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Recent approaches for controlling brown spot disease of Faba Bean in Egypt

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ABSTRACT

Commercial compounds of induced resistance (Bion, Starner, Boric acid and Salicylic acid) were used for controlling faba bean chocolate spot disease under greenhouse conditions. The tested compounds significantly reduced the disease severity and this was more pronounced in case of soaking faba bean seeds in Boric acid for 24 hrs at its double field dose that resulted in an efficacy of 87.61% controlling disease. The activities of peroxidase, catalase and polyphenoloxidase in the infected plants were markedly reduced in the treated infected plants. Applying tested compounds to the infected plants significantly increased total carbohydrate, total soluble protein and total phenols comparing to untreated infected plants. So it could be concluded that the used compounds could resist the detrimental effects of *Botrytis fabae* on the plant growth and yield.

Key words: Inducers compounds, Faba bean plants, Chocolate spot disease**.**

INTRODUCTION

Faba bean is considered the world's fifth food legume after dry bean, dry pea, chickpea and lentil (Adak *et al.,* 1998). This is due to its high nutritive value in both energy and protein contents. Therefore, increasing the plant crop production is one of the major targets of the agricultural policy in several countries.

In Egypt, faba bean is one of the main crop grown for seed; so it is an important food legume crop being the staple diet due to its high nutritive value (Nassib *et al.,* 1991). The annual faba bean production in Egypt is ranging from 0.56 to 0.58 million metric ton during 2003-2005. However, this production is not enough to meet domestic demand (ICARDA, 2005). Egypt is of the first dry broad bean importing countries; its importation ranged from 0.23-0.3 million metric tons at the last five years. This is a result for the huge consumption, limited area for growing faba bean and its high acceptability for many foliar diseases.

Chocolate spot disease of faba bean caused by *Botrytis fabae* was the most important limiting factor which causes great annual losses and sometimes complete crop failures (Koike, 1998).It is considered the most destructive disease affecting the crop causing serious damage to the plant and consequent decrease of the seed yield production especially in North Egypt (Abou Zeid and Hassanein, 2000).

At the last few years, many great efforts were carried out to save the environment from pollution. Application of pesticides is considered one of the most famous environmental pollutants (Dubey and Mall, 1972). Moreover, it causes several problems such as carcinogenicity (Epstein *et al.,* 1967), long persistence period (Beye, 1978), teratogenicity (Javoraska, 1978) and phytotoxicity (Ismail *et al.,* 1996).

These factors emphathsize the need for new methods to control diseases (Wilson *et al.,* 1987).

Ryals and Ward (1994) mentioned that all plants have the ability to defend themselves against pathogenic infection through a wide variety of mechanisms that can be either local or systemic, constitutive or inducible. These systemic acquired resistance (SAR) is a pathogen-inducible defense mechanism that dependent on salicylic acid (SA) and is associated with a system expression of a subset of defense genes, e.g. the acidic form of pathogenesis-related (PR) proteins (Ward *et al.,* 1991). Inducers are used to initiate defence reactions as they denote something that can activate the plant's defensive mechanisms. There are many different types of inducers including microorganisms (fungi, bacteria), certain chemicals, plant extracts and even ultraviolet light (Obradovic and Jones, 2005), which can be used more safely than chemicals.

If a suitable inducer is identified, it may help in protecting a plant against different types of diseases, caused by different kinds of pathogens (fungi, virus and bacteria). Furthermore, with the right inducer, it is possible to activate the defence in all cultivars of the plant, even the most susceptible. This is because many different kinds of defense reactions are activated by induced resistance compounds, some of which will be effective against the different kinds of pathogens, in all cultivars (Sticher *et al.,* 1997).

In this respect, Strobel and Kuc (1995) stated that peroxidant chemicals were used to evaluate the hypotheses that localized oxidative damage, which culminates in necrosis, can induce systemic acquired resistance (SAR). El-Hawa (1998) studied the effect of foliar application of hydroxyphaseolli (HP) on *Vicia faba* against chocolate spot disease (*B. faba*). He suggested that HP stimulated the formation of phytoalexins specific to the host in *V. faba*, in sufficient quantities that inhibit the parasite.

Csosze *et al.* (1999) explained that Bion 50 WG is a member of a novel class of inducers of systemic acquired resistance that activates gene expression and disease resistance. Field trials were carried out in which Bion 50 WB was applied to 50 varieties of wheat to investigate its ability to protect the plant against *Erysiphe graminis* f.sp. *tritici.* Bion 50 WB induced greater resistance than triadimefon (as Bayleton 25 WP). Oostendorp *et al*., (2001) revealed that the best-characterized signal pathway for systemically induced resistance is SAR that is activated by localized infections with necrotizing pathogen. It is characterized by protection against a broad range of pathogens through formation of set of induced proteins and by its dependence on salicylic acid (SA). They also suggested that the best-studied resistance activator is acibenzolar-5-methyl (Bion). In other work, Abou-Taleb (2001) mentioned that an aryl compound (benzylthydro peroxide) accumulated in cucumber plants after treatment with Bion 50 WG inhibited conidial germination and germ-tube growth of cucumber scab fungus *in vitro*.

The objective of this research is to prove that commercial inducer resistance can be used as alternative to fungicidal application to avoid its hazardous effect. Therefore, this study aimed to: (1) studying the role of selected compounds to reduce the faba bean chocolate spot disease, (2) finding an explanation for this role based on test trials, (3) evaluating the enhancement of the plant yield, (4) finding recommendation for controlling the fungal disease.

MATERIALS AND METHODS

The present work was carried out in Botany Department, Faculty of science, Tanta University, Tanta, Middle Delta, Egypt during 2005 and 2006. Grains of *V. faba* cultivar Giza 402, obtained from

the Agricultural Research Center (Giza, Egypt) served as sensitive host plants for *Botrytis fabae* causing chocolate spot disease of beans.

Preparation of *B. fabae* spore suspension:

The stock cultures of the isolated pathogen; Botrytis fabae (Sard.) was compared with a reference strain gifted from the Agricultural Research Center. Pathogenicity incoulum was prepared by growing on PDA medium for 5 days, then the fungus was homogenized and the spores were adjusted to be 2.5 x 106 CFU ml-1.

Tested inducers:

Four inducers were used throughout the present work. Table (1) illustrates the commercial and common names, chemical composition, and application rates of each inducer compound.

Table 1: Induces compounds used for the control of the studied diseases

Commercial name	*Common name*	*Chemical composition*	*Application rate*	
			Field dose (F.D)	**Double F.D.**
Boric acid	Orthoboric acid	H_3BO_3 or $B(OH)_3$	3 g/L	6 g/L
Starner	Starner	Oxalinic acid (20%) WP	2 g/L	4 g/L
Bion 50 wg (Bio.)	Acibenzolar-s-methyl.	3 benzol (1,2, 3) thia-diazole-7-carbothioic acid S methyl ester	0.18 g/L	0.36 g/L
Salicylic acid	Salicylic acid	O-hydroxybenzoic acid	0.32 g/L	0.64 g/L

Greenhouse experiment:

Greenhouse experiments was conducted to evaluate the effect of the tested compounds on susceptibility of faba bean plants (Giza 402) to chocolate spot disease , growth ,yield and some physiological activities of *V.faba* infected with *B.fabae*.The experiment was carried out in plastic pots (50 cm diameter) containing field soil (4 kg. soil / pot).

A) Shoot treatment:

Seeds of *V. faba* (cv. Giza 402) were disinfected in 2% (v/v) Na-hypochlorite for 10 min. followed by washing with sterile distilled water. Ten seeds were sown per pot (10 seeds /pot), and then thinned to three at 15 days after sowing. The sowing date was conducted on the 2nd of June (2005) and the experiment was continued for about 3 months. Pots were irrigated with tap water whenever needed but with equal amounts. NPK fertilizers were applied at rates of 0.6 gm. urea, 0.75 gm. Ca-super-phosphate and 0.25 g K-sulphate per pot. Phosphorus was added during soil preparation (before sowing). Each of N and K was applied in two equal doses, at thinning and two weeks after thinning.Faba bean plants were infected by spraying 30 ml of *B. fabae* water spore suspension, containing (2.5×10^6 spores ml^{-1}) with 1% Tween 80 by means of atomizer until the run-off point onto shoot of 30 days-old bean plants.. Thereafter, plants in each pot were left to be air-dried, sprayed with 15 ml distilled water and covered with plastic bags for two hours to maintain high humidity atmosphere around the leaves which is necessary for fungal infection, then infected plants were exposed to three successive sprays within 15 days intervals by each of the tested chemical compounds at both the field dose and double field dose (Table 1) at the rate of 30 ml/pot (3 pots were used for each dose).

The positive control plants were sprayed only with *B. fabae* spore suspension without any treatment, while the negative control plants treated with the chemicals compounds at each dose and left without any infection.

B) Seed treatment:

Faba bean seeds (*cv*. Giza 402) were treated with the tested chemical compounds by soaking at both field dose and double field dose separately (Table 1) for 24 hrs. Untreated control seeds were sown in the soil without any treatment serve as positive control. Then treated and untreated seeds were planted in plastic pots (50-cm.diameter) filled with field soil. After plants emergency, leaves were inoculated with *B. fabae* spore suspension and four pots left without infection serve as negative control. When chocolate spots appeared Abou-Zeid *et al*., (1979), examined the inoculated plants for spot score using the devised scale. Disease severity was calculated every 15 days using the following equation:

$$\% \text{ disease severity} = \frac{(n \times v)}{gN} X100$$

Where: n = number of plants in every sequence (v)

N = total number of examined plants.

G = maximum disease grade.

Then efficacy percentage (E %) of each compound in reducing disease, severity percentage of faba bean was assessed according to the equation adapted by Rewal and Jhooty (1985) as follow:-

$$\mathbf{E\ \%} = \frac{\%\text{disease severity in control} - \%\text{disease severity in treatment}}{\%\text{Disease severity in control}}$$

Growth criteria:

To determine the effect of different tested compounds on the growth parameters and seed yield of faba bean plants, the following parameters were measured for every treatment. At 2-month old, the numbers of flowers were counted for every plant. At 3-months old, plant samples were collected and were separated into shoots, leaves and pods. Shoot, length and fresh weight were measured, then leaves were oven dried at 70^{o}C to constant weights, then dry weight were recorded. The number of leaves and pods for every plant were also counted.

Metabolites Concentration:

Collected faba bean leaves for each treatment were prepared for the measurement of certain metabolites (total carbohydrate, phenol, and soluble protein) and some enzymes activities (catalase, peroxidase and polyphenol oxidase).The collected leaves were divided into two groups:

1-The first group was dried in oven at 60^{o}C to a constant weight then grinded to a fine powder and stored for the measurement of certain metabolites; (total carbohydrates and phenols).

2-The second group of collected leaves from every treatment were homogenized separately in a mortar with 0.1 M. sodium phosphate buffer at pH 7.1 at the rate of 2 ml/gm fresh weight leaves for 1 minute. Then these triturated tissues were strained through four layers of cheesecloth and the filtrates were centrifuged at 3000 rpm. for 20 minute at 6^{o}C.The clear supernatant was collected and considered as a crude extract for total soluble protein estimation and enzymes assay. Total carbohydrate was evaluated by anthrone method (Hodge and Hofreiter, 1962); Total phenols were estimated according to Malick and Singh (1980) method**,** while total soluble proteins were estimated by Bradford's method (1976).

Enzyme Assay

Peroxidase, catalase and pectinase enzymes were assayed at 26^{o}C and expressed as units in mg/ protein, where one unit is defined as the amount of enzymes converting one mole of substrate to product during 1 min.

Catalase enzyme:

Catalase enzyme was assayed according to the method of Kato and Shimizu (1987**)**.In the sample cuvette 0.1 ml crude extract was mixed with 0.5 ml. 0.2 M. sodium phosphate buffer (at pH 7.6) and 0.3 ml 0.5% H_2O_2, then the mixture brought to a final volume of 3 ml

with distilled water. The breakdown of H_2O_2 was followed by measuring the absorbance at 240 nm. Moreover, the enzyme activity was calculated according to the following equation:

Enzyme activity [unit (mg. protein)$^{-1}$] = K x (ΔA/min.).

Where: K (extension coefficient) is 40 mM / cm at 240 nm for H_2O_2.

ΔA/min. is the change in absorbency per minute.

Peroxidase assay:

Peroxidase activity was spectrophotometrically determined by measuring the oxidation of tetraguciaol in the presence of H_2O_2 at 470 nm. according to Kato and Shimizu (1987**)**. In the sample cuvette containing 0.5 ml. of 0.1 M. sodium phosphate buffer (pH 5.8), 0.3 ml. of 7.2 mM. tetraguaicol, 0.1 ml. of 11.8 mM. H_2O_2 and 0.3 ml crude extract were mixed and completed by distilled water to a final volume of 3.0 ml. The oxidation of tetraguciaol was followed by measuring the absorbance at 470 nm and the enzyme activity was calculated according to the following equation:

Enzyme activity [unit (mg. protein)$^{-1}$] = K x (ΔA/min.).

Where: K (extension coefficient) is 26.6 mM / cm at 470 nm for tetraguaiacol.

Polyphenoloxidase:

Polyphenoloxidase activity was determined according to Esterbaner *et al*., (1977). The reaction mixture contained 2 ml. enzyme extract, 1.0 ml. of 10^{-3} M. catechol and 1.0 l of 0.2 M. sodium phosphate buffer (at PH = 7) then the reaction mixture was brought to a final volume of 6.0 ml with distilled water. The activity of polyphenoloxidase was expressed according following equation:

Enzyme activity [unit (mg. protein)$^{-1}$] = K x (ΔA/min).

Where**: K** (extension coefficient) is 0.272 mM / cm at 490 nm for catechol. ***ΔA** is the change in the absorbance of the mixture every 0.5 minute for 5 minutes period at 490 nm.

RESULTS

Effect of the tested inducer compounds on faba bean chocolate spot incidence:

A) Shoot treatment experiments:

Table (2) illustrated the effect of certain growth inducer compounds used as foliar spray on faba bean chocolate spot disease severity under greenhouse conditions and indicated that the Boric acid at both field and double dose was the most effective compound against disease severity followed by Bion and then Salicylic acid. The efficacies of Boric, Salicylic, Bion and Starner at field dose were 71.9%, 71.29%, 66.98% and 83.99%, respectively.

The statistical analysis in table (2) was carried out using T-test which indicated that the variation in chocolate spot disease severity (%) was highly significant for F.D. and D.F.D. in the case of Boric acid, Bion and Salicylic acid ($P \leq 0.01$), while it was not significant in the case of Starner treatment.

B) Seed treatment experiments:

Results presented in Table (3) illustrated that, faba bean seeds treated with Boric acid at both field and double field dose, was more resistant to *B. fabae* infection followed by Bion, Salicylic acid and starner. They achieved disease-controlling efficacy at field dose of 83.59%, 78.16%, 71.9% and 70.09%, respectively. From Tables (2) and (3) it is evident that faba bean seeds treated with the tested inducer compounds before planting in the soil led to significant decreased in chocolate spot disease severity compared to chemicals compound foliar spray application As a step to control the pathogen infection before disease development, to applying the compounds as foliar spray and also to the untreated plants.

Table 2: Effect of certain growth inducer compounds used as foliar spray on faba bean chocolate spot disease severity under greenhouse conditions.

Treatments	***F.D***	*Disease severity percentage*	*Efficacy*	*T-test*	
	D.F.D		*Percentage*	**T-value**	**P-value**
Boric	**3.0**	**9.3±0.265**	***71.9***	**13.58**	**0.00****
acid	**6.0**	**5.3 ±0.364**	***83.99***		
Starner	**2.0**	**15.23 ±1.903**	***53.99***	**2.26**	**0.08**
	4.0	**12.63 ±0.568**	***61.84***		
Bion	**0.18**	**10.93 ±0.153**	***66.98***	**14.9**	**0.00****
	0.36	**6.63 ±0.473**	***79.97***		
Salicylic	**0.32**	**9.5 ±0.854**	***71.29***	**5.39**	**0.006****
acid	**0.64**	**6.33 ±0.551**	***80.88***		
Infected untreated plants		**33.1 ±1.433**			**----------**
Uninfected untreated plants		**11.4 ±0.844**		**----------**	
Treatments	**F.D**	Disease severity percentage	Efficacy	T-test	
	D.F.D		Percentage	**T-value**	**P-value**
Boric	**3.0**	**9.3±0.265**	***71.9***	**13.58**	**0.00****
acid	**6.0**	**5.3±0.364**	***83.99***		
Starner	**2.0**	**15.23±1.903**	***53.99***	**2.26**	**0.08**
	4.0	**12.63±0.568**	***61.84***		
Bion	**0.18**	**10.93±0.153**	***66.98***	**14.9**	**0.00****
	0.36	**6.63±0.473**	***79.97***		
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Starner	**2.0**	**15.23±1.903**	***53.99***	**2.26**	**0.08**
	4.0	**12.63±0.568**	***61.84***		
Bion	**0.18**	**10.93±0.153**	***66.98***	**14.9**	**0.00****
	0.36	**6.63±0.473**	***79.97***		
Salicylic	**0.32**	**9.5±0.854**	***71.29***	**5.39**	**0.006****
acid	**0.64**	**6.33±0.551**	***80.88***		
Infected untreated plants		**33.1±1.433**		**----------**	
Uninfected untreated plants		**11.4±0.844**		**----------**	

F.D = Field dose. D.F.D = Double field dose. * Significant at P ≤ 0.05 ** Highly Significant at P ≤ 0.01

Table 3: Effect of faba bean seeds soaking in certain growth inducers compounds on faba bean chocolate spot disease severity under greenhouse conditions.

Treatments	***F.D***	Disease severity percentage	*Efficacy*	*T-test*	
	D.F.D		*Percentage*	**T-value**	**P-value**
Boric	**3.0**	**5.43 ±0.193**	***83.59***	**10.00**	**0.001****
acid	**6.0**	**4.1 ±0.1**	***87.61***		
Starner	**2.0**	**9.9 ±0.624**	***70.09***	**2.088**	**0.105**
	4.0	**9.0 ±0.3**	***72.81***		
Bion	**0.18**	**7.23 ±0.231**	***78.16***	**8.459**	**0.001****
	0.36	**5.13 ±0.252**	***84.5***		
Salicylic	**0.32**	**9.3 ±0.436**	***71.9***	**2.640**	**0.058**
acid	**0.64**	**8.4 ±0.3**	***74.62***		
Infected untreated plants		**33.1 ±1.433**		**----------**	
Uninfected untreated plants		**13.7 ±0.844**		**----------**	

F.D = Field dose. D.F.D = Double field dose. * Significant at P ≤ 0.05 ** Highly Significant at P ≤ 0.01

The T-test statistical analysis in Table (3) indicated that the variation in chocolate spot disease severity (%) was highly significant for F.D. and D.F.D. in the case of Boric acid and Bion (P ≤ 0.01), while it was not significant in the case of Salicylic acid treatment.

Plant growth characteristics

Table (4) illustrates that treating faba bean plants with tested compounds significantly improved all tested parameters compared to the untreated infected plants and all parameters increased by increasing tested compounds field dose. In the case of shoot treatment, Salysilic acid was the most effective for all tested parameters (shoot length, shoot fresh weight and dry weight, leaves and flowers number and pods number) at both F.D. and D.F.D., while in the case of seed treatment, Bion achieved a good faba bean shoot length, shoot dry weight ,flowers number and pods number. In the meantime, Boric acid and Salicylic acid were the best for the plants fresh weight and leaves number, respectively.

Table 4: Effect of growth inducers on the growth parameters of infected faba bean (*B.fabae*).

	Treatment	*F.D*	*Shoot system*				*Flowers no. per plant*	*Pods no. per plant*
		D.F.D	Length (cm. plant^{-1})	*Fresh weight(gm. plant^{-1})*	Dry weight (gm. plant^{-1})	Leaves no. per plant		
Shoot treatment	**Boric acid**	3.0	**39.2** ±1.1	**9.9** ±0.77	**3.7** ±0.6	**16.** ±2.1	**3.0** ±0.0	**1.5** ±0.7
		6.0	**42.4** .7	**10.4** ±0.64	**4.2** ±0.1	**20.** ±1.4	**4.0** ±0.5	**1.5** ±0.1
	Starner	2.0	**30.8** ±0.9	**7.6** ±0.14	**3.0** ±0.1	**15.** ±2.1	**2.0** ±0.0	**1.0** ±0.7
		4.0	**32.0** ±3.9	**8.3** ±0.35	**.55** ±0.0	**16.**	**3.0** ±0.5	**1.5** ±0.0
	Bion	0.18	**33.3** ±0.1	**10.7** ±0.44	**.85** ±0.09	**17.** ±0.7	**3.5** ±1.4	**1.0** ±0.0
		0.36	**39.3** ±1.7	**13.2** ±0.76	**.45** ±0.34	**21.** ±1.4	**4.5** ±0.7	**1.5** ±1.4
	Salicylic acid	0.32	**43.8** ±1.6	**10.6** ±0.28	**.39** ±0.12	**18.** ±0.0	**4.0** ±0.5	**2.0** ±0.0
		0.34	**43.8** ±0.2	**12.8** ±0.64	**.25** ±0.2	**22.** ±1.4	**6.0** ±1.4	**2.5** ±0.7
Seed treatment	**Boric acid**	3.0	**45.8** ±3.4	**12.9** 0.5	**5.3** ±0.3	**23.** ±0.0	**4.0** ±0.4	**2.0** ±0.0
		6.0	**52.6** ±4.0	**13.9** 2.3	**5.8** ±0.4	**25.** ±0.1	**5.0** ±0.0	**3.5** ±0.0
	Starner	2.0	**23.9** ±1.5	**6.9** 0.7	**3.6** ±0.1	**16.** ±0.7	**2.0** ±0.1	**1.0** ±0.0
		4.0	**28.6** ±2.0	**9.4** 0.9	**3.6** ±0.3	**16.** ±0.9	**2.5** ±0.0	**1.0** ±0.0
	Bion	0.18	**52.0** ±2.6	**14.4** 0.8	**6.35** ±0.1	**24.** ±0.	**4.5** ±0.5	**2.5** ±0.0
		0.36	**54.3** ±3.0	**16.1** 0.7	**6.2** ±0.2	**26.** ±0.	**6.0** ±0.0	**4.0** ±0.0
	Salicylic acid	0.32	**38.6** ±2.0	**12.4** 0.4	**3.7** ±0.5	**24.** ±0.	**4.0** ±0.1	**1.0** ±2.1
		0.34	**43.2** ±1.9	**13.8** 0.5	**4.2** ±0.2	**26.** ±0.	**5.0** ±0.3	**3.0** ±0.7
Infected untreated plants			**33.9**).8	**10.5**).49	**2.8**).3	**15.0** 4	**0.0**).0	**0.0** ±0.0
Uninfected untreated plants			**39.8** 2.06	**11.8** 1.04	**3.3** ±0.3	**16.0** ±0.4	**1**).0	**0.0** ±0.0

Metabolites Accumulation in Plant Leaves

The data in Table (5) revealed that *B.fabae* had reduced all tested faba bean metabolites, while the tested compounds had led to increasing all tested metabolites. The most effective compounds are represented as fellows:

Using Shoot treatment: Treating faba bean plants with a foliar application of Bion at D.F.D. had led to increasing all tested metabolites; 0.805 mg/ml total carbohydrates, 0.345 mg/ml total soluble protein and 2.04 mg/100 gm total phenols (Table 5).

Using Seed treatment: Soaking faba bean seeds in Boric acid at D.F.D. before planting had led to increase in the tested metabolites; 0.838 mg/ml total carbohydrates, while soaking faba bean seeds in Bion increase both faba bean total soluble proteins (0.306 mg/ml) and total phenols (2.819 mg/100 gm) (Table 5).

Plant Defense against Spot Development indicated by tested enzymes:

Catalase, peroxidase and polyphenoloxidase of the infected faba bean plants were determined as affected by both treatments (shoot & seed treatments). The tested enzymes reached the highest levels in infected untreated faba bean leaves**.** The results in Table (6) showed that the activities of all tested enzymes recorded the lowest levels in

infected plants treated with different compounds. The most effective compounds are represented as fellows:
Using shoot treatment: Application of Salicylic acid had led to a significant decrease in catalase activity at D.F.D. (1.49 unit (mg/ protein)$^{-1}$), while Bion achieved the lowest peroxidase and polyphenoloxidase activities at D.F.D. (0.69 and 0.24 unit (mg/ protein)$^{-1}$, respectively).
Using seed treatment: The data revealed that treating faba bean seeds with Bion at D.F.D. before planting had led to a decrease in all tested enzymes activities; 0.89 unit (mg/ protein)$^{-1}$ catalase, 0.30 unit (mg/ protein)$^{-1}$ peroxidase and 0.21 unit (mg/ protein)$^{-1}$ polyphenoloxidase.

Table 5: Effect of certain growth inducers on some metabolic aspects of faba bean leaves infected with *B. fabae*.

	Treatment	F.D / D.F.D	Total carbohydrates (mg / ml)	Total Soluble protein (mg / ml)	Total Phenols (mg /100gm)
Shoot treatment	Boric acid	3.0	0.541 ±0.006	0.257 ±0.007	0.816 ±0.034
		6.0	0.644 ±0.023	0.288 ±0.008	1.814 ±0.062
	Starner	2.0	0.346 ±0.006	0.175 ±0.006	0.662 ±0.031
		4.0	0.379 ±0.006	0.229 ±0.057	0.735 ±0.021
	Bion	0.18	0.569 ±0.034	0.323 ±0.035	1.289 ±0.016
		0.36	0.805 ±0.022	0.345 ±0.036	2.042 ±0.12
Seed treatment	Salicylic acid	0.32	0.408 ±0.017	0.27 ±0.013	0.694 ±0.009
		0.34	0.454 ±0.052	0.311 ±0.012	1.301 ±0.035
	Boric acid	3.0	0.811 ±0.206	0.27 ±0.008	1.33 ±0.025
		6.0	0.838 ±0.009	0.29 ±0.013	1.972 ±0.039
	Starner	2.0	0.466 ±0.026	0.195 ±0.002	0.977 ±0.033
		4.0	0.536 ±0.107	0.215 ±0.011	1.041 ±0.039
	Bion	0.18	0.486 ±0.006	0.279 ±0.006	1.66 ±0.098
		0.36	0.568 ±0.029	0.306 ±0.011	2.819 ±0.115
	Salicylic acid	0.32	0.469 ±0.047	0.26 ±0.011	1.582 ±0.472
		0.34	0.541 ±0.047	0.271 ±0.004	2.056 ±0.078
Infected untreated plants			0.148 ±0.008	0.12 ±0.017	0.329 ±0.019
Uninfected untreated plants			0.195 ±0.07	0.17 ±0.013	0.413 ±0.047

Table 6: Effect of certain growth inducers on some enzyme activities [unit (mg protein^{-1})] of faba bean leaves infected with *B. fabae* under greenhouse conditions.

	Treatment	F.D. / D.F.D	Catalase	Peroxidase	Polyphenoloxidase
Shoot treatment	Boric acid	3.0	2.44).19	1.01 .09	0.32).025
		6.0	1.54).22	0.74 .01	0.32).047
	Starner	2.0	5.61).57	1.42 .16	0.44 0.07
		4.0	4.71).79	1.15 .11	0.43 0.06
	Bion	0.18	3.23 0.2	0.93 .06	0.32).036
		0.36	2.44).19	0.69 .03	0.24 0.055
Seed treatment	Salicylic acid	0.32	1.75).62	0.98 .05	0.35 0.04
		0.34	1.49).36	0.72 .02	0.27).015
	Boric acid	3.0	1.36 0.16	0.64 .03	0.24).021
		6.0	1.09 0.06	0.34).0	0.23).038
	Starner	2.0	5.23 1.39	1.12 .04	0.36).035
		4.0	4.35 0.47	0.91 .05	0.32).066
	Bion	0.18	1.09 0.49	0.54 .02	0.24 0.0252
		0.36	0.89 0.14	0.3 .03	0.21).021
	Salicylic acid	0.32	2.0 0.79	0.6 .07	0.27 0.01
		0.34	1.1 0.27	0.58 .05	0.26).015
Infected untreated plants			6.75).22	4.08 .03	0.79 .078
Uninfected untreated plants			3.5).38	1.7 04	0.57 .03

DISCUSSION

Faba bean cultural practice modifications using fungicides provided only partial crop protection without attention to subsidiary adverse effects of fungicides on the host plant. Chocolate spot disease caused by *Botrytis fabae* is considered one of the most serious diseases that devastates faba bean plantation in many parts of the world (Koike, 1998), as well as in Egypt (Heweidy, 1998). It is individually quite destructive and damaging disease due to its interaction with rust; yellow mosaic and/or bean leaf roll viral diseases (Omar *et al.,* 1985).

The present investigation concluded that the tested inducer compounds were highly effective in controlling chocolate spot disease incidence. However when applied as seed treatment before planting they were more effective than applied as foliar application, expect salicylic acid, which exhibited a high efficacy in controlling chocolate spot disease when applied as foliar spray on faba bean shoot system than seed treatment. Attitalla *et al.* (1998) found that applying salicylic acid to the root systems 3 weeks after sowing reduced disease incidence in plants which had been infected with *Fusarium* sp. Genetically Csosz *et al.* (1999) reported that Bion 50 WG as an inducer of systemic acquired resistance activated gene expression and disease resistance and it protected 50 varieties of wheat against *Erysiphe graminis f*.sp. *tritici.* Based on these conclusions new prospective were opened in the use of alternatives other than fungicides for controlling plant diseases. Inducer compounds indirectly control pathogens by inducing the internal defense system of plant. In addition, Ding *et al.,* (2002) suggested that the pre-treatment of tomato fruit with methyl salicylate induce the synthesis of some stress proteins, such as PR-proteins, which leads to increased chilling tolerance and resistance to pathogens and then decreasing the incidence of decay. When a pathogen attempts to infect such an induced plant, it will defend itself faster and stronger than a non-induced plant (Bakker *et al.,* 2003). Moreover, Gozzo (2004) mentioned that, the central role of salicylic acid (SA) as a signal transducer of systemic acquired resistance (SAR) was demonstrated in transgenic plants where it could not be detected. Later, Obradovic and Jones (2005) revealed that plants responded to a local infection treated with salicylic acid had led to systemic expression of broad spectrum and long-lasting disease resistance that is efficient against fungi, bacteria and viruses.

In the present work, it could be stated that most of the studied chemicals exerted stimulatory effect on faba bean plant growth and this effect increased with increasing inducer compounds comparing to untreated infected plants. These observations are in accordance with Hammad *et al.,* (2001) who investigated the effect of *B. fabae* on the growth parameters of *Vicia faba* and mentioned that the pathogen caused reduction in plant growth (shoot length, number of leaves, fresh and dry weight of shoot and root, and root length) and the mean values of growth parameters in case of the resistant variety were higher than those of the susceptible one. However Ismail (2004) studied the effect of nine chemicals compounds on the growth parameters of cotton plants after 45 days from planting under greenhouse conditions and found that Bion compound improved all tested growth parameters compared with untreated control and Starner enhanced height of cotton plants while it diminished the other growth parameters like fresh and dry weight of both shoot and root systems.

In addition, it is evident from the present data that all the tested chemical

compounds surpassed the control of all tested metabolic aspects. This could be attributed to the effect of these compounds, which may increase total carbohydrate content via increasing chlorophyll content, and the IAA hormone (Indol Acetic Acid) by inhibiting the enzyme IAA oxidase. This leads to the inhibition of chlorophyll breakdown as IAA protects chlorophyll, and thus increasing in chlorophyll content may increase the amount of CO_2 fixed during photosynthesis hence carbohydrates accumulation may also increase, as was reported by Krishnamoorthy (1981) and Abou Grab *et al.* (1997). Moreover, Ismail (2004) studied the effect of Bion and Starner on cotton plants leaves chlorophyll under the field conditions and reported that Bion increased chl. b, while Starner application led to a lower content of chl. b than the control. In the meantime, Mubarak (2003) showed that the best effective treatment which increased the content of *Phaseolus vulgaris* total carbohydrates content was salicylic acid 11.192 mg./100 gm in case of soil infested by *R. solani* + *M. phaseolina*.

Positive effects of all tested chemical compounds on soluble protein were mirrored on chocolate spot disease severity; increasing in total soluble protein may be involved in the induced resistance resulted from the application of the tested compounds. Similar results were obtained by Tuzun *et al.,* (1989) who studied induced systemic resistance to blue mold using some commercial inducers compounds i.e. fungi stop, photophore, Ecophote and Bion. They found that considerable increases of total protein were observed after the first and second spray of toppaco and suggested that these protein fraction or at least some of them were involved in the induced resistance resulted from the application of the tested compounds. In the meantime, Raskin (1992) reported that families of pathogenesis-related (PR) proteins were commonly associated with systemic resistance. Abou-Taleb (2001) reported that there is a considerable increase in the number of induced protein fractions that observed after the first and second spray with Bion compound. These increases ranged from 70.8% after the first spray to 96.6% after the second spray.

Phenols have been recorded to offer resistance to diseases and pests in plants, and grains containing high amount of polyphenols are resistant to several plant diseases (Malick and Singh, 1980).

The present investigation also revealed that all tested compounds recorded lowest activities of all tested host enzymes; catalase, peroxidase and polyphenoloxidase. The results also indicated that the activities of tested enzymes were higher in the leaves extracts of more diseased plants than less diseased plants. Generally, investigations of pathogen-host interaction problems often encountered where a number of factors play a part. The high activities of tested host enzymes recorded in infected untreated plants could be considered as antioxidant mechanism for protecting plants detrimental effects of pectinase on the plant cell walls. The close relationship between the rate of faba bean cell wall breakdown and the rate of cell injury supports the view that the cell wall breakdown is responsible for cell death (Basham and Bateman, 1975). Activities of oxidative enzymes in any infected plant tissues are known to contribute to disease resistance mechanisms through the oxidation of phenols (Tarrad *et al.,* 1993). The same authors reported that peroxidase activities increased in faba bean leaves that were infected by *B. fabae* compared to healthy leaf tissues; while polyphenoloxidase activities were not affected significantly. The increase in peroxidase and catalase activities in the infected untreated faba bean plants reflects the plant response to disease, where this increase could be higher

around the penetration sites of pathogen. The obtained results indicated significant differences in the activity of oxidative enzymes that in turn could influence the oxidation of phenolic compounds such as quinons as well as the accumulation of free radicals. It is well known that high levels of quinone are highly toxic for plants and inactivate the pectic enzymes secreted by the pathogen. In the present work, all tested inducer compounds affected the activity of oxidative enzymes. Nawar and Kuti (2003) mentioned that, peroxidase activity in leaves of resistant cultivars of broad bean infected with *Botrytis fabae* was 10 times higher than that of susceptible cultivars and eight times higher in uninfected leaves of the resistant than the susceptible cultivars. Later, Gozzo (2004) reported that Salicylic acid might interact with iron-based enzymes, either as a chelator of the metal ion or through binding to related proteins. The plants forming phenolic free radicals, resulting from the interaction with catalase or peroxidase has proposed to be involved in the induction of systemic acquired resistance (SAR).

REFERENCES

Abou-Grab,O.S.; Kady, F.A. and El-Kafrawy, A.M. (1997). Effect of some aromatic compounds as antitranspiration on growth yield, chlorophyll content, water consumptive use and leaf blight disease for maize plant. Zagazig J. Agric. Res., 24(3): 393-406.

Abou-Taleb, M. A. Mona (2001). Biochemical changes associated with the application of some resistance inducing compounds for controlling powdery mildew of cucumber. Egypt. J. Appl. Sci., 6(12): 387-405.

Abou-Zeid, N. M. and Hassanein, A.M. (2000). Biological control of chocolate spot disease (*Botrytis fabae* Sard.) in faba bean in Egypt. J. Plant Pathology Res. Inst., ARC, Giza, 90(6): 385-391.

Abou-Zeid, N. M. and Le-Normand, M. (1979). Variatal variability measurement of *Vicia faba* susceptibility to *Botrytis fabae* by a rapid method. ISSN0003-4177, 11(1): 134-135.

Adak, M. S.; Ulukan, H. and Guler, M. (1998). Determination of some agronomic traits in Turkish faba bean (*Vicia faba* L.) Lines. FABIS, News Letter, 42: 29-31.

Attitalla, I.; Quintanilla, P. and Brishammar, S. (1998). Induced resistance in tomato plants against *Fusarium* wilt invoked by *Fusarium* sp., Salicyclic acid and *Phytophthora cryptogea*. Acta Phytopathologica et Entomologica Hungarica, 33(1-2): 89-95.

Bakker, P.A.H.M.; Ran, L.X.; Pieterse, C.M.J. and Van Loon, L.C. (2003). Understanding the involvement of *Rhizobacteria* mediated induction of systemic resistance in biocontrol of plant diseases. Can. J. Plant Pathol., 25: 5-9.

Basham, H.G. and Bateman, D.F. (1975). Killing of plant cells by pectic enzymes. The lake of direct injurious interaction between pectic enzymes or their soluble reaction products and plant cells. Phytopathology, 65: 141-153.

Beye, F. (1978). Insecticides from the vegetable kingdom. PI. Res. Dev. 7: 13-31.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem., 72: 248-254.

Csosz, L.; Tyihak, E. and Manninger, S. (1999). First experience with Bion as a synthetic plant activator. Novenyvedelem, 35(7): 327-337.

Ding, C.K.; Wang, C.Y.; Gross, K.C. and Smith, D.L. (2002). Jasmonate and salicylate induce the expression of pathogenesis-related protein genes and increase resistance to chilling injury in tomato fruit, Planta J. 214(6): 895-901.

Dubey, S.C. (2000). Biological management of web blight of groundnut (*Rhizoctonia solani*). Mycol. and Plant Pathol. J. 30(1): 89-90.

El-Hawa, M.A. (1998). Induction of fungal disease resistance in leguminosae. Egyptian J. of Microbiol., 33(1); 147-154.

Epstein, S. S.; Andreae, M.; Jaffec, H.; Joshu, S.; Folk, H. and Natnel, N. (1967). Carcinogenicity of the herbicide

maleic hydrazide. Nature, 215: 1388-1390.

Esterbaner, H.; Schwarzl, E. and Hayn, M. (1977).In: Principles of Biochemistry (Publisher: Freeman. W. H.), 477: 486.

Gozzo, F. (2004). Systemic acquired resistance in crop protection. Outlooks on Pest Management, 20-23. pp.

Guillon, M.; Bompeix, G.; Leconte, F. and Dufretay, G. (1998). Development of an elicitor against fungal plant diseases. 21st, 22nd and 23rd January, 1998-Lille (France).

Hammad, I. A.; Migahed, F.F. and Nofel, A.M.M. (2001). Effect of *B. fabae* and lithium chloride on mitotic cell division and morphology of *Vicia faba*. African J. of Mycol. and Biotechnol., 9(1): 45-61.

Heweidy, M.A.M. (1998). Pathological studies on chocolate spot disease of faba bean (*Vicia faba* L.). Egypt. J. Appl. Sci., 13(5): 88-105.

Hodge, J.E. and Horfreiter, B.T. (1962). In: Methods in carbohydrate chemistry (eds. Whistler, R.L. and Be Miller, J.N.)Academic Press, New York.

ICARDA (2005). Faba bean pathology progress report 2003/04. Food Legume Improvement Program, ICARDA, Aleppo., Syria.

Ismail, A. A. (2004). Phytotoxic effects of some growth inducers, fungicides, and a biocide on cotton seedlings under several conditions. Agric. Res. J. Tanta Univ., 30(1): 132-146.

Javoraska, T. (1978). Effect of combined herbicides on the occurrence of morphoses in the spikes of spring barley. Agrochemia, 18: 37-42.

Kato, M. and Shimizu, S. (1987). Chlorophyll metabolism in higher plants. VII. Chlorophyll degradation in senescing tobacco leaves; phenolic-dependent peroxidative degradation. Can. J. Bot., 65: 729-735.

Koike, S.T. (1998). Severe outbreak of chocolate spot of faba bean, caused by *Botrytis fabae* in California. Plant Disease, 82(7): 831.

Liang, Z.Y.; Saxena, M.C.; Weigand, S. and Lang, L.J. (1993). Fungal diseases of faba bean in China. Faba bean production and research in China: Proceedings of an International Symposium, Hangzhou, China, 121-122 pp.

Malick, C.P. and Singh, M.B. (1980). In: Plant Enzymatology and Histoenzymology. Kalyani Publishers, New Delhi, p. 286.

Mubarak, H. M. E. (2003). Control of root rot and damping off disease of bean. Ph.D. Thesis, Fac. Sci., Tanta Univ., Egypt.

Nassib, A.M.; Ibrahim, A. and Abd El-Latif, A.H. (1983). On-farm trials in Egypt, In Faba bean in the Nile Valley. Report on the 1st phase of the ICARDA/FAO. Nile Valley Project (Saxena, M.C. and Stewart, R.A., eds.). Martinus Nijhoff Publisher for the ICARDA/FAO, Nile Valley project.

Nassib, A.M.; Khalil, S.A.; El-Borai, M.A. and Radi, M.M. (1991). In "Nile Valley Regional Program on Cool Season Food Legumes and Cereals".Annual Report, Egypt: ARC/ICARDA.

Nawar, H.F. and Kuti, J.O. (2003). Wyerone acid phytoalexin synthesis and peroxidase activity as markers for resistance of broad beans to chocolate spot disease. Phytopathol. J. 151(10): 564-570.

Obradovic, A. and Jones, J.B. (2005). Integration of biological control agents and systemic acquired resistance inducers against bacterial spot in tomato. Plant Disease, 89(7): 712-716.

Omar, S. A.; Chapman, G.P. and Bailtss, K.W. (1985). Interaction between virus and *Botrytis fabae* in *Vicia faba*. In Proc. 8th Botrytis Symp., Quad Vitic. Enol. Univ., Torino, pp. 243-248.

Oostendrop, M.; Kunz, W.; Dietrich, B. and Satub, T. (2001). Induced disease resistance in plants by chemicals. European Plant Pathology J., 107(1): 19-28.

Raskin, I. (1992). Role of salicylic acid in plants. Annu. Rev. Plant Physiol., 43: 439-463.

Rewal, H. S. and Jhooty, J. S. (1985). Differential response of wheat varieties to systemic fungicides applied to *Ustilago tritici* (Pers.). Rostr. Indian J. Agric. Sci., 55: 548-549.

Ryals, J.S.U.and Ward, E. (1994). Systemic acquired resistance. Plant Physiol., 104: 1109-1112.

Sticher, L.; Mauch, M.B. and Metraux, J.P. (1997). Systemic acquired resistance. Annu. Rev. Phytopathol., 35: 235-270.

Strobel, N. and Kuc, J. (1995). Chemical and biological inducers of systemic resistance to pathogens protect cucumber and tobacco plants from damage caused by paraquat and cupric chloride. Phytopathology, 85(10): 1306-1310.

Tarrad, A.M.; El-Hyatemy, Y.Y. and Omar, S.A. (1993). Wyerone derivatives and activities of peroxidase and phenoloxidase in faba bean leaves as induced by chocolate spot disease. Plant Sci., 89: 161-165.

Tuzun, S.; Rao, M.N.; Vogeli, U.; Schardl, C. L. and Kuc, J. (1989). Induced systemic resistance to blue mold: early induction and accumulation of beta-1,3-glucanase chitinase and other pathogenesis protein (*β*-proteins) in immunized tobacco. Phytopathol., 79: 979-983.

Ward, E. R.; Uknes, S. J.; Williams, S. C.; Dincher, S. S.; Wiederhold, D. L. and Ryals, J. A. (1991). Coordinate gene activity in response to agents that induce systemic acquired resistance. Plant Cell. 3: 1085-1094.

Wilson, C.L.; Faklin, J.D. and Otto, B.E. (1987). Fruit volatiles inhibitory to *Monilinia freuticola* and *Botrytis cinerea*. Plant Dis., 71(4): 316-319.

ARABIC SUMMARY

اتجاهات حديثة لمقاومة مرض التبقع البني في نبات الفول بمصر

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ناقش البحث دور المركبات الكيميائية التجارية (البايون، استارنر، وكلا من حامض البوريك والسلسيلك) في استحداث مقاومة لنبات الفول المصاب بمرض التبقع البني أو الشيكولاتي تحت ظروف البيوت الزجاجية.

استطاعت المركبات المختبرة أن تخفض شدة الإصابة لدرجة كبيرة وكان هذا جاليا عند نقع بذور الفول في حامض البوريك لمدة ٢٤ ساعة عند جرعة ضعف تلك التي تستخدم علي المستوي الحقلي بكفأه مقدارها ٨٧% تقريبا. أنخفض نشاط كلا من إنزيمات: البيروكسيدز، الكاتليز والفينول اوكسيديز في النباتات المصابة والمعاملة بالمركبات السابق زكرها.

أظهرت معاملة النباتات المصابة بالمركبات التجارية المختبرة زيادة جوهريه في محتوها الكلي من المواد الكربوهيدراتيه والبروتين والفينول عند مقارنتها بمثيلتها الغير مصابة.

وبناء علي ماسبق يمكن الاستنتاج أن المركبات الكيميائية المستخدمة يمكنها مقاومة التأثير المدمر لفطرة البوتريتس فابي علي مستوي النمو والأنتاجيه.