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Effect of Antioxidants on Growth, Yield and Favism Causative Agents in Seeds of *Vicia faba* L. Plants Grown under Reclaimed Sandy Soil

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Abstract: Two field experiments were conducted during the two winter seasons of 2002/2003 and 2003/2004 in a private farm with reclaimed sandy soil at El-Nagah village, South El-Tahrir provience El-Behaira Governorate Egypt. The experiments aimed to study the effect of foliar treatments with different concentrations of α-tocopherol, (100, 200 and 400 mg L^{-1}) ascorbic acid (100, 200 and 400 mg L^{-1}) or nicotinamide (25, 50 and 100 mg L^{-1}) on faba bean plants. The results indicated that, foliar spray with all treatments of antioxidants on faba bean plants induced increments regarding number of branches, leaves and leaves area/plant, fresh and dry weights of shoots as well as chlorophyll a, chlorophyll b and carotenoid contents as compared with the untreated plants after 75 days from sowing. Foliar application of antioxidants significantly increased plant height, number of branches, pods and seeds/plant; seed yield (g)/plant, 100 seed weight as well as seed and straw yield (ton/fed). Also total carbohydrate, crude protein, K, P and Ca contents in dry seeds were significantly increased. On the other hand, the results showed that, the different treatments in general induced marked reductions in vicine and convicine (the causative agents of favism) in yielded seeds. This reduction reached nearly to 42% as compared with those of the untreated plants. All the treatments of antioxidants induced significant decrease in lipid peroxidation and oxidative enzymes such as polyphenol oxidase, peroxidase, catalase and superoxide dismutase activities in faba bean shoots compared with the control values. The maximum yields of seed and straw (ton/fed) of faba bean were obtained in response to 50 mg L^{-1} of nicotinamide or 200 mg L^{-1} of α-tocopherol. In addition, the plants treated with 200 mg L^{-1} ascorbic acid, 50 mg L^{-1} nicotinamide and 400 mg L^{-1} ascorbic acid respectively recorded marked reduction in *vicine* and *convicine* contents.

Key words: Antioxidant, ascorbic acid, nicotinamide, α-tocopherol, carbohydrates, convicine, vicine, *Faba bean,* growth, protein, reclaimed sandy soil, *Vicia faba,* yield

INTRODUCTION

Faba beans (*Vicia faba* L.) are popular legume food with high yield capacity and high protein content (30% of their dry weight) which contain most of the necessary amino acids for human and animal nutrition and low sulphur amino acids concentrations[1].

Faba beans contain toxic glycosides as pyrimidine derivaties namely vicine [2, 6 diamino-4,5-dihydroxy pyrimidine, 5 (B-glycopyransoide)] and convicine [2,4,5-trihydroxy-6-amino pyrimidine, 5 (B-D gluco-pyranoside)]. The presence of vicine and convicine in faba bean decrease its nutritive value for humans and animals[2]. The toxic glycosides vicine and convisine are the factors responsible for favism for humans particularly young males that have a deficiency of erythrocytic enzyme glucose-6-phosphate dehydrogenase (G.6 PD) activity[3,4]. This disease constitutes a major health problem in countries throughout the Mediterranean countries. In Egypt, prevalence of favism among the population had been estimated to amount 26%[5].

The vicine and convicine contents were highest in fresh green cotyledons and gradually declined until a constant level was reached when seed dry matter percentage was around 40%[6,7]. The current of interest in reducing or eliminating legume antinutritional factors were carried out by both genetic and environmental factors[8]. The reductions of these compounds in Faba bean (*Vicia faba* L.) will benefits human nutrition through the elimination of the causative agents of favism. A few attempts to detoxify faba bean by treating the seeds or plants with different tools[9,10]. In this connection, Abd Allah *et al.*[11] found that soaking the faba bean seeds with sodium carbonate and acetic acid reduced the content of

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vicine to 38.4 and 61.3%, respectively. Moreover, Jamalian[2] found that soaking the seeds with 0.01M HCl or 0.013 M NaOH gave similar results. Gaber *et al.*[12] indicated that foliar sprayed and seed presoaked plants in GA_3, ABA or coumarin decreased the contents of vicine and convicine in the yielded seeds.

Some regions of the world, such as parts of the Sahara Desert of North Africa in Egypt is exposed to a combination of environmental stress conditions, including low water availability, high irradiance, temperature fluctuations and nutrient deprivation. Such stresses may lead to an imbalance between antioxidants defense and the amount of Activated Oxygen Species (AOS) resulting in oxidative stress[13,14]. The water stress may be alleviated by irrigation whenever possible[15] or by using certain antioxidants[16].

α-tocopherol is low molecular weight lipophilic antioxidants which protect membrane from oxidative damage[16]. Zhang *et al.*[17] showed positive correlation between α-tocopherol and shoot or root growth in the two grass species. Ascorbic acid is small water -soluble antioxidant molecule which acts as primary substrate in the cyclic pathway for enzymatic detoxification of hydrogen peroxide[18]. Ascorbic acid has been suggested as a bioregulator of plant growth and development[19]. Nicotinamide is a well characterized constituent of the pyridine dinucleotide coenzymes NADH and NADPH, which are involved in many enzymatic oxidations-reduction reactions in living cells[20]. In addition, nicotinamide improves the induction of defensive metabolism involving secondary metabolite biosynthesis as well as activation of peroxide and free radical-degrading enzymes[21]. Radi *et al.*[22] found that, nictoinamide treatment increased growth and yield of *Vicia faba* L. plants.

The aim of the present study was to assess the effect of ascorbic acid, nicotinamide and α-tocopherol on improving the growth, yield and quality of faba been plants. In addition, to reduce legume antinutritional factors, vicine and convicine under the newly reclaimed sandy soil.

MATERIALS AND METHODS

Two field experiments were carried out at El-Nagah village, South El-Tahrir region, El-Behaira Governorates, Egypt, during the two successive winter seasons of 2002/2003 and 2003/2004 to study the response of faba bean (*Vicia faba* L.) plants var. Giza blanka to foliar application of antioxidant on growth, yield and yield components, nutritional and antinutritional (vicine and convicine content) values of dry seeds of faba been. The experimental soil is sandy in texture and having the following characteristics: sand 94%, water holding capacity 10-15%, pH 8.3, organic matter 0.85%, $CaCO_3$ 0.35%, EC 0.07 m mhos cm^{-3}, total N 3.1 mg N/100 g and 1.7 mg P/100 g.

The experiments were laid in a Complete Randomized Block Design with four replicates. The treatments were; control and three antioxidants, at three concentrations α-tocopherol (vitamin E) 100, 200 and 400 mg L^{-1}, ascorbic acid (vitamin C) 100, 200 and 400 mg L^{-1}; nicotinamide, (vitamin B) 25, 50 and 100 mg L^{-1}. Seeds of faba bean were sown on the last week of October in both seasons in plots 12 m^2 (5 ridges, each 4 m in length and 0.6 m in width) in hills 20 cm apart. Organic manure at the rate of 10 m^3/fed was added during seed bed preparation time. Calcium superphosphate 15.5% P_2O_5 at the rate of 32 kg P_2O_5 was applied before ridgine. Three weeks after sowing the plants were thinned to two plants per hill, then 20 kg N/fed as ammonium nitrate 33% N were applied. Three concentrations of each vitamin were sprayed twice during plant growth period at 45 and 60 days after sowing. The random samples of ten plants were taken after 75 days from sowing to estimate the following, plant height, number of branches and leaves/plant, area of leaves/plant, fresh and dry weight/plant, photosynthetic pigment in leaves, lipid peroxidation and the activity of oxidative enzyme (peroxidase, polyphenol oxidase, catalase and superoxide dismutase).

At harvest date, two central rows were harvested and sub samples of ten plants were taken randomly to estimate the yield attributes as follows, plant height, number of branches/plant, number of pods/plant, number of seeds/pod, weight of seeds/plant and 100 seeds weight.

Whole plot was harvested and dried under upon condition to determine the yields of seed and straw ton/fed. Crop index was calculated as seed yield/straw yield per fed. and harvest index as seed yield/biological yield (above ground biomas)/fed.

The dried seed were finally ground the kept for total carbohydrate, crude protein, K, Ca and P percentage, vicine and convicine contents.

Chemical analysis: Photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) were determined spectrophotometerically[23]. Total carbohydrate percentage was determined in the yielded seeds as described by Younis *et al.*[24] Protein percentage was determined according to the method of AOAC[25] and K, Ca and P percentages by Chapmen and Pratt[26]. The separation and quantitative determination of vicine and convicine were detected as adopted by Marquardt and Frohlich[27].

The levels of lipid peroxidation were measured by determining the levels of malondialdehyde. Malondialdehyde is a product of lipid peroxidation and was assayed by thiobarbituric acid reactive substances (TBARS) contents[28]. Popolyphenol oxidase (PPO, EC 1.10.3.1) and catalase (CAT, EC 1.11.1.6) activities were determined following the method of Klapheck *et al.*[29] perioxidase (POX,EC 1.11.1.7) activity was determined following the method of Kar and Mishra[30] and superoxide dismutase (SOD EC 1.15.1.1) activity were made according to the method described by Marklund and Marklund[31] and calculated by Kong *et al.*[32]

Data were statistically analyzed according to Snedecor and Cochran[33]. Combined analysis of the two growing seasons was carried out. The means were compared by LSD test at level of 0.05 probability.

RESULTS AND DISCUSSION

Growth parameters and photosynthetic pigment: Growth parameters were generally increased significantly in response to the applied treatments with the different concentrations of α-tocopherol, ascorbic acid or nicotinamide on faba bean plants as compared to the control (Table 1). Maximum increase was obtained in response to 50 mg L^{-1} nicotinamide or 200 mg L^{-1} α-tocopherol. The obtained results are in agreement with those of Shorning *et al.*[34] and Stasolla and Yeung[35]. Zhang *et al.*[17] added that α-tocopherol or ascorbic acid increased the growth and development in different plant species. Also, Radi *et al.*[22] confirmed that nicotinamide treatment increased the growth characters of faba bean plants. The increase in the growth and development of faba bean plants in response to antioxidant treatments might be due to the enhancement of cell division and/or cell enlargement[36,37] and/or to influence DNA replication[20,38,21].

Application of α-tocopherol, ascorbic acid or nictoinamide on faba bean plants significantly increased chlorophyll a, chlorophyll b and corotenoid contents (Table 1). The magnitude of increase is much more pronounced by applying α-tocopherol. The increase in photosynthetic pigments in faba bean leaves may be due to the role of antioxidants (α-tocopherol and ascorbic acid) in protecting chloroplast from oxidative damage[39]. In addition, Sahu *et al.*[40] and Ghourab and Wahdan[41] indicated that ascorbic acid increased photosynthetic efficiency, leaf area and delayed leaf sensences. Similar results have been reported in response to nicotinamide application on wheat[42] and on *Tagetes minuta* L.[43]. The effect of nicotinamide on the biosynthesis of chlorophyll may be attributed to its activation of enzyme that regulate photosynthetic carbon reduction[44].

Yield and yield components: Foliar application of α-tocopherol, ascorbic acid or nicotinamide to faba bean plants significantly increased yield components as well as yields of seeds and straw (ton/feddan) as compared with those of the untreated plants (Table 2). Spraying faba bean plants, with 200 mg L^{-1} α-tocopherol produced the highest values for plant height, number of pods/plant, 100 seeds weight (g) and weight of seeds/plant followed by 50 mg L^{-1} nicotinamide and 400 mg L^{-1} α-tocopherol. In the mean time the maximum yields of seeds and straw (ton/fed) were obtained by applying 50 mg L^{-1} nicotinamide then 200 mg L^{-1} α-tocopherol followed by 200 mg L^{-1} ascorbic acid. Similar results were obtained in different plant species[22,43,45].

Thus, it can be concluded that the increment of seed yield/fed. in response to the applied treatments is mainly due to the increases in the number of branches, pods and seeds/plant and seeds weight/plant. The increases in yield and its components might be due to the effect of antioxidant on enhancing protein synthesis and delaying senescence[40,45].

Table 1: Effect of α- tocopherol, ascorbic acid or nictoinamide on growth parameters, and photosynthetic pigments of faba bean leaves after 75 days from sowing (combined analysis of 2002/2003 and 2003/2004 seasons)

Treatments (mg L^{-1})		Plant height (cm)	No. of branch/ plant	No. of leaves/ plant	Leaves area/ plant (cm^2)	Shoot fresh wt./ plant (g)	Shoot dry wt./ plant (g)	Photosynthetic pigments (mg/100 g dry weight)		
								Chl a	Chl b	Carotenoid
Control		45.67	4.00	37.00	394	148.3	33.46	960	227	239
α- tocopherol	100	48.62	6.00	54.67	699	204.4	52.71	1176	325	401
	200	58.41	8.33	66.33	791	254.6	58.99	1163	328	367
	400	52.23	7.33	55.67	702	221.8	54.82	1156	331	366
Ascorbic acid	100	49.83	4.67	40.67	420	169.0	39.17	1099	308	368
	200	55.33	5.67	54.69	612	206.1	50.82	1031	285	295
	400	49.98	5.30	49.33	617	176.7	43.49	1029	255	287
Nicotinamide	25	50.33	5.67	52.00	513	173.2	44.35	1040	273	370
	20	58.33	8.53	65.33	854	300.8	65.24	1051	290	333
	100	53.67	6.00	50.67	664	195.2	48.21	1046	289	319
LSD 5%		3.54	1.05	5.22	11.73	8.34	4.63	32.54	15.11	14.09

Table 2: Effect of α-tocopherol, ascorbic acid or nicotinamide on yield and yield components of faba bean plants (combined analysis of 2002/2003 and 2003/2004 seasons)

Treatments (mg L^{-1})		Plant height (cm)	No. of branches/ plant	No. of pods/ plant	No. of seeds/ plant	100 seed weight (g)	Weight of seeds/ plant (g)	Seed yield ton/fed	Straw yield ton/fed	Crop index (%)	Harvest index (%)
Control		95.3	6.67	21.0	71.7	72.52	52.62	2.57	4.37	58.8	37.0
α-tocopherol	100	105.4	9.00	32.7	92.0	86.71	81.23	3.07	5.19	59.2	37.2
	200	116.6	10.33	39.3	118.3	95.82	102.70	3.41	5.62	60.7	37.8
	400	108.3	9.67	37.7	106.3	90.34	96.24	3.21	5.40	59.7	37.3
Ascorbic acid	100	100.5	7.33	25.0	84.7	82.14	71.29	2.86	4.87	58.7	37.0
	200	108.4	9.14	31.6	90.7	87.04	81.13	3.25	5.47	59.4	37.3
	400	104.7	8.67	27.7	87.0	84.73	79.40	2.98	5.10	58.4	36.9
Nicotinamide	25	103.0	9.00	31.2	91.7	83.76	79.51	3.17	5.1	61.8	38.2
	50	110.0	11.00	38.00	121.3	94.32	100.22	3.46	5.80	59.7	37.4
	100	109.3	9.33	36.3	104.3	90.34	93.24	3.19	5.41	59.0	37.1
LSD 5%		4.70	1.16	5.7	5.09	4.69	4.16	0.23	0.38	NS	NS

Table 3: Effect of α- tocopherol, ascorbic acid or nicotinamide on the nutritional and antinutritional contents in faba bean yielded seeds (combined analysis of 2002/2003 and 2003/2004 seasons)

		Nutritional g/100 g dry wt.				Antinutritional (mg/100 g dry wt.)			
Treatments (mg L^{-1})		Carbohydrate (%)	Protein (%)	K (%)	Ca (%)	P (%)	Vicine	Convicine	Reduction (%)
Control		35.16	29.70	0.96	0.67	0.24	472	156	-
α-tocopherol	100	36.00	31.69	1.60	0.81	0.39	315	129	29.30
	200	37.49	31.00	1.56	0.80	0.38	365	115	23.57
	400	35.24	34.68	1.29	0.81	0.40	363	111	24.52
Ascorbic acid	100	42.68	31.13	1.62	0.98	0.25	347	123	25.16
	200	41.02	35.91	1.02	0.74	0.25	284	85	41.24
	400	40.84	32.82	1.10	0.72	0.24	341	89	31.53
Nicotinamide	25	43.68	37.00	1.19	0.70	0.25	354	135	22.13
	50	40.32	36.04	1.44	0.73	0.25	303	113	33.76
	100	39.84	30.72	1.65	0.88	0.28	366	93	26.91
LSD 5%		1.796	1.95	1.95	0.076	0.076	0.003	1.90	1.59	1.44

Table 4: Effect of α-tocopherol, ascorbic acid or nicotinamide on lipid peroxidation (TBARS) content (μmol/g fresh wt./h) and enzyme activities (g fresh wt./h) of faba bean shoots 75 days from sowing (combined analysis of 2002/2003 and 2003/2004 seasons)

Treatments (mg L^{-1})		TBARS	Polyphenol oxidase	Peroxidase	Catalase	Superoxide dismutase
Control		8.80	36.51	269.1	89.4	225.4
α-tocopherol	100	4.50	28.77	141.3	49.8	105.8
	200	4.48	26.61	140.9	40.2	119.1
	400	6.84	22.17	199.4	28.8	115.9
Ascorbic acid	100	6.26	24.42	240.5	64.0	202.9
	200	4.89	22.77	195.1	37.2	145.9
	400	4.50	23.25	179.2	27.2	80.2
Nicotinamide	25	5.47	31.23	211.7	48.0	175.0
	50	4.69	24.6	170.6	42.8	124.6
	100	4.89	24.53	136.4	16.2	125.8
LSD 5%		0.37	2.59	10.44	4.27	8.27

Seed quality: The percentage of carbohydrates in dry produced seeds was increased significantly by application of α- tocopherol, ascorbic acid or nicotinamide treatments (except 100 and 400 mg L^{-1} α-tocopherol). The highest increment of carbohydrate 24% was induced by 25 mg L^{-1} nicotinamide over the control value. Protein percentage significantly increased regarding all treatments used except 200 mg L^{-1} α-tocopherol and 100 mg L^{-1} ascorbic acid or nicotinamide. Maximum increase 21% was induced by 25 mg L^{-1} nicotinamide over the control value. Similar findings were obtained in different plant species in response to ascorbic acid application[46]. Mohamed *et al.*[47] found that nicotinamide increased the level of carbohydrates of wheat plant in different plant organs. In addition Gharib[43] found that folair application of nicotinamide increased total carbohydrates and crude protein of *Tagetes minuta* L. plant.

The contents of K, Ca and P in *faba* bean seeds were increased in general by α-tocopherol, ascorbic acid or nicotinamide treatments (Table 3). This may be due to the translocation of these elements from shoots to the storage organs (seeds). Several reports indicated that ascorbic acid or nicotinamide application increased mineral contents[46].

Perusal of the data in Table 3 showed that the foliar application of α-tocopherol, ascorbic acid or nicotinamide significantly decreased the contents of vicine and convicine. The most pronounced effect was observed at

200 mg L^{-1} ascorbic acid and 50 mg L^{-1} nicotinamide where the percentage of reduction reached to 41.2 and 33.5, respectively as compared with untreated plants. In this connection, Bjerg *et al.*[48] stated that both environmental and genetic factors seems to affect the concentrations of favism causative agents (vicine and convicine) in the seeds of *Vicia faba* plants. Also, Gaber *et al.*[12] indicated that foliar sprayed and seed presoaked plants with certain growth regulators decreased the contents of vicine and convicine in the seeds.

The reduction in the contents of vicine and convicine may be attributed to the effect of these factors on metabolic pathway of vicine and convicine precursor (orotic acid) formation which responsible for the formation of pyrimidine ring of these toxic constituents[49]. The purines and pyrimidines are regulator of the synthesis of secondary products[50].

Lipid peroxidation: Lipid peroxidation was decreased with in response to the application of different concentrations of α-tocopherol, ascorbic acid or nicotinamide (Table 4). In this respect, Asada[16] and Gupta and Datta[19] found that α- tocopherol application suppressed membrane lipid peroxidation and plasma membrane permeability. Shalata and Neumann[18] reported that ascorbic acid treatment in different plant species reduced lipid peroxidation by directly scavenging active oxygen species. Nicotinamide improves the induction of defensive metabolism involving secondary metabolite biosynthesis, as well as activation of peroxide and free radical-degrading enzymes[21].

The used antioxidant could be postulated as a key factor to control the oxidation at the membrane levels, limiting increase in hydroproxide and lipid radical content[16,21].

Antioxidant enzyme: Application of various concentrations of α-tocopherol, ascorbic acid or nictoinamide on faba bean plants induced significant reductions in the polyphenol oxidase, peroxidase, catalase and superoxide dismutase activities as compared with those of the untreated plants (Table 4). These reductions in enzyme activities could be attributed to antioxidants direct effects on scavenge superoxide radical (O^{-2}), hydrogen perioxide (H_2O_2) and singlet oxygen (O_2) and/or preventing the enhancement of the mentioned activated oxygen species[38,16]. In addition, Padh [51]reported that ascorbate plays an important role in preserving the activities of enzymes that contain prothetic-transition metal ions. Ascorbic acid acts as a primary substrate in the cyclic pathway for enzyme detoxification of hydrogen peroxide[18]. Berglund and Ohlsson[52] found that, 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.

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