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Investigations of growth promoting activity of some phenolic acids

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Abstract: The effect of phenolic acids as caffeic, ferulic or salicylic acids at low concentrations (up to 30 ppm) were investigated on faba bean growth, yield and some physiological processes. The experiments were carried out during the two growing seasons of 2014/2015 and 2015/2016 at the Research and Production Station, National Research Centre, Nubaria Province, Behaira Governorate, Egypt. Caffeic, ferulic or salicylic acids increased growth of faba bean plant over the untreated control. Caffeic acid at 20 ppm showed observable significant increase in dry weight of shoot. Regarding the effect of phenolic acids on some chemical compounds, caffeic acid is seemed to be having the greatest promotive effect on total photosynthetic pigments in fresh leaf tissues. The results also revealed that caffeic acid up to 30 ppm was superior concerning the total free amino acids. The increase in growth and photosynthetic pigments of faba bean plant was accompanied by increase in seed yield/plant and seed yield/feddan. Data also showed that all the tested phenolic acids increases in carbohydrate, protein and phenolic contents in the yielded seeds. The most optimum treatment was caffeic acid at 20 ppm. On the other hand, vicine content in yielded seeds was significantly decreased by all treatments than that of the untreated plants (control). The results suggested the possibility of using caffeic, ferulic or salicylic acids at low concentrations as growth promoters of faba bean plant.

Keywords: hydroxybenzoic acid, hydroxycinnamic acid, phenolic acids, vicia faba

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1 Introduction

Faba bean (*Vicia faba* L.) is one of the most important legumes in the Middle East countries and its cultivation leads to the increase of soil nitrogenous compounds. Faba bean seeds are excellent sources of proteins and carbohydrates (Alghamdi, 2009). On the other hand, seeds contain toxic glycosides as pyrimidine derivatives namely vicine (2, 6, diamino-4, 5-dihydroxypyrimidine, 5, B-glycopyranosoide) that decreased the nutritive value of faba bean and responsible for favism in humans (Jamalian, 1999). The highest vicine content was in fresh green cotyledons that gradually declined until the dry matter percentage of seeds reached about 40% (Burbano et al.,

1995).

Plant produce metabolites, which are inhibitor or stimulator depending on their concentrations and subsequently alter the growth and physiological functions of plants. These metabolites are mostly phenolic compounds (phenolic acids and flavonoids) and have been identified as allelochemicals which are widely spread throughout the plant kingdom (Singh et al., 2003, 2005). Phenolic compounds play significant role in regulation of plant metabolic processes and act as a substrate for many antioxidants enzymes (Khattab, 2007). In addition, the phenolic compounds has antioxidant role as free radical scavenger through their reactivity as electron or hydrogen donor, to stabilize and delocalize the unpaired electron, and from their role as transition metal ions chelator (Huang et al., 2005).

Phenolic acids are evaluated in two groups: hydroxybenzoic acid derivatives and hydroxycinnamic

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acid derivatives (Karamać et al., 2005; Mattila et al., 2005). Caffeic acid, chlorogenic acid, sinapic acid, ferulic acid, and *p*-coumaric acid are derivatives of hydroxycinnamic acid. These acids widely occur in bound forms because they are bound to structural compounds of the cell wall (Adom and Liu, 2002; Wang et al., 2013).

Caffeic acid is an early intermediate of phenylpropanoid metabolism and a precursor of polyphenols and many secondary compounds that regulate plant defense responses (Batish et al., 2008). Moreover, caffeic acid shows promotive effect on soybean plants even under salinity stress as reported by Klein et al. (2013, 2015).

Ferulic acid is a known allelochemical that affects the growth of several plant species (Cheng, 1995; Panteli and Voutsas, 2010). Using indole acetic acid-1-¹⁴C (IAA) and determination of the ¹⁴CO₂ evolved, it was found that ferulic acids synergize IAA-induced growth by counteracting IAA decarboxylation (Tomaszewski and Thimann, 1966).

Sharma et al. (2015) summarize the role of ferulic acid pre-treatment in augmenting the antioxidant response of wheat and suggest that phenolic acid at low concentration can be used for improving performance of wheat under various environmental constraints.

Caffeic and ferulic acids can promote or inhibit plant growth according to their concentration as have been concluded by Li et al. (1993) who found that low concentrations (below 10⁻³ M) of caffeic and ferulic acids promoted the elongation of lettuce hypocotyls, but higher concentrations (over 10⁻³ M) inhibited seedling growth and seed germination. In addition, caffeic acid and salicylic acid at 0.01 mM stimulated primary root length, number and length of secondary roots, and dry weight of *Deschampsia flexuosa* and *Senecio sylvaticus*, meanwhile caffeic acid and salicylic acid at 10 mM showed inhibition effect (Kuiters, 1989).

Salicylic acid is an endogenous growth regulator of phenolic nature and acts as potential non-enzymatic antioxidant that participates in the regulation of many physiological processes in plants, such as stomatal closure, photosynthesis, ion uptake, inhibition of ethylene biosynthesis, transpiration and stress tolerance (Khan et al., 2003; Arfan et al., 2007).

The effect of salicylic acid on the physiological processes was stated to be variable, promoting some processes and inhibiting others depending on its concentration, plant species, developmental stages and environmental conditions (El-Mergawi and Abd El-Wahed, 2004).

Dawood et al. (2012) stated that salicylic acid treatments had regulatory effect on growth, seed yield, total carbohydrate, phenolic content and the quality of the oil in favor of the increase of unsaturated fatty acids of sunflower plant grown under newly reclaimed sandy soil. Loría and Larqué-Saavedra (2015) showed that salicylic acid treatment significantly increased the length of roots and shoots of *Brosimum alicastrum* seedlings by 22% and 21%, respectively, in comparison with those of the control.

This work aimed to investigate the growth promoting activity of some phenolic acids (caffeic, ferulic or salicylic acids) at very low concentrations on growth of faba bean plants and its reflection on yield and yield components as well as some chemical constituents of the yielded seeds.

2 Materials and methods

2.1 Bioassay test

Primitive experiment was carried out in Petri dishes (Laboratory experiment) to determine at which concentrations of the three phenolic acids under investigation have stimulating effects (data are not shown).

2.2 Field experiments

Two field experiments were conducted at the Research and Production Station, National Research Centre, Nubaria Province, Behaira Governorate, Egypt. during the two growing seasons of 2014/2015 and 2015/2016 to study the effect of the three phenolic (caffeic, ferulic or salicylic acids) at low concentrations (10-30 ppm) on faba bean growth and yield as well as some biochemical changes. Seeds of faba bean cv. (Nubaria 4) were obtained from Legumes Research Department, Field Crop Institute, Agricultural Research

center, Giza, Egypt.

Faba bean seeds were soaked with caffeic, ferulic or salicylic acids for 12 hours then sown on 13 November in the two seasons. The experiments design was randomized complete block design with three replicates. Soil of the experimental site was sandy soil where mechanical and chemical analysis is reported in Table 1 according to Chapman and Pratt (1962). Soil preparation and cultural operations followed the normal practices of faba bean cultivation in the vicinity. The experimental land is divided into ten plots, each contained one treatment. The plot was ridged, four meters long, 50 cm apart, and hills were spaced at 20 cm distance, three seeds were sown in each hill. The plants were thinned to one plant per hill at 21 DAS (days after sowing). Regarding fertilization, P₂O₅ 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⁻¹ respectively. while nitrogen fertilizer as ammonium nitrate (33.5%) was added at the rate of 75 kg N fed⁻¹.

 Table 1
 Soil mechanical and chemical analysis characters

Character	Value	Character	Value
Sand %	88	К	10.18
Silt %	4.8	Ca mg/100 g	92.0
Clay %	7.2	Mg	18.4
Texture	Sandy	Na	12.36
pH (1: 2.5 water)	8.83	Fe	8.92
E.C (mmhos cm ⁻¹)(1:2.5)	0.12	Mn mg kg ⁻¹	8.34
CaCO ₃ %	4.8	Zn	0.13
O.M %	0.24	Cu	0.10
Р	0.22		

2.3 Data recorded

A random sample of ten plants was taken for investigation in each plot; total number of 30 plants was fixed for each treatment to study the morphological characters at the age of 60 days after sowing.

Morphological Characters:

- 1- Shoot height and root length (cm).
- 2- Number of leaves and branches/plant.
- 3- Shoot fresh and dry weight/plant (g).
- 4- Root fresh and dry weight/plant (g).

Yield Characters:

At harvest time, a random sample of ten plants was assigned for investigation in each plot; total number of 30 plants was fixed for each treatment to determine:

- 1- Number of pods and seeds per plant.
- 2- Number of seeds per pod.
- 3- Weight of pods and seeds per plant (g).
- 4- Weight of 100 seeds (g).
- 5- Seed yield (kg/Feddan).

2.3 Biochemical Studies

2.3.1 Determination of photosynthetic pigments

Photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) in the fresh leaves at 60 days from sowing were determined as the method described by Moran (1982).

2.3.2 Determination of free amino acids

Free amino acid content was determined with the ninhydrin reagent method according to Yemm et al. (1955).

2.3.3 Determination of Carbohydrates

Total soluble carbohydrates were determined as described by Slominski et al. (1993). Total carbohydrates were determined according to DuBois et al. (1956). Polysaccharides were calculated by the differences between total carbohydrates and total soluble carbohydrates.

2.3.4 Determination of protein

Nitrogen content of the yielded seeds was determined, and multiplied by 6.25 to calculate the crude protein content according to AOAC (1990).

2.3.5 Determination of total phenolic compounds

Total phenolic compounds were determined calorimetrically using Folin Ciocalteu reagent according to the method defined by Snell and Snell (1953).

2.3.6 Determination of vicine

Vicine content was determined according to the method described by Collier (1976).

2.4 Statistical analysis

Data on morphological and yield characters as well as on seed quality were subjected to conventional methods of analysis of variance according to Snedecor and Cochran (1990). Since the trend was similar in both seasons the homogeneity test Bartlet's equation was applied and the combined analysis of the two seasons was calculated according to the method of Gomez and Gomez (1984). Means were compared by using least significant difference (LSD) at 5% levels of probability.

3 Results

3.1 Faba bean growth parameters

The data in Table 2 reveal significant increases in faba bean shoot height, root length, fresh and dry weight of shoot and root due to soaking seeds with caffeic, ferulic or salicylic acids solutions up to 30 ppm in comparison to the untreated plants. Caffeic acid at 20 ppm exhibited great significant increases in the most of growth characters especially dry weight of both shoot and root/plant as compared to that untreated (control).

3.2 Photosynthetic pigments and free amino acids

Chlorophyll a, chlorophyll b and total photosynthetic pigments in faba bean leaf tissues significantly increased by all phenolic acids treatments (Table 3). Soaking faba bean seeds with caffeic acid at 20 and 30 ppm caused the highest significant increase in total photosynthetic pigments in comparison to the contents of the untreated plants (control).

Regarding free amino acids, the data recorded in Table 3 indicate that caffeic, ferulic or salicylic acids treatments induced significant increases in free amino acids in dry leaves of faba bean. Treated faba bean seeds with caffeic acid at 20 ppm measured the highest value content in total free amino acids in comparison to the contents in dry leaves of untreated control (Table 3).

3.3 Faba bean yield and yield components

The results in Table 4 indicate that caffeic, ferulic or salicylic acid acids at different concentrations (10-30 ppm) caused significant increases in number and weight of seeds/plant, weight of 100 seeds and seed yield/feddan (kg) over untreated control. Soaking faba bean seeds with caffeic acid treatment at 20 ppm recorded marked significant increase in seed yield/feddan followed by salicylic acid at 20 ppm.

Table 2 Effect of caffeic, ferulic or salicylic acids on some growth characters of faba bean plant grown under sandy soil conditions

Treatment	Concentration, ppm	Shoot height, cm	Root length, cm	Number of leaves/plant	Number of branches/plant	Fresh weight of shoot/plant, g	Fresh weight of root/plant, g	Dry weight of shoot/plant, g	Dry weight of root/plant, g
Ccontrol	0	27.80 D	11.60 G	12.00 CDE	1.20 BC	17.04 H	3.320 G	2.018 E	0.7880 E
	10	35.00 C	15.20 F	13.00 C	1.40 ABC	24.76 F	3.920 FG	3.406 C	0.8760 DE
Caffeic acid	20	35.00 C	19.40 BC	18.60 A	1.80 A	38.38 A	7.500 A	5.710 A	1.892 A
ueru	30	36.80 BC	18.60 CD	15.20 B	1.20 BC	30.82 C	6.560 B	4.432 B	1.700 B
Ferulic acid	10	37.80 B	21.40 A	12.60 CD	1.00 C	27.60 E	5.040 DE	4.084 B	1.274 C
	20	34.40 C	18.00 DE	10.80 E	1.00 C	18.68 H	4.380 EF	2.642 D	1.030 D
	30	38.00 B	20.40 AB	12.20 CDE	1.20 BC	28.70 DE	8.200 A	4.030 B	1.862 A
	10	34.40 C	15.20 F	11.20 DE	1.00 C	22.98 G	4.480 EF	3.048 CD	1.022 D
Salicylic	20	40.60 A	17.00 E	15.20 B	1.20 BC	33.90 B	5.68 CD	4.656 B	1.300 C
	30	41.00 A	17.40 DE	14.60 B	1.60 AB	30.30 CD	5.900 BC	4.040 B	1.244 C
LSI	D at 5%	2.27	13	0.48	1.72	1 31	0.8	0.59	0.16

Note: Means followed by the same letter for each tested parameter are not significantly different by L.S.D. test (P < 0.05)

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Traatmanta	Concentration,	Chlorophyll A Chlorophyll B		Carotenoids	Total photosynthetic pigments	Free amino acids,	
Treatments	ppm		mg/g dry leaves				
Control	0	4.37 E	1.97 E	0.71 B	7.04 C	16.70 G	
	10	5.12 D	2.42 CD	0.78 B	8.32 B	20.97 BC	
Caffeic acid	20	6.69 A	3.33 A	1.01 B	11.02 A	22.73 A	
	30	6.12 B	2.70 BC	2.13 A	10.94 A	21.73 B	
	10	5.84 BC	2.76 B	1.02 B	9.61 B	20.53 CD	
Ferulic acid	20	5.37 CD	2.37 D	0.85 B	8.58 B	19.83 DE	
	30	5.21 D	2.34 D	0.88 B	8.43 B	18.50 F	
	10	5.25 CD	2.30 D	1.04 B	8.59 B	16.73 G	
Salicylic acid	20	5.50 CD	2.41 CD	0.97 B	8.87 B	19.53 E	
	30	5.30 CD	2.82 B	0.68 B	8.80 B	19.50 E	
L.S.1	D. at 5%	0.55	0.29	0.66	1.21	0.82	

Note: Means followed by the same letter for each tested parameter are not significantly different by L.S.D. test (P<0.05).

Treatments	Concentration, ppm	Number of Pods/plant	Number of seeds/pod	Number of seeds/plant	Weight of seeds/Pod, g	Weight of seeds/plant, g	Weight of 100 seeds, g	Seed yield/feddan, kg
Control	0	7.500 F	3.033 B	22.67 F	2.363 D	17.77 H	78.87 G	472.7 G
	10	8.200 CDEF	3.333 AB	26.13 E	2.870 ABCD	24.43 F	89.00 DE	584.0 E
Caffeic	20	11.53 A	3.733 A	40.87 A	3.640 A	41.50 A	105.9 A	871.6 A
ueiu	30	10.47 AB	3.533 AB	34.53 BC	3.607 AB	33.53 C	102.8 A	704.7 C
Ferulic acid	10	9.467 BC	3.267 AB	34.13 BC	3.423 ABC	32.53 C	94.97 BC	682.4 C
	20	7.833 EF	3.200 AB	25.63 E	2.690 CD	22.13 G	84.47 F	494.7 FG
	30	8.933 CDE	3.267 AB	31.67 D	2.953 ABCD	27.80 E	91.47 CD	513.4 F
	10	8.133 DEF	3.167 AB	26.0 E	2.767 BCD	22.40 G	86.90 EF	490.1 FG
Salicylic acid	20	10.80 A	3.600 AB	35.10 B	3.623 A	36.03 B	102.2 A	756.9 B
	30	9.167 CD	3.267 AB	32.53 CD	3.107 ABCD	30.20 D	97.00 B	633.6 D
L.S.I	D. at 5%	1.185	0.5146	1.983	0.758	1.966	4.107	31.85

Table 4	Effect of caffeic,	ferulic or salicy	lic acids on y	vield and	vield com	ponents of faba	a bean grov	wn under sandy	soil conditions

Note: Means followed by the same letter for each tested parameter are not significantly different by L.S.D. test (P<0.05).

3.4 Nutritive value of the yielded seeds

All applied treatments caused marked increases in total carbohydrate; polysaccharide, protein and phenolic contents accompanied by decreases in soluble carbohydrate in the yielded faba bean seeds over that of untreated control (Table 5). The most optimum treatment was caffeic acid at 20 ppm. Regarding vicine content, the data indicated that caffeic, ferulic or salicylic acids up to 30 ppm decreased significantly vicine content in comparison to the untreated control. It was noted that ferulic acid at 10 ppm and 20 ppm followed by salicylic acid at 20 ppm and 30 ppm showed the highest significant decreases in vicine content of faba bean seeds.

Table 5 Effect of caffeic, ferulic or salicylic acids on some biochemical constituents of the yielded faba bean seeds

Treatments	Concentration,	Total carbohydrate	Soluble carbohydrate	Polysaccharides	Protein	Total phenolic compound	Vicine
	ppm		%			mg/100g	
Control	0	39.47 E	2.73 A	36.74 E	24.42 D	78.01 D	453.0 A
Caffeic acid	10	41.26 BC	2.04 C	39.22 BCD	25.64 BCD	105.10 B	407.0 BCDE
	20	42.28 A	1.72 E	40.56 A	28.12 A	106.20 B	420.5 B
Ferulic acid	30	41.05 CD	2.19 B	38.85 CD	25.26 CD	117.40 A	413.0 BCD
	10	40.22 DE	1.60 F	38.62 D	27.10 AB	91.83 C	336.0 G
	20	42.05 AB	1.74 DE	40.31 A	26.88 ABC	86.34 CD	385.5 EF
	30	41.58 ABC	1.85 D	39.73 ABC	26.26 BC	88.91 CD	396.0 CDEF
	10	41.98 ABC	1.52 F	40.14 AB	26.26 BC	82.10 CD	414.5 BC
Salicylic acid	20	41.67 ABC	1.84 D	40.15 AB	26.26 BC	85.48 CD	390.5 DEF
	30	41.09 CD	2.20 B	38.89 CD	26.88 ABC	92.04 C	381.5 F
L.S.I	D at 5%	0.29	0.11	0.89	1.81	10.07	21.62

Note: Means followed by the same letter for each tested parameter are not significantly different by L.S.D. test (P<0.05).

4 Discussion

Phenolic acids are evaluated in two groups: hydroxybenzoic acid derivatives as salicylic acid and hydroxycinnamic acid derivatives as caffeic acid and ferulic acid (Karamać et al., 2005; Mattila et al., 2005) that are widely distributed in plant tissues (Clifford 1999). The physiologic role of phenolic substances is argumentative. Some investigators dispute against any role of phenolic in plant growth regulation, because these compounds are localized in closed compartments of the cell, such as the vacuole, and hence faraway control of physiologic processes.

On the other hand, the results in Table 2 indicated that the three phenolic acids (caffeic, ferulic and salicylic) at concentrations up to 30 ppm increased the growth of faba bean characters. These results were supported by Kuiters (1989), Li et al. (1993), Cheng (1995), Panteli and Voutsas (2010). In this concern, Einhellig and Leather (1988) demonstrated that, the increase in growth parameters is attributed to the influence of phenolic compounds on physiological processes such as cellular expansion, membrane permeability, nutrient uptake and chlorophyll synthesis. Furthermore, Rice (1984) and Einhellig (1986) summarized that phenolic acids may interfere with indole-acetic acid metabolism, mitochondrial metabolism and respiration, photosynthesis, synthesis of proteins, and ion uptake and transport.

Kefeli and Kutjcek (1976) stated that caffeic and ferulic acid possess stimulatory properties and can promote growth because of their auxin-like properties. In accordance with Milborrow (1984), the increased growth by phenolic acids as ferulic acid may be attributed to the role of ferulic acid in counteracting IAA decarboxylation.

The increase in growth of faba bean (Table 2) was accompanied by increases in certain metabolic activity as enhancement in photosynthetic pigment and frees amino acids in leaves of faba bean (Table 3). Salicylic acids (100 ppm) increased chlorophyll contents in sunflower and maize leaves as mentioned by Dawood et al. (2012) and Rashad and Hussien (2014). Wan et al. (2015) stated that caffeic acid at 25 μ M increased the contents of proline in cucumber chilling-stressed leaves.

The increase in the growth of faba bean (Table 2) due to soaking faba bean seeds with caffeic, ferulic or salicylic acids (10-30 ppm) was accompanied by increase in yield which was represented by the number of pods, weight of pods, seed yield/plant and weight of 100 seeds (Table 4). Similar results were obtained on pepper plants by Abdul Qados (2015) who found that salicylic acids increased fruit setting, fruit yield, and fresh and dry weights of pepper fruits. Dawood et al. (2012) mentioned that 100 ppm salicylic acid significantly increased sunflower seed yield and its yield components. Moreover, El-Awadi et al. (2016) stated that 30 mg L⁻¹ benzoic acid or trans-cinnamic acid significantly increased lupine seed yield and yield components.

Regarding the promotive effect of three phenolic acids on the nutritive value of the yielded seeds, Dawood et al. (2012) stated that salicylic acid treatments had regulatory effect on total carbohydrate, protein and phenolic content of sunflower plant grown under newly reclaimed sandy soil. El-Awadi et al. (2016) concluded that either benzoic acid or trans-cinnamic acid at 30 mg L^{-1}

are the most promising treatments in increasing quality and quantity of lupine plants grown under sandy soil condition.

These increments in carbohydrate and protein contents in the yielded seeds (Table 5) may be attributed to the increases in vegetative growth parameters (Table 2) as well as photosynthetic pigments and free amino acid in leaf tissues (Table 3).

The increase in total phenolic contents may be attributed to the increases in carbohydrate synthesis as reported by Youssef (1993) or may be due to the phenolic nature of hydroxybenzoic acid derivatives as salicylic acid and hydroxycinnamic acid derivatives as caffeic acid and ferulic acid (Karamać et al., 2005; Mattila et al., 2005). Moreover, Dawood and Sadak (2007) showed that the increase in total phenolic contents was concur with the increase in IAA contents and led to the suggestion that most of phenolic compounds are diphenols and polyphenols which may inhibit IAA oxidase activity and leading to auxin accumulation, and reflected in stimulating the growth and yield of plant.

On the other hand, all applied treatments decreased the content of vicine in the yielded seeds than that of the untreated control. In this connection, Bjerg et al. (1985) stated that both environmental and genetic factors seem to affect the contents of favism causative agents in faba bean seeds. Furthermore, Gaber et al. (2000) indicated that foliar application or seed presoaking with certain growth regulators decreased the contents of vicine in the yielded seeds. The reduction in the contents of vicine may be attributed to the effect of these factors on metabolic pathway of vicine precursor (orotic acid) formation, which responsible for the formation of pyrmidine ring of these toxic constituents (Brown and Roberts, 1972).

5 Conclusion

The results suggest that pre-treatment faba bean seed with low concentrations of caffeic ferulic and salicylic acids can be used for improving performance, yield and nutritive value of the yielded seeds. It is worthy to indicate that the most optimum treatment was caffeic acid at 20 ppm.

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