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Physiological and biochemical responses of thyme plants to some antioxidants

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Abstract. *Orabi SA, Talaat IM, Balbaa LK. 2014. Physiological and biochemical responses of thyme plants to some antioxidants. Nusantara Bioscience 6: 118-125.* Two pot experiments were conducted to investigate the effect of tryptophan, nicotinamide and α-tocopherol (each at 50 and 100 mg/L) on plant growth, essential oil yield and its main constituents. All treatments significantly promoted plant height, and increased fresh and dry mass (g/plant) of thyme (*Thymus vulgaris* L.). The treatment with 100 mg/L nicotinamide showed increasing in total potassium mainly in the first cut. Total soluble sugars, oil percentage and oil yield and protein recorded increments with tryptophan treatments. Treatment of *Thymus* plants with 100 mg/L nicotinamide observed the highest percentage of thymol (67.61%). Oxygenated compounds recorded the highest value with 50 mg/L α-tocopherol treatment, while the maximum non-oxygenated ones resulted from the application of 100 mg/L nicotinamide. All treatments under study significantly affected the activity of oxidoreductase enzymes (POX and PPO). Nicotinamide at the concentration of 100 mg/L recorded the highest increments in APX and GR and the lowest values in oxidoreductase enzyme activities added to the lowest values of lipid peroxidation to enhance the best protection of thyme plants.

Key words: antioxidants, essential oil, GLC, medicinal and aromatic plants, *Thymus vulgaris*

INTRODUCTION

Thyme (*Thymus vulgaris* L.) belongs to the Lamiaceae family and is an aromatic and medicinal plant of increasing economic importance. Thyme volatile phenolic oil has been reported to be among the top 10 essential oils, showing antibacterial, antimycotic, intoxicative, natural food preservative and mammalian age delaying properties (Jackson and Hay 1994; Letchamo et al. 1995). Schwarz et al. (1996) reported that the main essential oil components of *Thymus vulgaris* L. were thymol, carvacrol and ρ-cymene. Thymol showed strong effect on common respiratory tract (Inouye et al. 2001).

In winter, when plants are subjected to low temperature, during the different stages of growth and development, show several biochemical and physiological dysfunctions due to reactive oxygen species (ROS) including singlet oxygen, superoxide radicals, and alkyl peroxy radicals (Berger et al. 1993). Such disturbances include the loss of membrane integrity, enhancement of peroxidation of membrane lipids, leakage of solutes from cells, the accumulation of metabolic intermediates and the bleaching of chlorophyll (Wang 1982). These changes led to reduction of growth and poor quantity and quality of the yield. In winter, thyme plants are cultivated in Egypt, in warmed green houses. However, the high humidity inside these houses is a suitable medium for encouraging several plant infections which necessitate the application of pesticides, which creates health hazards and is not environmentally accepted. It is very important to alleviate the harmful effect of low temperature on thyme plants in the open field by the use of some specific treatments such as antioxidants which have a protective role in this respect.

Little information is available about the role of vitamins in the regulation of the biosynthesis of essential oils in plants. Nicotinamide (niacin) is considered as one of growth regulating substances which in minute quantities can alter some physiological aspects of plants (Bearder 1980). Robinson (1973) reported that nicotinamide acts as coenzyme in the enzymatic reactions by which carbohydrates, fats and proteins are metabolized and involved in photosynthesis and respiration. Nicotinamide is a well characterized constituent of the pyridine dinucleotide coenzymes NADH and NADPH, which are involved in many enzymatic oxidation-reduction reactions in living cells (Berglund 1994). In addition, nicotinamide improves the induction of defensive metabolism involving secondary metabolite biosynthesis as well as activation of peroxide and free radical-degrading enzymes (Bartoli et al. 1999).

Aberg (1961) indicated that amino acids can act as growth factors of higher plants since they are the build blocks of protein synthesis, which could be important for enzymes metabolic activities. Moreover, amino acids as organic nitrogenous compounds are the building blocks in the synthesis of proteins (Davies 1982). Amino acids are particularly important for cell growth stimulation. They act as buffers which help to maintain favorable pH value within the plant cell. Amino acids also function in the synthesis of other organic compounds, such as protein, amines, purines and pyrimidines, alkaloids, vitamins, enzymes, terpenoids and others (Goss 1973; Abd El-Aziz and Balbaa 2007). Tryptophan is the major precursor of IAA in most organisms (Ramaih et al. 2003). Steif (1988) found that stress decreased the tryptophan synthesis alpha

monomers, which was gradually dissociated from oligomers. This in turn produced less active isoenzymes, reduced biosynthesis of L-tryptophan and consequently that of IAA. Martens and Frankenberger (1994) indicated that the tryptophan treatment increased growth rate and yield of wheat and *Zea mays*, respectively. Wyszkowska (1999) found that tryptophan increased K^+, N and Ca^{2+} in different plant organs.

Alpha-tocopherol (vitamin E) is low molecular weight lipophilic antioxidant which mainly protect membrane from oxidative damage. Zhang et al. (2000) showed positive correlation between α-tocopherol and shoot or root growth in the two grass species of tall fescue and creeping bentgrass. El-Bassiouny et al. (2005) also reported that foliar spray with α-tocopherol on faba bean plants induced increments in growth parameters and yield components. α-tocopherol helps to maintain the integrity of the photosynthetic membranes under oxidative stress (Munné-Bosch and Alegre 2002a).

The best characterized and probably most important function of tocopherols is to act as recyclable chain reaction terminators of polyunsaturated fatty acid free radicals generated by lipid oxidation. From a biosynthetic perspective, tocopherols are members of a large, multifunctional family of lipid soluble compounds called prenylquinones that also include tocotrienols, plasto-quinones, and phylloquinones (vitamin K1). Tocopherols are thought to be important for free radical scavenging and protection from oxidative stress (Berger et al. 1993). In plants, tocopherols are presumed to function as membrane-associated antioxidants and as structural components of membranes, although evidence supporting this role is limited (Norris et al. 1998). Tocopherols are believed to protect chloroplast membranes from photooxidation and help to provide an optimal environment for the photosynthetic machinery (Berger et al. 1993). Many of the proposed tocopherol functions in animals and plants are related to their antioxidant properties, the most prominent of which is protection of polyunsaturated fatty acids from lipid peroxidation by quenching and scavenging various reactive oxygen species (ROS). Regulation of tocopherol biosynthesis in senescing and stressed plants may occur at multiple steps of the pathway. The enzyme *p*-hydroxyphenyl pyruvate dioxygenase (HPPD) activity limits tocopherol synthesis in non-stressed *Arabidopsis* plants (Tsegaye 2002).

The main aim of this work is to study the effect of the antioxidants tryptophan, nicotinamide and α-tocopherol on growth, yield, antioxidant enzymes and oil content of *Thymus vulgaris* L.

MATERIALS AND METHODS

Plant material and growth conditions

Two pot experiments were carried out during two successive seasons of 2010/2011 and 2011/2012 in the greenhouse of National Research Centre, Dokki, Giza, Egypt to study the effect of the antioxidants tryptophan, nicotinamide and α-tocopherol on growth, yield and essential oil of thyme. Seeds of thyme were provided from "SEKEM" company and cultivated on 10th of November, for both seasons. Thyme seeds were put into the pots (30 cm in diameter), with 3 replicates for each treatment. Each replicate includes 6 pots. Freshly prepared aqueous solutions of Tryptophan and nicotinamide were dissolved in distilled water, while α-tocopherol was dissolved in minimum amount of potassium hydroxide solution, then completed with distilled water. Untreated plants sprayed with distilled H_2O which serve as control. Tepol was added (1 mL/L) as a wetting agent to the prepared solutions before spraying on foliage till running using plastic automizer. Plants were foliar sprayed with the different treatments under study at 10th May and 10th August, respectively in both seasons. All the plants were fertilized by the following fertilizers (given per pot): 4 g ammonium nitrate (33.5% N), 4 g calcium superphosphate (16% P_2O_5), 1 g potassium sulphate (48% K_2O). These amounts of fertilizers were added in two doses, the first was given one month before the first cutting and the second dose was added after week of first cutting. Water requirements and other agricultural practices were regularly fulfilled according to the weather conditions during growing the plants in both experiments. Two cuts were taken during the growing seasons (the first one on 10th July and the second on 10th October 2011 and 2012, respectively). The following parameters were recorded at each cut: plant height (cm), fresh and dry mass of herb (g/plant), essential oil percentage and mineral ions content. The percentage of the volatile oil of air dried herb for each treatment was determined by hydro-distillation according to Guenther (1973).

Essential oil isolation

The essential oil was extracted by hydro-distillation for 3h using a Clevenger type apparatus (Clevenger 1928), dehydrated over anhydrous sodium sulfate and kept in refrigerator till Gas Liquid Chromatography (GLC) analysis.

Gas Liquid Chromatography (GLC) analysis

GLC analysis of volatile oil of each treatment in the second cut was performed separately with a Hewlett-Packard model 5890. A fused silica capillary column (Carbowax 20 M measuring 20m x 0.32 mm internal diameter, film thickness of 0.17im) was used. The temperature program adopted was maintained at 75°C for 5 min. with an increase of 4°C min-1 until 220 °C (10 min). The carrier gas was Helium and the working flow rate was 1.0 mL/min, detector was 9144 HP. The identification of the compounds was achieved by matching their retention times with those of authentic samples injected under the same conditions.

Biochemical constituents determination

Enzymes activities

Fresh leaves were collected for estimation the activity of antioxidant enzymes (Ascorbate peroxidase (APX, EC 1.11.1.11), guaiacol peroxidase (POX, EC 1.11.1.7), Glutathione reductase (GR, EC 1.6.4.2), polyphenoloxidase (PPO, EC 1.14.18.1) and lipid peroxidation. Extraction of the antioxidant enzymes POX, GR and APX were determined according to the method of Mukherjee and

Choudhuri (1983). The POX and APX activities were determined according to Nakano and Asada (1981) and GR activity was determined according to Zanetti (1979). Lipid peroxidation was determined by measuring Malondi-aldehyde (MDA) content as described by Dhindsa et al. (1982). Polyphenoloxidase (PPO) activity was determined according to the modified method of Taneja and Sachar (1974). The oxidizing capacity of the enzyme extract was determined spectrophotometrically against pyrogallol.

Determination of total soluble sugars

Total soluble sugars were determined by using the method described by Dubois et al. (1956). Potassium and phosphorous and nitrogen contents were determined according to the method described by Chapman and Pratt (1961). Crude protein percentage was calculated by multiplying the total nitrogen percentage by the factor 6.25 as used by Tripathi et al. (1971).

Statistical analysis

The average of the obtained data of the two growing seasons were subjected to statistical analysis using F-test according to the procedure outlined by Snedecor and Cochran (1980). Least significant difference (LSD) at 0.05 level were calculated, to compare the mean values of each determined criteria for different treatments in this study.

RESULTS AND DISCUSSION

Effect of antioxidants on plant growth

Data presented in Table 1. indicate that foliar application of tryptophan significantly increased plant height, fresh and dry weights of herb of thyme plants, especially in plants treated with 100 mg/L. These results hold true for both cuttings.

Similar results were obtained by Talaat (2005) who reported that exogenous application of both tryptophan and putrescine on periwinkle transplants considerably increased plant growth at successive developmental stages. The effect was more pronounced with 10^{-3} M tryptophan or putrescine. Tryptophan is considered as one of the three primary metabolites of periwinkle (*Catharanthus roseus*) indole alkaloids. The role of tryptophan is well known in this respect. Phillips (1971) stated that, several alternative routes of IAA synthesis exist in plants, all starting from the amino acid tryptophan. Attoa et al. (2002) reported that spraying *Iberis amara* L. plants with the amino acid tryptophan at 75 ppm increased plant height, number of branches, leaves fresh and dry weights, number of coryomb and coryomb fresh weight per plant.

El-Bassiouny (2005) found that the increase in growth of wheat plant in response to tryptophan treatment relative to untreated plants might be a result of increased levels of endogenous hormones consequently stimulation of cell division and/or cell enlargement and subsequently growth. In addition, Abdel-Monem et al. (2010) reported that the exogenous application of tryptophan improved tolerance of two sunflower cultivars (Hysun 336 and Euroflor) grown under different saline conditions through increasing photosynthetic pigments, endogenous promoters, especially IAA or increasing the uptake of K, Ca and Mg which in turn alleviate the harmful effect of oxidative stress. Nicotinamide treatments significantly increased plant height, fresh and dry weights of herb of thyme plants, especially in plants treated with 100 mg/L. These results hold true for both cuttings (Table 1).

These results are in good agreement with those obtained by Foda (1978) and Deyab (1989) on wheat plants. In addition Hathout et al. (1993a,b) found that the application of 10, 40 and 80 mg/L nicotinamide as foliar spray on tomato plants caused stimulatory effects on growth, yield and endogenous promoters (auxins and gibberellins). Similar findings were also obtained by Tarraf et al. (1999) who reported that foliar application of nicotinamide to lemongrass plants significantly promoted vegetative growth as well as essential oil percentage and yield per plant, total carbohydrates and crude proteins. Several studies found that α-tocopherol and/or nicotinamide improved growth in different plants (Zhang et al. 2000; El-Bassiouny et al. 2005; Hassanein et al. 2009; Sadak et al. 2010) who reported that α-tocopherol or nicotinamide may act as growth stimulants which can play a role in mitigating the adverse effect of oxidative stress on metabolic activities relevant to growth through increasing IAA content. That was further cooperated by the significantly higher levels of carbohydrates observed generally in the treated plants (Shi et al. 2000).

Data shown in Table 2. indicate that all treatments significantly increased total soluble sugars content (mg/g fresh weight), oil percentage and oil yield (mL/plant), except plants treated with 100 mg/L nicotinamide in the second cut, which slightly decreased oil percentage. Treatment of thyme plants with α-tocopherol significantly increased essential oil percentage (0.45% in the first cut and 0.74% in the second cut), especially in plants treated with 100 mg/L α-tocopherol (Table 2). Treatment of thyme plants with 100 mg/L tryptophan recorded the highest increments in total proteins percentage in both cuttings (Table 3). The lower concentration of all treatments under study markedly increased phosphorous percentage (Table, 3). Treatment with nicotinamide showed increasing in total potassium and phosphorous contents mainly in the first cut. On the other hand, the highest phosphorous content was recorded in plants treated with 50 mg/L tryptophan in the second cut (Table 3).

These results are in agreement with those obtained by Talaat (2005) who found that foliar application of tryptophan at the rate of 10^{-5} M to periwinkle plants promoted alkaloidal content in the leaves, while treating periwinkle plants with 10^{-4} M and 10^{-3} M tryptophan were more effective in affecting total alkaloids content. These results hold true for both flowering and fruiting stages, respectively.

Attoa et al. (2002) also reported that spraying *Iberis amara* L. with tryptophan at 75 ppm increased carbo-hydrates, nitrogen and phosphorus contents. El-Bassiouny et al. (2005) found that the contents of K and P in faba bean seeds were increased in general by α-tocopherol or nicotinamide treatments. Moreover, Abdel-Monem et al. (2010) mentioned that spraying sunflower plants previously

sown in soil of EC 7.83 dsm^{-1} salt concentration, with either tryptophan or Prozac at concentrations of 2.5, 5 mg/L induced significant increments in both total carbohydrates and total protein contents in shoots as compared to control.

Table 1. Effect of some antioxidants on growth of thyme plants.

Treatment	Plant height (cm)		Fresh wt of herb (g/plant)		Dry wt of herb (g/plant)	
	First cut	Second cut	First cut	Second cut	First cut	Second cut
Tr 50 ppm	23.00	31.33	37.92	67.09	3.74	18.98
Tr 100	27.00	33.67	47.11	70.59	5.03	22.46
Ni 50	26.00	34.67	32.71	68.86	3.61	23.37
Ni 100	28.00	35.33	54.00	77.08	8.54	24.68
α-T 50	23.33	30.00	38.37	74.80	4.27	23.53
α-T 100	24.00	32.00	52.22	85.32	7.2	25.68
control	20.00	29.33	29.31	49.59	2.65	16.67
LSD (5%)	3.02	2.30	3.81	3.82	0.47	4.72

Note: wt = weight

Table 2. Effect of some antioxidants on total soluble sugars, oil percentage and oil yield of thyme plants.

Treatment	Total soluble sugars (mg/g fresh weight)		Oil percentage		Oil yield (mL/plant)	
	First cut	Second cut	First cut	Second cut	First cut	Second cut
Tr 50 ppm	11.17	6.80	0.42	0.61	0.16	0.41
Tr 100	12.01	7.08	0.43	0.96	0.20	0.67
Ni 50	10.07	5.69	0.44	0.62	0.14	0.35
Ni 100	10.62	6.60	0.53	0.56	0.28	0.43
α-T 50	9.37	5.90	0.44	0.70	0.17	0.52
α-T 100	8.96	5.28	0.45	0.74	0.23	0.63
Control	7.92	4.10	0.41	0.61	0.12	0.31
LSD (5%)	0.65	0.77	0.05	0.23	0.03	0.07

Table 3. Effect of some antioxidants on protein, phosphorous and potassium contents of thyme plants.

Treatment	Total protein		Total phosphorous		Total potassium	
	%	As % of control	%	As % of control	%	As % of control
First cut						
Tr 50 ppm	14.22	148.21	0.31	106.90	2.50	116.28
Tr 100	16.22	169.06	0.29	100.00	2.80	130.23
Ni 50	11.63	121.17	0.35	120.69	2.85	132.56
Ni 100	16.13	168.08	0.32	110.34	3.00	139.53
α-T 50	10.06	104.89	0.33	113.79	2.35	109.30
α-T 100	11.44	119.22	0.24	82.76	2.55	118.60
Control	9.59	100.00	0.29	100.00	2.15	100.00
Second cut						
Tr 50 ppm	13.63	152.92	0.43	179.17	2.40	126.32
Tr 100	16.72	187.64	0.29	120.83	2.70	142.11
Ni 50	10.41	116.79	0.28	116.67	2.15	113.16
Ni 100	11.09	124.51	0.22	91.67	2.70	142.11
α-T 50	9.09	102.06	0.31	129.17	1.95	102.53
α-T 100	13.91	156.07	0.13	54.17	2.15	113.16
Control	8.91	100.00	0.24	100.00	1.90	100.00

Bassiouny et al. (2008) and Park et al. (2009) concluded that the marked increase in total carbohydrate and protein contents due to tryptophan treatment not only play a hormonal role by alleviating the inhibitory effect of oxidative stress via osmotic adjustment or act as protective agent by conferring some desiccation resistance to plant cell, but also stimulated the accumulation of carbohydrates and nitrogen contents. Sadak et al. (2010) mentioned that soaking sunflower seeds in different concentrations of α-tocopherol or nicotinamide under different salinity levels increased total carbohydrates, total protein percentage and oil yield/feddan (ton) as compared to the corresponding salinity levels for both tested cultivars (Hysun 336 and Euroflor). Meanwhile, foliar application of ascorbic acid or α-tocopherol caused significant increases in total carbohydrates and proteins, mineral ions (Mg^{+2}, K^{+}, Ca^{+2} and P) and oil percentage of the flax cultivars (Sakha 3, Giza 8 and Ariane) (Sadak and Dawood 2014). Abdelhamid et al. (2013) reported that nicotinamide led to the increase of the concentrations of (P, K, Ca, Zn) ions in the shoots of the stressed faba bean plants through its role in increasing osmotolerance and/or through regulating various processes including absorption of nutrients from soil solution. Talaat and Aziz (2005) reported that essential oil percentage and total oil yield (mL/plant) were significantly increased as a result of foliar spray of chamomile plants with glutathione, nicotinic acid or ascorbic acid. They also mentioned that total sugars and total nitrogen appeared to follow the same trend.

Generally, vitamins could alleviate the inhibitory effects of oxidative stress by activating protein synthesis where vitamins might act as activators for protein synthesis (Kodandaramaiah 1983; Abdelhamid et al. 2013). The accumulation of soluble sugars or soluble protein (soluble osmolytes) was more prominent in leaves of plants grown under cold or salt stress (Orabi 2004; Orabi et al. 2013) since protection of cell structure against oxidative damage and maintaining the structure of proteins and membranes are the main functions of compatible solutes (Hajihashemi et al. 2006; Thakur and Dev-Sharma 2005; Sidari et al. 2008) to reduce osmotic potential in the cytoplasm and contributes to maintaining water homeostasis among several cellular compartments (Sairam and Tyagi 2004).

Enzyme activity

Data presented in Table 4. indicate that foliar application of nicotinamide to thyme plants resulted in significant increase in the activity of both ascorbate peroxidase (APX) and glutathione reductase (GR) enzymes, especially in plants treated with 100 mg/L concentration. Treatment with tryptophan and α-tocopherol were less effective. Concerning peroxidase (POX) activity, it was significantly increased in plants treated with all treatments under study. Meanwhile, the highest POX activity was obtained in plants treated with 100 mg/L α-tocopherol, followed by 50 mg/L α-tocopherol and 100 mg/L tryptophan.

All treatments under study significantly decreased the activity of Malondialdehyde (MDA) content, especially plants treated with 100 mg/L nicotinamide, followed by

plants treated with 100 mg/L α-tocopherol. In this respect, Abdelhamid et al. (2013) studied the interactive effect of salinity stress and nicotinamide and concluded that nicotinamide could improve the physiological and biochemical parameters by decreasing MDA level of faba bean plants.

Data presented in Table 4. also indicated that the highest polylphenoloxidase (PPO) activity as pyrogallol substrate was obtained in plants treated with 100 mg/L tryptophan, followed by those treated with 50 mg/L tryptophan.

Antioxidant metabolism plays an important role in protecting plants from a wide variety of environmental stresses, such as drought, salinity extreme temperatures, pollutants, ultraviolet radiation and high levels of light (Orabi 2004; Amirjani 2010; Orabi et al. 2010; Siringam et al. 2011; Orabi et al. 2013). The antioxidant systems, including antioxidants and anti-oxidant enzymes, can ameliorate the deleterious effects of ROS in vivo and in vitro. Antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPX) function, by catalyzing the decomposition of oxidants and free radicals. The ROS concentration in the tissues directly exposed to salt are strongly influenced by the coordinated action of different antioxidative enzymes (Munns and Tester 2008).

El-Bassiouny (2005) and Abdel-Monem et al. (2010) reported that nicotinamide, tryptophan and Prozac could enhance plant tolerance. El-Bassiouny et al. (2005) found that α-tocopherol and nicotinamide suppressed lipid peroxidation and plasma membrane permeability. Nicotinamide improves the induction of defensive metabolism involving secondary metabolite biosynthesis, as well as activation of peroxide and free radical-degrading enzymes (Bartoli et al. 1999). Nicotinamide is a well characterized constituent of the pyridine dinucleotide coenzymes (NADH and NADPH) which are involved in many enzymatic oxidation-reduction reactions in living cells (Berglund and Ohlsson 1995).

Numerous reports are available where the attempt has been made to reduce oxidative stress in plants by exogenous application of tocopherols via the foliage of plants (Foyer et al. 1994a). Plant cells can be protected against oxidant stress by various radical-scavenging systems, including low-molecular-weight antioxidants such as ascorbate, glutathione, α-tocopherol, and carotenoids, as well as by antioxidant enzymes such as superoxide dismutases, peroxidases, and glutathione reductases (Foyer et al. 1994b). Protection against phytotoxic peroxidation processes in lipophilic environments may be achieved by antioxidants, like α-Tocopherol (Vitamin E), which is assumed to be the most effective radical-chain-breaking substance in the membranes. Therefore, many attempts have been made to reduce oxidative stress in plants by exogenous application of this vitamin (Foyer et al. 1994a).

Table 4. Effect of some antioxidants on enzyme activity in the second cut.

Treatment	APX (µmol/g Fwt)	GR (nmol/g Fwt)	POX (µmol/g Fwt)	MDA (µmol/g Fwt)	PPO (Unit/g Fwt)
Tr 50 ppm	3.16	370.86	14.32	22.06	5.24
Tr 100	3.55	386.29	15.60	21.30	5.73
Ni 50	4.20	408.62	13.40	18.12	2.82
Ni 100	4.88	448.82	13.91	17.88	4.02
α-T 50	2.30	288.14	16.26	21.59	3.42
α-T 100	2.66	339.41	18.89	19.00	4.55
Control	2.15	245.62	13.07	26.07	2.90
LSD (5%)	0.24	57.42	1.55	1.36	0.48

Table 5. Effect of some antioxidants on essential oil constituents in the second cut.

Treatments (mg/L) constituents (%)	Tr 50	Tr 100	Ni 50	Ni 100	α-T 50	α-T 100	Control
α-Pinene	1.32	1.39	0.29	0.09	0.39	0.65	0.79
Myrcene	1.60	1.19	2.30	1.47	1.16	1.41	1.50
ρ-cymene	11.90	14.82	8.03	9.09	15.93	9.10	14.71
ϒ-terpinene	2.17	2.56	0.61	1.64	3.58	2.51	0.96
Linalool	1.35	1.32	3.34	1.98	1.41	2.50	2.48
Camphor	0.06	0.12	4.07	4.34	0.15	3.21	1.84
Borneol	4.23	2.91	0.49	1.74	3.12	0.51	2.21
Terpineol	0.33	0.18	2.74	1.13	1.54	2.12	0.98
Thymol	65.12	64.55	63.20	67.61	58.45	65.64	61.49
Carvacrol	0.10	0.57	4.22	4.70	6.00	4.99	4.78
β-bourbonene	0.82	0.57	1.12	0.55	0.75	0.89	0.84
Trans-caryophyllene	1.02	0.19	1.31	0.14	0.18	0.15	0.11
Cadinene	0.16	0.16	0.11	0.16	0.19	0.14	0.12
Germacrene-D	0.54	0.15	0.18	0.95	0.06	1.04	0.08
Caryophyllene oxide	0.16	0.08	0.13	0.09	0.44	0.09	0.59
Oxygenated compounds	22.96	24.48	21.87	21.47	27.30	22.03	25.48
Non-oxygenated compounds	67.93	66.27	70.27	74.20	66.07	72.95	68.01
Total identified compounds	**90.89**	**90.76**	**92.14**	**95.68**	**93.36**	**94.97**	**93.48**

Data presented in Table 5. indicate that thymol is the main essential oil constituent (58.45-67.61%), followed by ρ-cymene (8.03-15.93%). Treatment of plants with 100 mg/L nicotinamide recorded the highest thymol content and treatment of plants with 50 mg/L nicotinamide recorded the lowest ρ-cymene content. Oxygenated compounds recorded the highest value with 50 mg/L α-tocopherol treatment, while the maximum non-oxygenated ones resulted from the application of 100 mg/L nicotinamide.

It is clear that increasing nicotinamide concentration increased the biosynthesis of thymol, while the opposite was true with p-cymene. It is well known that p-cymene transforms to thymol or carvacrol and the environmental conditions affect the rate of transformation (Foyer et al. 1994a,b). Increasing nicotinamide concentration increased the biosynthesis of thymol. On the other hand, increasing nicotinamide concentration decreased p-cymene content. Tryptophan is considered as one of the three primary metabolites of *C. roseus* indole alkaloids. The role of tryptophan is well known in this respect. Phillips (1971) stated that, several alternative routes of IAA synthesis exist in plants, all starting from the amino acid tryptophan. These results are in agreement with the findings of Harridy (1986), who mentioned that tryptophan applied to *C. roseus* plants increased IAA concentration. The most effective concentration was 25 ppm. The increase of indoles may be attributed to the conversion of tryptophan to IAA as stated by Phillips (1971).

In plants, tocopherol levels and composition vary in different tissues and fluctuate during development and in response to abiotic stresses. Significant increases in leaf α-tocopherol levels are observed during aging and senescing of plants (Molina-Torres and Martinez 1991; Munné-Bosch and Alegre 2002b), possibly to protect cellular components from increased oxidative stress (Munné-Bosch and Alegre 2002b). Enhanced tocopherol accumulation also occurs in response to a variety of abiotic stresses including high light, drought, salt, and cold and may provide an additional line of protection from oxidative damage (Berger et al. 1993; Havaux et al. 2000).

CONCLUSION

Applying 100 mg/L tryptophan resulted in the highest essential oil percentage while applying 100 mg/L nicotinamide resulted in the best percentage of thymol (the major compound) and the highest increments in the activities of the antioxidant enzymes APX and GR the two evolved in ascorbate-glutathione pathway which play a main role in scavenging H_2O_2. The beneficial role of using these antioxidants could help thyme plants to increase their tolerance against oxidative stress through mainly the decrease in membrane damage symptoms by lowering of lipid peroxidation beside increments in the different studied traits.

REFERENCES

Abd El-Aziz NG, Balbaa LK. 2007. Influence of tyrosine and zinc on growth, flowering and chemical constituents of *Salvia farinacea* plants. J Appl Sci Res 3: 1479-1489.

Abdelhamid MT, Sadak Mervat Sh, Schmidhalter URS, El-Saady AM 2013. Interactive effects of salinity stress and nicotinamide on physiological and biochemical parameters of faba bean plant. Acta Biol. Colomb. 18: 499-510.

Abdel-Monem AA, El-Bassiouny HMS, Rady MM., Gaballah, MS. 2010. The role of tryptophan and Prozac (5-Hydroxy tryptophan) on the growth, some biochemical aspects and yield of two sunflower cultivars grown in saline soil. IJAR 2: 254-262.

Aberg B. 1961. Vitamins as growth factors in higher plants. Encycl Plant Physiol 14: 418-449.

Amirjani MR. 2010. Effects of salinity stress on growth, mineral composition, proline content, antioxidant enzymes of soybean. Am J Physiol 5: 350-360.

Attoa GE, Wahba, HE, Farahat, AA. 2002. Effect of some amino acids and sulphur fertilization on growth and chemical composition of *Iberis amara* L. plant. Egypt J Hort 29: 17-37.

Bartoli CG, Simontacchi M, Tambussi E, Beltrano J, Montaldi E Puntarulo S. 1999. Drought and watering dependent oxidative stress: effect on antioxidant content in *Triticum aestivum* L. leaves. J Exp Bot 50: 375-383.

Bassiouny FM, Hassanein RA, Baraka DM, Khalil RR. 2008. Physiological effects of nicotinamide and ascorbic acid on Zea mays plant grown under salinity stress. II-Changes in nitrogen constituents, protein profiles, protease enzyme and certain inorganic cations. Austr J Basic Appl Sci 2: 350-359.

Bearder IR. 1980. Plant hormones and other background, structure and occurance. In: Mac Millan J. (ed.). Hormonal regulation and development. Encyclopedia of plant physiology. New Series. Vol. 9, Springer, Berlin.

Berger S, Ellersiek U, Westoff P, Steinmuller K. 1993. Studies on the expression of NDH-H, a subunit of the NAD(P)H-plastoquinone-oxidoreductase of higher plant chloroplasts. Planta 190: 25-31.

Berglund T, Ohlsson AB. 1995. Defensive and secondary metabolism in plant tissue cultures, with special reference to nicotinamide, glutathione and oxidative stress. Plant Cell Tissue Organ Cult 43: 137-145.

Berglund T. 1994. Nicotinamide, a missing link in the early stress response in eukaryotic cells: a hypothesis with special reference to oxidative stress in plants FEBS Lett 351: 145-149.

Chapman HD, Pratt PF. 1961. Methods of analysis for soils and water. Univ., Calif. Div. Agric. Sci., 2nd Print. Chapter, 17: 150-161.

Clevenger JF. 1928. Apparatus for determination of essential oil. J Amer Pharm Assoc 17: 346-349.

Davies DD. 1982. Physiological aspects of protein turnover. In: Boulter D, Parthier B, eds. Nucleic Acids and Proteins in Plants. I. Encyclopedia of Plant Physiology. New series, Vol. 14A. Springer, Berlin.

Deyab MA. 1989. Studies on some enzymic and hormonal activities in germinating grains and seeds of some graminaceous and leguminous plants. [Ph.D. Dissertation]. Faculty of Science, Tanta University, A.R.E.

Dhindsa RS, Plumb-Dhindsa P, Reid DM. 1982. Leaf senescence and lipid peroxidation. Effects of some phytohormones and scavengers of free radicals and singlet oxygen. Physiol Plant 56: 453-457.

Dubois MKA, Gilles JK, Hamilton PA, Repers Smith F. 1956. Colorimetric method for determination of sugars and related substances. Anal Chem 28: 350-356.

El-Bassiouny Hala MS, Gobarah Mirvat E. Ramadan, Amany, A. 2005. Effect of antioxidants on growth, yield and favism causative agents in seeds of *Vicia faba* L. plants grown under reclaimed sandy soil. J Agron 4: 281-287.

El-Bassiouny HMS. 2005. Physiological responses of wheat to salinity alleviation by nicotinamide and tryptophan. Inter J Agric Biol 7: 653-659.

Foda EAA. 1978. Growth dynamics of *Triticum vulgare*. [M.Sc. Thesis]. Faculty of Science, Tanta Univ., A.R.E.

Foyer CH, Descourvieres P, Kunert KJ. 1994b. Protection against oxygen radicals: an important defense mechanism studied in transgenic plants. Plant Cell Environ 17: 507-523.

Foyer CH, Lelandais M, Kunert KJ. 1994a. Photooxidative stress in plants. Physiol Pl 92: 696-717.

Goss JA. 1973. Amino Acid Synthesis and Metabolism. Physiology of Plants and Their Cell. Pergamon, New York.

Guenther E. 1973. The Essential Oils. D. Van Nostrand, New York.

Hajihashemi S, Kiarostami K, Enteshari S, Saboora A. 2006. The effects of salt stress and paclobutrazol on some physiological parameters of two salt tolerant and salt sensitive cultivars of wheat. Pak J Biol Sci 9: 1370-1374.

Harridy IMA. 1986. Physiological studies on periwinkle plant, *Catharanthus roseus* G. Don. [Ph.D. Dissertation]. Faculty of Agriculture, Cairo University, Egypt

Hassanein RA, Bassiouny FM, Baraka DM, Khalil RR. 2009. Physiological effects of nicotinamide and ascorbic acid on *Zea mays* plant grown under salinity stress. I. Changes in growth, some relevant metabolic activities and oxidative defense systems. Res J Agric Biol Sci 5: 72-81.

Hathout TA, Shetawi SA, Khallal SM. 1993b. Effect of mode of application of some growth regulators on physiology of tomato plants. IV. Effect of nicotineamide on the endogenous hormones contents. Egypt J Physiol Sci 2: 201-221.

Hathout TA, Shetawi SA, SM. Khallal SA. 1993a. Effect of mode of application of some growth regulators on physiology of tomato plants. III. Effect of Nicotineamide on morphology, growth, metabolism and productivity. Egypt J Physiol Sci 172: 183-200.

Havaux M, Bonfils J, Lütz C, Niyogi KK. 2000. Photodamage of the photosynthetic apparatus and its dependence on the leaf developmental stage in the *npq1* Arabidopsis mutant deficient in the xanthophyll cycle enzyme violaxanthin de-epoxidase. Plant Physiol 124: 273-284.

Inouye S, Takizawa T, Yamaguchi H. 2001. Antibacterial activity of essential oils and their major constituents against respiratory tract pathogens by gaseous contact. J Antimicrobiol Chemother 47: 565-573.

Jackson SAL, Hay RKM. 1994. characteristics of varieties of thyme (*Thymus vulgaris* L.) for use in the U.K., Oil content, composition and related characters. Ann Hort Sci 69: 275-281.

Kodandaramaiah J. 1983. Physiological studies on the influence of B-vitamins on leaf and fruit metabolism in cluster beans *Cyamopsis tetragonoloba* L. Tanb. [Ph.D. Dissertation]. Srivenkateswera Univ. Tirupati, India.

Letchamo W, Xu HL, Gosseline A. 1995. Variation in photosynthesis and essential oil in thyme. J Plant Physiol 147: 29-37.

Martens DA, Fankenberger WTJr. 1994. Assimilation of exogenous 2, - 14C - indole -3 -acetic acid and 3, - 14C tryptophan exposed to the roots of three wheat varieties. Plant Soil 166: 281-90

Molina-Torres J, Martinez ML. 1991. Tocopherols and leaf age in *Xanthium strumarium* L. New Phytol 118: 95-99.

Mukherjee SP, Choudhari MA. 1983. Implications for water stress-induced changes in the levels of endogenous ascorbic acid and hydrogen peroxide in *Vigna* seedlings. Plant Physiol 58: 166-170.

Munné-Bosch S, Alegre L. 2002a. The function of tocopherols and tocotrienols in plants. Crit Rev Plant Sci 21: 31-57.

Munné-Bosch S, Alegre L. 2002b. Interplay between ascorbic acid and lipophilic antioxidant defences in chloroplasts of water-stressed *Arabidopsis* plants. FEBS Lett 524: 145-148.

Munns R, Tester M. 2008. Mechanisms of salinity tolerance. Annu Rev Plant Biol 59: 651-681.

Nakano Y, Asada K. 1981. Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. Plant Cell Physiol 22: 867-880.

Norris S, Chen X, DellaPenna D. 1998. Complementation of the Arabidopsis pds1 mutation with the gene encoding ρ-hydroxyphenyl-pyruvate dioxugenase. Plant Physiol 117: 1317-1323.

Orabi SA, Mekki, BB, Sharara Faida A. 2013. Alleviation of adverse effects of salt stress on faba bean (*Vicia faba* L.) plants by exogenous application of salicylic acid. WASJ 27: 418-427.

Orabi SA, Salman SR, Shalaby Magda AF. 2010. Increasing resistance to oxidative damage in cucumber (*Cucumis sativus* L.) plants by exogenous application of salicylic acid and paclobutrazol. World J Agric Sci 6: 252-259.

Orabi SA. 2004. Physiological impacts of cold injury on cucumber (*Cucumis sativus* L.) plant. [Ph.D. Dissertation]. Faculty of Science, Cairo Univ, Cairo.

Park S, Kang K, Lee K, Choi D, Kim YS, Back, K. 2009. Induction of serotonin biosynthesis is uncoupled from the coordinated induction of tryptophan biosynthesis in pepper fruits (*Capsicum annum*) upon pathogen infection. Planta 230: 1197-1206.

Phillips IDJ. 1971. Introduction to the Biochemistry and Physiology of Plant Growth Hormones. Mc Grow-Hill Book Co., New York.

Ramaih S, Guedira M, Paulsen GM. 2003. Relationship of indoleacetic acid and tryptophan dormancy and preharvest sprouting of wheat. Funct. Plant Biol 30: 939-45.

Robinson FA. 1973. Vitamins. In: Miller LP (ed.) Phytochemistry. Vol. III. Van-Nostrand Reinhold Co., New York.

Sadak Mervat Sh, Dawood Mona G. 2014. Role of ascorbic acid and α tocopherol in alleviating salinity stress on flax plant (*Linum usitatissimum* L.). J Stress Physiol Biochem 10: 93-111.

Sadak MSh, Rady MM, Badr NM, Gaballah MS. 2010. Increasing sunflower salt tolerance using nicotinamide and α-tocopherol. Int J Acad Res 2: 263-270.

Sairam RK, Tyagi A. 2004. Physiology and molecular biology of salinity stress tolerance in plants. Curr Sci 86: 407-421.

Sarwar M, Frankenberger WTJr. 1994. Influence of L- tryptophan and auxin applied to the rhizosphere on the vegetative growth of *Zea mays* L. Plant Soil 160: 97-104.

Schwarz K, Emst H, Temes W. 1996. Evaluation of antioxidative constituents from thyme. J Sci Food Agric 70: 217-223.

Shi W, Yang Z, Sun H, Lancero H, Tong L. 2000. Phenotypic analyses of frz and dif double mutants of *Myxococcus xanthus*. FEMS Microbiol Lett 192: 211-215.

Sidari M, Mallamaci C, Muscolo A. 2008. Drought, salinity and heat differently affect seed germination of *Pinus pinea*. J For Res 13: 326-330.

Siringam K, Juntawong N, Cha-Um S, Kirdmanee C. 2011. Salt stress induced ion accumulation, ion homeostasis, membrane injury and sugar contents in salt-sensitive rice (*Oryza sativa* L. spp. *indica)* roots under isoosmotic conditions. African J Biotechnol 10: 1340-1346.

Snedecor GM, Cochran WG. 1980. Statistical Methods. 7th ed., Iowa State College Press, Amer Iowa, USA.

Steif M. 1988. Isoenzymes tryptophan synthase (Serine hydrolase adding indolglycerol phosphate) of higher plants and their role in growth regulation. Agron Rost 49: 83-107

Talaat IM, Aziz EE. 2005. Physiological response of *Matricaria chamomilla* L. plants to Glutathion, nicotinic acid and ascorbic acid. Egypt J Appl Sci 207: 218-231.

Talaat IM. 2005. Physiological Response of *Lavandula angustifolia* L. Plants to Cysteine and Nicotineamide. J Agric Sci Mansoura Univ 1573-1582.

Taneja SR, Sachar RC. 1974. Stimulation of polyphenoloxidase (monophenoloxidase) activity in wheat endosperm by gibberellic acid, cycloheximide and actinomycin D. Planta (Berl.) 116: 133.

Tarraf ShA, Gamal El-Din, KM, Balbaa LK. 1999. The response of vegetative growth, essential oil of lemongrass (*Cympopogon citratus* L.) to foliar application of ascorbic acid and nicotinamide and some microelements. Ann Agric Sci Ain Shams Univ Cairo 7: 247-259.

Thakur M, Dev SA 2005. Salt-stress-induced proline accumulation in germinating embryos: Evidence suggesting a role of proline in seed germination. J Arid Environ 62: 517-523.

Tripathi RD, Srivastava GP, Misra MS, Pandey SC. 1971. Protein content in some varieties of legumes. The Allah Abad Farmer 16: 291-294.

Tsegaye Y, Shintani DK, DellaPenna D. 2002. Over-expression of the enzyme *p*-hydroxyphenyl-pyruvate dioxygenase in *Arabidopsis* and its relation to tocopherol biosynthesis. Plant Physiol Biochem 40: 913-920.

Wang CY. 1982. Physiological and biochemical responses of plants to chilling stress. HortScience 17 (2):173-186.

Wyszkowska J. 1999. Modification of faba bean chemical composition caused by precursors of plant growth regulators and soil microorganism I. Effect of L-tryptophan and beta-indolyl acetic acid. Biuletyn-Naukowy 5: 55-63.

Zanetti G. 1979. Rabbit liver glutathione reductase, purification and properties. Arch Biochem Physiol 198: 241-246.

Zhang R, Schmidt E, Zhang XZ. 2000. Hormone containing products impact on antioxidant status of tall fescue and creeping betagrass subjected to drought. Crop Sci 40: 1344-1349.