Induction of Faba Bean Resistance to Chocolate Spot and Rust Diseases and Improving the Productivity using Amino and Humic Acids K.M. Abd El-Hai*; A.M. El-Ghamry** and K.M. Ghoneem***

Dept. Leguminous and Forage Crops Dis., Plant Pathol. Res. Inst., ARC, Egypt.

Soils Dept., Fac. Agric., Mansoura Univ., Egypt.

*** Dept. Seed Pathol. Res., Plant Pathol. Res. Inst., ARC, Egypt.

Field experiments were conducted in 2007-2008 and 2008-2009 winter seasons to study the effect of amino acids (AA) and humic acids (HA) and their interactions in control of chocolate spot and rust diseases, growth enhancement, chemical changes and chlorophyll content of faba bean plants. The maximum reduction of disease severity of chocolate spot at 55 days from planting was recorded with the interaction between AA plus HA at1000 ppm each then HA at 1000 ppm, while, at 75 days the maximum reduction in both disease severity and disease incidence occurred by AA at 3000 ppm followed by the treatment of HA at 1000 ppm. HA at 3000 ppm followed by the interaction between AA 1000 and HA 1000 ppm then the treatment with HA 2000 ppm were the most effective in reducing rust disease severity of faba bean plant. All morphological traits (plant height, no of branches and leaves plant and yield components (pods No./plant and weight of 100 seed) as well as macronutrients content (N, P, K in seeds and straw) and chlorophyll content significantly increased by the application of AA (2000 ppm) interacted with HA (2000 ppm). The maximum plant seed yield per plant occurred under the application of AA plus HA (2000 ppm each) followed by AA (3000 ppm) then AA (1000 ppm). On the other hand, number of seeds pod-1 did not significantly affected.

The present investigation recommended using AA at 1000 ppm and HA at 1000 ppm as foliar application to decrease the damage of chocolate spot and rust diseases as well as improve growth and mineral content in addition the advantages as environmental safety and coast effective.

Keywords: Amino acids, chocolate spot, faba bean, growth enhancement, humic acid and rust diseases.

Faba bean is a major fabaceous legume crop in Egypt. Due to its high nutritive value in both energy and protein contents, it is a primary source of protein in the diet of masses. Therefore, increasing the crop production is one of the most important targets of agricultural policy in several countries. This strategic crop is suffering from many destructive diseases. It is attacked by more than 100 pathogens in the Mediterranean region (Hebblethwaite, 1983).

Amino acids (AA), which account for the majority of organic nitrogen in soils and humic substances, impact plant growth and serve to explain how organic matter promotes soil productivity (Schnitzer, 2001). However, foliar nutrition in the form of hydrolysed amino acids through foliar spray provides readymade building blocks for protein synthesis. The amino acid is absorbed by the cells as such, and is simply fed into the metabolic machinery of the cell. Evidently, the cells absorb the glutamate faster than it is metabolized, as the glutamate eventually is found in glutamine, glutathione, and protein. The subsequent utilization of these pools of free glutamate in various synthetic and derogative processes leaves little doubt that at least some plants can incorporate amino acids directly into their metabolic pathways.

Application of humic acids (HA) has several benefits and agriculturists all over the world are accepting humic acids as an integral part of their fertilizer program. It can be applied directly to the plant foliage in liquid form or to the soil in the form of granules alone or as fertilizer mix. Humic acid is one of the major components of humus. Humates are natural organic substances, high in humic acid and containing most of known trace minerals necessary to the development of plant life. Humic substances are an important soil component because they constitute a stable fraction of carbon and improve water holding capacity, pH buffering and thermal insulation (McDonnell et al., 2001). Studies of the positive effects of humic substances on plant growth have demonstrated the importance of optimum mineral supply, independent of nutrition (Yildirim, 2007).

Chocolate spot caused mainly by Botrytis fabae Sard. and to some extent by B. cinerea pers ex fr (Rahman et al., 2002), and rust (Uromyces vicia fabae pers. Schroet.) diseases are the most important limiting factors which cause great annual losses and sometimes complete crop failures (Mohamed, 1982; Hebblethwait, 1983 and Hanounik and Bisri, 1991). Chocolate spot occurs mainly on leaves, but stems and flowers may also be infected under favourable conditions. Under optimum conditions of temperature (18 to 20°C) and relative humidity (90 to 100%), the infection becomes aggressive. Also, under prolonged wet conditions, the disease may reach epidemic proportions with heavy crop losses (Harrison, 1988 and Bernier et al., 1993).

The infection by *Uromyces vicia faba* first appears as minute, slightly raised, white to cream coloured spots on leaves and to a lesser extent on stems. As spots enlarge the epidermis ruptures, releasing masses of dark brown spores (urediospores) to form characteristic pustules (uredia). The pustules are often surrounded by a ring of yellow tissue. On highly susceptible cultivars, rust can build up rapidly until most of the leaves are covered with pustules. Severely infected leaves rapidly dry up and premature defoliation may occur (Benier et al., 1993).

Due to the widespread occurrence of fungicide resistance in species of *Botrylis* and Uromyces, many chemicals traditionally used to control chocolate spot and rust diseases are less effective (Harrison, 1988), giving only partial disease control, high cost of their use and adverse environmental effects as well as on the accompanying microflora (Khaled *et al.*, 1995). So, there is a growing need to develop alternative approaches for controlling plant diseases. HA is a suspension, based on potassium-

humates, which can be applied successfully in many areas of plant production as a plant growth stimulant or soil conditioner for enhancing natural resistance against plant diseases and pests (Scheuerell and Mahaffee, 2006), stimulation plant growth through increased cell division, as well as optimized uptake of nutrients and water (Atiyeh et al., 2002 and Chen et al., 2004). Moreover, HA stimulated the soil microorganisms (Atiyeh et al., 2002 and Qualls, 2004). Several reports indicated the efficiency of HA in reducing some plant diseases. In this respect, Scheuerell and Mahaffee (2006) reported that the most effective treatments for suppression gray mould disease caused by Botrytis cinerea in Geranium plants was compost tea plus kelp extract and HA. Moreover, HA at concentrations of 6 or 8 ml/L as foliar spray reduced root rot and Alternaria leaf spot diseases in bean plants (Abd El-Kareem, 2007). The role of AA in plant diseases may be due to the correlation between these acids and plant health. AA are used both for the production of new cell biomass and to produce energy. Followed by deamination into the keto acid which enter into the tri carboxylic acid cycle, which play important role in plant resistance (Bush, 1993).

The present study was amid to study the influence of AA and HA and their combination on some morphological and physiological characteristics, yield and its components and chemical composition. In addition to the alleviation of damage of chocolate spot and rust diseases on faba bean.

Materials and Methods

Chemicals and faba bean seeds:

Amino acids (Peptone; containing 6% free amino acid + 12% organic nitrogen + 3.5% K_2O) and humic acids (Hammr; containing 86% humic acid + 6% K_2O) were kindly obtained from Egyptian Fertilizer Development Centre, El-Mansoura, Egypt. while, faba bean seeds cv. Giza 3 were obtained from Field Crops Res. Instit., Leguminous Res. Dept., Agric. Research Centre, Giza, Egypt.

Field evaluation of amino and humic acids on faba bean:

The soil analysis of Tag EL-Ezz, Agric. Res. Station, Dakahlia, Egypt is presented in Table (1). Particle size distribution was determined using the international pipette method as described by Piper (1950). Electrical conductivity in 1:2.5 soil: water extract, pH values, OM, available NPK, CaCO₃ and real density were determined according to Jackson (1967), Hesse (1971), Dewis and Freitas (1970) and Anonymous (1990).

Table 1. Physical and chemical analysis of the soil

		Physi	ical ch	aracte	ristics				
Soil texture	Coarse sand (%)	Fine sand (%)		Clay (%)		EC dSm ⁻¹	Field capacity (%)	Real densit (g/cm³)	
Clay loam	6.2	32.6	24.7	35.5	2.4	0.32	34.3	2.66	
		Chem	ical cl	haracte	eristics				
pН	Organic matter	CEC			Availa	ble nut	rients (ppm)		
soil paste	, 0			N		P		K	
7.6	1.42	35.2		32.3			14.4		

The experiment was carried out under natural infection during the two successive growing seasons of 2007/2008 and 2008/2009. In addition to, kocide 101 (2.5 g/l) as a fungicide of chocolate spot disease as well as plantvax 20% EC (3.5 ml/L) as a fungicide for rust disease, AA (Peptone; containing 6% free amino acid + 12% organic nitrogen + 3.5% K_2O) and HA (Hammr; containing 86% humic acid + 6% K_2O) and their interactions were also, used as foliar treatments as follows:

- · Check (tap water).
- AA at 1000 ppm.
- AA at 2000 ppm.
- AA at 3000 ppm.
- HA at 1000 ppm.
- HA at 2000 ppm.
- HA at 3000 ppm.
- AA at 1000 ppm+ HA at 1000 ppm.
- AA at 2000 ppm+ HA at 2000 ppm.
- kocide 101 2.5 g/l.
- Plantvax 20% EC at 3.5 ml/l.

Seeds were sown 10th and 18th November in 2007/2008 and 2008/2009 seasons, respectively. Each plot was 3.5x3 m. All agricultural practices were carried out according to the recommendation of Ministry of Agric., Egypt. Complete randomized block design with three replicates was allocated.

Disease assessment:

The plants developed from each assigned treatment were sprayed with individual AA, HA and its interactions three times with 15-day intervals beginning from 30-day after sowing using a hand atomizer till dripping. Plants sprayed with tap water only served as check. The plants were rated for disease incidence (DI) and disease severity (DS), the former as the presence or absence of disease (percentage of infected leaves on the plant) and the latter as the severity percentage of disease damage.

The disease severity (DS) of chocolate spot disease was estimated 55 and 75 days after sowing under natural infection by using the scale of Bernier *et al.* (1993) as follows:

- 1= No disease symptoms or very small specks (highly resistance).
- 3= few small discrete lesions (resistant).
- 5= some coalesced lesions with some defoliation (moderately resistant)
- 7= large coalesced sporulating lesions, 50% defoliation and some dead plant (susceptible).
- 9= Extensive lesions on leaves, stems and pods, severe defoliation, heavy sporulation, stem girdling, blackening and death of more than 80% of plants (highly susceptible).

The disease severity of rust was recorded at 90 days from sowing according to the standard scale suggested by Bernier et al. (1993) as follows:

1= No pustules or very small non sporulating flecks (highly resistant).

3= few scattered pustules covering less than 1% of leaf area, and few or no pustules on stem (resistant).

5= pustules common on leaves covering 1-4% of leaf area, little defoliation and some pustules on stem (moderately resistant).

7= pustules very common on leaves covering 4-8% of leaf area, some defoliation and many pustules on stem (Susceptible).

9= Extensive pustules on leaves, petioles and stems covering 8-10% of leaf area, many dead leaves and severe defoliation (highly susceptible).

Percentage of chocolate spot and rust diseases severity were calculated using the formula adopted by (Hanounik, 1986):

Disease severity (%) =
$$\frac{\sum (NPC \times CR)}{(NIP \times MSC)} \times 100$$

Whereas: NPC = No. of plants in each class rate

CR = class rate

NIP = No. of infected plants.

MSC= Maximum severity class rate.

The disease incidence (DI) of chocolate spot as a disease percentage was determined after 55 and 75 days from sowing according to the following formula:

The investigated parameters of faba bean:

At 70 days of the cultivation, growth parameters i.e., plant height (cm), no. of leaves and branches plant was determined.

70 days-old samples of faba bean plants were collected from the blade of the third leaf from plant tip (terminal leaflet) to determine photosynthetic pigments (chlorophyll a, b and carotenoids). Photosynthetic pigments were extracted by methanol 90% for 24h at room temperature after adding traces of sodium carbonate (Robinson and Britz, 2000). Then photosynthetic pigments were determined spectrophotometrically by the equations of Mackinney (1941) at the wave lengths 452.5, 650 and 665 nm.

At harvest stage, yield components {No. of pods plant⁻¹, No. of seed pod⁻¹ and seed index (weight of 100-seed in gram) were determined.

For the determination of N, P and K, samples of straw and seeds were dried to fine powder and 0.2 gm was wet digested with a mixture of sulphuric and perchloric acids according to Jackson (1967). Nitrogen was estimated by semi-micro-kjeldahle method (Block et. al., 1965), phosphorus was determined spectrophotometrically (Pratt, 1961) and potassium was determined flame photometrically (Hesse, 1971).

Statistical analysis:

Data were subjected to statistical analysis of variance of randomized complete block design by Gomez and Gomez (1984). Mean values of treatments were compared (Steel and Torrie, 1980).

Results

Disease assessments:

Data of DS of chocolate spot disease of faba bean were recorded in Table (2). At 55 days the application of Kocide 101 led to the maximum reduction of DS of chocolate spot followed by AA plus HA (1000 ppm each), then HA (1000 ppm) in both seasons. At 75 days the maximum reduction in DS after the application of kocide 101 was recorded with the foliar application of AA at 3000 & 1000 ppm and combination with AA at 1000 plus HA (1000 ppm each).

Data in Table (2) showed that there is no significant decrease in disease incidence at 55 days as affected by amino and humic acids in both seasons while at 75 days there is highly significant decrease in DI. The maximum reduction after 75 days recorded with Kocide 101 followed by AA (3000 ppm) in the first season and AA plus HA(1000 each) in the second. In contrast, the application of HA at 3000 ppm increased significantly this parameter compared with other treatments in the first season.

Fig. (1) show that, spraying faba bean with amino and humic acids at any used dose decreased significantly the DS of rust, irrespective to plantvax 20% EC treatment. The maximum reduction was recorded for HA (3000 ppm) followed by AA plus HA(1000 ppm each) then HA (2000 ppm).

Table 2. Effect of amino and humic acids on severity and incidence of chocolate spot disease of faba bean

		2007	/2008		2008/2009					
Treatment	55	days	75	days	55	days	75 days			
	DS	DI	DS	DI	DS	DI	DS	DI		
Check	42.5	16.0	64.4	27.3	34.1	13.9	53.2	21.5		
AA 1000	12.4	10.4	30.1	16.4	17.1	10.4	23.8	17.4		
AA 2000	22.0	12.8	43.0	19.7	25.7	11.2	37.2	18.3		
AA 3000	16.0	13.1	20.9	10.1	19.1	11.9	26.1	19.3		
HA 1000	11.7	10.1	57.9	19.8	14.4	9.9	19.7	15.9		
HA 2000	25.5	14.6	46.3	16.7	27.6	12.9	41.8	21.3		
HA 3000	16.7	9.28	62.0	25.4	20.5	8.2	26.3	14.0		
AA (1000) + HA (1000)	10.5	7.9.	24.1	14.3	13.7	6.8	17.1	10.9		
AA (2000) + HA (2000)	18.7	10.6	47.5	19.0	23.7	10.7	33.2	17.8		
Kocide 101	6.5	6.7	11.3	5.1	4.7	3.7	6.5	5.5		
LSD at 5%	8.4	- Carper	12.7	3.7	6.3	On all talls	10.8	2.3		

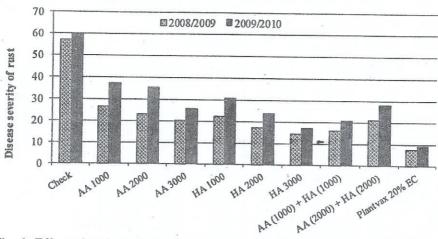


Fig. 1. Effect of amino and humic acids on rust disease severity in faba bean after 90 day from sowing.

In comparison with AA plus HA(1000 each), using AA plus HA at 2000 ppm each led to marked induction in disease parameters of chocolate spot and rust diseases. Accordingly, the higher concentration of both AA and HA, *i.e.* 3000+3000ppm is not recommended to be applied on plants, taking into account the expected rise in expenses when using higher concentrations.

Morphological trails:

After 70 days from sowing, the response of faba bean plants growth to the foliar application of amino and humic acids and their combinations was determined by means of measuring height and branches and leaves number per plant. Data in Table (3) reveal that, the majority of amino and humic acids treatments significantly and differently increased growth parameters. In this respect, application of AA plus HA at 2000ppm each recorded superiority in increasing branches and leaves number per plant while AA3000ppm give the highest value of plant height in both seasons. On the other hand, no significant differences were observed in all faba bean growth parameters with the fungicide kocide 101 application as compared to check treatment.

Chlorophyll and carotenoids content:

The photosynthetic pigments content in fresh plant were determined as chlorophyll a, b and carotenoids as shown in Table (4). In chlorophyll content, all treatments of AA and HA gave significant increase except, AA (1000ppm) or HA (3000ppm) which led to insignificant decrease in chlorophyll b. In this respect, highest increase in total chlorophyll was observed with the combination of AA plus HA (2000 ppm each), followed by AA at3000 ppm in both seasons. The maximum increase in carotenoids content occurred under the application of AA plus HA (2000 each) followed by AA at 2000ppm. Fungicides applications had no significant effects on these parameters.

Table 3. Effect of amino and humic acids on morphological traits of faba bean plants

		2007/200	08	2008/2009				
Treatment	Plant height (cm)	Leaves No. plant ⁻¹	No of branches plant ⁻¹	Plant height (cm)	Leaves No. plant ⁻¹	No of branches plant ⁻¹		
Check	61.3	43.0	4.7	72.0	37.7	3.7		
AA 1000	73.0	45.0	5.7	85.0	48.0	5.3		
AA 2000	76.3	48.0	4.7	90.0	54.7	4.0		
AA 3000	84.3	48.0	6.0	98.0	56.0	5.7		
HA 1000	77.0	48.0	6.3	89.0	54.0	6.7		
HA 2000	79.7	55.0	6.0	92.0	- 59.0	5.3		
HA 3000	60.0	39.0	5.3	71.7	36.7	5.3		
AA (1000) + HA (1000)	68.3	40.0	6.0	83.0	42.0	5.7		
AA (2000) + HA (2000)	84.0	56.0	6.7	96.0	61.0	7.0		
Kocide 101	62.7	44.0	4.7	74.0	38.3	3.7		
Plantvax 20% EC	68.0	45.0	5.0	82.0	50.0	4.3		
LSD at 5%	6.3	6.7	0.8	5.3	4.3	0.9		

Table 4. Chlorophyll and carotenoids contents of faba bean as affected by amino and humic acids

12.45 - F 1		20	007/200	08	2008/2009					
Treatment	Chl.	Chl.	Total Chl.	Caroten.	Chl.	Chl.	Total Chl.	Caroten.		
Check	0.86	0.31	1.17	0.38	0.99	0.40	1.39	0.23		
AA 1000	0.86	0.29	1.15	0.39	0.98	0.38	1.36	0.23		
AA 2000	0.93	0.37	1.31	0.48	1.10	0.43	1.52	0.31		
AA 3000	1.23	0.42	1.65	0.37	1.41	0.48	1.89	0.23		
HA 1000	0.94	0.38	1.32	0.38	1.11	0.44	1.55	0.25		
HA 2000	1.09	0.35	1.45	0.44	1.15	0.42	1.56	0.29		
HA 3000	0.90	0.29	1.19	0.34	1.00	0.38	1.38	0.24		
AA (1000) + HA (1000)	1.13	0.39	1.51	0.46	1.22	0.46	1.67	0.30		
AA (2000) + HA (2000)	1.30	0.48	1.78	0.53	1.53	0.51	2.05	0.32		
Kocide 101	0.89	0.30	1:19	0.39	0.99	0.39	1.39	0.24		
Plantvax 20% EC	0.91	0.36	1.28	0.43	1.06	0.42	1.48	0.26		
LSD at 5%	0.22	0.10	0.26	0.09	0.16	0.10	0.21	0.03		

Yield components:

Data in Table (5) show that treatments of tested amino and humic acids had direct effect on faba bean net yield. Spraying faba bean plants with AA plus HA (2000 ppm each) significantly improved pods number/ plant and seed index. Both treatments of AA at 3000 and 2000 ppm came next in increasing seed index. On the other hand, application of amino and/or humic acids or one of fungicides to faba bean plants had no significant effect on number of seeds/plant as compared to check treatment.

				components	

		2007/200	8	2008/2009				
Treatment	No of pods plant-1	No of seed pod ⁻¹	Seed index (g)	No of pods plant-1	No of seed pod-1	Seed index (g)		
Check	23.00	2.67	52.67	29.33	3.00	53.33		
AA 1000	44.67	3.33	62.33	41.00	3.33	56.33		
AA 2000	33.67	3.33	63.33	32.33	3.33	58.67		
AA 3000	59.67	3.67	64.00	51.00	3.67	61.33		
HA 1000	54.00	3.33	48.67	44.33	3.00	49.00		
HA 2000	37.33	3.33	62.67	35.33	3.00	56.67		
HA 3000	42.67	3.67	51.00	39.67	3.67	52.33		
AA (1000) + HA (1000)	41.00	3.00	58.67	36.67	3.33	56.00		
AA (2000) + HA (2000)	67.33	3.67	66.33	53.33	3.67	63.00		
Kocide 101	34.33	2.67	56.33	34.67	3.00	54,00		
Plantvax 20% EC	37.00	3.33	60.67	37.33	3.33	54.67		
LSD at 5%	7.52		10.03	6.65		8.13		

Fig. (2) show that all of AA and HA concentrations and their interactions increased plant seed yield, high increase occurred after the application of AA plus HA (2000 ppm each) followed by AA (3000 ppm) then AA (1000 ppm).

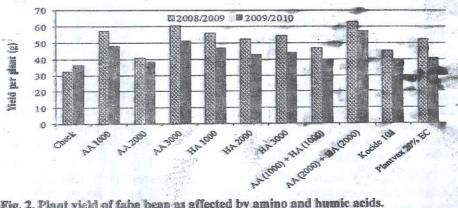


Fig. 2. Plant yield of faba bean as affected by amino and humic acids.

Amino and humic acids vis N.P.K content of faba bean:

Data in Table (6) show that high significant increase of nitrogen content in seed and straw of faba bean was observed as response to amino and humic acids applications. In seed, the highest increase was recorded either with AA plus HA (2000 each) or with AA (2000 ppm) while the highest increase of nitrogen in straw was recorded with AA plus HA (2000 each) treatment then AA plus HA (1000ppm each) in both seasons. The same trend was observed with both P and K content of faba bean seed and straw. On the other hand, no significant increase of N and K content in seed and straw with fungicides applications as compared to check treatment while P content increased significantly under both fungicides application.

Table 6. Effect of amino acids and humic substances on macronutrients in faba

bea	n p	lan	ts

	2007/2008							2008/2009						
Treatment	N%		P%		K%		N%		P%		K%			
	Seed	Straw	Seed	Straw	Seed	Straw	Seed	Straw	Seed	Straw	Seed	Straw		
Check	2.97	1.61	0.31	0.14	1.83	1.73	2.92	1.71	0.32	0.16	1.95	1.77		
AA 1000	4.04	2.21	0.75	0.36	2.12	2.11	4.20	2.37	0.80	0.40	2.18	2.15		
AA 2000	5.41	2.36	0.77	0.50	2.14	2.21	5.70	2.45	0.82	0.52	2.26	2.30		
AA 3000	5.01	2.51	0.91	0.58	2.18	2.31	4.93	2.63	0.98	0.61	2.30	2.45		
HA 1000	3.84	1.75	0.48	0.22	1.92	1.75	3.95	1.86	0.52	0.29	1.96	1.80		
HA 2000	3.95	1.89	0.52	0.30	2.02	1.82	4.10	2.01	0.60	0.32	2.06	1.86		
HA 3000	4.46	1.92	0.59	0.35	2.06	1.92	4.52	2.11	0.64	0.38	2.11	1.98		
AA (1000) + HA (1000)	5.12	2.57	0.97	0.78	2.25	2.50	5.38	2.76	1.03	0.81	2.36	2.53		
AA (2000) + HA (2000)	5.77	2.67	1.04	0.99	2.51	2.80	5.93	2.85	1.11	0.96	2.63	2.76		
Kocide 101	3.06	1.63	0.37	0.18	1.86	1.73	3.18	1.72	0.41	0.20	1.96	1.78		
Plantvax 20% EC	2.93	1.65	0.41	0.16	1.88	1.74	3.10	1.72	0.49	0.18	1.98	1.80		
LSD at 5%	0.60	0.04	0.04	0.01	0.05	0.04	0.80	0.05	0.05	0.04	0.04	0.06		

Discussion

Application of AA plus HA (1000 ppm each) gave the maximum reduction of chocolate spot severity at 55 days, but at 75 days the highest reduction was recorded with AA at 3000 ppm. The highest reduction in rust disease severity occurred at 3000 ppm of HA. Amino acids have a chelating effect on micronutrient when applied, that make the absorption and transportation of micronutrients inside the plant easier due to its effect on cell membrane permeability. Some of these micronutrients play roles in plant resistance by regulating the levels of auxin in plant tissues by activating the auxin oxidase system (Maschner, 1986) and by it appears to be required in synthesis of intermediates in the metabolic pathway, through tryptophan to auxin (Ohki, 1978). Consequently, auxin lead to increase in total phenol, calcium content and activity of chatechol oxidase, these materials protect plants against pathogen stress (Chowdhury, 2003).

Root uptake of amino acids is an energy driven process whereby the outwardly directed plasma membrane H⁺-ATP-ase generates the proton motive gradient to drive inwardly directed amino acid H⁺-contra sport (Fischer et al., 1998). Following

of AA deamination and introduction of the keto acids into the TCA cycle, many antioxidant such as citric, succicnic, oxaloacetic and pyruvic acids are produced, which in turn play important role of the plant defence activity (Bush, 1993).

Anxino acids help in increasing chlorophyll concentration in plant leading to higher degree of photosynthesis. Any factor causes increase in photosynthetic pigments is expected to increase carbohydrate content. Carbohydrates are the main repository of photosynthetic energy, they comprise structurally polysaccharides of plant, principally cellulose, hemicelluloses and pectin and lignin that considered an important structural compound of plant. Also associated with phenolic compounds, which play a major role in plant defence (Hahlbrock and Scheel, 1989).

The role of HA in overcoming the harmful effects of chocolate spot and rust diseases in faba bean plant may be due to the increase in chitinase activity (Abd El-Kareem, 2007) and stimulation plant growth through increased cell division, as well as optimized uptake of nutrients and water (Atiyeh et al., 2002 and Chen et al., 2004) also, regulate hormone level, improve plant growth and enhance stress tolerance (Piccolo et al., 1992). HA is a suspension, based on potassium humates, which can be applied successfully in many areas of plant production as a plant growth stimulant or soil conditioner for enhancing natural resistance against plant diseases and pests (Scheuerell and Mahaffee, 2006) which consequently increase yield of plant. Foliar application of HA (25% active HA) consistently enhanced antioxidants such as α-tocopherol, β-carotene, superoxide dismutases, and ascorbic acid concentrations in turf grass species (Zhang, 1997). These antioxidants may play a role in the regulation of plant development, flowering and chilling of disease resistance (Ziadi et al., 2001; Dmitrier et al., 2003 and Achuo et al., 2004).

Yield components, morphological traits and chemical contents of faba bean plants records significant increases especially (AA at 2000+ HA at 2000 ppm), except number of seeds/pod, with AA and HA treatments. Amino acids are fundamental ingredients in the process of protein synthesis. About 20 important amino acids are involved in the process of each function. Studies have proved that amino acid can directly or indirectly influence the physiological activates of the plant. Because of the amino acid pool is only a small portion of the total dissolved organic nitrogen pool, which generally contains less than 10% free amino acids in temperate ecosystems (Qualls and Haines 1991 and Yu et al., 2002). So, several studies have shown that plants can uptake N in forms of amino acids without relying on microbial mineralization (Lipson and Nasholm, 2001).

The increment in growth parameter and yield may be due to that HA are extremely important component because they constitute a stable fraction of carbon, thus regulating the carbon cycle and release of nutrients, including nitrogen, phosphorus, and sulfur, which decreasing the need for inorganic fertilizer for plant growth. HA stimulate plant growth by the assimilation of major and minor elements, enzyme activation and/or inhibition, changes in membrane permeability, protein synthesis and finally the activation of biomass production (Ulukan, 2008). Moreover, Russo and Berlyn (1990) reported that, humates (granular and liquid forms) can reduce plant stress that involved plant diseases as well as enhance plant

nutrient uptake. HA contributes significantly to water retention and metal/solute binding and release, and they are necessary for safe plant nutrition (Stevenson, 1994, and McCarthy et al., 1990). In addition, HA can be used as a growth regulator by regulate endogenous hormone levels (Fragbenro and Agboola, 1993 and Piccolo et al., 1992).

Plant growth is influenced indirectly and directly by humic substances. Positive correlations between the humus content of the soil, plant yields and product quality have been published in many different scientific journals. Indirect effects, previously are those factors which provide energy for the beneficial organisms within the soil, influence the soil's water holding capacity, influence the soil's structure, release of plant nutrients from soft minerals, increased availability of trace minerals, and in general improved soil fertility. Direct effects include those changes in plant metabolism that occur following the uptake of organic macromolecules, such as humic acids, fulvic acids. Once these compounds enter plant cells several biochemical changes occur in membranes and various cytoplasmic components of plant cells (Yildirim, 2007).

Humic acids and fulvic acids are excellent foliar fertilizer carriers and activators. Application of humic acids or fulvic acids in combination with trace elements and other plant nutrients, as foliar sprays, can improve the growth of plant foliage, roots, and fruits. By increasing plant growth processes within the leaves an increase in carbohydrates content of the leaves and stems occurs. These carbohydrates are then transported down the stems into the roots where they are in part released from the root to provide nutrients for various soil microorganisms on the rhizoplane and in the rhizosphere. The microorganisms then release acids and other organic compounds which increase the availability of plant nutrients. Other microorganisms release "hormone like" compounds which are taken up by plant roots.

Humic substances will maximize the efficient use of residual plant nutrients, reduce fertilizer costs, and help release those plant nutrients presently bound is minerals and salts (Atiyeh et al., 2002; Chen et al., 2004).

Such improvement in yield and growth parameters, especially in a crop like faba bean in Egypt, is seriously needed since all parts of faba bean plants are hundred percent in use regardless fresh or dry. Moreover, humic and amino acids are natural components. So, they are safe for use from an antifungal point of view. In addition to the alleviation of harmful effect of chocolate spot and rust diseases. Their practical use in plants under field conditions is easy and possible. Further studies are highly needed to elucidate the mode of action of HA and AA, both on plant host and on the pathogen.

References

Abd El-Kareem, F., 2007. Induced Resistance in Bean Plants Against Root Rot and Alternaria leaf spot Diseases Using Biotic and Abiotic Inducers under Field Conditions. Res. J. Agric. and Biol. Sci., 3 (6): 767-774.

- Achuo, E.A.K.; Audenaert, Meziana H. and Hofte, M. 2004. The salicylic acid dependent defense pathway is effective against different fungi in tomato and tobacco. *Plant Pathol.*, 53: 65-72.
- Anonymous, 1990. Official Methods of Analysis Association of Official Analytical Chemists. 15th Ed. Inc. Washington D.C., USA.
- Atiyeh, R.M.; Lee, S.; Edwards, C.A.; Arancon, N.Q. and Metzger, J.D. 2002. The influence of humic acids derived from earthworm processed organic wastes on plant growth. *Bioresource Technology*, 84: 7-14.
- Bernier, C.C.; Hanounik, S.B.; Hussein, M.M. and Mohamed, H.A. 1993. Field Manual of Common Faba Bean Diseases in the Nile Valley. Internat. Centre for Agric. Res. in the Dry Areas (ICARDA), Information Bulletin No. 3.
- Block, R.J.; Durrum, E.L. and Zweig, G. 1965. A manual Paper Chromatography and Paper Electrophoreses. 2nd Ed., Academic Press, Inc. Publishers, New York.
- Bush, D.R. 1993. Proton coupled sugar and amino acid transporters in plants. Ann. Rev. of Plant Physiol. and Plant Mole. Biol., 44: 513-542.
- Chapman, H.D. and Pratt, P.F. 1961. Methods of analysis for soils, plants and waters. Univ. California, Div. Agric. Sci., Riverside, CA. USA.
- Chen, Y.; De Nobili, M. and Aviad, T. 2004. Stimulatory effect of humic substances on plant growth. Pages: 103-130. In: Soil Organic Matter in Sustainable Agriculture. Magdoff, F. and Weil, R.R. (eds.). Boca Raton, FL., USA.
- Chowdhury, A.K. 2003. Control of Sclerotium blight of groundnut by plant growth substances. *Crop Res.*, 25: 355-357.
- Dewis, J. and Freitas, F. 1970. Physical and Chemical Methods of Soil and Water and Analysis. Food and Agric. Organiz. of United Nations, Rome.
- Dmitrier, A.; Tena, M. and Jorrin, J. 2003. Systemic acquired resistance in sunflower (Helianthus annuus L.). Tsitologiya-1-Genetika, 37 (3): 9-15.
- Fischer, W.N.; Andre, B.; Rentsch, D.; Krolkiewicz, S.; Tegeder, M.; Breitkreuz, K. and Frommer, W.B. 1998. Amino acid transport in plants. *Trends in Plant Science*, 3: 188-195.
- Fragbenro, J.A. and Agboola, A.A. 1993. Effect of different levels of humic acid on the growth and nutrient uptake of teak seedling. J. Plant Nutrit., 16: 1465-1483.
- Gomez, K.A. and Gomez, A.A. 1984. Statistical Procedures for Agricultural Research. 2nd Ed. John Wiley and Sons. Pp. 229-308.
- Hahlbrock, K. and Scheel, D. 1989. Physiology and molecular biology of phenyl propanoid metabolism. *Annu. Rev. Plant Physiol. Plant Mol. Biol.*, 40: 347-369.
- Hanounik, S.B. and Bisri, M. 1991. Status of diseases of faba bean in the Mediterranean region and their control. Ciheam Options Mediter., 10: 59-66.

- Hanounik, S.B. 1986. Screening techniques for disease resistance in faba bean. Internat. Centre for Agric. Res. in the Dry Areas (ICARDA). Syria, 59 pp.
- Harrison, J.G. 1988. The biology of *Botrytis* spp. on Vicia beans and chocolate spot disease, a review. *Plant Pathol.*, 37 (2): 168-201.
- Hebblethwaite, P.D. 1983. The Faba Bean Butterworths. London. UK., 573pp.
- Hesse, P.R. 1971. A Text Book of Soil Chemical Analysis. John Murray (Pub.) Ltd., London.
- Jackson, M.L. 1967. Soil Chemical Analysis. Prentice-Hall of India, New Delhi.
- Khaled, A.A.; Abd El-Moity, S.M.H. and Omar, S.A.M. 1995. Chemical control of some faba bean diseases with fungicides. *Egypt J. Agric. Res.*, 73(1): 45-56.
- Lipson, D.A. and Nasholm, T. 2001. The unexpected versatility of plants: organic nitrogen use and availability in terrestrial ecosystems. *Oecologia*, 128: 305-316.
- McCarthy, P.; Bloom, P.R.; Clapp, C.E. and Malcolm, R.L. 1990. Humic substances in soil and crop sciences: An overview. Pages: 261-271. In: *Humic Substances in Soil and Crop Seines: Selected Reading*. McCarthy, P.; Clapp, C.E.; Malcolm, R.L. and Bloom, P.R. (eds.). American Society of Agronomy and Soil, Science Society of America, Madison, Wisconsin, USA.
- Mackinney, G. 1941. Absorption of light by chlorophyll solution. J. Bio. Chem., 140 (2): 315-322.
- Maschner, H. 1986. Mineral Nutrition of Higher Plants. Academic Press, London.
- McDonnell R.; Holden, N.M.; Ward, S.M.; Collins, J.F.; Farrell, E.P. and Hayes, M.H.B. 2001. Characteristics of humic substances in health land and forested peat soils of the Wick-low mountains. *Biol. and Environ.*, 101 (3): 187-197.
- Mohamed, H.A.R. 1982. Major disease problems of faba beans in Egypt. Pages: 213-225. In: Faba Bean Improvement. Hawtin, G.C. and Webb, C. (eds.). Martinus Nijhoff Publishers. The Hague. The Netherlands.
- Ohki, K. 1978. Zinc concentration in soybean as related to growth, photosynthesis, and carbonic anhydrase activity. *Crop Sci.*, 18: 79-82.
- Piccolo, A.; Nardi, S. and Concheri, G. 1992. Structural characteristics of humic substances as regulated to nitrate uptake and growth regulation in plant systems. *Soil Biochem.*, 24: 373-380.
- Piper, C.S. 1950. Soil and Plant Analysis. Univ. Adelaide, Australia.
- Qualls, R. and Haines, B.L. 1991. Geochemistry of dissolved organic nutrients in water percolating through a forest ecosystem. Soil Sci. Soc. Amer. J., 55: 1112-1123.
- Rahman, M.Z.; Honda, Y.; Islam, S.Z. and Arase, S. 2002. Effect of metabolic inhibitors on red light-induced resistance of broad bean (Vicia faba L.) against Botrytis cinerea. J. Phytopathol., 150: 463-468.

- Robinson, J.M. and Britz, S.J. 2000. Tolerance of a field grown soybean cultivars to laved ozone level is concurrent with higher leaflet ascorbic acid level, higher ascorbate dehydrogenase redox status and long term photosynthetic productivity. *Photosynthesis Res.*, **64**: 77-87.
- Russo, R.O. and Berlyn, G.P. 1990. The use organic biostimulants to help low input sustainable agriculture. *J. of Sust. Agric.*, 1 (2): 19-42.
- Scheuerell, S.J. and Mahaffee, W.H. 2006. Variability associated with suppression of gray mould (*Botrytis cinerea*) on geranium by foliar applications of no aerated and aerated compost teas. *Plant Dis.*, 90: 1201-1208.
- Schnitzer, M. 2001. The chemistry of nitrogen in soils. Pages: 117-129. In: Chemistry for the 21st Century. Keinan, E. and Schechter, I. (eds.). Wiley-VCH GmbH, Weinheim.
- Steel, R.G.D. and Torrie, J.H. 1980. Principles and Procedures of Statistics. 2nd Ed. McGraw Hill International Book Company, New York, USA.
- Stevenson, F.J. 1994. Humus Chemistry: Genesis, Composition and Reactions. 2nd Ed. Wiley Interscience, New York.
- Ulukan, H. 2008. Effect of soil applied humic acid at different sowing times on some yield components in wheat (*Triticum* spp.) hybrids. *Int. J. Bot.*, 4 (2): 164-175.
- Yildirim, E., 2007. Foliar and soil fertilization of humic acid affect productivity and quality of tomato. *Acta Agric. Scandinavia. Section B. Soil and Plant Sci.*, 57 (2): 182-186.
- Yu, Z.; Zhang, Q. and Kraus, T.E.C. 2002. Contribution of amino compounds to dissolved organic nitrogen in forest soils. *Biogeochemistry*, 61: 173-198.
- Zhang, X. 1997. Influence of plant growth regulators on turfgrass growth, antioxidant status, and drought tolerance. Ph.D. Thesis, Fac. of Virginia Polytechnic (Institute and State University), 131 p.
- Ziadi, S.; Barbedette, S.; Godard, J.F.; Monoti, C.; Corre, L.E.D.; Silue, D. and Lecorre, D. 2001. Production of pathogenesis related protein in the cauliflower (*Brassica oleacea* var. *botrytis*) downy mildew (*Peronospora parasitica*) pathosystem treated with acidbenzolar-5-methyl. *Plant Pathol.*, 50 (5): 579-586.

(Received 17/09/2011; in revised form 18/10/2011) استحثاث المقاومة لأمراض التبقع البنى والصدأ وتحسين الإنتاجية في الفول باستخدام الأحماض الامينية وأحماض الهيوميك

قمر محمد عبد الحي ، أيمن محمد الغمرى ** ، خالد محمد غنيم ***

* قسم بحوث أمراض البقوليات والعلف ـ معهد بحوث أمراض النباتات ـ مركز البحوث الزراعية ـ الجيزة ـ مصر.

: * قسم الأراضي - كلية الزراعة جامعة المنصورة - مصر

*** قسم بحوث أمراض البذور _ معهد بحوث أمراض النباتات _ مركز البحوث الزراعية _ الجيزة _ مصر.

تم اقلمة تجربتين حقليتين بمحطة البحوث الزراعية بتاج العز _ دقهلية في موسمى ٢٠٠٨/٢٠٠٧ ـ ٢٠٠٩/٢٠٠٨ لدراسة تأثير الأحماض الامينية وأحماض الهيوميك وتداخلاتهما على أمراض التبقع الشيكولاتي والصدأ وكذلك النمو والمحتوى الكيمياني ومحتوى الكلوروفيل في نبات الفول البلدي. أدت معاملة التداخل بين الأحماض الامينية بتركيز ١٠٠٠ جزء في المليون وأحماض الهيوميك ٠٠٠ جزء في المليون إلى تقليل شدة الإصابة بالتبقع الشيكولاتي بعد ٥٠ يوم من الزراعة. بينما أدت المعاملة بالأحماض الامينية بتركيز ٢٠٠٠ جزء في المليون إلى أقل شدة ونسبة إصابة بعد ٧٥ يوم تبعتها المعاملة باحماض الهيوميك بتركيز ١٠٠٠ جزء في المليون. وقد سجلت المعاملة بلحماض الهيوميك ٢٠٠٠ جزء في المليون أقل نسبة إصابة بالصدأ تبعتها معاملة التداخل بين الأحماض الامينية . • • ١ جزء في المليون + احماض الهيوميك • • • ١ جزء في المليون ثم المعاملة باحماض الهيوميك ٢٠٠٠ جزء في العليون. وأدت المعاملة بالأحماض الامينية ٢٠٠٠ جزء في المليون + أحماض الهيوميك ٢٠٠٠ جزء في المليون إلى زيادة معنوية في جميع الصفات المورفولوجية ومكونات المحصول وكذلك محتوى العناصر الكبرى (نيتروجين وفوسفور وبوتاسيوم) وأيضا محتوى الكلوروفيل كما ادت نفس المعاملة إلى الحصول على أعلى محصول بذرة تبعتها المعاملة بالأحماض الامينية ٢٠٠٠ جزء في المليون ثم المعاملة بالأحماض الامينية ١٠٠٠ جزء في المليون. بينما لم يتأثر عدد البذور بالقرن معنويا. وتوصى الدراسة باستخدام الأحماض الامينية متداخلة مع أحماض الهيوميك بتركيز ١٠٠٠ جزء في المليون رشا على المجموع الخضري لتقليل الإصابة بالتبقع الشيكولاتي والصدا في القول البلدي بالإضافة لأثرهما الايجابي على البيئة والاقتصاد.