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Effect of Some Growth Regualtors and Vitamins on Essential Oil, Phenolic Content and Activity of Oxidoreductase Enzymes of *Thymus vulgaris* L.

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Abstract: A pot experiment was conducted in the Experimental Area of Botany Department, National Research Centre, Dokki, Giza, Egypt. Seeds of thyme plants were presowing soaked in gibberellic acid, indole butyric acid, benzyleaminopurine, ascorbic acid, thiamine-HCl and nicotinamide, each at 30 and 60 mg L^{-1}, in addition to control (i.e. distilled water). Three cuttings were periodically sampled of each treatment. The obtained results revealed the effectiveness of certain growth regulators or vitamins at each cutting for increasing photosynthetic pigments and promoting growth, the activity of peroxidase and polyphenoloxidase. Favourable alternation in the contents of phenolic compounds and essential oil, as well as their composition of the main components respectively, were determined. The results revealed that certain level of either vitamins especially vit. C or growth regulators significantly increased essential oil (as percent or as yield per plant) and phenolic compounds at the three cuttings.

Key words: Thyme · potosynthetic pigments · total phenols · phenolic compounds · PPO · POD · enzyme · essential oil · oil composition

INTRODUCTION

Thymus vulgaris, L., Family Lamiaceae, is an important aromatic medicinal plant. It possesses antispamodic, antiseptic, exportant, carminative, antitussive, antimicrobial and antioxidant activities [1].

Growth regulators and vitamins are known to affect plant growth through regulating primary and secondary metabolism [2-4]. Usha and Swany [5] reported that benzylaminopurine induced more multiple shoots of *Artemisia anana* L., grown on MS media. Buettner and Jurkiewicz [6] mentioned that soaking lettuce seeds in 1000 ppm ascorbic acid stimulated plant growth. Begley *et al.* [7] reported that nicotinamide is an essential cofactor in all living systems and function as hydride donors (NADH and NADPH) in biochemical redox reactions. These abovementioned positive effects of growth regulators and vitamins motivate, therefore, to determine the response of photosynthetic pigments, essential oil and phenolic compounds (quality and quantity) as well as the activity of peroxidase (POD) and polyphenol oxidase of *Thymus vulgaris* to presowing application of growth regulators (gibberellic acid, indole-3-butyric acid and benzyladenine) and vitamins (ascorbic acid, thiamine and nicotinamide) in three successive cuttings of the herb.

MATERIALS AND METHODS

Seeds of thyme (*Thymus vulgaris* L.), Family Lamiaceae were secured from Medicinal and Aromatic Plants Dept., Agricultural Research Centre, Ministry of Agriculture, Giza, Egypt. Seeds were soaked for 95 min., in two concentrations (30 and 60 mg L^{-1}) of gibberellic acid (GA_3, Merck), indole butyric acid (IBA, aldrich) benzyl aminopurine (BAP, Fluka), ascorbic acid (vit.C Merk) thiamine-HCl (vit. B_1) and nicotinamide (NA, Sigma). Control seeds were soaked in distilled water. Then, the seeds were sown in 50 cm top diameter pots filled with loamy clay soil (mechanical analysis sand 30.33%, silt 25.72 % and clay 40.74 %). The experiment was carried out in the Experimental Farm of National Research Centre, Dokki, Giza, Egypt. The design was complete randomized with three replicates per each treatment. Each pot was fertilized with 2.0 g nitrogen as ammonium nitrate (33.5% N), 1.5 g superphosphate (15 % P_2O_5) and 1.0 g potassium sulphate (48 % K_2O) after one month from sowing and after every cutting. Water requirements and other agricultural practices were regularly fulfilled according to the weather conditions and local recommendations during the plant growth. The experiment sown in Feb. 2000 and terminated April 2001.

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The contents of photosynthetic pigments, chlorophyll a,b and carotenoids, in the leaves were determined by the method described by Smith and Benitez [8] and with some more details in AOAC [9].

Essential oil percent of thyme was determined in the air dried aerial vegetative parts of plants (100 g) of each treatment according to British Pharmacopeia [10]. The components of thyme essential oil were identified by gas liquid chromatography using GLC (HP 6890 series GC-system, USA).

Total phenols content of shoot fresh mass was determined using Folin Ciocalteu assay as detailed by Singleton and Rossi [11] using pyrogallol as a standard. The method of Zheng and Wang [12] was used to obtain the different fractions of phenolic compounds in the fresh thyme samples using HPLC (Shimadzu, Japan).

Polyphenoloxidase (EC 1.10.3.2) activity was determined according to the modified method of Taneja and Sachar [13]. The oxidizing capacity of the enzyme extract was determined spectrophotometrically against pyrogallol and catechol. Peroxidase (EC 1.11.1.7) activity was assayed spectrophotometrically according to Amako *et al*. [14] method.

The activity of α-amylase (EC 3.2.1.1) enzyme was determined spectrophotometrically following Hofmann and Galler [15] method using Starch-Iodine according to Smith and Roe [16].

Statistical analysis: Analysis of variance of the obtained data and LSD at 0.05 level for significant f-test were calculated according to Snedecor and Cochran [17]. Duncan's Range test was used to compare between means of treatments, Waller and Duncan [18] at probability 5 %.

RESULTS AND DISCUSSION

Photosynthetic pigments: Data presented in Table 1 indicate that in cutting (I), chlorophyll (a) content was significantly increased at both levels of benzylaminopurine (BAP), while chlorophyll (b) content was significantly increased at 30 mg L^{-1} IBA and both levels of GA_3. Carotenoids content was increased at all treatments of growth regulators. In cutting (II), chlorophyll (a) content was significantly increased at both levels of IBA, 60 mg L^{-1} vit. B_1 and 20 mg L^{-1} NA. While, Chlorophyll (b) content was significantly increased as a result of presowing seeds treatment with vit. C and NA at 30 mg L^{-1}. Carotenoids content was slightly increased in plants treated with BAP at 60 mg L^{-1}.

In cutting (III), treatment with vit. C at both levels significantly increased chlorophyll (a) content. All treatments caused variable decreases in chloropohyll (b) content. Treatment with GA_3 at 60 mg L^{-1} caused significant increase in carotenoids content followed by treatment with vit. C at 30 mg L^{-1}. Supporting these

Table 1: Photosynthetic pigments content (mg g^{-1} F.W) of *Thymus vulgaris* L. herb influenced by soaking of seeds in some growth regulators and vitamins at three cuttings

Treatment		Photosynthetic pigments (mg g^{-1} FW)								
		Cutting (I)			Cutting (II)			Cutting (III)		
(mg l^{-1})		Chl (a)	Chl (b)	Carot.	Chl (a)	Chl (b)	Carot.	Chl (a)	Chl (b)	Carot.
		Growth regulators								
Control (H_2O)		0.61^{de}	0.46^{c}	0.27^{abc}	0.29^{abc}	0.08^{b}	0.18^{ab}	1.52^{bc}	0.61^{a}	0.68^{de}
GA_3	30	0.65^{bcd}	0.50^{ab}	0.29^{abc}	0.22^{de}	0.05^{cd}	0.17^{ab}	1.09^{fg}	0.39^{cd}	0.54^{h}
	60	0.68^{abc}	0.52^{a}	0.32^{ab}	0.20^{e}	0.08^{bcd}	0.14^{bc}	1.23^{ef}	0.56^{ab}	0.80^{a}
IBA	30	0.69^{ab}	0.52^{a}	0.32^{ab}	0.29^{ab}	0.07^{bcd}	0.18^{ab}	1.07^{fg}	0.36^{cd}	0.41^{j}
	60	0.69^{ab}	0.37^{d}	0.32^{ab}	0.33^{a}	0.06^{bcd}	0.13^{c}	1.52^{bc}	0.57^{ab}	0.72^{bcd}
BAP	30	0.71^{ab}	0.45^{c}	0.33^{a}	0.26^{bcd}	0.04^{d}	0.09^{d}	1.37^{cde}	0.51^{b}	0.75^{abc}
	60	0.71^{a}	0.48^{bc}	0.32^{ab}	0.29^{abc}	0.08^{bc}	0.20^{a}	1.07^{fg}	0.44^{c}	0.59^{gh}
		Vitamins								
Vit. C	30	0.63^{cde}	0.48^{bc}	0.27^{abcd}	0.26^{bcd}	0.12^{a}	0.15^{bc}	1.60^{b}	0.58^{ab}	0.78^{ab}
	60	0.59^{ef}	0.46^{c}	0.25^{cd}	0.29^{abc}	0.07^{bcd}	0.12^{cd}	1.84^{a}	0.56^{ab}	0.72^{cde}
Vit. B_1	30	0.62^{de}	0.50^{ab}	0.27^{bcd}	0.23^{cde}	0.06^{bcd}	0.13^{c}	1.50^{bcd}	0.55^{ab}	0.68^{de}
	60	0.63^{cde}	0.48^{bc}	0.21^{d}	0.30^{ab}	0.07^{bcd}	0.11^{cd}	1.33^{de}	0.58^{ab}	0.67^{ef}
NA	30	0.55^{f}	0.46^{c}	0.23^{cd}	0.30^{ab}	0.13^{a}	0.17^{ab}	0.94^{g}	0.34^{d}	0.48^{i}
	60	0.63^{cde}	0.48^{bc}	0.27^{abcd}	0.28^{abc}	0.05^{d}	0.17^{ab}	1.25^{ef}	0.43^{c}	0.62^{fg}

Chl (a): Chlorophyll a, Chl (b): Chlorophyll b and Carot: Carotenoids (as mg g^{-1} fresh wt), Figures followed by the same letters are not-significant, GA_3: Gibberellic acid, IBA: Indole butyric acid, BAP: Benzylaminopurine, Vit. C: L-ascorbic acid, Vit. B_1: Thiamine and NA: Nicotinamide

Table 2: Oil percent of *Thymus vulgaris* L. herb influenced by soaking of seeds in some growth regulators and vitamins at three cuttings

Treatment (mg l^{-1})		Cutting (I)		Cutting (II)		Cutting (III)	
		Oil (%)	As percent of control	Oil (%)	As percent of control	Oil (%)	As percent of control
		Growth regulators					
Control (H_2O)		1.04^{cd}	100.00	0.80^{bcde}	100.00	0.96^{d}	100.00
GA_3	30	1.21^{ab}	116.35	0.77^{cde}	95.65	0.71^{e}	74.24
	60	0.80^{f}	76.92	0.92^{ab}	114.55	1.04^{d}	108.90
IBA	30	1.10^{bcd}	105.77	0.83^{abcde}	103.73	1.30^{abc}	136.13
	60	1.13^{bc}	108.65	0.95^{a}	117.66	1.25^{bc}	130.89
BAP	30	0.80^{f}	76.92	0.94^{a}	117.41	1.29^{abc}	135.08
	60	0.88^{ef}	84.62	0.64^{f}	79.48	1.25^{bc}	130.89
		Vitamins					
Vit. C	30	1.27^{a}	122.12	0.88^{abc}	109.83	1.42^{ab}	148.69
	60	0.85^{ef}	81.73	0.95^{a}	118.28	1.12^{cd}	117.28
Vit. B_1	30	1.07^{bcd}	102.88	0.78^{cde}	97.51	1.00^{d}	104.29
	60	0.85^{ef}	81.73	0.70^{ef}	86.69	1.46^{a}	152.88
NA	30	0.75^{f}	72.12	0.84^{abcd}	104.60	1.42^{ab}	148.69
	60	0.96^{de}	92.31	0.73^{def}	90.92	0.96^{d}	100.10

Figures followed by the same letters are not-significant, Mean values of the results of the three replicates, GA_3: Gibberellic acid, IBA : Indole butyric acid, BAP: Benzylaminopurine, Vit. C: L-ascorbic acid, Vit. B_1: Thiamine and NA: Nicotinamide

Table 3: Oil yield (ml/dry wt./plant) and dry weight (g/plant) of *Thymus vulgaris* L. as affected by seed soaking with some growth regulators and vitamins at three cuttings

Treatment (mg l^{-1})		Cutting (I)			Cutting (II)			Cutting (III)		
		Oil (yield)	As percent of control	DW	Oil (yield)	As percent of control	DW	Oil (yield)	As percent of control	DW
		Growth regulators								
Control		0.07^{e}	100.0	6.79^{e}	0.16^{ef}	100.0	19.54^{de}	0.29^{d}	100.0	30.48^{de}
GA_3	30	0.16^{b}	232.9	13.52^{be}	0.16^{ef}	101.9	20.78^{de}	0.24^{e}	83.2	34.24^{bc}
	60	0.13^{c}	178.6	15.65^{e}	0.24^{a}	154.8	26.42^{a}	0.38^{b}	129.2	36.14^{a}
IBA	30	0.15^{bc}	211.4	13.49^{bc}	0.20^{bc}	128.0	24.22^{bc}	0.40^{b}	137.1	30.73^{de}
	60	0.16^{b}	227.1	14.03^{abc}	0.24^{a}	152.9	25.32^{ab}	0.44^{a}	149.5	34.80^{abc}
BA	30	0.09^{de}	124.3	10.82^{d}	0.19^{cde}	120.4	20.05^{de}	0.32^{c}	111.0	25.10^{g}
	60	0.13^{c}	185.7	14.77^{ab}	0.16^{ef}	100.6	24.66^{bc}	0.44^{a}	151.5	35.28^{ab}
		Vitamins								
Vit.C	30	0.19^{a}	267.1	14.72^{ab}	0.23^{ab}	145.9	25.89^{ab}	0.42^{a}	145.7	29.87^{e}
	60	0.13^{c}	190.0	15.67^{a}	0.25^{a}	161.8	26.81^{a}	0.38^{b}	128.9	33.52^{c}
Vit.B_1	30	0.13^{c}	190.0	10.99^{d}	0.17^{def}	105.1	19.25^{e}	0.30^{cd}	104.1	20.65^{b}
	60	0.09^{d}	132.9	12.47^{cd}	0.13^{f}	85.4	21.08^{d}	0.30^{cd}	103.4	30.52^{de}
NA	30	0.10^{d}	141.4	13.25^{bc}	0.19^{cd}	122.9	23.05^{c}	0.39^{b}	132.6	27.19^{f}
	60	0.14^{c}	195.7	14.32^{abc}	0.18cde	114.6	24.67^{bc}	0.30^{cd}	103.8	31.63^{d*}

Treatments and letters of statistics are as in Table 2,* DW = Dry weight of overground parts per plant

results, Bai and Kastori [19], reported that treatment of sunflower seeds with BAP before sowing caused pronounced increases in chlorophyll (a) and (b) as well as carotenoids contents in the chloroplasts. Sanai and Ota [20] reported that nicotinamide (20 or 80 mg L^{-1}) stimulated chlorophyll (b) formation in treated rise seedlings. The present results are also in agreement with those obtained by Noctor and Foyer [21] who reviewed the role of ascorbate in the regulation of photosynthesis process and its importance in photoprotection. They stated that not only did the chloroplast ascorbate pool detoxified H_2O_2, thereby preventing enzyme inactivation and the generation of more dangerous radicals, but it also allowed flexibility in the production of photosynthetic assimilatory power and that ascorbate is implicated in the regulation of photosynthetic light harvesting processes.

Essential oil content: Data presented in Table 2 indicate that in cutting (I), the highest increase in oil percent was obtained in plants treated with vit. C at 30 mg L^{-1} followed by GA_3 at 50 mg L^{-1}. In cutting (II), oil percent was significantly increased in treatments with vit. C, IBA

Table 4: Percentage composition of essential oil in *Thymus vulgaris* L. herb influenced by soaking of seeds in some growth regulators and vitamins at cutting (II)

Compounds	R t	RRt	Control	GA_3 (30 mg l^{-1})	GA_3 (60 mg l^{-1})	IBA (30 mg l^{-1})	IBA (60 mg l^{-1})	BAP (30 mg l^{-1})	BAP (60 mg l^{-1})
	Growth regulators								
α-pinene	6.50	0.35	0.66	0.99	1.45	1.44	1.65	1.25	0.26
β-pinene	6.83	0.37	1.32	1.02	1.49	1.27	1.78	1.76	0.79
P-Cymene	7.92	0.43	11.93	17.68	25.00	19.43	20.31	26.66	1.24
Camphor	10.44	0.57	1.91	3.13	2.08	2.54	2.56	3.03	2.43
Borneol	12.19	0.66	0.49	0.90	0.75	0.50	0.36	0.56	0.75
Linalool	13.02	0.71	1.75	1.12	1.35	1.74	1.76	0.92	1.15
Thymol	18.44	1.00	57.36	47.70	44.14	47.39	44.79	37.61	75.97
Carvacrol	18.66	1.01	4.59	2.74	3.14	2.64	3.57	6.65	6.30
	Vitamins		Control	Vit. C 30 mg L^{-1}	Vit. C 60 mg L^{-1}	Vit. B1 30 mg L^{-1}	Vit. B1 60 mg L^{-1}	NA 30 mg L^{-1}	NA 60 mg L^{-1}
α-pinene	6.49	0.35	0.66	1.47	1.85	1.00	1.26	1.15	0.86
β-pinene	6.82	0.37	1.32	1.49	2.15	0.72	1.98	0.62	-
P-Cymene	7.88	0.43	11.93	25.28	24.99	17.08	19.53	14.38	7.03
Camphor	10.42	0.57	1.91	1.21	2.23	3.31	3.01	3.44	1.74
Borneol	12.14	0.66	0.49	2.10	0.43	0.90	0.69	0.86	0.77
Linalool	13.00	0.70	1.75	0.37	1.00	3.08	2.23	1.32	1.70
Thymol	18.21	0.99	57.36	43.27	37.01	50.04	46.08	56.13	45.41
Carvacrol	18.55	1.01	4.59	2.39	2.70	3.80	3.43	4.93	4.44

GA_3: Gibberellic acid, IBA: Indole butyric acid, BAP: Benzylaminopurine, Vit. C: L-ascorbic acid, Vit. B ¡ Thiamine and NA: Nicotinamide, PN: Peak number, Rt: Retention time and RRt: Relative retention time

Table 4: Percentage composition of essential oil in *Thymus vulgaris* L. herb influenced by soaking of seeds in some growth regulators and vitamins at cutting (III)

Compounds	R t	RRt	Control	GA_3 (30 mg l^{-1})	GA_3 (60 mg l^{-1})	IBA (30 mg l^{-1})	IBA (60 mg l^{-1})	BAP (30 mg l^{-1})	BAP (60 mg l^{-1})
	Growth regulators								
α-pinene	6.17	0.39	1.65	0.60	1.40	1.07	0.56	1.36	1.03
β-pinene	6.45	0.41	2.37	0.28	2.12	2.45	2.18	1.63	2.17
P-Cymene	7.50	0.47	25.35	6.71	33.65	23.63	16.26	21.79	17.92
Camphor	9.53	0.60	2.88	4.95	1.80	1.90	2.10	3.29	1.97
Borneol	11.63	0.73	2.14	0.71	0.72	1.75	1.81	0.68	1.02
Linalool	13.69	0.86	0.35	1.86	1.86	5.10	0.12	0.15	0.22
Thymol	15.86	1.00	30.00	55.73	30.15	32.46	45.73	42.53	49.94
Carvacrol	16.02	1.01	1.76	2.48	1.31	1.36	1.49	2.75	2.34
	Vitamins		Control	Vit. C 30 mg L^{-1}	Vit. C 60 mg L^{-1}	Vit. B1 30 mg L^{-1}	Vit. B1 60 mg L^{-1}	NA 30 mg L^{-1}	NA 60 mg L^{-1}
α-pinene	6.43	0.41	1.65	1.63	1.36	1.02	1.45	1.37	1.14
β-pinene	6.75	0.43	2.37	2.28	2.32	2.17	2.66	2.51	2.20
P-Cymene	8.10	0.51	25.35	24.44	29.52	24.89	25.26	27.84	20.69
Camphor	10.38	0.65	2.88	1.77	2.40	2.22	2.69	2.55	3.14
Borneol	12.06	0.76	2.14	2.05	1.09	0.37	4.09	1.24	1.86
Linalool	12.96	0.82	0.35	2.87	3.22	2.47	0.12	0.32	1.40
Thymol	18.49	1.17	30.00	25.18	29.50	27.04	30.50	32.81	36.19
Carvacrol	18.65	1.18	1.76	1.50	1.64	1.06	1.72	1.86	1.87

GA_3: Gibberellic acid, IBA: Indole butyric acid, BAP: Benzylaminopurine, Vit. C: L-ascorbic acid, Vit. B_1: Thiamine and NA: Nicotinamide, PN: Peak Number, Rt: Retention time and RRt: Relative retention time

(60 mg L^{-1}) and BAP (30 mg L^{-1}). In cutting (III), the highest oil percent was obtained in plants treated with vit. B_1 at 60 mg L^{-1} followed by vit. C or NA at 30 mg L^{-1}., then by IBA or BAP treatments at 30 mg L^{-1}.

Essential oil yield: Data presented in Table 3 also indicate that in cutting (I), the highest oil yield was obtained as a result of treatment with vit. C at 30 mg L^{-1} followed by GA_3 at 30 mg L^{-1}, then by IBA at 60 mg L^{-1}. In cutting (II), the highest oil yield was significantly increased in vit. C treatment at 60 mg L^{-1} followed by GA_3 and the IBA at 60 mg L^{-1}. In cutting (III), the greatest oil yield was obtained in treatment with BAP, IBA at 60 mg L^{-1} and vit.C at 30 mg L^{-1}.

Table 5: Total phenolic content (mg g^{-1} F.W) of *Thymus vulgaris* L. herb influenced by soaking of seeds in some growth regulators and vitamins at three cuttings

Treatment		Total phenolic content (mg g^{-1} F.W)		
(mg l^{-1})		Cutting (I)	Cutting (II)	Cutting (III)
		Growth regulators		
Control (H_2O)		1.075^{bc}	1.370^{ab}	1.470^{a}
GA_3	30	0.829^{f}	1.253^{de}	1.173^{bc}
	60	1.017^{de}	1.342^{abc}	1.177^{bc}
IBA	30	1.201^{a}	1.232^{e}	0.975^{e}
	60	1.131^{b}	1.391^{a}	1.174^{bc}
BAP	30	1.001^{e}	1.389^{a}	1.219^{b}
	60	1.206^{a}	1.401^{a}	1.155^{c}
	Vitamins			
Vit. C	30	0.981^{e}	1.277^{cde}	1.179^{bc}
	60	0.969^{e}	1.374^{ab}	1.122^{cd}
Vit. B_1	30	1.186^{a}	1.226^{e}	1.135^{cd}
	60	1.086^{bc}	1.319^{abcd}	1.086^{d}
NA	30	1.095^{bc}	1.318^{abcd}	1.135^{cd}
	60	1.059^{cd}	1.303^{bcde}	1.121^{cd}

Figures followed by the same letters are not-significant, Mean values of the results of the three replicates, GA_3: Gibberellic acid, IBA: Indole butyric acid, BAP: Benzylaminopurine, Vit. C: L-ascorbic acid, Vit. B_1: Thiamine and NA: Nicotinamide

These increases in oil yield in plants of cutting (I) with treatments vit. C and GA_3 could be interpreted as both oil % and dry weights (Table 3) of the herb increased at the same treatments. Buettner and Jukiewicz [6] mentioned that the maximum lettuce growth was attained by seed soaking with 1000 mg L^{-1} ascorbic acid. Supporting the present results, seed treatment with 100 ppm GA_3 and longdays increased the essential oil content in the leaves of *Mentha arvensis* [22]. In addition, Balbaa and Refaat [23] reported that the application of thiamine at 40 mg L^{-1} to *Tagetes minuta* plants had a promotive effect on oil percentage as well as oil yield.

Main components content of essential oil: Data presented in Table 4 indicate that in cutting (I), the highest thymol and carvacrol percents were obtained in treatments with BAP (60 mg L^{-1}) followed by that of GA_3 (30 mg L^{-1}). However, p-cymene percent decreased as a result of the same treatments. In cutting (I) vitamin treatments caused decreases in the main essential oil constituents. In cutting (II) presowing seed soaking in BAP at 60 and 30 mg L^{-1} resulted in the highest thymol and carvacrol percents respectively, and the lowest p-cymene content in the thyme herb. In cutting (III), the highest thymol percent was attained in treatment of GA_3 at 30 mg L^{-1} followed by BAP treatment at 60 mg L^{-1}. The highest carvacrol content was obtained in plants treated with BAP at 30 mg L^{-1}, while p-cymene percent was decreased as a result of the same treatment.

The present results could be interpreted on the basis that growth regulators affected many aspects of biochemical processes [4]. In addition, vitamins used here were known to act as coenzymes in the essential physiological processes mainly photosynthesis and respiration. Pandarikakshudu and Bhavsar [24] reported that ascorbic acid increased oil yield and quality of *Anethum sowa*. Tawfik *et al*. [25] reported that BAP significantly increased total ketones such as borneol present in the essential oil of *Salvia officinalis* L. shoot grown on MS medium.

Total phenolic content: Data presented in Table 5 indicate that in thyme herb, total phenolic content of cutting (I) was significantly increased in treatments with BAP (60 mg L^{-1}), IBA and vit. B_1 (30 mg L^{-1}). In cutting (II), total phenolic content was significantly increased as a result of treatments with BAP (both levels), IBA and vit. C at 60 mg L^{-1}. From these results, it could be concluded that the obtained increases in phenolic content in treated thyme plants by growth regulators or vitamins are in favour for raising the validity of thyme as potential antioxidant agent. However, in cutting (III), total phenolic content was significantly decreased at all treatments. In this respect, kadioglu and Atalay [26] found that IAA and GA_3 application decreased the level of total phenolic substances in *Diospyros lotus* fruits.

Fractionation of main phenolic compounds: Data presented in Table 6 indicate that, in all treatments caffeic acid, leutolin and rosmarinic acid were detected. Rosmarinic acid percent was increased at both levels of vit. B_1 followed by BAP at 30 mg L^{-1}, while the highest caffeic acid percent was obtained at both levels of NA. The greatest leutolin percent was obtained at 30 mg L^{-1} vit. C.

These results are in agreement with those obtained by Reda and Gamal El-Din [27] who reported that application of thiamine and ascorbic acid on *Chamomilla recutita* L. caused significant increases in its total phenols content at 150 mg L^{-1} thiamine and 100 mg L^{-1} ascorbic acid. Zheng and Wang [27] reported that rosmarinic acid was one of the predominant phenolic compounds in the herb of *Thymus vulgaris* L. consequently, it could be deduced from the present results that both vit. B_1 and BAP at 30 and 60 mg L^{-1}

Table 6: Percentage composition of phenolic compounds (HPLC peak area %) in *Thymus vulgaris L.* herb influenced by soaking of seeds growth regulators and vitamins at cutting (II)

Treatment (mg l^{-1})		Area % a (Phenolic compounds)				
		Rt	RRt	Caffeic acid	Leutolin	Rosmarinic acid
		Growth regulators				
Control (H_2O)		6.58	0.25			
		6.97	0.26	1.404	2.142	5.163
		26.40	1.00			
GA_3	30	6.51	0.25			
		7.05	0.27	1.744	8.578	9.754
		27.54	1.04			
	60	6.60	0.25			
		7.10	0.27	8.581	2.859	6.307
		26.84	1.02			
IBA	30	6.58	0.25			
		7.28	0.28	6.778	3.154	8.953
		27.30	1.03			
	60	6.62	0.25			
		7.19	0.27	7.250	2.882	3.316
		26.30	1.00			
BAP	30	6.14	0.23			
		6.52	0.25	7.011	8.311	20.600
		26.30	1.00			
	60	6.56	0.25			
		7.15	0.27	2.159	6.581	8.640
		27.69	1.05			
		Vitamins				
Vit. C	30	6.94	0.26			
		7.12	0.27	8.200	12.665	5.425
		26.78	1.01			
	60	6.45	0.24			
		7.04	0.27	3.095	8.049	4.937
		28.87	1.09			
Vit. B_1	30	6.38	0.24			
		7.11	0.27	4.853	1.754	22.613
		27.33	1.04			
	60	6.45	0.24			
		6.78	0.26	1.678	1.145	21.269
		28.21	1.07			
NA	30	6.71	0.25			
		7.04	0.27	11.462	6.211	5.124
		28.99	1.10			
	60	6.60	0.25			
		7.14	0.27	11.696	6.505	11.797
		27.30	1.03			

a: Identification based on a very good match Rt of available reference compound, GA_3: Gibberellic acid, IBA: Indole butyric acid, BAP: Benzylaminopurine, Vit. C: L-ascorbic acid, Vit. B_1: Thiamine and NA: Nicotinamide

Table 7: Polyphenoloxidase activity as pyrogallol or catechol (units g^{-1} F.W/min) of *Thymus vulgaris* L. herb influenced by soaking of seeds in some growth regulators and vitamins at three cuttings

Treatment		Cutting (I)		Cutting (II)		Cutting (III)	
(mg l^{-1})		PPO activity as pyrogallol	PPO activity as catechol	PPO activity as pyrogallol	PPO activity as catechol	PPO activity as pyrogallol	PPO activity as catechol
		Growth regulators					
Control (H_2O)		7.65^{b}	17.68^{d}	3.25^{e}	6.90^{h}	5.35^{cd}	3.50^{cd}
GA_3	30	5.80^{cde}	21.95^{b}	3.93^{de}	9.53^{efg}	4.78^{de}	6.40^{a}
	60	4.00^{g}	17.98^{d}	4.58^{cd}	10.23^{cdef}	6.55^{b}	3.48^{cd}
IBA	30	5.08^{ef}	16.33^{ef}	4.55^{cd}	10.78^{cd}	3.85^{fg}	6.05^{a}
	60	7.73^{b}	22.65^{b}	5.78^{a}	11.93^{ab}	3.63^{fgh}	3.70^{e}
BAP	30	8.50^{a}	22.38^{b}	4.78^{bc}	12.13^{a}	2.83^{I}	6.18^{a}
	60	5.50^{de}	22.83^{b}	4.83^{bc}	11.85^{ab}	6.03^{bc}	4.60^{b}
	Vitamins						
Vit. C	30	6.25^{cd}	17.38^{de}	3.73^{e}	9.35^{fg}	3.45^{ghi}	3.03^{de}
	60	5.78^{cde}	16.08^{f}	4.53^{cd}	10.95^{bc}	2.95^{hi}	2.98^{de}
Vit. B_1	30	8.16^{ab}	25.25^{a}	5.43^{ab}	10.50^{cde}	6.05^{bc}	2.55^{ef}
	60	8.83^{a}	20.10^{c}	5.65^{a}	8.68^{g}	7.98^{a}	3.45^{cd}
NA	30	4.43^{fg}	12.83^{g}	4.45^{cd}	9.83^{def}	7.55^{a}	3.48^{cd}
	60	6.38^{c}	16.15^{f}	4.88^{bc}	10.10^{cdef}	4.30^{ef}	2.10^{f}

Figures followed by the same letters are not-significant, Mean values of the results of the three replicates, GA_3: Gibberellic acid, IBA: Indole butyric acid, BAP: Benzylaminopurine, Vit. C: L-ascorbic acid, Vit. B_1: Thiamine and NA: Nicotinamide

Table 8: Specific activity of peroxidase (EU mg^{-1} protein min^{-1}) of *Thymus vulgaris* L. herb treated with foliar spray of some bioregulators and vitamins at three cuttings (Season, 2000-2001)

Treatments		Specific activity of peroxidase (EU mg^{-1} protein min^{-1})		
(ppm)		Cutting I	Cutting II	Cutting III
		Bioregulators		
Control (H_2O)		5.34^{b}	6.21^{b}	6.21^{de}
GA_3	30	4.82^{cd}	5.41^{e}	5.98^{ef}
	60	5.05^{bc}	5.67^{d}	8.11^{b}
IBA	30	5.96^{a}	6.01^{bc}	6.16^{de}
	60	4.33^{e}	6.96^{a}	6.29^{d}
BAP	30	4.41^{de}	4.63^{g}	5.64^{g}
	60	4.86^{cd}	6.94^{a}	5.59^{g}
		Vitamins		
Vit. C	30	5.35^{b}	5.38^{e}	5.97^{ef}
	60	5.86^{a}	4.93^{f}	6.86^{c}
Vit. B1	30	5.18^{bc}	5.29^{e}	6.61^{c}
	60	5.21^{bc}	7.01^{a}	8.83^{a}
NA	30	4.47^{de}	5.95^{c}	5.87^{fg}
	60	5.12^{bc}	5.46^{e}	5.69^{g}

Figures followed by the same letters are not-significant, Mean values of the results of the three replicates, GA_3 = Gibberellic acid, IBA = Indole Butyric Acid, BAP = Benzylaminopurine, Vit. C = Ascorbic acid, Vit. B1 = Thiamine and NA = Nicotinamide

favoured the accumulation of rosmarinic acid, whether in primary steps of biosynthesis or interconversion of one phenolic compound to another.

Oxidoreductase enzyme activities [polyphenoloxidase (PPO) and peroxidase (POD)]: Data given in Table 7 indicate that, in cutting (I), the highest PPO activity as pyrogallol substrate was obtained in plants treated with BAP at 30 mg L^{-1} and with vit. B_1 at 60 mg L^{-1}. Also, the application of vit. B_1 at 30 mg L^{-1} followed by BAP at 60 mg L^{-1} resulted in the highest activity of PPO as catechol substrate. In cutting (II), the highest PPO activity was obtained in treatments with IBA and vit. B_1 (60 mg L^{-1}) as pyrogallol and with BAP at 30 mg L^{-1} followed by vit. C at 60 mg L^{-1}as catechol substrate. In cutting (III), the highest PPO activity was obtained as pyrogallol in plants treated with vit. B_1 at 60 mg L^{-1} and NA at 30 mg L^{-1}. Meanwhile, PPO activity assayed as catechol was significantly increased in treatments with GA_3, followed by BAP (30 mg L^{-1} each). Other treatments, especially of vitamins showed variable decreases in PPO activity.

Peroxidase (POD) activity was significantly increased in plants treated with GA_3 at 30 mg^{-1} (Table 8) at cutting (I). However, in cutting (II), all treatments caused decreases in peroxidase activity. In cutting (III), peroxidase activity was significantly increased in

treatment 60 mg^{-1}, GA_3, followed by vit. B_1 at the same concentration. In this respect, Kavrayan and Aydemir [28] reported that ascorbic acid was one of the potent inhibitors of PPO activity. These results could be supported by those obtained by Halim and Montgomery [29] who found that the inhibition of d'Arijon pears PPO was 100 % when 10 μM ascorbic acid was used. In addition, at low ascorbic acid concentration, inhibitory effects on crude taro PPO activity were observed (Duangmal and Owusu-Apenten [30].

On the other hand, Kapehina-Toteva and Yakimove [31] reported that peroxidase could be among enzymes expressing antioxidative functions, its activity was increased in single mode of rose transferred to media containing BAP (1.0-2.2 μM). They concluded that this enzyme activity controlled the level of H_2O_2 and the rate of cell division. Wang *et al.* [32] concluded that there was an inverse relationship between the activities of polyphenoloxidase and peroxidase and that the phenolic substances could modify the activity of these enzymes, as both inhibitors and stimulators. Lee-Hosun *et al.* [33] studied the effect of gibberellins on the dormancy breaking, physiological activity and growth of Hanabu (*Hanabusaya asiatica* L.) and found that peroxidase and catalase activities were increased after dormancy breaking by GA_3 treatment and resulted in taller plants with less chlorophyll content than that of the control.

In conclusion, applied plant growth regulators (GA_3, IBA and BAP) and vitamins (vit. B_1, vit. C and NA) at certain level had positive and favourable effects on photosynthetic pigments, dry matter accumulation, essential oil (percent and yield), phenolic compounds and activities of PPO and POD enzymes.

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