Improving performance and quality of faba bean plant via folic acid and α- tocopherol application

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Abstract: Field experiment was carried out at the experimental station of Agricultural Production and Research Station, National Research Centre, El Nubaria Province, El Behaira Governorate, Egypt during two successive winter seasons (2014/2015) and (2015/2016) to evaluate the effect of foliar application of folic acid and α-tocopherol at different concentrations (200, 400, 600 mg/l) on the growth and seed quantity and quality of two faba bean cultivars (Maser 3 and Nubaria 2). Results show that all applied treatments, either folic acid or α-tocopherol at different concentrations had positive effect on vegetative growth parameters, photosynthetic pigments, seed yield, yield components, nutritive value of the yielded seeds of two faba bean cultivars. α-tocopherol treatments had more pronounced effect than folic acid treatments. α-tocopherol at 600 mg/l was the most effective treatment followed by folic acid at 600 mg/l. it is worthy to mention that, under control treatment, growth of faba bean plants belong to Nubaria 2 cultivar showed more adaptable to sandy soil conditions than that of Misr 3.
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1. Introduction

Faba bean (*Vicia faba* L.) is a popular legume food with high protein content (30% of their dry weight) which contains most of the necessary amino acids for human and animal nutrition and contributes to soil fertility through biological nitrogen fixation (Gaber et al., 2000; Cazzato et al., 2012).

Recently, a great attention has been focused on the possibility of using safe substances in order to improve plant growth. In this regard, vitamins have synergistic effects on growth, yield and yield quality of many plant species. These compounds have beneficial effects on catching free radicals or active oxygen species that produced during photosynthesis and respiration processes (Foyer *et al*., 1991; Fard et *al*., 2008). Vitamins are organic bio-regulator compounds which are required in trace amount to maintain normal plant growth and development. These compounds act as coenzyme systems and take part in the regulation of metabolism (Hassanein *et al*., 2009). The most familiar vitamins are folic acid (vitamin B9) and α-tocopherol (vitamin E) that affect plant growth and development (Samiullah *et al*., 1988).

In order to understand the enhancement role of folic acid and α-tocopherols as two promising vitamins on plant, it is necessary to make an intensive research on the optimal rate, method and time of application and its wide different responses.

Folic acid (B9) is water-soluble vitamin. According to the Lehninger et al. (2005); Burguieres et al. (2007); Bailey et al. (2009), folic acid is active in plants only in its reduced form, as tetra-hydro-folic acid (tetra-hydro-folate) and tetra-hydro-folic coenzymes. These folic acid derivatives have multiple functions (Stakhova et al.,2000) as involved in photosynthesis (Grunert et al., 2002), biochemical conversions of nitrogen, carbon, and sulfur as well as synthesis and catabolism of protein amino acids (Metzler and Metzler, 2003) and nucleic acids (Litwack, 2008; Andrew *et al*., 2000). Several researchers indicated that folic acid treatment stimulated transport of amino acids to the appropriate location in protein chain creation (Kelly, 1988), regulation of cell division and cell elongation (Naheif and Mohamed, 2013), photosynthesis (Andrew *et al*., 2000) and yield improvement in different crops (Andrew *et al*., 2000; Abd El-Naeem and Abd El-Hakim, 2009; Emam *et al*., 2011; Raeisi et al., 2017). Furthermore, folic acid is considered the central cofactor for one-carbon transfer reactions which are involved in many cellular reactions such as synthesis of purines, nucleic acids, metabolism of amino acids, a glycine to serine conversion, synthesis of methionine and the formation of lignin, chlorophyll and choline and also in the photorespirations cycle (Andrew *et al.,* 2000; Hanson and Roje, 2001; Jabrin *et al.,* 2003). The folic acid is an ancillary initiator of glutamic acid synthesis and induces synthesis of other amino acids, because glutamate is an allosteric activity regulator for multiple-enzyme systems (Popova et al., 1995).

α-Tocopherol is low molecular weight lipophilic membrane-located antioxidant. Photosynthetic organisms are the only source of tocopherols production for all other organisms. Tocopherols play a regulatory role in a range of different physiological phenomena including plant growth and development, senescence, preventing lipid peroxidation and interact with the signal cascade that convey abiotic and biotic signals (Sattler *et al.,* 2004; Baffel and Ibrahim, 2008; and Soltani *et al.,* 2012). The powerful antioxidant properties of α-tocopherol is mainly due to their ability to donate their phenolic hydrogens to lipid free radicals (Bagheri and Sahari, 2013). α-tocopherol scavenges cytotoxic H_2O_2 and reacts non-enzymatically with other ROS and protectes cell membranes from oxidative damage and poly unsaturated fatty acids from lipid peroxidation (Asada, 1999; Krieger-Liszkay and Trebst, 2006) stabilizes membrane structures (Blokhina et al., 2003), improves membrane permeability to small ions and molecules (Foyer, 1992; Maeda and Della Penna, 2007), modulates signal transduction (Noctor 2006), controls certain gene expressions (Munné-Bosch, 2005(.

This work aimed to evaluate the effect of foliar application of folic acid and α-tocopherol at different concentrations on faba bean growth and seed yield quality and quantity of faba bean plant grown under sandy soil conditions.

2. Materials and Methods

Plant materials and experimental conditions

Two field experiments were conducted at the Research and Production Station, National Research Centre, El-Nubaria Province, El-Behira Governorate, Egypt, during two successive winter seasons of 2014/2015 and 2015/2016. The seeds of two faba bean cultivars (Maser 3 and Nubaria 2) were obtained from the Legumes Crops Research Department, Ministry of Agriculture, Egypt and selected for uniformity in size and colour.

Regarding fertilization, P_2O_5 as calcium super-phosphate (15.5%) and K_2O as potassium sulphate (48%) were added during seed bed preparation at the level of 31 and 24 kg/fed respectively, while nitrogen fertilizer as ammonium nitrate (33.5%) was added at the rate of 75 kg N/fed. Seeds of faba bean were sown in hill spaced 20 cm apart at both sides of ridge on the middle of November during the two growing seasons. Thinning was carried out at 15 days after sowing to leave two plants per hill. The experiments were laid out in factorial experiment in complete randomizedblock design withfour replicates/treatment. The experimental unit was 10.5 m^2 (1/400 fed.) 3 meter long and 3.5 meter wide and 60 cm apart between rows. The plants were sprayed twice at 30 and 45 days after sowing with freshly prepared solutions of folic acid at and tocopherol at 200,400,600 mg/l. Meanwhile, untreated plants were sprayed by distilled water to serve as control. Irrigation was carried out using the sprinkler irrigation system where plants were watered every 5 days for two hours**.**

Data recorded

Plant samples were collected after 60 days from sowing for measurement of some growth parameters (plant height, leaves and branches number/plant, dry weight of plant) and photosynthetic pigments in fresh leaf.

At harvest the following characters were recorded at random of ten guarded plants from each treatment: number and weight of pods / plant, number of seeds /plant, seed yield / plant (g), weight of straw/plant (g). The yielded seeds were cleaned and crushed to determine total carbohydrate, total protein, vicine content, and N, P, K elements.

Chemical analysis

Chlorophyll a, chlorophyll b and carotenoids were determined using method described by Lichtenthaler and Buschmann (2001). Phosphorus (P), potassium (K) and nitrogen (N) were measured in the yielded seeds. The seeds were digested using an acid mixture consisting of nitric, perchloric and sulfuric acids in the ratio of 8:1:1 (v/v). Phosphorus and potassium were estimated photometrically using flame photometer method described by the methods described by Chapman and Pratt (1978). Total nitrogen (N) was determined using micro-Kjeldahl method, as described by AOAC (2000). The phenol-sulphuric acid method was used for the determination of total carbohydrates (Dubois *et al.,* 1956). Total protein was calculated by multiplying N% by 6.25. Vicine content was determined according to Collier (1976).

3. Results

Vegetative growth parameters

Vegetative growth parameters of two faba bean cultivars (Misr 3 and Nubaria 2) were significantly and gradually increased by increasing the concentration of applied treatments (Table 1). α-tocopherol treatments were more effective than folic acid treatments in increasing the most investigated parameters. α-tocopherol at 600mg/l increased dry weight of Misr 3 by 42.56% and Nubaria 2 by 42.23% relative to corresponding controls followed by folic acid treatment at 600mg/l. It was noted that plant dry weight of Nubaria 2 (7.15g) was significantly higher than plant dry weight of Misr 3(6.39 g) under control treatment. Hence, the growth of Nubaria 2 was more adaptable to sandy soil conditions than Misr 3.

Photosynthetic pigments

Total photosynthetic pigments of both cultivars were significantly increased by folic acid and α-tocopherol treatments at 400 and 600 mg/l (Table 2). Whereas, both vitamins at 200 mg/l caused non significant increases relative to corresponding control.

The most pronounced treatment was 600 mg/l α-tocopherol, since, it increased total photosynthetic pigments of Misr 3 by 27.33% and Nubaria 2 by 17.76% relative to control. Non significant difference appeared between total photosynthetic pigments of both cultivars under control treatments.

Table (1): Effect of folic acid and α-tocopherol on some growth parameters of faba bean

Treatments	Concentration (mg/l)	Plant height (cm)	Leaves number/plant	Branches number/plant	Plant dry weight (g)
Misr 3					
Control	0	48.33 g	12.67 j	1.67 c	6.39 g
Folic acid	200	52.33 de	17.00 g	2.00 b	6.63 g
	400	54.00 cd	18.33ef	2.00 b	7.12 f
	600	59.33 ab	19.33 d	2.00 b	8.19 cd
tocopherol	200	51.00 ef	14.67 i	2.00 b	6.54 g
	400	59.33 ab	14.67 i	2.00 b	8.03 d
	600	61.33 a	15.67 h	2.00 b	9.11 b
Nubaria 2					
Control	0	41.00 h	12.33 j	1.33 d	7.15 f
Folic acid	200	55.00 c	17.92 f	1.67 c	7.72 e
	400	57.67 b	18.50 e	2.00 b	8.38 c
	600	59.33 ab	21.00 c	2.00 b	9.32 b
tocopherol	200	50.00fg	21.58 b	1.67 c	7.21 f
	400	54.67 c	21.83 b	2.00 b	7.95 de
	600	61.00 a	22.58 a	2.33 a	10.17 a

Values in the same column with same letters are not significantly different (*p*<0.05).

Table (2): Effect of folic acid and α-tocopherol on photosynthetic pigments (mg/g) of fresh leaf tissue of faba bean

Treatments	Concentration (mg/l)	Chlorophyll a	Chlorophyll a	carotenoid	Total photosynthetic pigments
Misr 3					
Control	0	1.09 de	0.31 c	0.22 c	1.50 e
Folic acid	200	1.14 de	0.34 bc	0.22 c	1.59 de
	400	1.24 c	0.37 bc	0.25 a	1.73 bcd
	600	1.25 bc	0.41 ab	0.26 a	1.83 ab
tocopherol	200	1.32 ab	0.35 bc	0.23 bc	1.71 bcd
	400	1.25 bc	0.37 bc	0.25 ab	1.81 ab
	600	1.37 a	0.45 a	0.26 a	1.91 a
Nubaria 2					
Control	0	1.08 e	0.32 c	0.19 d	1.52 e
Folic acid	200	1.11 de	0.33 c	0.23 bc	1.55 e
	400	1.16 d	0.35 bc	0.21 cd	1.63 cde
	600	1.27 bc	0.36 bc	0.23 bc	1.76 bc
tocopherol	200	1.13 de	0.34 bc	0.21 cd	1.55 e
	400	1.26 bc	0.35 bc	0.22 c	1.73 bcd
	600	1.32 ab	0.37 bc	0.25 ab	1.79 ab

Values in the same column with same letters are not significantly different (*p*<0.05).

Seed yield and its components

Table 3 shows that all applied treatments significantly increased seed yield and yield components of both faba bean cultivars except 200 mg/l folic acid and 200 mg/l α-tocopherol showed non significant increases in some investigated parameters. α-tocopherol treatments were more pronounced than folic acid treatments. 600mg/l α-tocopherol was the most effective treatments followed by 600 mg/l folic acid. Where, 600 mg/l α-tocopherol significantly increased seed yield of Misr 3 by112.78% and Nubaria 2 by 133.56%. Meanwhile, 600 mg/l folic acid significantly increased seed of Misr 3 by 93.78% and Nubaria 2 by 68.83% relative to corresponding controls. It was noted that, seed yield and yield components of Nubaria 2 were superior to that of Misr 3 under control treatments. Hence, we can say that growth and yield of faba bean plants belong to Nubaria 2 were more adaptable to the conditions of sandy soil conditions than faba bean plants belong to Misr 3 under control conditions.

Nutritive value of the yielded seeds

It was noted that most applied treatments significantly increased carbohydrate and protein contents of both cultivars accompanied by significant decreases in vicine content relative to control (Table 4). 600 mg/l α-tocopherol was the most effective treatment followed by 600 mg/l folic acid in increasing carbohydrate and protein contents of both cultivars and decreasing vicine content. Where, 600 mg/l α-tocopherol decreased vicine content by 23.95 % in Misr 3 and by 36.19 % in Nubaria 2 relative to corresponding controls. It was noted that Misr 3 cultivar was characterized by higher carbohydrate and protein contents and lower vicine content under control treatment.

Regarding mineral content of the yielded seeds, all applied treatments increased N, P, K contents of the yielded seeds of both cultivars relative to control. 600 mg/l α-tocopherol was the most effective treatment in increasing mineral content of both cultivars followed by 600 mg/l folic acid.

Table (3): Effect of folic acid and α- tocopherol seed yield and yielded components of faba bean

Treatments	Concentration (mg/l)	Number of Pods/plant	Weight of Pods/plant	Number of seeds/plant	Weight of Seeds/plant	Weight of straw/plant
Misr 3						
Control	0	4.34 f	10.5 e	9.43 g	8.21 d	11.24 e
Folic acid	200	4.52 f	11.90 de	9.53 g	8.94 cd	11.27 e
	400	7.72 cd	20.54 b	13.20 f	15.43 b	14.98 c
	600	10.60 a	21.04 b	13.35 f	15.91 b	15.44 c
tocopherol	200	7.14 de	21.36 b	11.50 fg	15.41 b	18.32 b
	400	8.42 bc	21.53 b	16.94 cd	17.02 b	19.26 ab
	600	11.50 a	21.79 b	18.58 bcd	17.47 b	20.27 a
Nubaria 2						
Control	0	6.19 e	13.57 d	13.88 ef	9.98 cd	12.47 d
Folic acid	200	7.63 cd	16.24 c	16.38 de	11.06 c	18.33 b
	400	10.50 a	21.01 b	16.83 cd	15.78 b	18.47 b
	600	10.66 a	22.57 b	20.29 b	16.85 b	19.60 ab0
tocopherol	200	8.89 b	17.49 c	19.72 bc	15.01 b	14.29 c
	400	10.44 a	21.59 b	20.23 b	16.84 b	19.32 ab
	600	10.50 a	30.14 a	24.17 a	23.31 a	20.48 a

Values in the same column with same letters are not significantly different ($p<0.05$).

Table (4): Effect of folic acid and α- tocopherol on the nutritive value of the yielded faba bean seeds

Treatments	Concentration (mg/l)	Total carbohydrate %	Protein content %	Vicin content	N	P	K
Misr 3							
Control	0	54.24 cd	23.21 d	304.17 g	3.84 e	0.29 e	1.37 e
Folic acid	200	53.81d	23.62 cd	284.16 h	4.11 cd	0.33 de	1.62 d
	400	54.81 bcd	24.46 b	244.31 k	4.25 b	0.35 cde	1.78 c
	600	55.51 ab	25.13 a	219.02m	4.37 a	0.37bcd	1.84 c
tocopherol	200	54.24 cd	23.21 d	304.17 g	4.04 d	0.32 de	1.56 d
	400	55.36 abc	24.27 bc	257.07 j	4.22 bc	0.34 de	1.65 d
	600	56.12 a	25.70 a	231.30 l	4.47 a	0.37 cde	1.77 c
Nubaria 2							
Control	0	48.98 g	21.33 f	465.12 a	3.71 f	0.32 de	1.64 d
Folic acid	200	49.86 fg	21.31 f	374.88 c	3.71 f	0.35 cde	1.80 c
	400	52.24 e	21.97 ef	319.55 f	3.82 ef	0.42abc	2.04 b
	600	53.74 d	23.44 d	268.38 i	4.08 d	0.45 a	1.84 c
tocopherol	200	50.18 f	22.20 e	409.35 b	3.86 e	0.37 cd	1.85 c
	400	52.28 e	23.06 d	338.98 e	4.01 d	0.42abc	2.02 b

	600	54.27 cd	24.17 bc	296.75 g	4.20 bc	0.43 ab	2.19 a

Values in the same column with same letters are not significantly different ($p<0.05$).

4. Discussion

Vegetative growth parameters

The positive effects of folic acid and α-tocopherol on growth and development of faba bean plant illustrate their importance role on biochemical and physiological processes in plant cells. Folic acid application improves agronomic performance and the other morphological traits of faba bean (Table 1). Foliar application of folic acid significantly increased growth parameters of flax (Emam *et al.*, 2011) and dry weight of wheat shoots (Esfandiari *et al*., 2012). The increase in vegetative growth characters caused by folic acid may be attributed to its content of the most prominent of B complex vitamins besides its essential biochemical function in amino acid metabolism and nucleic acid synthesis (Andrew et al., 2000).

The enhancement effect of α-tocopherol on growth parameters of faba bean plants (Table 1) are in a good agreement with those reported by Hussein *et al.* (2007) on cowpea; Sadiq et al. (2016) on mung bean. Moreover, Mahdi et al. (2017) Rahmawati and Damanik (2018); Sadiq et al. (2018; 2019) stated that α-tocopherol treatments improved growth attributes to *Glycine max* and mung bean plants under water deficit and salt stress conditions. These increments in plant growth may be due to the enhancement effects of α tocopherol on cell division or cell enlargement, DNA replication (Bartoli *et al.,* 1999) and endogenous phytohormones in plant (Baffel and Ibrahim, 2008).

Photosynthetic pigments

The increments in all components of photosynthetic pigment due to folic acid application (Table 2) may be attributed to the role of folic acid as a central cofactor for one-carbon transfer reactions which are involved in many cellular reactions such as synthesis of chlorophyll and in the photorespirations cycle (Hanson and Roje, 2001; Jabrin *et al.,* 2003). Folic coenzymes involve in the synthesis of glycine and high amount of free glycine initiates synthesis of plant porphyrins and their derivatives, chlorophylls (Metzler and Metzler, 2003). In this respect, Stakhova *et al.* (2000); Ibrahim et al. (2015) reported that exogenous folic acid increased chlorophyll content and their continuance function in pea, barely and potato and attributed the increase in chlorophyll to the role of folic acid in activation the biosynthesis of glycine, which is involved in the synthesis of porphyrins and chlorophyll in chloroplast membranes.

Regarding positive effect of α-tocopherol on photosynthetic pigment of faba bean (Table 2), Munne´-Bosch and Algere (2005); Liu et al. (2008); Collin et al. (2008); Dawood et al. (2016) mentioned that α-tocopherols are believed to protect chloroplast membranes in plants from photo oxidation and help to provide an optimal environment for the photosynthetic machinery and may provide an additional line of protection from oxidative damage. α-tocopherol plays a major role in chloroplastic antioxidant network of plants, contributes to preserve an adequate redox station in chloroplasts, prevents lipid peroxidation and protects chloroplast membranes from photo-oxidation and assists the transport of electrons in photosystem-II (Farouk 2011; Hassan et al., 2013), maintains thylakoid membrane structure and function during plant development (Munne´-Bosch and Alegra, 2002; Munne´-Bosch, 2005).

Seed yield and its components

The increase in seed yield due to folic acid and α-tocopherol application (Table 3) could be attributed to the increase in nutrient uptake and/or assimilation due to vitamin application (Samiullah *et al*., 1988). Stakhova et al. (2000) described that folic acid application stimulates the synthesis of the dependent amino acids and increases the yield and quality of the seeds of pea (*Pisum sativum* L.) and barley (*Hordeum vulgare* L.). Likewise, in strawberry, Li *et al.* (2015) reported that folate is one of the most important micronutrients and has many forms, but only folic acid form has cofactor activity. In this respect, a few literatures reported that exogenous folic acid has positive effect on growth, yield and quality of some plants such as flax (Emam *et al.,* 2011), faba bean (Zewail *et al.,* 2011), winter wheat (Vician and Kovacik, 2013) white button mushroom (Dahmardeh *et al.,* 2015; Raeisi et al., 2017).

The increases in faba bean seed yield and yield components under the effect of α-tocopherol treatments might be attributed to its role as antioxidant and enhancing protein synthesis and delaying senescence as suggested by Sadak *et al.* (2010) Dawood et *al.* (2014). In line with our findings, Mohammed and Tarpley (2011) mentioned that rice plants treated with α-tocopherol showed 6% increases in grain yield as a result of decreases in respiration and increases in membrane integrity. In addition, Sadak and Dawood (2014) mentioned that α-tocopherol (0.46 and 0.93 mM) caused marked increases in seed yield and yield attributes of three flax cultivars either in plants irrigated with tap water or saline solution as compared to corresponding control. Likewise, the increase in seed yield is probably referred to the role of the applied plant antioxidant in improving the nutrient uptake from the soil and subsequently increasing the plant growth, increasing the photosynthetic rate and consequently seed yield (Dawood et al., 2016).

Nutritive value of the yielded seeds

Both folic acid and α-tocopherol treatments increased nutritive value of the yielded seed (Table 4). Improving the seed biochemical constituents by the foliar application of folic acid was emphasized earlier by many researchers (Emam *et al.,* 2011; Esfandiari *et al*., 2012; Dahmardeh *et al.,* 2015). Stakhova et al. (2000) reported that folic acid plays an important role in the regulation of metabolism of plant cells. They added that different crops show various responses to folic acid treatments. Barley responded to folic acid treatment higher than that of pea where pea seeds responded to folic acid via amino acids and proteins while barley seeds responded to folic acid treatments through carbohydrate accumulation (Leegood et al.,2000). El-Metwally, and Dawood. (2017) stated that foliar spraying with folic acid at the rate of 30 mg L^{-1} enhanced growth, yield and chemical composition of faba bean seeds and attributed these increases to the regulating role of folic acid in plant metabolism which reflected on the nutritive value of the yielded seed.

Concerning the promotive effect of α-tocopherol on the nutritive value, Maeda and Della Penna (2007) indicated that α-tocopherol plays crucial role in maintaining the photo-assimilate substances and affects the sugar transport from the source (leaves) to sink (seeds) because of its effect on the plant metabolism processes. Sakuragi *et al.* (2006) reported that absence of tocopherols dramatically impacts primary carbohydrate metabolism in plant, and added that accumulation of α-tocopherol elevated the levels of sugars. Gilliland *et al.* (2006) provide an insight into the regulation and/or metabolism of vitamin E in plants and clear ramifications for improving the nutritional content of crops. Sadak et al. (2010) demonstrated that application of α-tocopherol on sunflower plants led to the accumulation of total carbohydrates, stimulation of protein synthesis and delaying senescence of sunflower plant. α-tocopherol has positive effects on many physiological processes including the regulation of growth, differentiation and metabolism of plants under saline conditions and increasing physiological availability of water and nutrients (Semida et al., 2014).

Regarding mineral content of the yielded seeds, both folic acid and α-tocopherol treatments increased N, P, K content of the yielded seed a (Table 6). Xudan, (1986) stated that folic acid caused increases in the nutrient uptake and increased the plant performance. Folic acid can be used as a convenient and affordable organic fertilizer to increase the efficiency of the plant and preserve its nutrients (Poudineh *et al.*, 2015).

Application of α-tocopherol increased ions content of the seed through their role in increasing osmo-tolerance and/or through regulating various processes including absorption of nutrients from soil solution (Sadak and Dawood, 2014).

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