.5
Indole -3- butyric acid (60)	1.30	118.2	1.35	103.9	1.35	103.9
Benzylaminopurine (30)	1.50	136.4	1.10	84.6	1.25	96.2
Benzylaminopurine (60)	1.30	118.2	1.00	76.9	1.00	76.9
Ascorbic acid (30)	1.30	118.2	0.95	73.1	1.25	96.2
Ascorbic acid (60)	1.25	113.6	1.00	76.9	1.00	76.9
Thiamine (30)	1.25	113.6	1.30	100.0	1.20	92.3
Thiamine (60)	1.30	118.2	1.00	76.9	1.00	76.9
Nicotinamide (30)	1.40	127.3	1.35	103.9	1.30	100.0
Nicotinamide (60)	1.30	118.2	1.00	76.9	1.10	84.6
L.S.D. at 0.05 level	0.18	-	0.19	-	0.19	-

Table II. Percentage of volatile oil compounds in the herb of thyme plants as affected by foliar application of some bioregulators and vitamins at cutting I.

Compounds	Control	GA3 (30)	GA3 (60)	IBA (30)	IBA (60)	BA (30)	BA (60)
α– Pinene	1.91	2.78	2.55	2.95	2.80	2.21	0.81
B- Pinene	3.13	2.73	0.95	2.80	3.61	1.56	0.77
P- Cymene	29.47	32.76	40.66	32.45	38.54	27.53	17.17
Camphor	4.17	2.78	2.64	2.36	2.57	2.16	3.11
Borneol	1.13	1.73	1.96	1.52	1.85	2.13	1.95
Linalool	1.76	2.29	2.29	2.33	1.91	2.64	1.62
Thymol	49.26	36.81	40.30	35.96	41.57	43.09	53.96
Carvacrol	4.46	3.61	4.11	3.50	3.31	3.33	5.22
Compounds	**Control**	**ASc (30)**	**ASc (60**	**Thiamine (30)**	**Thiamine (60)**	**Nicotina-mide (30)**	**Nicotina-mide (60)**
α– Pinene	1.91	2.88	2.82	2.92	2.53	3.02	3.31
B- Pinene	3.13	3.85	2.63	4.14	3.90	4.33	3.17
P- Cymene	29.47	31.26	44.62	38.87	32.53	36.32	34.99
Camphor	4.17	2.54	2.35	2.15	2.69	2.39	2.62
Borneol	1.13	1.62	1.46	1.45	1.34	2.06	1.39
Linalool	1.76	1.68	2.39	1.13	1.14	1.57	2.00
Thymol	49.26	40.61	34.21	40.29	39.61	35.27	34.93
Carvacrol	4.46	3.61	3.57	4.08	3.70	3.03	3.21

Figures in parenthesis indicate dose in mg L^{-1}; GA3 = Gibberellic acid, IBA = Indole -3- butyric acid, BA = Benzyladenine and ASc = Ascorbic acid

time (Piccaglia & Marotti, 1993; Omer *et al*., 1994; Omer, 1998).

It could be concluded that treating with BA treatment (60 mg L^{-1}) for cutting I, nicotinamide (30 mg L^{-1}) for cutting II and thiamine (60 mg L^{-1}) for cutting III favored the biosynthesis of some of the oil components and relatively increased amounts of thymol and carvacrol in thyme grown under the climatic conditions of Egypt. This conclusion is in agreement with other previous published reports (Tarraf *et al*., 1999; Balbaa *et al*., 2002).

Total phenols content. Total phenol contents of all treatments at cutting I were great than those of cuttings II and III (Table V). This result could be interpreted to the notion that samples of cutting II and III were collected from plants grown at relatively higher or lower air temperature,

Table III. Percentage of volatile oil compounds in the herb of thyme plants as affected by foliar application of some bioregulators and vitamins at cutting II.

Compounds	Control	GA3 (30)	GA3 (60)	IBA (30)	IBA (60)	BA (30)	BA (60)
α– Pinene	3.01	2.08	1.52	1.90	1.75	1.83	2.00
B- Pinene	1.91	2.46	1.14	2.05	1.18	1.09	2.50
P- Cymene	37.34	35.20	42.74	41.03	48.36	51.96	36.29
Camphor	6.38	3.68	4.74	2.37	1.97	2.84	4.06
Borneol	1.79	1.73	3.32	1.30	2.06	3.10	1.90
Linalool	2.82	5.60	4.43	4.61	3.65	2.81	4.24
Thymol	37.26	37.95	33.98	36.04	28.62	20.87	37.83
Carvacrol	4.18	3.03	3.77	3.88	3.23	2.93	4.38
Compounds	**Control**	**ASc (30)**	**ASc (60)**	**Thiamine (30)**	**Thiamine (60)**	**Nicotina-mide (30)**	**Nicotina-mide (60)**
α– Pinene	3.01	1.95	1.78	1.79	1.38	0.51	2.18
B- Pinene	1.91	0.90	2.06	0.93	0.77	0.45	1.16
P- Cymene	37.34	44.15	38.56	46.28	40.67	23.56	51.02
Camphor	6.38	3.01	5.13	3.59	2.65	2.59	2.12
Borneol	1.79	3.18	2.00	3.05	1.99	2.67	1.13
Linalool	2.82	2.87	2.85	1.46	2.28	4.10	3.11
Thymol	37.26	34.00	36.84	32.12	40.36	53.14	26.52
Carvacrol	4.18	2.65	3.72	2.90	4.29	6.73	3.66

Figures in parenthesis indicate dose in mg L^{-1}; GA3 = Gibberellic acid, IBA = Indole -3- butyric acid, BA = Benzyladenine and ASc = Ascorbic acid

Table IV. Percentage of volatile oil compounds in the herb of thyme plants as affected by foliar application of some bioregulators and vitamins at cutting III.

Compounds	Control	GA3 (30)	GA3 (60)	IBA (30)	IBA (60)	BA (30)	BA (60)
α– Pinene	1.70	1.68	2.28	2.07	1.83	1.98	1.24
B- Pinene	1.21	1.31	1.11	1.83	1.64	1.70	1.20
P- Cymene	24.25	20.96	23.48	23.57	24.24	32.57	36.35
Camphor	3.15	1.47	1.80	1.02	1.65	2.43	2.79
Borneol	1.82	3.40	1.12	1.45	2.99	1.85	1.32
Linalool	2.43	2.78	1.43	1.86	2.02	1.18	0.86
Thymol	39.91	36.75	39.74	36.71	34.64	25.27	34.29
Carvacrol	3.18	2.28	2.74	2.21	1.86	1.87	3.41
Compounds	**Control**	**ASc (30)**	**ASc (60)**	**Thiamine (30)**	**Thiamine (60)**	**Nicotina-mide (30)**	**Nicotina-mide (60)**
α– Pinene	1.70	1.68	1.41	1.69	1.57	1.67	1.99
B- Pinene	1.21	1.67	1.25	1.49	1.21	1.60	1.10
P- Cymene	24.25	28.23	25.14	26.72	20.56	26.74	34.82
Camphor	3.15	2.28	3.09	1.28	2.45	2.21	1.54
Borneol	1.82	3.40	2.53	2.48	1.94	2.17	2.56
Linalool	2.43	1.01	1.49	1.44	1.16	1.87	1.04
Thymol	39.91	34.32	39.06	34.69	49.13	38.57	31.10
Carvacrol	3.18	2.49	2.48	1.84	3.34	2.27	2.68

Figures in parenthesis indicate dose in mg L^{-1}; GA3 = Gibberellic acid, IBA = Indole -3- butyric acid, BA = Benzyladenine and ASc = Ascorbic acid

respectively, than plants of cutting I. Thiamine at 60 mg L^{-1} resulted in the most significant total phenols content at cutting I as compared with other bioregulators and vitamins. At cutting II, 60 mg L^{-1} of GA_3 or thiamine produced the most significant increase in total phenol content. At cutting III, BA or nicotinamide at 60 mg L^{-1} was more effective in increasing the total phenols contents. Kintzio *et al*. (1996) reported that rosmarinic acid production in *Salvia officinalis* and *S. fruticosa* increased with callus growth on media supplemented with 2,4-D at 4.5 mM combined with kinetin at 4.5 mM. However, Kadioglu and Atalay (2002) found that IAA and GA_3 application decreased the level of total

phenolic substances in *Diospyros lotus* fruits.

Polyphenoloxidase activity. The activity of polyphenol oxidase (PPO) as pyrogallol was significantly increased in the herb extracts at the following treatments arranged in a descending order for the amounts of activity, IBA at 30 and 60 mg L^{-1}, GA_3 at 30 mg L^{-1}, ascorbic acid at 60 mg L^{-1} and thiamine at 30 mg L^{-1} at cutting I (Table IV). These effects were true in case of expressing the activity of PPO as units catechol but not significant. Similar results have been reported in wheat and barley. Taneja and Sachar (1974) reported that GA_3 stimulates PPO activity in wheat cultivars. Jennings and Duffus (1977) indicated that phenoloxidase activity in de-embryonated wheat and barley grains was enhanced by addition of GA_3. Martinez and Whitaker (1995) and Whitaker and Lee (1995) reported that PPO catalyzed the oxidation of phenolic substrates to O-quinones, whilst ascorbic acid converted O-quinones back to phenolic compounds.

At cutting II, the activity of PPO (as pyrogallol) was significantly increased at 60 mg L^{-1} BA, IBA, (Table VI). However, when PPO activity was expressed as units of catechol, 60 mg L^{-1} of most bioregulators treatments, in particular nicotinamide (60 mg L^{-1}) caused the most significant increase in this enzyme activity at the cuttings II and III. Working on other plant species, changes in PPO of wheat during kernel growth and maturation indicated that up to 12 PPO isozymes were located in different parts of the kernel and PPO activity increased upon germination (Kruger, 1976). In addition, Udayasekhara and Deosthale (1987) reported an increase in PPO activity on germination in blackgram seeds.

Main phenolic compounds. The major phenolic compounds in Thyme at cutting II, of all treatments were caffeic acid, leutolin and rosmarinic acid. The content of caffeic acid increased by most treatments, 60 mg L^{-1} of GA_3 or IBA was more effective (Table VII). For vitamins treatments, the increases in caffeic acid were less as compared with those of GA_3 or IBA. However, both treatments of benzyladenine (BA) decreased caffeic acid content as compared with the control. Leutolin and rosmarinic acid contents were increased by applied bioregulators and vitamins, more so with 60 mg L^{-1}. However, 30 mg L^{-1} of IBA decreased leutolin content. Rosmarinic acid content was the predominant phenolic compound in control plants and its content increased by 60 mg L^{-1} ascorbic acid or GA_3, as well as 30 mg L^{-1} BA and nicotinamide. Zheng and Wang (2001) reported that rosmarinic acid was one of the predominant phenolic compounds in *Salvia officinalis* and *Thymus vulgaris*. In their study, total phenolic compounds content was not affected by the applied bioregulators except GA_3. Our results indicated that the bioregulators and vitamins used did not affect all phenolic compounds in the same way. This could be interpreted by the interconversion of one phenolic compound to another as bioregulators enhanced the biosynthesis and activities of most enzyme systems. Therefore, the foliar application of the bioregulators could be in favor for the increase in certain important phenolic compounds as shown in Table VII. These phenolic compounds could increase the quality value of thyme herb because of their roles as antioxidant (Osawa, 1994; Velioglu *et al*., 1998).

Table V. Total phenol content (mg Pyrogallol/g F.M) of *Thymus vulgaris* L. as affected by foliar application of some bioregulators and vitamins at three cuttings

Bioregulators and vitamins Treatments (mg L^{-1})	Cutting I	Cutting II	Cutting III
Control (H2O)	1.71	1.35	1.42
Gibberellic acid (30)	1.77	1.38	1.39
Gibberellic acid (60)	1.79	1.48	1.31
Indole -3- butyric acid (30)	1.51	1.39	1.38
Indole -3- butyric acid (60)	1.56	1.35	1.35
Benzylaminopurine (30)	1.62	1.36	1.19
Benzylaminopurine (60)	1.78	1.35	1.45
Ascorbic acid (30)	1.74	1.40	1.24
Ascorbic acid (60)	1.73	1.34	1.36
Thiamine (30)	1.75	1.31	1.34
Thiamine (60)	1.90	1.49	1.30
Nicotinamide (30)	1.79	1.35	1.30
Nicotinamide (60)	1.79	1.35	1.45
L.S.D at 0.05 level	0.09	0.08	0.09

Table VI. Polyphenoloxidase activity (units/g F.W/min) of *Thymus vulgaris* L as affected by foliar application of some bioregulators and vitamins at cuttings III (Pyro.= as Pyrogallol; Cat.= Catechol)

Bioregulators and vitamins	Cutting I		Cutting II		Cutting III	
Treatments (mg L^{-1})	Pyro.	Cat.	Pyro.	Cat.	Pyro.	Cat.
Control (H2O)	1.65	16.85	4.00	3.35	1.50	5.00
Gibberellic acid (30)	1.90	17.90	3.35	2.25	1.56	11.50
Gibberellic acid (60)	1.15	14.45	4.51	4.35	1.67	17.10
Indole -3- butyric acid (30)	2.45	18.88	3.45	7.55	2.10	13.90
Indole -3- butyric acid (60)	2.40	18.65	5.25	5.10	2.35	10.00
Benzylaminopurine (30)	1.80	17.90	3.60	7.20	1.54	16.80
Benzylaminopurine (60)	1.25	13.60	5.35	4.10	1.80	21.00
Ascorbic acid (30)	1.75	16.10	2.75	5.15	2.50	20.20
Ascorbic acid (60)	2.30	18.70	3.45	3.05	2.15	19.10
Thiamine (30)	2.10	13.10	2.90	2.40	1.80	15.60
Thiamine (60)	1.45	13.75	3.50	2.30	2.10	20.95
Nicotinamide (30)	1.35	11.80	2.70	5.05	2.05	22.25
Nicotinamide (60)	1.65	19.25	2.45	7.40	2.15	29.55
L.S.D. at 0.05 level	0.24	2.49	0.74	0.36	0.29	1.33

Table VII. Main phenolic compounds content identified by HPLC in Thymus vulgaris L extracts of cutting II in response to bioregulators and vitamins

Bioregulators and vitamins treatments (mg L^{-1})	Caffeic acid	Leutolin	Rosmarinic acid
Control (H2O)	2.81	2.66	5.69
Gibberellic acid (30)	5.97	4.04	6.80
Gibberellic acid (60)	7.34	2.03	9.93
Indole -3- butyric acid (30)	11.19	1.23	8.04
Indole -3- butyric acid (60)	14.09	2.09	6.96
Benzyladenine (30)	2.09	4.67	10.59
Benzyladenine (60)	1.72	6.44	4.00
Ascorbic acid (30)	4.24	3.85	8.32
Ascorbic acid (60)	3.28	3.28	15.49
Thiamine (30)	3.97	4.03	3.90
Thiamine (60)	3.62	4.45	8.64
Nicotinamide (30)	6.71	6.91	10.47
Nicotinamide (60)	3.66	4.85	8.63

In conclusion, bioregulators and vitamins at certain level increased thyme essential oil quantitatively and qualitatively. The quality value of thyme phenolic compounds was increased and thus improved the oil flavor and aroma.

REFERENCES

Abo–Taleb, N.S.A., S.M. Hassan, A.E. Omer and A.M. Eissa 1997. Foliar application of urea, phosphorus, and gibberellin on the growth, essential oil and nutrients contents of *Thymus vulgaris* L. *Ann. Agric. Sci.,* 42: 537–55

Angelini, L.G., G. Carpanese, P.L. Cioni, I. Morelli, M. Macchia and G. Flamini, 2003. Essential oils from Mediterranean *Lamiaceae* as weed germination inhibitors. *J. Agric. Food Chem*., 51: 6158–64

Balbaa, L.K. and A.M. Refaat, 2002. The response of vegetative growth, essential oil of *Tagetes minuta* L. to foliar application of Thiamine and nicotinamide. *Egyptian J. Appl. Sci.*, 9: 287–304

British Pharmacopoeia, 1980. *Vol. II; Volatile oil in drugs.* A 108–A112. The Univ. Press, Cambridge, England

El–Keltawi, N.E. and R. Croteau, 1987. Salinity depression of growth and essential oil formation in spearmint and marjoram and its reversal by foliar applied cytokinin. *Phytochem.* 26: 1333–4

El–Khateeb, M.A., 1989. Effect of some growth regulators on the vegetative growth and essential oil of *Rosmarinus officinalis* L. Plant. *Bull. Fac. Agric.*, 40: 333–46

Farag, R.S., A. Badei, F.M. Hewedi and G.S.A. Elbaroty, 1989a. Antioxidant activity of some spice essential oils on linoleic acid oxidation in aqueous media. *J. Am. Oil Chem. Soc*., 66: 792–9

Farag, R.S., A., Badei and G.S.A. Elbaroty, 1989b. Influence of thyme and clove essential oils on cottonseed oil oxidation. *J. Am. Oil Chem. Soc.*, 66: 800–4

Farag, R.S.F., Z.Y. Daw, F.M. Hewedi and G.S.A. El–Baroty, 1989c. Antimicrobial activity of some Egyptian spice essential oils. *J. Food Prot.*, 52: 665–7

Gastaldo, P.M., P. Modenesi and C. Vanzo, 1979. Observation on treatment of *Thymus vulgaris* with gibberellic acid and Maleic Hydrazide. *Rievut Oil, 28:* 119–22

Ghosh, M.L., S.K. Chatterjee, D. Palevitch, J.E. Simon and A. Mathe, 1993. Physiological and biochemical of synthesis of essential oil in *Mentha spp*. grown in India. *Acta Hort.*, 331: 351–6

Heldt, H.W., 1997. *Plant Biochemistry and Molecular Biology,* pp. 396–413. Oxford Univ. Press, England

Ibrahim, M.E., Sh. Tarraf, E.A. Omer and K.A. Turky, 1992. Response of growth, yield and essential oil of *Mentha piperital* L. to some growth regulators. *Zagazig. J. Agric. Res*., 19: 1855–8

Inouye, S., H. Yamaguchi and T. Takizawa, 2001. Antibacterial effects of essential oil and their major constituents against respiratory tract pathogens by gaseous contact. *J. Antibicrob. Chemother*, 47: 565–73

Jennings, P.H. and C.M. Duffus, 1977. Effect of gibberellic acid on polyphenoloxidase activity in de–embryonated wheat and barley grains. *New Phytol.*, 78: 383–9

Kadioglu, A. and F. Atalay, 2002. Effect of GA3 and IAA on major biochemical changes in *Diospyros lotus* fruits. *Biologia (Bratislava)*, 57: 125–30

Kintzio, S., A. Nokolaou, M. Skoula, J. Drossopoulos, C. Holevas and F. Pank, 1996. Somatic embryogenesis and *in vitro* rosmarinic acid production from *Salvia officinalis* and *S. fruticosa* biotypes collected in Greece. *Beitrage – Zur zuchtungs forschung – Bundesanstalt – Für – Zuchtungsforschung an kultur Pflanzen*, 2: 282–5

Kruger, J.E., 1976. Changes in the polyphenol oxidases of wheat during kernel growth and maturation. *Cereal Chem*., 53: 201–13

Lee, K.G. and T. Shibamoto, 2002. Determination of antioxidant potential of volatile extracts isolated from various herbs and spices. *J. Agric. Food Chem.*, 50: 4947–52

Martinez, M. and J.R. Whitaker, 1995. The biochemistry and control of enzymatic browning. *Trends Food Sci. Technol*., 6: 195–200

Mohr, H. and P. Schopfer, 1995. *Plant Physiology,* pp. 275–84 and 383–408. Springer–Verlag, Berlin

Mok, D.W.S. and M.C. Mok, 2001. Cytokinin metabolism and action. *Annu. Rev. Plant Physiol. Plant Mol. Biol.,* 52: 89–118

Naguib, N. and M.Y. Khalil, 2002. Studies on the effect of dry yeast thiamine and biotin on the growth and chemical constituents of blackcumin (*Nigella sativa*, L.). *Arab Univ. J. Agric. Sci.,* 10: 919–37

Normanly, J., J.P. Slovin and J.D. Cohen, 1995. Rethinking auxin biosynthesis and metabolism, *Pl. Physiol*., 107: 323–9

Omer, E.A., H.E. Ouda and S.S. Ahmed, 1994. Cultivation of sweet majoram, *Majorana hortnsis*, in newly reclaimed lands of Egypt. *J. Herbs, Spices and Med. Pl.*, 2: 9–16

Omer, E.A., 1998. Response of wild Egyptian oregano to nitrogen fertilization in sandy soil. *Egyptian J. Hort*., 25: 295–308

Osawa, T., 1994. Novel natural antioxidants for utilization in food and biological systems. *In:* Uritani, I. Garcia and V.E.M. Medoza, (eds.), *Post–harvest Biochemistry of Plant Food Materials,* pp. 241–51. Japan Scientific Societies Press, Tokyo, Japan.

Plaza, P., R. Torres, J. Usall, N. Lamarca and I. Viasa, 2004. Evalauiton of the potential of commercial post–harvest application of essential oils to control citrus decay. *J. Hort. Sci. Biotech.,* 79: 935–40

Piccaglia, R. and M. Marotti, 1993. Characterization of several aromatic plants grown in Northern Italy. *Flav. Fagr. J*., 8: 115–22

Reda, F., S. Tarraf; E.A. Abdel–Rahim, A.S. Afify and H.S. Ayad, 1999. The response of growth and some chemical constituents of chamomile plants to benzyladenine (BAP). *J. Agric. Sci.*, 24: 2205–22

Schwarz, K., H. Ernst and W. Ternes, 1996. Evaluation of antioxidative constituents from thyme. *J. Sci. Food Agric*., 70: 217–23

Singleton, V.L. and J.A. Rossi, 1965. Colorimetry of total phenolics with phosphomolybdic phoshotungstic acid reagents. *Am. J. Enol. Vaticult*, 16: 144–58

Snedecor, G.W. and W.G. Cochran, 1980. *Statistical methods*, 7th Ed. Iowa State Univ. Press, Iowa, U.S.A.

Taneja, S.R. and R.C. Sachar, 1974. Stimulation of polyphenoloxidase (monophenoloxidase) activity in wheat endosperm by gibberellic acid, cycloheximide and actinomycin D. *Planta (Berl)*, 116: 133–9

Tarraf, Sh. A., K.M. Gamal El–Din and L.K. Balbaa, 1999. The response of vegetative growth, essential oil of lemongrass (*Cymbopogon citratus* Hort) to foliar application of ascorbic acid, nicotinamide and some micronutrients. *Arab Univ. J. Agric. Sci.,* 7: 247–59

Udayasekhara, R.P. and Y.G. Deosthale, 1987. Polyphenoloxidase activity in germinated legume seeds. *J. Food Sci*., 52: 1549–51

Velioglu, Y.S., G. Mazza, L. Gao and B.D. Oomah, 1998. Antioxidant activity and total phenolics in selected fruits, vegetables and grain products. *J. Agric. Food Chem*., 46: 4113–7

Whitaker, J.R. and C.Y. Lee, 1995. Recent advances in chemistry of enzymatic browning. An overview. *In:* Chang, Y.L. and J.R. Whitaker, (eds.) *Enzymatic Browning and Its Prevention*, pp. 2–7 ACS Symposium Series 600. Amer. Chem. Soc., Washington, D.C.

Yanishlieva, N.V., E.M. Marinova, M.H. Gordon, and V.G. Raneva, 1999. Antioxidant activity and mechanism of action of thymol and carvacrol in two lipid systems. *Food Chem*., 64: 59–66

Youssef, A.A. and I.M. Talaat, 2003. Physiological response of rosemary plants to some vitamins. *Egyptian Pharm. J.,* 14: 81–93

Zheng, W. and S.Y. Wang, 2001. Antioxidant activity and phenolic compounds in selected herbs. *J. Agric. Food. Chem*., 49: 5165–70

(Received 10 July 2005; Accepted 20 August 2005)