Plant Pathology Journal

ISSN 1812-5387

Plant Pathology Journal 9 (4): 169-178, 2010
ISSN 1812-5387

Improving the Performance of Faba Bean and Controlling of Chocolate Spot Disease using Bio-compounds

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Abstract: There is an urgent need to improve faba bean yield since this plant remains an important part of the human diet. So, experiments have been designed to determine the effect of some bio-compounds on faba bean growth, chemical composition and chocolate spot disease. *In vitro*, results revealed that Bio Zeid and Bio ARC completely inhibited the linear growth, mycelia dry weight and sporulation of *Botrytis fabae*. Plant oils (cumin, cornation, garlic and rocket oils) completely inhibited the sporulation only. *In vivo*, results (2007/2008 and 2008/2009 seasons) confirmed the positive return of the tested compounds in which, the highest increase in fresh weight was recorded with Bio ARC, while in dry weight the highest increase was recorded with rocket oil. Plant height, number of branches, pods/plant and seeds/pod increased significantly with Acadian-C plant. The weight of 100 seeds reached to the maximum with aminogreen. In addition to the general improvements in N, P, K, Zn, Fe and Mn content. Total chlorophyll reached to the maximum values when using Bio Zeid, Bio ARC, Acadian-C plant and rocket oil. Bio ARC, Bio Zeid and garlic oil revealed the most superior positive effect among other additions on total phenolic compounds. Bio ARC and Bio Zeid led to maximum reduction of chocolate spot disease severity. Bio-, organic and natural additives were found to be most effective in increasing growth, chemical composition and yield parameters of faba bean and decrease the disease severity of chocolate spot disease.

Key words: Faba bean, growth parameters, chemical composition, chocolate spot, essential oil extracts, bio-compounds

INTRODUCTION

Faba bean (*Vicia faba*) is one of the main pulse crops grown for seed in Egypt. Due to its high nutritive value in both energy and protein contents, it is a primary source of protein (Nassib *et al.*, 1991). This strategic crop is suffering from many destructive diseases, it is attacked by more than 100 pathogens in the Mediterranean region (Hebblethwaite, 1983). Chocolate spot cause mainly by *Botrytis faba* and to some extract by *B. cinerea* is an important disease worldwide occurring almost in all regions where faba bean are grown (Rahman *et al.*, 2002).

The disease appears as lesions on flowers, leaves and stems with oblong elliptical of reddish to chocolate brown color, with darker margins that are fairly defined and often a concentric circular pattern. As lesions increase in number, generally on the upper leaf side, they may remain small expand or merge (Harrison, 1988) The spots led to harmful effects on growth, physiological activities and yield (Khaled *et al.*, 1995).

Biological control of *B. fabae* by different bio-agents is reported by Cook and Baker (1983), Abd-El-Moiety and Abu-Zeid (1985) and Omar *et al.* (1987). Essential oil extracts have been considered as natural preservatives or food additives and can be used for controlling pathogens (Naidu, 2000), because of their biocidal effects on bacteria, fungi, viruses, protozoa, insects and plants (Kalemba and Kunicka, 2003).

The production of faba bean in Egypt is still limited and fails to face the increasing local consumption of the crop. Therefore, increasing the crop production is one of the major targets of the agricultural policy and can be realized by increasing the cultivated area with faba bean through introduction the crop. The problem of adequately

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protecting plant against the fungus by using fungicides has been complicated by the development of fungicidal resistance and/or adverse effect on growth and productivity of the host plant as well as on the accompanying microflora (Khaled *et al*., 1995).

Because of the hazards of fungicide on public health and environmental balance, it was hypothesized that bio, organic and natural compounds might reduce or nullify the negative effects of chocolate spot on the growth, photosynthesis pigments, mineral constituents and yield and yield components of faba bean. Therefore, this research aimed to study the effect of bio, organic and natural additives on some pathological, morphological, physiological traits and productivity of faba bean.

MATERIALS AND METHODS

Source of tested materials and faba bean seeds: Seeds of faba bean seeds (Giza 3) were obtained from Legumes Dept., Field Crops Res. Institute, Agric. Res. Center, Giza, Egypt. The types and composition of the used bio-compounds are presented in Table 1:

- Two kinds of bio-agents were used, the first Bio Zeid which contains the fungus of *Trichoderma album* (2.5%, w/w) and the second Bio ARC which contains the bacterium; *Bacillus megaterium* (6%, w/w)
- Three kinds of organic additives were used, the first (Acadian-C Plant) is a marine algae extract the second (Amino green) is a kind of amino acids and the third (Novaful) is another also kind of amino acids
- The extractions of four plants were used as natural additives (cumin, carnation, garlic and rocket oils)

Isolation of chocolate spot causal pathogen: *B. fabae* was isolated from naturally infected faba bean leaves collected from different fields of Dakhlia governorate. Leaves were surface sterilized (with 0.5% sodium hypochlorite solution for 1-2 min) and plated on faba bean dextrose agar medium (400 g of faba bean leaves (autoclaved and filtered to obtain faba infusion), 20 g of dextrose and 18 g agar) at 20°C (Haggag *et al*., 2006). Isolates were purified and identified according to Morgan (1971).

***In vitro* evaluation of the tested materials:** This experiment aimed to study the effect of bio, organic and natural addition on linear growth, fungal dry weight and sporulation of chocolate spot causal pathogen (*Botrytis fabae*). The tested materials at 2.5 g L^{-1} were, separately, mixed with PDA medium before solidification, then poured in a sterile Petri dishes. Four plates for each concentration were inoculated with fungal disc which had been cut from the periphery of 7 days old culture of *B. fabae*. The plates were incubated at 20±1°C,

Table 1: Types and composition of the used bio-compounds

Treatments	Composition	Types
Bio Zeid	Trichoderma album 2.5% (w/w)	Bio-agents
Bio ARC	Bacillus megaterium 6% (w/w)	
Acadian-C plant (marine algae extract)	N 0.33% - P2O5 0.3% - K2O 4% Zn 15 ppm - Fe 44 ppm - Mn 2 ppm Mg 0.1% - Cu 9 ppm - Ca 0.05% B 30 ppm - S 0.3% - Na 1% Its contains also cytocynin, auxin and gibrlin	Organic
Amino green (amino acid)	Contents (w/v) Total organic acid + Amino acid 20% Fe 2.9%, Zn 1.4%, Mn 0.7%	
Novaful (Amino acid)	Amino acids 29.7% N 4.30% - K2O 5.17% - P2O5 618 ppm	
Cumin oil (*Cuminum cyminum*)	Cumin seeds (Nutritional value per 100 g) Energy 370 kcal 1570 kJ - Carbohydrates 44.24 g Fat 22.27 g-Protein 17.81 g Water 8.06 g – Vit. A 64 μg - Vit. B1 0.628 mg - Vit. B2 0.327 mg - Vit. B3 4.579 mg - Vit. B6 0.435 mg - Vit. B9 10 μg - Vit. C 7.7 mg - Vit. E 3.33 mg - Vit. K 5.4 μg - Ca 931 mg - Fe 66.36 mg - Mg 366 mg - P 499 mg - K 1788 mg - Na 168 mg - Zn 4.8 mg	Natural
Carnation oil (*Dianthus caryophyllus* L.)	Nitrogen (3.33-4.19 % N) - Sulphur (0.27-0.35 % S)- Phosphorus (026-0.40 % P) - Magnesium (0.29-0.39 % Mg) - Calcium (1.13-1.64 % Ca) - Sodium (0.10-0.50 % Na) - Potassium (2.79-4.00 % K) - Manganese (50-250 ppm Mn) - Zinc (20-60ppm Zn) - Copper (6-10 ppm Cu) - Boron (30-100 ppm B) - Molybdenum (0.10-2.10 ppm Mo) - Iron (51-120 ppm Fe)	
Garlic oil (*Allium sativum* L.)	Appearance: Bright yellow oil – Odour: Strong odor of garlic - AV 1.08 - Ajoene 0.65 mg g^{-1} - Vinyl Dithiins 4.5 mg g^{-1} - Allyl Sulfides 40 mg g^{-1}- Specific gravity 0.9-0.94 - Purity 99% - Ash% 2.0% Max - Arsenic <2 ppm - Heavy metals (Pb) <1 ppm - Foreign materials None - Pesticide residues None	
Rocket oil (*Eruca sativa* Mill)	Nutritional value per 100 g - Energy 20 kcal 70 kJ - Carbohydrates 3.40 g - Fat 0.10 g - Protein 0.68 g - Vit. B1 0.012 mg - Vit. B2 0.039 mg - Vit. B3 0.254 mg– Vit. B5 0.165 mg – Vit. B6 0.071 mg 5% - Vit. B9 25 μg – Vit. C 14.8 mg - Ca 25 mg – Fe 0.34 mg – Mg 10 mg – P 20 mg - K 233 mg - Zn 0.28 mg	

linear growth (cm) of the tested pathogenic fungus measured when particular control filled Petri dishes with the fungal mycelia.

The influence of bio, organic and natural additives on dry weight and sporulation of *B fabae* were tested on Czapk's broth medium in conical flask containing 20 mL broth medium amended individually with all tested concentration of each addition at 2.5 g L^{-1}. A set of similar flasks containing chemical free medium served as check. Four flasks for each concentration were inoculated with fungal disc (0.6 mm diameter) of *B. fabae* taken from 7 days old culture for each flask. After 10 days of inoculation at 20±1°C, flasks were shacked (150 rpm) for 1 h, then 1 mL from each flask was take to determine the number of spores using haemocytometer. The rest of the flask was filtered through pre weighted whatman No. 1 filter paper, washed with distilled water and dried at 70±2°C in a vacuum oven to constant weights.

Field experiment: Field experiment was carried out at Tag El-Ezz Research Station, Dakahlia Governorate, Egypt during winter seasons of 2007/2008 and 2008/2009. Soil is clayey loam in texture containing 35.5% clay, 24.7% silt and 38.8% sand. The Field capacity = 34.3% and the real density = 2.66 g cm^{-3}. EC_e in soil paste = 0.32 dS m^{-1}, pH in soil paste = 7.6, organic matter = 1.42%, CEC = 35.2 meq 100 g^{-1} and $CaCO_3$ = 2.4%. Available N, P, K, Zn, Mn and Fe were 32.3, 14.4, 215, 1.6, 7.3 and 12.2 ppm, respectively.

The 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, O.M., available NPK, microelements Fe, Zn and Mn, $CaCO_3$ and Real density were determined according to Jackson (1967), Hesse (1971), Dewis and Freitas (1970) and AOAC (1990).

Seeds of faba bean were sown in 20th November 2007 and 30th November 2008. All chemical fertilizers were added as a recommended dose of ministry of agriculture, Egypt. All bio-, organic and natural additives were added at two equal doses; the first after 40 days of planting and the second additions after 55 days of planting. Each of them was used at the rate of 250 g 100 L^{-1} water and sprayed over the plant till dripping using small pressure pump in the presence of wetting. All other agricultural processes were done. A complete randomized block design with three replicates was used. The experimental plot contains 5 ridges occupying an area of 10.5 m^2 (3.5×3 m).

Chemical analysis of faba bean shoot: At the 75th day from sowing, samples of shoot system were dried at 70°C then finally ground to fine powder and 0.2 g was wet digested with a mixture of sulphuric and perchloric acids according to Jackson (1967). N, P and micronutrients Fe, Mn and Zn were determined according to Chapman and Pratt (1965), while K was determined according to Hesse (1971).

Determination of photosynthetic pigments: At the 75th day from planting, the blade of the 3rd leaf from plant tip (terminal leaflet) was taken to determine photosynthetic pigments using methanol 90% for 24 h at room temperature after adding traces of sodium carbonate. Then photosynthetic pigments were determined spectrophotometrically and calculated using the equations of Mackinney (1941).

Determination of total phenolic compounds: Total phenolics were determined after 75 days from sowing in fresh shoot using the Folin-Ciocalteau reagent (Singleton and Rossi, 1965). Samples (2 g) were homogenized in 80% aqueous ethanol at room temperature and centrifuged at 10000 rpm for 15 min under cooling. The residues were re-extracted twice with 80% ethanol and supernatants were pooled, put into evaporating dishes and evaporated to dryness at room temperature. Residues were dissolved in 5 mL of distilled water. One-hundred microliters of this extract were diluted to 3 mL with water and 0.5 mL of Folin-Ciocalteau reagent was added. After 3 min, 2 mL of 20% of sodium carbonate was added and the contents were mixed thoroughly. The developed color was measured spectrophotometrically at 650 nm after 60 min, using catechol as a standard. The results were expressed as mg catechol/ 100 g fresh weight material.

Disease assessment: The disease severity of chocolate spot disease was recorded at the 75th day from sowing using the scale of Bernier *et al.* (1993), as follows:

- 1 = No disease symptoms or very small specks (highly resistance)
- 3 = Few small disease lesions (resistant)
- 5 = Some coalesced lesions, with some defoliation (moderately resistant)
- 7 = Large coalesced sporulating lesions, 50% defoliation and some dead plants (susceptible)
- 9 = Extensive, heavy sporulation, stem girdling, blackening and death of more than 80% of plants (highly susceptible)

The formula adopted by Hanounik (1986) was used to estimate the percentage of chocolate spot severity;

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

Where:
NPC = No. of plants in each class rate
CR = Class rate
NIP = No. of infected plants
MSC = Maximum severity class rate

Nodulation and nitrogenase activity: Samples of faba bean roots were collected after 80 days from sowing for the determination of number and dry weight (at 70°C until constant weight) of nodules. Nitrogenase activity was determined (Lethbridage *et al*., 1982) in the second season.

Morphological characters and yield components: Samples were taken at 90 days from planting to estimate growth parameters as: plant height, number of branches/plant, fresh and dry weight of plant shoot. At harvest, number of pods/plant, seed/pod and weight of 100 seeds were recorded.

Statistical analysis: All data were subjected to the proper statistical analysis of variance of randomized complete block design by Gomez and Gomez (1984). Mean values of treatments were differentiated using LSD according to procedure outlined by Steel and Torrie (1980).

RESULTS

Growth and sporulation of *B. fabae* as affected by bio-compounds: Marked variations in the linear growth and mycelial dry weight as well as sporulation of the pathogen (*B. fabae*) were recorded with the different types of the tested compounds (Fig. 1), Bio Zeid and Bio ARC completely inhibited the linear growth, fungal dry weight and sporulation compared with check. Garlic oil also has a best effect on inhibited the linear growth more than other treatments. In general, plant oils completely inhibited sporulation of *B. fabae*. Aminogreen and Novaful recorded moderate effects. On the other side, Acadian C plant did not have any inhibitory effect, inversely, it induced the growth and sporulation of *B. fabae* compared to control.

Field evaluation of the tested bio-compounds on faba bean
Effect on NPK content of faba bean shoot: According to the results of Table 2, all tested compounds reached to the level of significancy and recorded high content of NPK in faba bean shoot compared with the control, in the two growing seasons. The nitrogen content of faba bean shoot recovered from garlic oil treatment reached to the maximum (3.67 and 3.72%). Whereas, the maximum P (0.36 and 0.38%) and K (1.99 and 1.97%) were recovered from plants treated with rocket oil, in both seasons, respectively. Of bioagents, Bio ARC is more effective, than Bio Zeid.

Table 2: Effect of the tested bio-compounds on macronutrients in faba bean shoot

	2007/2008			2008/2009		
	N	P	K	N	P	K
Treatment	(%)					
Control	2.47	0.28	1.44	2.51	0.26	1.40
Bio Zeid	2.87	0.31	1.53	2.90	0.30	1.51
Bio ARC	2.73	0.33	1.59	2.75	0.34	1.57
Acadian-C plant	3.13	0.29	1.49	3.17	0.30	1.46
Aminogreen	3.20	0.35	1.60	3.25	0.36	1.57
Novaful	3.07	0.32	1.83	3.10	0.33	1.79
Cumin oil	2.67	0.31	1.77	2.71	0.32	1.74
Cornation oil	3.30	0.32	1.94	3.35	0.34	1.91
Garlic oil	3.67	0.34	1.84	3.72	0.36	1.81
Rocket oil	3.47	0.36	1.99	3.49	0.38	1.97
LSD (5%)	0.12	0.01	0.05	0.02	0.02	0.02

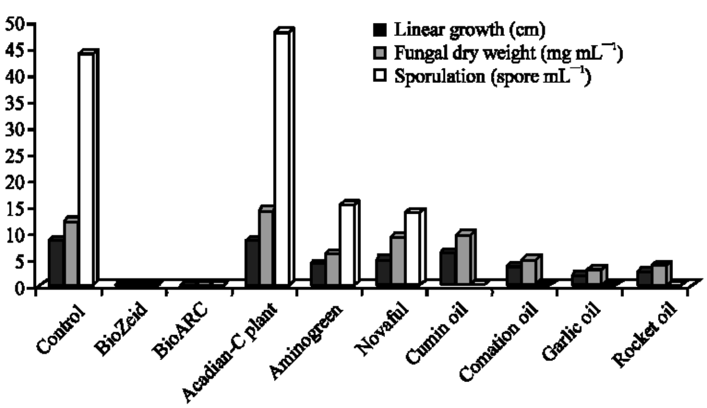

Fig. 1: Effect of the tested bio-compounds on linear growth, fungal dry weight and sporulation of *B. fabae*

Table 3: Effect of the tested bio-compounds on micronutrients in faba bean shoot

Treatment	2007/2008 Zn (ppm)	2007/2008 Fe (ppm)	2007/2008 Mn (ppm)	2008/2009 Zn (ppm)	2008/2009 Fe (ppm)	2008/2009 Mn (ppm)
Control	16.67	41.00	20.00	16.33	42.67	17.67
Bio Zeid	18.33	45.33	23.33	18.62	46.67	23.00
Bio ARC	20.67	49.00	26.00	21.00	51.33	26.67
Acadian-C plant	24.33	46.67	30.67	25.00	48.00	32.33
Aminogreen	20.00	52.33	28.00	19.67	54.33	33.00
Novaful	26.00	51.00	29.33	26.67	53.00	30.33
Cumin oil	24.67	49.00	31.33	24.67	51.33	33.33
Cornation oil	28.33	61.67	30.33	29.33	63.67	33.00
Garlic oil	27.33	60.00	33.33	27.33	62.33	35.67
Rocket oil	29.67	63.33	29.33	30.00	64.67	30.67
LSD (5%)	1.55	1.58	1.21	0.98	1.03	3.06

Table 4: Response of chlorophyll A, B, carotenoid and total phenol content of faba bean to the tested bio-compounds

Treatment	2007/2008 Chl. A (mg g^{-1} fresh weight)	Chl. B (mg g^{-1} fresh weight)	Carotenoid (mg g^{-1} fresh weight)	Total phenol (mg 100 g^{-1} fresh weight)	2008/2009 Chl. A (mg g^{-1} fresh weight)	Chl. B (mg g^{-1} fresh weight)	Carotenoid (mg g^{-1} fresh weight)	Total phenol (mg 100 g^{-1} fresh weight)
Control	1.13	0.35	0.29	530.67	1.21	0.46	0.16	578.00
Bio Zeid	1.78	1.07	0.21	712.33	1.81	0.99	0.16	851.67
Bio ARC	1.64	0.93	0.22	757.67	1.70	0.86	0.18	870.00
Acadian-C plant	1.76	1.50	0.07	574.00	1.77	1.21	0.127	720.00
Aminogreen	1.56	0.59	0.28	591.00	1.60	0.61	0.29	739.67
Novaful	1.78	1.15	0.23	559.67	1.80	1.03	0.23	695.33
Cumin oil	1.64	0.58	0.37	542.67	1.68	0.53	0.31	664.00
Cornation oil	1.58	0.62	0.39	614.67	1.63	0.64	0.35	754.67
Garlic oil	1.75	0.75	0.33	686.67	1.73	0.71	0.27	808.67
Rocket oil	1.79	1.19	0.21	648.33	1.87	1.07	0.18	788.33
LSD (5%)	0.03	0.01	0.02	7.10	0.03	0.02	0.02	9.27

Effect on some micronutrients content of faba bean shoot: The response of faba bean plants to the application of different compounds in the form of micronutrients was recorded in Table 3 in which both seasons followed the same trend. All tested compounds excreted significant effect on Zn, Fe and Mn content. The highest concentration of Zn and Fe were observed with rocket oil, while the highest content of Mn was found when garlic oil. Among bio-agents, Bio ARC is more effective than Bio Zeid in recovering Zn, Fe and Mn. While of organic additives, amino green was better than novaful on Fe concentration, while novaful is more effective than aminogreen with Zn and Mn. In general, garlic and rocket oils effectively induced the accumulation of N, P, K, Zn, Fe and Mn in faba bean shoot.

Photosynthetic pigments and total phenol content: The photosynthetic pigments content as chlorophyll a, b and carotenoid as well as total phenol in fresh plants were determined. As shown in Table 4, the bioagent Bio Zeid was more effective than Bio ARC on Chl. A and B, while Bio ARC is better than of Bio Zeid on carotenoid and total phenol content in both seasons.

Rocket oil had the highest significant increase on Chl. A, while in case of Chl. B the highest significant increase was with Acadian C. plant. The application of any of such compounds improved the photosynthetic apparatus of faba bean. The improvements varied according the applied compound.

Disease assessment: Data on disease severity of chocolate spot disease of faba bean was recorded in Fig. 2. The maximum reduction of disease severity of chocolate spot was recorded by Bio ARC followed by Bio Zeid and garlic oil. Slight increase in disease severity was mentioned by cumin oil and novaful, while, there is no significant difference in disease severity of chocolate spot between Acadian-C plant, aminogreen and novaful.

Nodulation and nitrogenase activity: It was found to extend the tested parameters to include one of the most important criteria in faba bean, i.e., nodulation status. Only the best compound from each group was analyzed. As plotted in Fig. 3 the tested compounds showed remarkable improvement in number and dry weight of nodules compared with the control treatment. Nitrogenase activity recorded similar trend.

Effect on morphological characteristics: Data in Table 5 of field study show that the two types of bio-agents (Bio Zeid and Bio ARC) recorded the highest significant increase in fresh and dry weights. Bio ARC appeared to

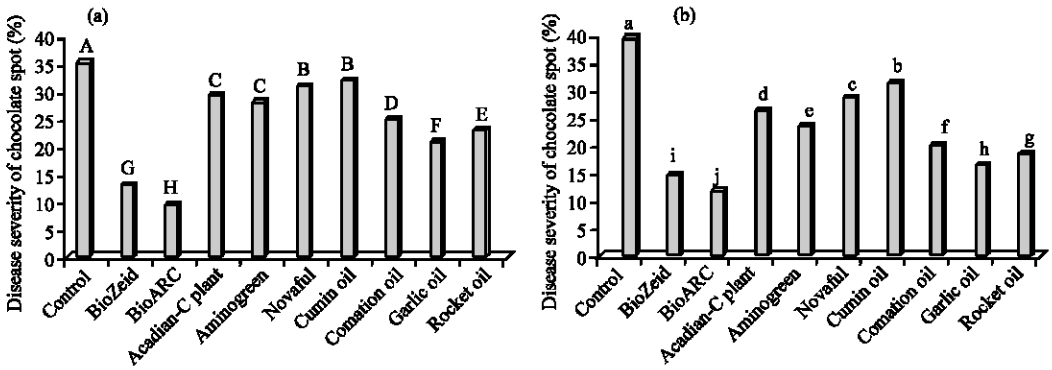

Fig. 2: Effect of the tested bio-compounds on disease severity of chocolate spot in faba bean. In each season, column superscripted with the same letter is not significantly differed. (a) season one and (b) season two

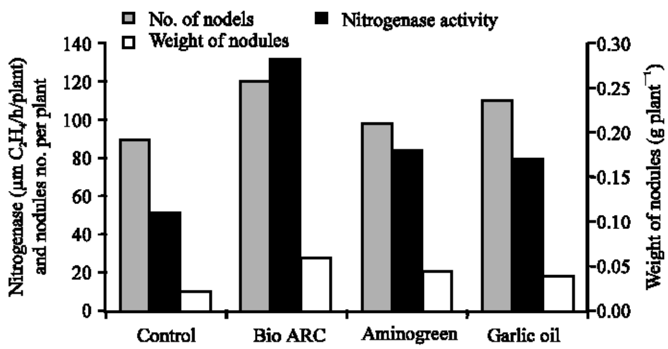

Fig. 3: Nodulation status and nitrogenase activity of faba bean as affected with bio-, organic and natural additives

Table 5: Effect of the tested bio-compounds on growth parameter of faba bean under field conditions

	2007/2008				2008/2009			
Treatment	Fresh weight (g)	Dry weight (g)	Plant height (cm)	Branches No./plant	Fresh weight (g)	Dry weight (g)	Plant height (cm)	Branches No. /plant
Control	59.12	32.85	74.67	4.33	53.70	28.34	70.33	4.00
Bio Zeid	80.82	47.25	86.00	7.67	75.17	42.47	82.33	6.67
Bio ARC	113.56	50.54	83.33	7.33	102.04	47.61	75.00	6.33
Acadian-C plant	94.42	44.90	104.33	9.67	88.32	40.46	96.67	8.33
Aminogreen	62.37	39.66	88.67	9.67	59.43	35.79	85.67	8.00
Novaful	62.38	35.19	84.33	5.33	60.07	31.83	80.67	4.67
Cumin oil	93.81	47.09	83.67	4.33	83.41	43.04	77.00	4.00
Cornation oil	61.56	40.01	88.67	5.67	64.07	37.43	84.33	5.00
Garlic oil	74.17	41.21	99.00	7.67	71.55	38.37	89.67	6.67
Rocket oil	72.40	55.29	76.00	6.00	64.53	50.74	72.67	5.33
LSD (5%)	4.79	3.92	2.68	1.12	6.26	1.48	1.76	0.76

be superior over the other compounds (bio, organic and natural additions) in improving fresh weight, while in dry weight the highest significant increase was recorded with rocket oil. On the other hand, Table 5 showed that there is no significant difference between two kinds of amino acids (aminogreen and novafull) in fresh weight and also no significant difference between garlic oil and rocket oil in fresh weight. In dry weight, there is no significant difference between cornation oil and garlic oil. The best treatment of plant oils was cumin oil on fresh weight and rocket oil on dry weight.

As mentioned in Table 5 there is highly significant increase in plant height as affected by bio, organic and natural additions, in which, the highest increment was recorded with Acadian C plant followed by garlic oil. Of bio-agents, Bio Zeid was better than Bio ARC. Moreover, of organic compounds, amino green was more effective than novaful.

Number of branches/plant as a morphological character was affected by different additions. The superiority of all additions referred to Acadian C plant. Bio Zeid has a better effect than Bio ARC in all treatments

Table 6: Effect of the tested bio-compounds on yield parameters of faba bean

	2007/2008			2008/2009		
Treatment	100-seed wt. (g)	Pods No./plant	Seeds No./Pod	100-seed wt. (g)	Pods No./plant	Seeds No./Pod
Control	54.83	32.33	3.00	55.57	30.33	3.00
Bio Zeid	68.83	44.67	3.33	65.84	37.67	3.00
Bio ARC	65.54	38.67	3.00	66.37	34.67	3.00
Acadian-C plant	68.76	64.67	4.00	65.95	55.33	4.00
Aminogreen	70.17	51.67	2.67	68.33	45.00	3.00
Novaful	59.19	37.33	3.00	58.44	33.33	2.67
Cumin oil	64.36	39.67	2.67	62.67	34.33	3.00
Cornation oil	62.54	50.00	3.00	60.83	42.00	3.00
Garlic oil	58.19	56.67	3.67	57.13	50.33	3.33
Rocket oil	57.72	46.67	2.67	55.97	38.33	2.67
LSD (5%)	4.52	2.37	0.72	0.97	1.16	0.54

and amino green was better than novafull on number of branches/plant. On the other hand, the highly significant increase in number of branches/plant as affected by plant oils was occurred with garlic oil.

Effect on yield parameters: Data in Table 6 show the values of 100-seed weight as affected by different bio-, organic and natural additives during the two growing seasons. There is highly significant increase in all treatments as compared to control. The highest increase of all treatments was recorded with aminogreen followed by Bio Zeid. There is no significant difference between cumin oil and cornation oil and also between garlic oil and rocket oil.

Concerning the effect of bio, organic and natural additions on number of pods/plant and number of seeds/pods, Bio Zeid was more effective than Bio ARC on number of pods/plant. Aminogreen recorded high significant increase than novaful. The same trend was noticed with number of seeds/pods. With respect to plant oils, the best effect was occurred with garlic oil, there is no significant difference between cumin oil and rocket oil.

DISCUSSION

Concerning to the effect of bio, organic and natural additions on faba bean growth and reducing chocolate spot disease in faba bean. The results of this study showed that all growth parameters and yield components increased significantly with Acadian C plant, while the weight of 100 seeds reached to the highest by amino green, on the other hand Bio ARC and Bio Zeid were the most effective in reducing the disease severity of chocolate spot in faba bean plant.

It has been known for many years that *Trichoderma* sp. inhibit the fungal growth by three mechanisms: competition (for space and nutrients), parasitism (deriving nutrients from the host) and antibiosis (production of an inhibitory metabolite or antibiotic) (Harman, 2006). The mycoparasitism and competition and/or fungicidal action is because of the capacity of *Trichoderma* to produce antibiotics or hydrolytic enzymes (Lorito *et al*., 1994). *Trichoderma harzianum* was considered as bio-control agent against phytopathogenic fungi, but the mechanism of this effect is not clearly understood. Proposed mechanisms of biocontrol are antibiosis (Ghisalberti *et al*., 1990).

Certain strains of *Bacillus* appear to be effective as a biological control agent, by inhibiting the mycelial growth of plant pathogenic fungi (Mahmoud, 2004) and dry weight of the fungus as well as sporulation as in the present study (Fig. 1). Additionally, *Bacillus subtilis* can induce resistance in plant to disease by stimulation of phytoalexins production and increasing the activity of lytic enzymes (Sailaja *et al*., 1998).

The increasing in growth and yield due to application of Bio Zeid (which contains *Trichoderma album* as the main organism) can be explained by increasing of mineral levels (N, P and K) and both chlorophyll biosynthesis and photosynthetic activity, which in turn led to the accumulation of metabolites (carbohydrate and proteins) consequently increase plant resistance to *B. fabae* as well as plant growth and productivity (Mahmoud *et al*., 2004). Phosphorus is probably the most limiting nutrient for production of leguminous crop, possibly by its influence on the activity of rhizobium bacteria and nodule formation (Mengel and Kirkby, 1982).

The positive effect of Acadian-C plant and amino acids may be due to their contents of some nutrients such as NPK, Zn, Mn, B and also cytokinin, auxin and gibberellin, each of these contents has an essential role in plant growth (plant height) and its vital functions as well. Gibberellin causes increase in cell division and cell enlargement (Bruce, 1990; Deotale *et al*., 1998), number of internodes/plant and length of the internodes (Castro and Vello, 1983). Treated plants with growth regulators showed an increase in total phenol, calcium content and increase of the activity of chatechol oxidase, these materials protect plants against pathogen stress (Chowdhury, 2003). Manganese, which found in

Acadian-C plant, activates a number of enzymes. It is known to induce a cycle of reaction within the plant. The role of Mn in photosynthesis is clearly identified through is absolute necessary for water photolysis during photosynthesis (El-Naggar *et al*., 1994). Manganese also, regulates the levels of auxin in plant tissues by activating the auxin oxidase system (Marschner, 1986).

The data show that all of used treatments increased chl. a and chl. b contents in the leaves of faba bean plant. Bio Zeid, Noval and Roket oil were the most effective in this respect. Carotenoids which known as a major endogenous plant antioxidant were increased due to cumin oil, carnation oil and garlic oil treatments. It is well known that chlorophyll and phenolic compound are good parameters reflecting the health condition of any plant.

The increase in photosynthetic pigments led to carbohydrates accumulation, hence increase carbohydrate contents in faba bean plants. Carbohydrates are the main repository of photosynthetic energy, they comprise structural polysaccharides of plant cell walls and principally cellulose, hemicellulose and pectin, also associated with the structural polysaccharides are phenolic compounds. Which play an important role in plant defense such phenols that are essential for biosynthesis of lignin, which is considered an important structural component of plant cell walls (Hahlbrock and Scheel, 1989).

The high content of Zn, Mn and Fe in the treated faba bean plants may stimulate several physiological activities since they are Co-factors of super oxide dismutase (enzymatic antioxidant) which alleviate the harmful effect of reactive oxygen species (free radicals) caused by botrytis pathogens stress. The role of zinc in enhancement the vegetative growth which followed by stimulation superioxidase dismutase activity, in addition to the marked effect on auxin synthesis (Ohki, 1978), which in turn encourage the meristemic activity of the plant leading to more cell division and cell enlargement (Devlin and Witham, 1983). Iron plays a key role in several enzyme systems in which haemin functions as the prosthetic group. These heam enzyme systems comprise the catalases, peroxidases and several cytochromese (El-Naggar *et al*., 1994). Additionally, the tested compounds improved the performance of nitrogen fixation process. Similar results were obtained by Saber *et al*. (2009).

The tested oils decreased the harmful effects of *B. fabae*. This is agreement with Radwan (1980) stated that garlic juice inhibited growth of *Fusarium manilifarm*, *Helminthosporium oryzae*, *Alternaria citri*, *Scleratium bataticola*, *Aspergillus niger* and *Penicillium italicum*, also clove juice suppressed the formation of *Macrophomin phoseolina* sclerotia around the paper disk. Tansey and Appleton (1975) mentioned that aqueous extract of garlic inhibited the fungal growth of *Asbolus lineolatus*, *Coniphora suffocate*, *Favolus* sp. and *Phycomyces blakesleanus*. Fahmy (1994) tested oils of curaway, cumin, coriander and fennel for their antifungal activity, he found that mycelial growth of *Rhizoctonia solani*, *Fussarium oxysporum* and *Sclerotium rolficii* were completely inhibited with all the tested oils, except coriander oil. Farag *et al.* (1989) found that essential oils of thyme, cumin, clove and rosemary caused complete inhibition of *Asperigillus parasiticus* mycelial growth and aflatoxin production.

It could be concluded that the promotive effect of Acadian-C plant, amino green, cumin oil, carnation oil, garlic oil and rocket oil may be due to the antioxidant material which found as a major content of these compounds. Such antioxidants alleviate and mitigate the harmful effect of botrytis pathogen stress. In this study, it may be recommended to use Bio ARC, aminogreen and garlic oil as a foliar spray after 40 and 55 days from planting to alleviate the harmful effect of chocolate spot disease and maximizing yield of faba bean plant.

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