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## INDUCTION OF RESISTANCE AGAINST DAMPING-OFF AND ROOT ROT DISEASES IN FABA BEAN

[39]

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### **ABSTRACT**

Faba bean (*Vicia faba* L.) seeds cv. Misr 1 were treated with Bion (5 mM), salicylic acid (5 mM) as chemical inducers as well as *Paenibacillus polymyxa* and *Trichoderma harzianum* as biotic inducers to study their effect on the infection with *Rhizoctonia solani* and *Fusarium solani* f. sp. *fabae* under greenhouse and field conditions.

Under greenhouse conditions (Agricultural Research Centre, Giza) all treatments induced reduction in the percentages of pre- and postemergence damping off caused by R. solani or F. solani f. sp. fabae compared to untreated control. The highest percentage of survived plants was achieved from the inducer Bion (92 % and 88%) compared with untreated control (40% and 36%) in the presence of R. solani or F. solani f. sp. fabae respectively. Meantime, there was no significant difference between the Bion and the fungicide Rizolex -T (3 g/Kg seeds) treatments. However, all treatments caused significant increase in the figures of plant height, shoot and root dry weight, nodules number/plant and nodules dry weight over the control treatment grown in infested soil by R. solani or by F. solani f. sp. fabae. The highest values of all growth parameters under study were recorded on faba bean plants check (healthy plants) grown in disinfested soil followed by Bion and Rizolex -T treatments in the presence of R. solani or F. solani f. sp. fabae. There was no significant difference between Bion and Rizolex-T treatments with exception of nodules number and nodules dry weight /plant in the presence of *R. solani*. On the contrary, there was significant difference between Bion and Rizolex-T treatments with exception of plant height in the presence of *F. solani* f. sp. fabae.

Under field conditions at Giza (Giza Governorate) and Etay El-Baroud (El- Behira Governorate) Agricultural Research Stations during winter season 2013-2014, all the treatments significantly decreased the percentage of pre- and postemergence damping-off and increased the percentage of survived plants compared with untreated control in two locations with exception of postemergence percentage figures at Giza Agricultural Research Station. Regarding survived plants, there were no significant differences between the Bion (89.5% and 86.5%) as well as Rizolex-T (90.9% and 88.8%) treatments compared with untreated control (68.3% and 64.3%) at Giza and Etay El-Baroud Agricultural Research Stations respectively. However, all inducer treatments significantly improved growth parameters (i.e. plant height, number of pods/plant, seed weight /plant and one hundred seed weight) as well as yield compared to the untreated control in the two locations. Higher increase in seed yield (ton /feddan) was obtained with Rizolex-T and Bion treatments (62.2% and 57.9%) respectively, followed by salicylic acid and P. polymyxa with (39.4% and 28.4 %) respectively. increasing over the untreated control calculated as means of the two locations.

Activities of peroxidase (PO), polyphenol oxidase (PPO) enzymes and phenol content were determined in faba bean plants treated with different inducers. Bion treatment showed the highest increase in PO and PPO activity, total and free phenols followed by salicylic acid treatment in the presence of *R. solani* or *F. solani* f. sp. fabae.

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#### INTRODUCTION

Faba bean (*Vicia faba* L.) is an important winter legume crop originated in between the oriental Mediterranean countries and Afghanistan (**Cubero, 1974**). In Egypt, faba bean cultivated area decreased from 123796 hectare in 1995 to 77149 hectare in 2013. However, imported value of broad bean increased from 40,347,000 \$ in 1995 to 279,356,000 \$ in 2013 (**Anonymous, 2015**).

However, faba bean roots infected with many fungal pathogens which caused a considerable yield losses. In Egypt, *Rhizoctonia* spp., *R. solani, F. oxysporum* f. sp. fabae, *F. solani* f.sp. fabae, *F. avenaceum, F. subglutinans, F. moniliforme, Macrophomina phaseolina* and *Sclerotium rolfsii* were reported as pathogens on faba bean seedlings (Omar, 1986; Abou-Zeid et al 1997; Hugar, 2004; Eisa (Nawal) et al 2006; Abdel-Razik (Sahar) et al 2012 and Abdel-Monaim, 2013).

The control of damping off and root rot diseases of faba bean has mainly depended upon using of fungicides. Although the application of fungicides is always effective, their non-target environmental impact and the development of pathogen resistance have led to search for alternative methods. Resistant varieties are few and not available (Assuncao et al 2011), and use of cultural practices such as crop rotation however the wide host range exhibited by many of these pathogens and its ability to survive in the soil in the absence of hosts complicates management. Induction of resistance by application of elicitors is one of the alternatives, either alone or as a part of an integrated control strategy. Systemic acquired resistance (SAR) and induced systemic resistance (ISR) are two forms of systemic resistance. In both SAR and ISR, plant defences are released by previous elicitors; thus, biotic and abiotic factors play a role in resistance; most inducers reduce disease in the infected plants by 20%-85% (Walters et al 2013). Commonly tested chemical elicitors which, depends on the salicylic acid (SA) responsiveness in systemic acquired resistance (SAR) are salicylic acid, methyl salicylate, benzothiadiazole, benzoic acid, chitosan, and so forth which affect production of phenolic compounds and activate various defence-related enzymes in plants (Thakur and Sohal, 2013).

Induced systemic resistance (ISR) can be defined by induction of defences in plants against many pathogens via application of plant growth-promoting microorganisms in the soil (Maksimov

et al 2011). ISR depends on two pathways that respond to ethylene (ET) and jasmonic acid (JA) (Verhagen et al 2004). The species of the genus Trichoderma, which contain a group of plant growth-promoting fungi, can colonize the intercellular parts of plant roots and stimulate systemic resistance in all parts of the plant; the actions of these fungi suppress some plant diseases by direct mycoparasitism or antibiosis, as well as indirect induced resistance (Sharma & Sain 2004). Trichoderma spp. stimulates ISR through hormonal and molecular pathways in a JA/ET-dependent manner (Shoresh et al 2010). Many studies in the past have proved the potential of *Trichoderma* spp. as biocontrol agents of several soilborne plant pathogens such as R. solani, Sclerotium rolfsii, Pythium aphanidermatum, and Fusarium spp. (Hadar et al 1979; Katan, 1980; Sivan et al 1984; Samuels, 1996; Basak & Basak, 2011 and Kim & Knudsen, 2013).

Plant growth promoting rhizobacteria (PGPR) are root-associated bacteria in the rhizosphere of many plant species that increase plant growth and suppress plant disease (Ryu et al 2006). In addition to suppressing plant pathogens by secretion of antibiotics and production of allelopathic compounds in the rhizosphere some PGPR can also elicit mechanism of induced systemic resistance (ISR) against a broad range of pathogens, (Jain et al 2013). One of the reported plant growth promoting rhizobacteria (PGPR) is Paenibacillus polymyxa which has a range of reported properties, including nitrogen fixation (Bal and Chanway, 2012); soil phosphorus solubilization (Wang et al 2012); production of antibiotics and other hydrolytic enzymes (Raza et al 2008), and chitinase (Mavingui and Heulin, 1994).

The objective of this study was to evaluate the disease management potential of biotic and abiotic inducers against damping off and root rot diseases caused by *Rhizoctonia* and *Fusarium* under greenhouse and field conditions.

### **MATERIALS AND METHODS**

### Plant material

Faba bean seeds (*Vicia faba* L.), cultivar Misr 1 were obtained from the Legume Res. Dept., Field Crops Res. Inst., ARC, Giza, Egypt.

### **Pathogens**

The fungi *R. solani* Kühn and *F. solani* f. sp. *fabae* were kindly provided by Legume and Forage

Dis. Res. Dept., Plant Pathol. Res. Inst., Agric. Res. Centre, Giza, Egypt. The fungi were isolated from naturally infected faba bean plants, showing damping off and root rot symptoms. Their pathogenicity were previously confirmed and identified on the basis of cultural properties and microscopic morphological characters according to **Sneh et al** (1991) and **Booth** (1971).

### Preparation of pathogens inocula

Inocula of R. solani and F. solani f. sp. fabae were prepared by growing the fungi in glass bottles 500 cc containing 100 gram sterilized sorghum grains medium. The bottles were inoculated with actively growing of equal five disks (0.5 cm) for each bottle of four days old R. solani or seven days old F. solani cultures. The bottles were incubated at 25 ± 1°C for 18 days; during this period the bottles were vigorously shaken daily for the first 4 days to encourage more rapid and uniform colonization of the sorghum grains then shaken every three days to ensure uniform distribution of the fungal growth. After incubation period, the glass bottles were then evacuated and its content was air dried at room temperature, and crushed in a mill to pass through a 3-mm sieve. The dried crushed inocula stored in paper bags at 4 ± 1°C until added to soil within one week (Gaskill, 1968 and Leslie & Summerell, 2006).

### **Chemical inducers**

Bion® wettable granule (WG) 50%, Benzothiadiazole, (Syngenta Crop Protection, InC); and Salicylic acid (Sigma Aldrich, USA) were used in this study.

### **Growing of biotic inducers**

A- Paenibacillus polymyxa: The culture of the bacteria P. polymyxa (isolate 9D14), previously isolated by Shehata et al (2006) and belongs to the collection of Plant Pathol. Dept., Fac. of Agric., Ain Shams Univ. was kindly provided. The culture of the bacteria was activated on fresh slants and, after 24 hrs was transferred to many 250 ml Erlenmeyer flasks with 50 ml of nutrient yeast dextrose broth (NYDB) medium (per litre: nutrient broth 8 g, yeast extract 5 g and dextrose 10 g). The flasks were placed on a rotary shaker to grow at 120 rpm for 66 hrs at 24±1°C.

B- Trichoderma harzianum: The fungus T. harzianum which isolated from rhizosphere of faba bean by Elsaid et al (2005) and belongs to the collection of Plant Pathol. Dept., Fac. of Agric., Ain Shams Univ. was kindly provided. Its identification was previously confirmed on the basis of cultural properties and microscopic morphological characters according to Rifai (1969). The fungus was kept under a phosphate buffer (pH 6.5) at 4± 0.5°C for long-time storage (Boeswinkel, 1976). Formulation of T. harzianum was prepared by growing the fungus in glass bottles 500 cc containing 100 gram sterilized sorghum grains medium (Rini and Sulochana, 2007). The bottles were inoculated with actively growing 0.5 cm diameter mycelia disc of 7 days old T. harzianum culture. Five discs for each bottle were used for inoculation. The bottles were incubated at 27 ± 1°C for 18 days and were vigorously shaken daily for the first 4 days to encourage more rapid and uniform colonization of the sorghum grains. At the end of incubation period, the colonised sorghum grains by mycelium and conidia of T. harzianum were removed from the bottles and air dried in shade at room temperature, and fine grinded in a mill then sieved through 60mesh (0.25 mm) sieve (Tewari and Bhanu, 2004). The amount of inoculum in the sorghum grains was determined on potato dextrose agar (PDA) supplemented with Rosebengal 25 mg/L by dilution plate technique (Rini and Sulochana, 2007). One gram of air dried fine grinded sorghum grains was contained 3.9x 10<sup>9</sup> colony-forming unit (CFU) of *T. harzianum*. The grinded sorghum grains were kept in polythene bag and treated as formulated Trichoderma within one week.

### Seed and soil treatments

Apparently healthy uniformity seeds of faba bean were surface disinfected by immersing in sodium hypochlorite (1%) for 2 min, and washed several times with sterilized water, then left to dry on screen cloth with paper towel underneath to absorb the excess water at room temperature for approximately two hour.

A) Chemical inducers treatments: The disinfected faba bean seeds were soaked in aqueous solutions of the inducers (Bion or salicylic acid) for 6 hours on previous day of sowing at the rate of 5 mM then the treated seeds were air-dried for 15 hrs until sowing time.

- B) The bacterial inducer treatment: After growth of P. polymyxa, the liquid cultures media were then centrifuged under cooling (4°C) at 10000 rpm for 10 min. Then, the disinfected faba bean seeds were soaked in supernatant for 6 hours on previous day of sowing. Cells of P. polymyxa were collected in 20 cm Petri dish and bacterial slurry was obtained by adding 1ml of 1% methyl cellulose (Sigma-Aldrich, Milwaukee, WI, USA) in sterile distilled water to bacterial cells harvested from each Erlenmeyer flask. Healthy seeds of faba bean that previously were soaked in supernatant, were coated with bacterial slurry, then spread on screen cloth with paper towel underneath to absorb the excess liquid, then the coated seeds were airdried for 15 hrs until sowing time. Enumeration of bacteria coated on seeds was performed by plate dilution method on the basis of colony forming unit (CFU/seed) on nutrient yeast dextrose agar (NYDA) medium.
- C) The *T. harzianum* treatment: Air dried fine grinded sorghum grains which contained 3.9x 10<sup>9</sup> (CFU) of *T. harzianum* (formulated *Trichoderma*) was used on previous day of sowing to coat the disinfected faba bean seeds moistened with 1% methyl cellulose in sterile distilled water as sticker, then the coated seeds were air-dried for 15 hrs until sowing time.
- D) Fungicide treatment: seed dressing was carried out to the disinfected faba bean seeds by applying the Rizolex-T 50% WP (Tolclofosmethyl-thiram), Sumitomo Chemical Company Ltd. at the recommended dose (3 g/kg) to the 1% methyl cellulose (as sticker) moistened seeds in polyethylene bags and shaking well to ensure even distribution of the fungicide.
- E) Root-nodule bacteria treatment: Formulation of *Rhizobium leguminosarum* biovar *viciae* (Faba bean), was kindly obtained from Biofertilizers Production Unit, Soils Water and Environment Res. Inst., (SWERI), Agric. Res. Centre (ARC), Giza, Egypt, was used to inoculate potted soils (infested or not-infested with pathogenic fungus) or field soil. Five grams of *R. leguminosarum* bv. *viciae* formulation were mixed in each pot during sowing or 800 g of *Rhizobium* formulation was mixed with approximately 50 kg of moistened fine sandy soil and added to field soil into the seed furrow during sowing, at rate of 800 g *Rhizobium* formulation/feddan.

F) Control, The disinfected faba bean seeds were soaked in sterilized water for 6 hours on previous day of sowing then air-dried for 15 hrs until sowing time.

### **Greenhouse experiment**

The trials were carried out in the greenhouse of Plant Pathology Research Institute, Agricultural Research Centre, Giza. Pots (30 cm in diameter) with a bottom drainage hole were sterilized by dipping in 5% formalin solution for 15 minutes, and left for one week until complete formalin evaporation. Pots were filled with steam disinfested sandy clay soil 1:2 (V/V). Soil infestation was achieved by mixing the inoculum of R. solani or F. solani f. sp. fabae with the soil at the rate of 2% of soil weight (Papavizas & Davey, 1962). Sterilized uninoculated grounded sorghum grains were added to the disinfested soil at the same rate for used as healthy control. The infested soil was mixed thoroughly and watered every 2 days for a week before planting to stimulate the fungal growth and ensure its distribution in the soil. Five seeds of treated faba bean seeds, as mentioned before, were sown in each pot and pots were irrigated directly. Five replicated pots were used for each particular treatment. All pots were irrigated when necessary, and watered once a week to near field capacity with a 0.1% 15:15:15 (N: P: K) fertilizer solution in the first month and kept in a greenhouse under natural conditions. Other agricultural procedures were performed according to normal practice. The treatments were as follows: (1) Bion at 5 mM; (2) Salicylic acid at 5 mM; (3) P. polymyxa; (4) T. harzianum; (5) Rizolex-T. Soaking in water served as untreated control for both infested and non infested soil. Eight plants (four replicates each of two plants) were uprooted 60 days after sowing; plant height (cm) was measured and the stem was cut at the soil line. Roots were washed under running water to remove soil particles. Numbers of nodules were recorded. Shoots, roots and nodules placed in a paper bags and oven dried at 70°C for 48h, then weighed.

### Disease assessment

The disease incidence (DI) % was determined by recording pre-emergence damping-off, postemergence damping off and the percentage of survived plants 15, 30 and 45 days after sowing, respectively according to the following formulas:  $Pre-emergence\% = \frac{Total\ No.\ of\ un-germinated\ seeds}{Total\ No.\ of\ planted\ seeds} x100$ 

 $Post-emergence\%\\ = \frac{Total\ No.\ of\ rotted\ seedlings}{Total\ No.\ of\ planted\ seeds} x100$  Survived seedlings % $= \frac{Total\ No.\ of\ survived\ seedlings}{Total\ No.\ of\ planted\ seeds} x100$ 

Reduction or increasing % over the infected control was also calculated according to the following formula:

Reduction or increasing%  $= \frac{DI \ of \ control - DI \ of \ treatment}{DI \ of \ control} x100$ 

The field experiments was carried out during the winter growing season 2013 - 2014 at two location, Giza Agricultural Research Station, Giza Governorate and Etay El-Baroud Agricultural Research Station, El- Behira Governorate, Egypt, in field known to have root rot history, in order to investigate the effect of chemical and biotic inducers for controlling damping-off and root rot diseases. The disinfected faba bean seeds were treated by the same manner in a greenhouse experiment. In the control treatment, seeds were soaked in distilled water as mentioned before. The treated faba bean seeds were sown in the field on October 29 and 30, 2013 at Giza and Etay El-Baroud respectively. The field trial (24 plots) was designed in complete randomized block with four replicates. The area of each plot was 10.5 m<sup>2</sup> consisted of five rows; each row was 3.5 m length and 0.6 m width. All treatments were sown in hills 20 cm apart on both sides of the row ridge, with one seed per hill. Calcium super-phosphate (15 % P<sub>2</sub>O<sub>5</sub>) at 150 kg /feddan was added on rows during the soil preparation. Potassium sulphate (48% K2O) at 50 Kg/feddan was applied as soil application at the first irrigation after sowing. Ammonium sulphate (20.5% N) at rate of 75-100 kg/feddan was added at the sowing time as a starter dose of nitrogen. All other recommended agricultural practices were followed according to the recommendations of the Egyptian Ministry of Agriculture and Land Reclamation. The treatments were as follows: (1) Bion 5 at mM; (2) Salicylic acid at 5 mM; (3) P. polymyxa; (4) T. harzianum; (5) Rizolex-T, and (6) Water (control). The disease incidence (DI) % was determined as mentioned before. Random samples of ten faba bean plants were collected (from the inner rows) at harvest stage from each plot. Plant growth parameters of plant height (cm), number of branches, number of pods per plant, weight of one hundred seed and seed weight per plant were recorded as well as seed yield ton/ feddan were calculated.

Effect of faba bean seed treatment with inducers on activity of oxidative enzymes and phenol content

An experiment was carried out to determine activity of oxidative enzymes and phenol content. Faba bean plants were grown as mentioned before in greenhouse experiment. Fifteen days after sowing, activity of peroxidase (PO), polyphenol oxidase (PPO) and phenol contents was determined in tissue extracts of soybean plants surviving from the following treatments: (1) Bion at 5 mM; (2) Salicylic acid at 5 mM; (3) *P. polymyxa*; (4) *T. harzianum*; (5) Water in infested soil (Control, infected) and (6) Water in non-infested soil (Control, healthy).

### Assay of enzymes activities

### A) Assay of peroxidase (PO)

Extraction and assay of peroxidase (PO) activity were carried out according to Chakraborty and Chatterjee (2007). For extraction of the peroxidase enzyme, 4.0 g of plant tissue from each treatment were separately homogenized in 20 ml of chilled potassium phosphate buffer (0.1M at pH 7.0) at 0°C. A pinch of neutral sand was added to facilitate crushing. The extracts were obtained by filtering off the debris with a clean cloth and centrifuged under cooling (4°C) at 3000 rpm for 15 minutes. The supernatants (crude enzyme extract) were stored at -20°C or immediately used for determination of PO. Five ml of freshly prepared pyrogallol reagent (prepared by mixing 10 ml of 0.5 M pyrogallol solution and 12.5 ml of 0.66 M phosphate buffer and volume was made to 100 ml with distilled water) and 1.5 ml of the enzyme extract were mixed in a cuvette of a spectrophotometer and the mixture was immediately adjusted to zero absorbance. 0.5 ml of 1% H<sub>2</sub>O<sub>2</sub> solution was added to it and inverting the tube mixed the content. The reaction was initiated by the addition of H<sub>2</sub>O<sub>2</sub>. Enzyme activity was recorded as the change in absorbance per minute at 430 nm immediately after the addition of substrate using a Milton Roy 601 UV-Vis spectrophotometer.

Similarly, control on non-enzymatic oxidation was maintained at different times by heating the extract at 100°C for 10 minutes. The activity was always measured zero indicating its complete inactivation by the heat treatment.

### B) Assay of polyphenol oxidase (PPO)

Extraction and assay of polyphenoloxidase enzyme (PPO) were carried out according to Sadasivam & Manickam (1996). For extraction of the polyphenol oxidase enzyme, 2.0 g of plant tissue from each treatment were separately homogenized with a pinch of neutral sand in 6.0 ml of phosphate buffer (0.1M at pH 7.0) at 0°C. The extracts were obtained by filtering off the debris with a clean cloth and centrifuged under cooling (4°C) at 3000 rpm for 15 minutes. The supernatants (crude enzyme extract) were stored at -20°C or immediately used for determination of PPO. Two ml of the enzyme extract and 3.0 ml of 0.1M phosphate buffer were mixed together in a cuvette and the sample was adjusted to zero absorbance at 495 nm using a Milton Roy 601 UV-Vis spectrophotometer. 1.0 ml of 0.01 M catechol in 0.1 M phosphate buffer (0.4 mg/ml) was added to the above mixture and the reactants were quickly mixed. The enzyme activity was measured as the change in absorbance per minute up to 10 minute at 495 nm immediately after the addition of catechol solution, which initiated the reaction. Control in similar manner was maintained at different times by heating the extract at 100°C for 10 minutes. The activity was always measured zero indicating complete inactivation of the enzyme by this heat treatment.

### **Determination of phenolic compounds**

Extraction of phenolic compounds was carried out according to Sutha et al (1998). Five grams of plant tissue from each treatment were separately homogenized with 30 ml of 80 % ethanol using pestle and mortar. The contents were shaken well at 50°C for 30 min and centrifuged at 10,000 rpm for 10 min. The pellet was extracted twice at 50°C. The supernatants were pooled and treated with equal volume of petroleum ether and shaken well and allowed to stand for 5 min and the petroleum ether layer containing chlorophyll was discarded. The alcohol fraction was evaporated to dryness under vacuum using rotary evaporator at 45°C. The residue was dissolved and quantitatively transferred into 5 ml of isopropanol and stored in vials at -20°C till used for determination of total and

free phenols. Phenolic compounds were determined using methods of analysis described by **Snell and Snell (1953).** 

The total phenolic content was determined by mixing 0.5 ml of the sample extract with 0.25 ml HCl and boiled in water bath for 10 minutes then left to cool. One ml of the Folin Ciocaltea's reagent and 6 ml of Na<sub>2</sub>CO<sub>3</sub> 20% were added. The mixture was diluted to 10 ml with warm distilled water (30-35°C). After 30 minutes standing in dark, the optical density of the developed blue colour was measured at 520 nm using a Milton Roy 601 UV-Vis spectrophotometer against a reagent blank. Free phenols content was determined by adding 1 ml of the reagent and 3 ml of a 20% Na<sub>2</sub>CO<sub>3</sub> solution to 0.5 ml of the sample diluted to 10 ml with warm distilled water, let to stand for 30 minutes in dark and the optical density of the developed blue colour was measured at 520 nm using a Milton Roy 601 UV-Vis spectrophotometer against a reagent blank.

Conjugated phenols content was determined by subtracting the amount of free phenols content from that of total phenols. The total phenolic contents and free phenols content were calculated on the basis of the calibration curve of Catechol and expressed as catechol equivalents in milligrams per gram fresh weight.

### Statistical analysis

Completely randomized design (CRD) and randomized blocks design (RBD) were conducted in greenhouse experiment and field experiment, respectively. The obtained data were subjected to computer statistical software (ASSISTAT) originated by **Silva & Azevedo (2009).** Data analyzed using analysis of variance (ANOVA), and mean values were compared using Duncan's multiple range test at a significance level of  $P \le 0.05$ .

### **RESULTS**

## 1- Enumeration of bacterium and fungus coated on seeds

Enumeration of bacterium or fungus coated on seeds was performed by plate dilution method on the basis of colony forming unit (CFU/seed) on nutrient yeast dextrose agar (NYDA) medium for *P. polymyxa*, or potato dextrose agar (PDA) supplemented with Rosebengal (25 mg per litre) for *T. harzianum*. Colony forming units (cfu) were counted after 24 and 48 hrs of incubation for *P. polymyxa* and *T. harzianum* respectively (**Rini and** 

Sulochana, 2007). Population density of P. polymyxa were  $3.7 \times 10^5$ ;  $3.2 \times 10^5$  and  $3.4 \times 10^5$  CFU per seed of faba bean and propagule density of T. harzianum were 3.9 x  $10^4$ ; 3.4 x  $10^4$  and 3.6 x  $10^4$ CFU per seed of faba bean in greenhouse experiment, field experiments and the experiment of oxidative enzymes activity determination, respective-

### 2- Greenhouse experiment

### I- Effect of some inducers on the incidence of faba bean Rhizoctonia or Fusarium dampingoff diseases

In this experiment, faba bean seeds were soaked in aqueous solutions of Bion or salicylic acid for 6 hours on previous day of sowing as

chemical inducers, or soaked in culture filtrate of P. polymyxa for 6 hours and coated with bacterial cells slurry as biotic inducer or coated with T. harzianum (formulated Trichoderma) to study their effect on the incidence of Rhizoctonia or Fusarium damping- off diseases in pots. Results in Table (1) indicate that all treatments induced reduction in the percentages of pre- and post-emergence damping off caused by R. solani or F. solani f. sp. fabae compared to untreated control. Bion and salicylic acid treatments gave the highest reduction effect for pre-emergence damping off. However, all treatments significantly increase survived plants compared to untreated control. Bion, Rizolex -T and salicylic acid treatments gave highest increase effect followed by P. polymyxa treatment In the presence of R. solani or F. solani f. sp. fabae.

Table 1. Effect of some chemical and biotic inducers as well as Rizolex-T as seed treatments on the percentage of damping- off disease of faba bean plants grown in artificial infested soil2 by Rhizoctonia solani (A) or Fusarium solani f. sp. fabae (B) under greenhouse conditions

### (A) R. solani:

		Damp	ing- off		Survived		
Treatments	Pre-emergence		Post- en	nergence	plants	Increasing	
rreatments	Incidence	Reduction	Incidence	Reduction	piants %	%	
	%	%	%	%	/0		
Bion 5mM	0.0	100.0	4.0	66.7	92.0 a	130.0	
Salicylic acid 5mM	4.0	88.9	4.0	66.7	88.0 a	120.0	
P. polymyxa <sup>3</sup>	8.0	77.8	4.0	66.7	88.0 a	120.0	
T. harzianum⁴	12.0	66.7	4.0	66.7	84.0 a	110.0	
Rizolex –T <sup>5</sup>	4.0	88.9	0.0	100.0	92.0 a	130.0	
Control <sup>6</sup> ( <i>R. solani</i> )	36.0	0.0	12.0	0.0	40.0 b	0.0	
Control <sup>6</sup> healthy		1.0	0	^	06	200	
(non infested soil)	2	+.0	0.0		96.0 a		

### (B) F. solani f. sp. fabae:

(=) 11 ceram mep. 14.0						
		Damp		Survived		
Tractments	Pre-emergence		Post- emergence			Increasing
Treatments	Incidence	Reduction	Incidence	Reduction	plants %	%
	%	%	%	%	70	
Bion 5mM	4.0	87.5	4.0	75.0	88.0 a	144.4
Salicylic acid 5mM	4.0	87.5	4.0	75.0	88.0 a	144.4
P. polymyxa <sup>3</sup>	8.0	75.0	4.0	75.0	84.0 a	133.3
T. harzianum⁴	12.0	62.5	0.0	100.0	84.0 a	133.3
Rizolex –T <sup>5</sup>	4.0	87.5	4.0	75.0	88.0 a	144.4
Control <sup>6</sup> ( <i>F. solani</i> )	32.0	0.0	16.0	0.0	36.0 b	0
Control <sup>6</sup> healthy	1	.0	0	. 0	06	i.0 a
(non infested soil)	4	.0	"	0.0	90	o.0 a

<sup>1-</sup> Faba bean seeds (cv. Misr 1) were soaked in aqueous solutions of Bion and salicylic acid for 6 hours on previous day of sowing then air dried.

<sup>2-</sup> Soil infestation was achieved by mixing the inoculum of R. solani (A) or F. solani f. sp. fabae (B) with the soil at the rate of 2% of soil weight.

<sup>3-</sup> Faba bean seeds were soaked in culture filtrate for 6 hours on previous day of sowing, and then seeds were coated with bacterial cells slurry of Paenibacillus polymyxa.

<sup>4-</sup> Faba bean seeds moistened with 1% methyl cellulose were coated with formulated Trichoderma harzianum.

<sup>5-</sup> Seed dressing by fungicide was carried out at the recommended dose (3 g/kg).6- Faba bean seeds were soaked in sterilized water for 6 hours on previous day of sowing then air dried.

<sup>7-</sup> Means in each column followed by the same letter are not significantly different according to Duncan's multiple range test, (p = 0.05)

ı	Induction	٥f	rocietanco	against	damning.	off and	l root r	ot diseases	in fa	ha	hoan
ı	inauction	OΤ	resistance	adainst	aambina.	orr and	i root r	ot diseases	ın ta	ıba	bean

## II- Effect of some inducers on some growth parameters of faba bean plants infected with *R. solani* under greenhouse condition

Results presented in Table (2) reveal that Bion, salicylic acid, P. polymyxa and T. harzianum treatments caused a significant increase in the figures of plant height, shoot and root dry weight, nodules number/plant and nodules dry weight over the control treatment grown in infested soil by R. solani. The highest values of all growth parameters under study were recorded on faba bean plants control (healthy plants) grown in disinfested soil followed by treatments with Bion and Rizolex-T treatments. There was no significant difference between the treatments with Bion and Rizolex-T with exception of nodules number/plant and nodules dry weight. Meanwhile, there was no significant difference between the treatments with P. polymyxa and T. harzianum regarding to plant height, shoot dry weight and nodules number/plant. However, there was significant difference between Bion treatment and treatments by P. polymyxa as well as T. harzianum regarding to shoot and root dry weight, and nodules number/plant in faba bean plants grown in infested soil by R. solani. Finally, results indicate that there was no significant difference between the treatments with salicylic acid and P. polymyxa with exception of shoot and root dry weight. Also, results show that there was no significant difference between the treatments with salicylic acid and *T. harzianum* with exception of shoot dry weight and nodules dry weight in faba bean plants grown in infested soil by R. solani.

# III- Effect of some inducers on some growth parameters of faba bean plants infected with *F. solani* f. sp. *fabae* under greenhouse condition

Results in **Table (3)** show that Bion, salicylic acid, *P. polymyxa* and *T. harzianum* treatments caused a significant increase in the figures of plant height, shoot and root dry weight, nodules number/plant and nodules dry weight over the control treatment grown in infested soil by *F. solani* f. sp. fabae. The highest values of most growth parameters under study were recorded on faba bean plants control (healthy plants) grown in disinfested soil followed by treatments with Bion, Rizolex-T, salicylic acid, *P. polymyxa* and *T. harzianum* treatments. However, there was significant difference between the treatments with Bion and Ri-

zolex-T with exception of plant height. Also there was significant difference between the Bion treatment compare to salicylic acid treatment regarding to all growth parameters under study in faba bean plants grown in infested soil by *F. solani* f. sp. *fabae*. Meantime there was significant difference between the treatments with *P. polymyxa* and *T. harzianum* with exception of nodules number/plant.

### 3- Field experiments

## I- Effect of some inducers on the incidence of faba bean damping- off disease

In these experiments, the effect of treatments by Bion 5mM, salicylic acid 5mM, P. polymyxa, T. harzianum and the fungicide Rizolex-T on damping- off incidence and survived plants of faba bean under field conditions at Giza and Etay El-Baroud Agricultural Research Stations during winter season 2013-2014 was studied. Results in Table (4) exhibited that all the treatments significantly decreased the percentages of pre and postemergence damping-off compared with untreated control in two locations with exception of postemergence figures at Giza Agricultural Research Station. The highest percentage of pre-emergence damping- off reduction over the control was obtained from treatments with Rizolex-T and Bion. Meantime, there were no significant differences between the treatments with salicylic acid, P. polymyxa, and T. harzianum in two locations. For the percentage of post-emergence damping-off reduction over the control, Rizolex-T treatment was more efficient but significantly not different from other treatments. Also, results showed that all the tested treatments significantly increased the percentage of survived plants compared with the control. There were no significant differences between the treatments with Bion and Rizolex-T, whilst the treatments with salicylic acid P. polymyxa and T. harzianum significantly were less effective regarding survived plants.

### II- Effect of some inducers on growth parameters and yield of faba bean plants

Under field condition, inducers treatments significantly improved growth parameters and yield compared to the untreated control treatment in the two locations (**Table 5, A & B**). Recorded results indicate that:

### Plant height

The plant height was affected by seed treatments with different inducers. In two locations, all

**Table 4.** Effect of some chemical and biotic inducers as well as Rizolex-T as seed treatments on the percentage of damping- off disease of faba bean plants grown under field conditions at Giza Agricultural Research Station (A) and Etay El-Baroud Agricultural Research Station (B) during winter growing season 2013 - 2014 (natural infection).

(A): Giza Agricultural Research Station

		Damp				
	Pre-emergence		Post- emergence		Survived	Increasing
Treatments	Incidence	Reduction	Incidence	Reduction	plants %	%
	%	%	%	%	76	
Bion 5mM	6.0 c	76.9	2.0 ab	42.9	89.5 a	31.0
Salicylic acid 5mM	11.0 b	57.7	2.3 ab	34.3	84.2 b	23.3
P. polymyxa <sup>2</sup>	12.7 b	51.2	2.0 ab	42.9	83.3 b	22.0
T. harzianum <sup>3</sup>	11.9 b	54.2	2.7 ab	22.9	83.3 b	22.0
Rizolex –T <sup>4</sup>	5.9 c	77.3	1.5 b	57.1	90.9 a	33.1
Control <sup>5</sup>	26.0 a	0.0	3.5 a	0.0	68.3 c	0.0

### (B): Etay El-Baroud Agricultural Research Station

		Dampi		Survived		
Tooleyees	Pre-emergence		Post- emergence			Increasing
Treatments	Incidence	Reduction	Incidence	Reduction	plants	%
	%	%	%	%	%	
Bion 5mM	9.0 c	64.3	2.7 b	49.1	86.5 a	34.5
Salicylic acid 5mM	13.2 b	47.6	1.0 b	81.1	84.0 b	30.6
P. polymyxa <sup>2</sup>	12.0 b	52.4	2.3 b	56.6	83.0 bc	29.1
T. harzianum³	14.0 b	44.4	2.7 b	49.1	81.2 c	26.3
Rizolex –T <sup>4</sup>	7.2 c	71.4	2.7 b	49.1	88.8 a	38.1
Control <sup>5</sup>	25.2 a	0.0	5.3 a	0.0	64.3 d	0.0

<sup>1-</sup> Faba bean seeds (cv. Misr 1) were soaked in aqueous solutions of Bion and salicylic acid for 6 hours on previous day of sowing then air dried.

<sup>2-</sup> Faba bean seeds were soaked in culture filtrate for 6 hours on previous day of sowing, and then seeds were coated with bacterial cells slurry of *Paenibacillus polymyxa*.

<sup>3-</sup> Faba bean seeds moistened with 1% methyl cellulose were coated with formulated Trichoderma harzianum.

<sup>4-</sup> Seed dressing by fungicide was carried out at the recommended dose (3 g/kg).

<sup>5-</sup> Faba bean seeds were soaked in sterilized water for 6 hours on previous day of sowing then air dried.

<sup>6-</sup> Means in each column followed by the same letter are not significantly different according to Duncan's multiple range test, (p = 0.05)

**Table 5.** Effect of some chemical and biotic inducers as well as Rizolex-T as seed treatments on some growth parameters of faba bean plants grown under field conditions at Giza Agricultural Research Station (A) and Etay El-Baroud Agricultural Research Station (B) during winter growing season 2013 - 2014 (natural infection)

(A): Giza Agricultural Research Station

Treatments	Plant height (cm)	Number of branches/ plant	Number of pods/plant	Seed weight/ Plant (g)	100 seed weight (g)	Seed yield (ton/fed)
Bion 5mM	113.8 b	4.8 a	19.2 a	39.3 b	76.4 a	1.524 a
Salicylic acid 5mM	107.0 c	3.8 b	18.0 b	36.6 c	74.7 b	1.362 b
P. polymyxa <sup>2</sup>	107.3 c	3.0 bc	17.6 b	35.3 c	73.6 b	1.252 bc
T. harzianum <sup>3</sup>	104.8 c	2.8 c	16.0 c	33.0 d	73.4 b	1.198 c
Rizolex –T <sup>4</sup>	117.5 a	4.5 a	19.0 a	43.9 a	77.7 a	1.578 a
Control <sup>5</sup>	89.0 d	2.2 cd	12.9 d	25.1 e	68.8 c	0.957 d

(B): Etay El-Baroud Agricultural Research Station

D). Liay Li Baroaa Ag	riountarar reco	our orr otatio	•			
Treatments	Plant height (cm)	Number of branches/ plant	Number of pods/ plant	Seed weight/ Plant (g)	100 seed weight (g)	Seed yield (ton/fed)
Bion 5mM	109.7 a	4.5 a	18.8 a	38.3 a	77.8 a	1.463 a
Salicylic acid 5mM	104.5 b	3.8 ab	17.9 ab	34.2 b	74.3 b	1.275 b
P. polymyxa <sup>2</sup>	104.6 b	2.8 c	17.3 b	32.2 b	73.0 b	1.176 bc
T. harzianum³	102.3 b	2.8 c	15.6 c	31.9 b	72.9 b	1.155 c
Rizolex –T <sup>4</sup>	111.5 a	4.5 a	19.0 a	39.4 a	78.4 a	1.490 a
Control <sup>5</sup>	86.2 c	2.0 d	12.5 d	23.7 с	68.2 c	0.934 d

- 1- Faba bean seeds (cv. Misr 1) were soaked in aqueous solutions of Bion and salicylic acid for 6 hours on previous day of sowing then air dried.
- 2- Fába bean seeds were soaked in bacterial culture filtrate for 6 hours on previous day of sowing, and then seeds were coated with bacterial cells slurry of *Paenibacillus polymyxa*.
- 3- Faba bean seeds moistened with 1% methyl cellulose were coated with formulated Trichoderma harzianum.
- 4- Seed dressing by fungicide was carried out at the recommended dose (3 g/kg).
- 5- Faba bean seeds were soaked in sterilized water for 6 hours on previous day of sowing then air dried.
- 6- Means in each column followed by the same letter are not significantly different according to Duncan's multiple range test, (p = 0.05)

treatments significantly increased plant height as compared with untreated control. The maximum plant height was recorded with Rizolex-T and Bion treatments at Etay El-Baroud Agricultural Research Station and it differed from rest of all treatments followed by *P. polymyxa*, salicylic acid and *T. harzianum* treatments. The maximum plant height was recorded with Rizolex-T treatment followed by Bion treatment at Giza Agricultural Research Station. However, in two locations there were no significant differences among the treatments with salicylic acid, *P. polymyxa and T. harzianum* regarding to plant height.

### Number of branches per plant

Number of branches per plant was affected by seed treatments with some different inducers. All treatments in the two locations significantly showed an increase in number of branches except the treatments with *P. polymyxa and T. harzianum* at Giza Agricultural Research Station as compared with untreated control. Highest significant increase in the number of branches per plant was recorded with Bion and Rizolex-T treatments followed by salicylic acid treatment in the two locations.

### Number of pods per plant:

All treatments significantly increased number of pods per plant as compared with untreated control. The maximum number of pods per plant was recorded with Bion and Rizolex-T treatments which significantly differed from other treatments except the treatment with salicylic acid at Etay El-Baroud Agricultural Research Station. The minimum figures of pods per plant were recorded in *T. harzianum* treatment. In two locations, it was no significant difference between salicylic acid and *P. polymyxa* treatments. Meantime, it was significant difference between *P. polymyxa and T. harzianum* treatments.

### Seed weight per plant

In two locations, all treatments significantly increased seed weight per plant as compared with untreated control. At Etay El-Baroud Agricultural Research Station, the maximum seed weight per plant was recorded with Rizolex-T and Bion treatments, and it significantly differed from the rest of all treatments. Meantime, there were no significant differences among the treatments with salicylic acid, *P. polymyxa and T. harzianum*. At Giza Agricultural Research Station, the maximum seed weight per plant was recorded with Rizolex-T treatment followed by Bion treatment. Meantime, there was no significant difference between salicylic acid and *P. polymyxa* treatments regarding to seed weight per plant.

### The weight of one hundred seed

In two locations, all treatments significantly increased the weight of one hundred seed as compared with untreated control. The seed treatments with Rizolex-T and Bion significantly increased the weight of one hundred seed and it differed from the rest of all treatments followed by salicylic acid, *P. polymyxa and T. harzianum* treatments. Meantime, in two locations there were no significant differences among the treatments with salicylic acid, *P. polymyxa and T. harzianum* regarding to the weight of one hundred seed.

### Seed yield

The two locations showed nearly similar results which indicated that all treatments significantly increased the seed yield as compared with untreated control. The maximum seed yield was recorded from Rizolex-T and Bion treatments which

significantly differed from the rest of all treatments followed by salicylic acid treatment. Meantime, there were no significant differences between the salicylic acid and *P. polymyxa* treatments or between *P. polymyxa* and *T. harzianum* treatments. Whereas, minimum seed yield was recorded in *T. harzianum* treatment compared with untreated control in the two locations.

### 4- Effect of faba bean seed treatment with inducers on activity of oxidative enzymes and phenol content

### I- Activity of oxidative enzymes

Activities of peroxidase (PO) and polyphenol oxidase (PPO) enzymes of faba bean plants were evaluated with the different inducer treatments in the presence of R. solani (Table 6A) or F. solani f. sp. fabae (Table 6B). Results denote that all treatments were effective in eliciting the enzyme activities (peroxidase and polyphenol oxidase). The maximum activities as compared to the untreated control of the two enzymes were recognized with Bion treatment either in the presence of R. solani or F. solani f. sp. fabae. Meantime, salicylic acid treatment showed a considerable increase in the two enzymes but it had less effect as compared to Bion treatment. However, less increase enzyme activities were observed when biotic inducers P. polymyxa and T. harzianum were applied and their treatments in decreasing order had comparatively lower effect (Table 6).

### **II- Phenol content**

The content of total phenols was highly enhanced in faba bean plants treated with different inducers compared with untreated plants in the presence of R. solani (Table 7A) or F. solani f. sp. fabae (Table 7B). The maximum increase in the content of total and free phenolic compounds was recorded with Bion treatment followed by salicylic acid and P. polymyxa compared with untreated control in the presence of R. solani or F. solani f. sp. fabae. Whereas the less increases were recognized in total and free phenols content, when T. harzianum was applied compared with untreated control in the presence of R. solani or F. solani f. sp. fabae. As for conjugated phenols in the presence of R. solani, salicylic acid and T. harzianum treatments gave the highest increase over untreated control (99.6% and 87.8% respectively) followed by Bion and P. polymyxa (83.8% and 78.8% over untreated control).

**Table 6.** Effect of some chemical<sup>1</sup> and biotic inducers as seed treatments on the peroxidase and polyphenol oxidase activity in faba bean plants grown in artificial infested soil<sup>2</sup> by *Rhizoctonia solani* (A) or *Fusarium solani* f. sp. *fabae* (B) under greenhouse conditions

### (A) R. solani:

Treatments		dase activity <sup>3</sup> nce at 430 nm)	Polyphenol oxidase activity <sup>3</sup> (absorbance at 495 nm)		
reatments	Activity	Increasing over control %	Activity	Increasing over control %	
Bion 5 mM	2.93	171.3	0.262	178.7	
Salicylic acid 5 mM	2.76	155.6	0.213	126.6	
P. polymyxa <sup>4</sup>	1.92	77.8	0.201	113.8	
T. harzianum⁵	1.76	63.0	0.189	101.1	
Control <sup>6</sup> ( <i>R. solani</i> )	1.08	0.0	0.094	0.0	
Control <sup>6</sup> healthy (non-infested soil)	0.75		0.078		

### (B) F. solani f. sp. fabae:

Treatments		dase activity <sup>3</sup>	Polyphenol oxidase activity <sup>3</sup> (absorbance at 495 nm)		
reatments	Activity	Increasing over control %	Activity	Increasing over control %	
Bion 5 mM	2.64	161.4	0.210	141.4	
Salicylic acid 5 mM	2.07	105.0	0.198	127.6	
P. polymyxa <sup>4</sup>	1.72	70.3	0.161	85.1	
T. harzianum⁵	1.65	63.4	0.149	71.3	
Control <sup>6</sup> ( <i>F. solani</i> )	1.01	0.0	0.087	0.0	
Control <sup>6</sup> healthy (non infested soil)		0.75	0.0	78	

<sup>1-</sup> Faba bean seeds (cv. Misr 1) were soaked in aqueous solutions of Bion and salicylic acid for 6 hours on previous day of sowing then air dried.

<sup>2-</sup> Soil infestation was achieved by mixing the inoculum of *R. solani* (A) or *F. solani* f. sp. fabae (B) with the soil at the rate of 2% of soil weight.

<sup>3-</sup> Enzyme activity is expressed as change in absorbance/minute/g fresh weight.

<sup>4-</sup> Faba bean seeds were soaked in culture filtrate for 6 hours on previous day of sowing, and then seeds were coated with bacterial cells slurry of *Paenibacillus polymyxa* thereafter the seeds dried out aerobically.

<sup>5-</sup> Faba bean seeds moistened with 1% methyl cellulose were coated with formulated Trichoderma harzianum.

<sup>6-</sup> Faba bean seeds were soaked in sterilized water for 6 hours on previous day of sowing then air dried.

**Table 7.** Effect of some chemical and biotic inducers as seed treatments on levels of phenolic compounds in faba bean plants grown in artificial infested soil by *Rhizoctonia solani* (A) or *Fusarium solani* f. sp. *fabae* (B) under greenhouse conditions.

### (A) R. solani:

( <del>/                                    </del>								
		Phenolic contents (mg/g fresh weight)						
Treatments	Total phenols	Increase over control %	Free phenols	Increase over control %	Conjugated phenols	Increase over control %		
Bion 5 mM	8.833	101.0	4.072	125.7	4.761	83.8		
Salicylic acid 5 mM	8.593	95.6	3.423	89.7	5.170	99.6		
P. polymyxa <sup>3</sup>	7.662	74.4	3.031	68.0	4.631	78.8		
T. harzianum⁴	7.125	62.2	2.261	25.3	4.864	87.8		
control <sup>5</sup> ( <i>R. solani</i> )	4.394	0.0	1.804	0.0	2.59	0.0		
control <sup>5</sup> healthy (non infested soil)	1.9	947	1.0	019	0.9	28		

(B) F. solani f. sp. fabae:

(B) 11 00/4/1/1: 0p: 14b								
		Phenolic contents (mg/g fresh weight)						
Treatments	Total phenols	Increase over control %	Free phenols	Increase over control %	Conjugated phenols	Increase over control %		
Bion 5 mM	8.172	111.7	4.293	172.6	3.879	69.7		
Salicylic acid 5 mM	7.608	97.0	3.691	134.3	3.917	71.3		
P. polymyxa <sup>3</sup>	7.566	96.0	3.545	125.1	4.021	75.9		
T. harzianum⁴	7.243	87.6	2.531	60.7	4.712	106.1		
control <sup>5</sup> ( <i>F. solani</i> )	3.861	0.0	1.575	0.0	2.286	0.0		
control <sup>5</sup> healthy (non infested soil)	1.9	947	1.	019	0.9	28		

<sup>1-</sup> Faba bean seeds (cv. Misr 1) were soaked in aqueous solutions of Bion and salicylic acid for 6 hours on previous day of sowing then air dried.

However, in the presence of *F. solani* f. sp. *fabae*, *T. harzianum* and *P. polymyxa* treatments gave the highest increase of conjugated phenols over untreated control followed by salicylic acid and Bion treatments. Moreover, the least values in total, free and conjugated phenols were recorded in healthy control treatment (**Table 7 A and B**).

### DISCUSSION

Faba bean (*Vicia fabae* L.) is one of the most important winter legume crop grown in northern eastern Africa i.e. Egypt, Sudan and Ethiopia. It is

a valuable protein-rich food since it contains approximately 250 g protein/kg seed (**Multari et al 2015**). However, roots rot diseases caused by *R. solani* Kühn and *F. solani* (Mart.) Sacc. f. sp. *fabae* Yu & Fang are a major cause of yield instability and losses for faba bean production in most areas of the world (**Yu and Fang, 1948; Abdel-Monaim, 2013**; and **Eshetu et al 2013**). Plant pathologists make great efforts to minimise the use of synthetic fungicides and to optimise the use of alternative management strategies to control soil-borne pathogens. Acquired resistance that increases plant resistance to subsequent pathogen attack, by us-

<sup>2-</sup> Soil infestation was achieved by mixing the inoculum of *R. solani* (A) or *F. solani* f. sp. *fabae* (B) with the soil at the rate of 2% of soil weight.

<sup>3-</sup> Faba bean seeds were soaked in culture filtrate for 6 hours on previous day of sowing, and then seeds were coated with bacterial cells slurry of *Paenibacillus polymyxa* thereafter the seeds dried out aerobically.

<sup>4-</sup> Faba bean seeds moistened with 1% methyl cellulose were coated with formulated Trichoderma harzianum

<sup>5-</sup> Faba bean seeds were soaked in sterilized water for 6 hours on previous day of sowing then air dried

ing biotic (microorganisms) or abiotic (chemicals) agents as inducers seem to be one of alternatives to substitute for, or at least to decrease the use of fungicides in plant disease control. Resistance induced by these agents (resistance elicitors) has broad spectrum against numerous pathogens and long lasting, but rarely provides complete control of infection, as many resistance elicitors provide between 20 and 85% disease control (Kuc, 2001; da Rocha & Hammerschmidt, 2005; Walters et al 2005 & 2013).

In the present work, seed treatment with Bion {benzothiadiazole, (BTH)} and salicylic acid (SA) induced systemic resistance in susceptible faba bean plants (cv. Misr 1). The treatments enhanced resistance against pre- emergence and postemergence damping- off caused by *R. solani* and *F. solani* f. sp. *fabae* and highest percentage of survived plants under green house and field conditions, compared with the control. Furthermore, the treatments increased the vegetative and seed growth parameters of faba bean plants under field conditions.

In this respect, the BTH previously showed to be an efficient broad-spectrum resistance inducer against bacterial, fungal and viral diseases in different monocot and dicot crops (Walters et al 2013). Early, it was shown to induce a typical SAR response in wheat and tobacco, effective against different pathogens and resulting in induced expression of Systemic Acquired Resistance "SAR" genes (Gorlach et al 1996 and Friedrich et al 1996). Benzothiadiazole (BTH) is widely reported to induce resistance against a broad spectrum of pathogens in many plant species for example in common bean against Uromyces appendiculatus (Iriti and Faoro, 2003); in pea against Uromyces pisi (Barilli et al 2010a and 2010b); in faba bean against Uromyces viciae-fabae and Orobanche crenata (Sillero et al 2012). The mechanisms of BTH-induced plant resistance have been shown to involve the activation of SAR mechanisms based on the salicylic acid (SA) pathway (Friedrich et al 1996), with consequent up-regulation of defence genes (Bovie et al 2004) and accumulation of phenolic compounds (Iriti et al 2004); induced synthesis of chitinase and β-1,3-glucanase isozymes (Burketova et al 1999); also, activating resistance by increasing the activity of peroxidase (POD) (Sarma et al 2007) and the accumulation of pathogenesis-related (PR) proteins chitinase and H<sub>2</sub>O<sub>2</sub>, some of which possess antimicrobial properties (Sauerborn et al 2002). The beneficial effect of BTH in reducing the extent of fungal colonization in the root tissues is primarily associated with a massive accumulation of structural barriers i.e. wall appositions (**Benhamou**, **1996**).

However, in greenhouse and field experiments, the highest percentage of disease reduction over the control followed Bion (5mM) treatment was obtained from treatment with salicylic acid (5mM). The role of salicylic acid in plants was recorded for the first time in 1979 (White, 1979). Treatment with SA and its derivative induced expression of pathogenesis-related (PR) proteins (Malamy et al 1990 and Gaffney et al 1993). So salicylic acid as a key plant hormone plays an important role in induction of plant defence against a variety of biotic and abiotic stresses through morphological, physiological and biochemical mechanisms (Kumar, 2014). It regulates the activities of various enzymes such as, peroxidase (POD), polyphenol oxidase PPO, phenylalanine ammonia lyase (PAL) etc., which are the major components of induced plant defence against biotic and abiotic stresses (Idrees et al 2011).

On the other side, treatment with P. polymyxa, the genus which was created by Ash et al in 1993 to accommodate the former 'group 3' of the genus Bacillus, had also a significant effect on damping off reduction and significantly enhanced the vegetative and seed growth parameters of faba bean plants under greenhouse and field conditions compared with untreated control, although, its treatment was less effective than Bion. Previous reports have shown that P. polymyxa controls many soil and foliar pathogens in the greenhouse and in the field (Dijksterhuis et al 1999; Beatty & Jensen, 2002; Ryu et al 2006; Raza et al 2009; Phi et al 2010; and Raza et al 2015). P. polymyxa is known for its ability to produce antimicrobial compounds (produced by some but not all strains) act against fungi, bacteria and actinomycetes including gavaserin and saltavidin (Pichard et al 1995); polyxin (Piuri et al 1998); fusaricidins (Beatty & Jensen, 2002); polymyxins and lantibiotics (He et al 2007); and LI-F antibiotics (Deng et al 2011). More than that, P. polymyxa strains are capable of producing several hydrolytic enzymes, including \$1,3glucanases and chitinases which are considered key enzymes in control of fungal plant diseases (Mavingui & Heulin, 1994 and Jung et al 2003 and Raza et al 2009) as well as some isolates have siderophore-producing capabilities. The production of these antibiotics could provide an advantage for establishing the population during the germination of seeds. Plant growth promotion may be an indirect effect of this antibiotic production

through the suppression of plant diseases in disease-carrying soil. One of the possible explanations for growth promotion by *P. polymyxa* which have also been reported that it produces many plant growth stimulators, including auxin as indole-3-acetic acid (**Lebuhn et al 1997 and da Mota et al 2008**); cytokinin (**Timmusk et al 1999**); and 2,3-butanediol (**Nakashimada et al 2000**). So, application of *P. polymyxa* in seed pelleting can be used to manage pre- and post-emergence damping-off in plants (**Choong-Min et al 2006**).

Meantime, in this study, the Trichoderma treatment had also a significant effect on dampingoff reduction and significantly enhanced the vegetative and seed growth parameters of faba bean plants under greenhouse and field conditions compared with untreated control. To some extent, there was no significant difference between the treatments by P. polymyxa and T. harzianum. The genus Trichoderma has been known since at least the 1920s for its ability to act as bio-control agents against plant pathogens (Samuels, 1996). Many T. harzianum isolates were previously found to control the development of R. solani and F. solani diseases of many crops under greenhouse and field conditions (Gallou et al 2009; Basak & Basak, 2011 and Kim & Knudsen, 2013). T. harzianum was demonstrated to be very efficient producer of a wide range of extracellular enzymes and some of these were implicated in the biological control of plant diseases (Almeida et al 2007). Some Trichoderma biological control agents (BCAs) produce highly efficient siderophores that chelate iron and stop the growth of other fungi (Chet and Inbar, 1994). Substantial information provides support that the extraordinary capacity of *T. harzianum* to attack the structures of phytopathogenic fungi and sclerotial degradation by mycoparasitism which has been observed for R. solani (Almeida et al 2007). Meanwhile, T. harzianum is well known producers of antibiotic (produced by some but not all strains) that are toxic for phytopathogenic fungi, including 6-pentyl-a-pyrone (Scarselletti and Faull 1994); Koninginins A, B, D, E and G (Almassi et al 1991 and Ghisalberti & Rowland, 1993); Harzianopyridone (Dickinson et al 1989 and Vinale et al 2006); Harzianic acid (Vinale et al 2009); Azaphilones (Vinale et al 2006); Harzianolide (Almassi et al 1991; Claydon et al 1991 and Vinale et al 2006); and Trichorzianines A (ElHajji et al 1987). Strains of Trichoderma are always associated with plant roots and root ecosystems. However, some authors have defined Trichoderma strains as plant symbiont opportunistic avirulent organisms, able to colonize plant roots by mechanisms similar to those of mycorrhizal fungi and to produce compounds that stimulate growth and plant defense mechanisms (Harman et al 2004). Trichoderma strains that produce cytokinin-like molecules, e.g. zeatyn and gibberellin GA3 or GA3-related, have been detected (Benitez et al 2004). Furthermore, Strains of Trichoderma added to the rhizosphere protect plants against numerous classes of pathogens, e.g. those that produce aerial infections, including viral, bacterial and fungal pathogens, which points to the induction of resistance mechanisms similar to the hypersensitive response (HR), systemic acquired resistance (SAR), and induced systemic resistance (ISR) in plants (Harman et al 2004).

However, the present results indicated that treatments with Bion, salicylic acid and P. polymyxa as seed treatments were effective in eliciting the enzyme activities (peroxidase PO and polyphenol oxidase PPO) in the presence of R. solani or F. solani f. sp. fabae, and the maximum activities of the two enzymes were observed with Bion treatment. The increase of activity was associated with increasing resistance against infection by many diseases (Wang et al 2000). For many years, the role of oxidative enzymes and their metabolic products in the plant defence mechanism has been extensively studied (Hammerschmidt et al 1982). The oxidation of phenols is mediated by the enzymes PO and PPO and the resulting quinones are effective inhibitors of SH group of enzymes which may be inhibiting to the pathogen (Goodman et al 1967). Peroxidase is reported to have an important function in secondary cell wall biosynthesis by polymerizing hydroxy and methoxycinnamic alcohols into lignin and forming rigid cross-links between cellulose, pectin, hydroxyproline-rich glycoproteins (HRGP) and lignin (Grisebach, 1981). Therefore, Peroxidase may be directly associated with the increased ability of systemically protected tissue to lignify which may restrict the penetration (Gross, 1979). Meantime, polyphenoloxidase (PPO) is a widespread enzyme found in plant cells, located in the chloroplast thylakoid membranes and plays an important role in plant resistance. However, it indicates the highest activity toward hydroxylation of monophenols to diphenols, and is capable of dehydrogenating of odiphenols to produce o-quinones (antimicrobial compounds) as well as lignifications of plant cells during microbial invasion (Meyer, 1987). Polyphenoloxidases are suggested to be indirectly involved in auxin biosynthesis because the o-quinones pro-

duced then can react with tryptophan to form indole-3-acetic acid (Mayer and Harel, 1979). Furthermore, the treatments led to increase the phenolic compounds content compared with the untreated control. In this respect, the role of phenolic compounds in disease resistance was postulated by many authors (Nicholson and Hammerschmidt, 1992). They indicated that, phenols are oxidized to quinones or semi-quinones which are more toxic and play a great role as antimicrobial substances on the invaded pathogen (Farkas & Kiraly, 1962). From the obtained results, it can be concluded that treatments with Bion, salicylic acid and P. polymyxa as seed treatments increased plant resistance against the infection by R. solani, or F. solani f. sp. fabae, improved plant growth, yield, accumulation of some antimicrobial substances such as phenolic compounds and increasing activity of defence related enzymes. Such these treatments may be used as a part of integrated disease management for field crops in order to avoiding the use of fungicides.

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