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Induction of Resistance against Soybean Damping-off caused by *Rhizoctonia solani*

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Soybean seeds (cv. Giza 22) were treated with Bion, salicylic acid and saccharin as chemical inducers as well as *Paenibacillus polymyxa* and *Pseudomonas fluorescens* as biotic inducer to study their effect on the infection by *Rhizoctonia solani* under greenhouse and field conditions. Under greenhouse conditions, all treatments induced significant reduction in the percentages of pre- and post-emergence damping-off compared to untreated (check) treatment. The highest survived plants was achieved 84% by the inducers Bion (3 mM) and salicylic acid (5 mM) 82% as compared to 90% by the fungicide Rizolex-T (3g/kg seeds) treatment which achieved. Significant increases in plant height and shoot dry weight of soybean plants were recorded with Bion and fungicide treatments followed by salicylic acid and *P. polymyxa* treatments compared with the check treatment. Under field conditions during 2013 and 2014 growing seasons, all treatments significantly reduced pre- and post-emergence damping-off and increased the percentage of survived plants compared with the check treatment at the two growing seasons. However, the highest survived plants (%) of the two seasons were recorded in case of Rizolex-T (84.6%) and Bion (83.5%) followed by salicylic acid (77%) and *P. polymyxa* (76.2%) treatments compared with the check treatment (62.9%). Meantime, Bion, salicylic acid and *P. polymyxa* inducers significantly improved growth parameters, *i.e.* stem length, number of branches/plant, number of pods/plant and seeds weight/plant, compared to the check treatment throughout the two seasons. Also higher increase in seed yield (kg/feddan) was recorded throughout the two seasons by Bion and fungicide treatments (88.9% and 81.4 %, respectively), followed by salicylic acid and *P. polymyxa* (69.7% and 58.4%, respectively), compared to the check treatment.

Concerning the plant enzyme activity, PO activity increased in Bion treatment by 64.1% followed by salicylic acid by 31.6%. Also, Bion treatment showed the highest increase (118%) in PPO activity over the check treatment. On the other hand, the content of total phenols increased in plants treated with different inducers, compared with untreated plants. The maximum increase (141.2%) in the content of phenolic compounds was recorded with Bion treatment over check treatment, followed by salicylic acid and *P. polymyxa* treatments (being 50% and 49.6%, respectively).

Keywords: Bion, *Paenibacillus polymyxa*, *Pseudomonas fluorescens*, *Rhizoctonia solani*, Rizolex-T, saccharin, salicylic acid and soybean.

Soybean (*Glycine max* L. Merrill) is an important crop, now it is an essential and dominant source of protein and oil with numerous uses in feed, food and industrial applications (Lee *et al.,* 2007). Recent nutritional studies claim that consumption of soybean reduces cancer, blood serum cholesterol, osteoporosis, and heart disease (Birt *et al.,* 2004). In Egypt, during 2013 the total cultivated area of this crop is 8000 hectare, producing 23000 tonnes, but during 2011 Egypt imported additional 1,115,797 tonnes with value of $ 936,340,000 (Anonymous, 2014).

Rhizoctonia root-rot, caused by *Rhizoctonia solani* Kuhn, is a major disease of soybean (Yang, 1999). Damping-off, root-rot and hypocotyls-rot are diseases caused by *R. solani* AG-4 on soybean (Anderson, 1982). Losses in soybeans yield due to infections of *R. solani* reached as much as 48% in small plots (Tachibana *et al.,* 1971). The fungus *R. solani* AG-4 infects soybean, alfalfa, broad bean, common bean, canola, peas, sugar beet, red clover, tomato and potato and its inoculum can survive in their residues (Yang and Li, 2012). The wide host range exhibited by this pathogen complicates management strategies. Moreover, there are no commercial soybean cultivars resistant to *R. solani* (Bradley *et al.,* 2001). Control of this disease has traditionally depended upon chemical control. Growing public concern over the health and environmental hazards associated with pesticides use has generated an urgent need for the development of non-chemical methods for controlling plant diseases. Therefore, induced resistance could be proposed as an alternative, non-conventional and ecologically-friendly approach for plant protection. Its introduction into agricultural practices could minimize the scope of chemical control, thus contributing to the development of sustainable agriculture (Edreva, 2004).

Induction of systemic acquired resistance (SAR) has been shown to protect plants against viral, bacterial, fungal and nematode pathogens among a range of crops (Zhao and Guo, 2003). The protection afforded by SAR is frequently non-specific and long-lasting (Kessmann *et al.,* 1994). Systemic acquired resistance against pathogens can be induced by several synthetic chemical agents, such as salicylic acid, methyl salicylate, benzothiadiazole, *β*-aminobutyric acid, isonicotinic acid, benzoic acid, chitosan, saccharin and so forth which affect production of phenolic compounds and activation of various defence-related enzymes in plants (Thakur and Sohal, 2013 and Walters *et al.,* 2013). Salicylic acid (SA) was the first synthetic compound shown to induce enhanced activation of a variety of defence responses against major pathogens on various crops. However, benzothiadiazole (BTH) stimulates the salicylic acid defence pathway and was recently identified in 1996 by scientists at Novartis as a novel disease-control compound and have been reported to induce SAR in a variety of plants against a wide range of microbial pathogens without possessing direct antimicrobial activity (Görlach *et al.,* 1996 and Thakur and Sohal, 2013). On the other hand, saccharin is a metabolite of probenazole which induces SAR, and has been used in Asia for more than 30 years to control rice blast. Siegrist *et al.* (1997) were the first to identify saccharin as an inducer of systemic resistance. Previous observations have highlighted the potential of saccharin to activate SAR against many diseases (Boyle and Walters, 2005 & 2006 and Srivastava *et al.,* 2011).

However, Plant growth-promoting rhizobacteria (PGPR) are free-living or root-associated bacteria in the rhizosphere of many plant species that enhance plant growth, productivity and often elicit plant immunity against multiple plant pathogens (Ryu *et al*., 2006 and Ahemad and Kibret, 2014). One of the reported plant growth promoting rhizobacteria (PGPR) is *Bacillus polymyxa*, now named *Paenibacillus polymyxa* (Ash *et al*., 1993). Earlier work showed that *P. polymyxa* was active against *Rhizoctonia bataticola* causing charcoal rot disease in soybean (Senthilkumar *et al*., 2007). Strains of *P. polymyxa* have been shown to produce a wide variety of secondary metabolites, including different antibacterial and⁄or antifungal compounds; therefore, the antagonistic effect of these strains upon microbial growth suggests a potential application as biological control agents (Kajimura and Kaneda 1997 and Mageshwaran *et al*., 2010). Specific strains of *Paenibacillus* spp. are known to elicit induced systemic resistance (ISR) similar to that of *Pseudomonas* spp. which leads to the stimulation of host defence mechanisms against multiple pathogens on diverse crop plants. Fluorescent pseudomonads are non-pathogenic rhizobacteria which suppress the soil-borne pathogens through rhizosphere colonization, antibiosis, iron chelation by siderophore production and ISR (Vanitha and Ramjegathesh, 2014).

The objective of this study is to investigate the capability of Bion (BTH), SA, saccharin and plant growth promoting rhizobacteria to protect soybean plants against infection by *R. solani* under greenhouse and field conditions.

Materials and Methods

Plant materials:

Soybean seeds (*Glycine max* L. Merrill), cv. Giza 22, were obtained from the Legume Res. Dept., Field Crops Res. Inst., ARC, Giza, Egypt.

The pathogen:

Rhizoctonia solani Kühn, isolated from naturally infected soybean plants showing damping-off and root-rot symptoms, was kindly provided by Legume and Forage Dis. Res. Dept., Plant Pathol. Res. Inst., ARC, Giza, Egypt. Its pathogenicity was previously confirmed and identified on the basis of cultural properties and microscopic morphological characters according to Sneh *et al.* (1991). The culture maintained on malt extract agar (Malt extract 20g; Peptone 3g and 18g Agar) slants under a phosphate buffer (pH 6.5) at 4±0.5°C (Boeswinkel, 1976).

Preparation of pathogen inoculum:

Inoculum of *R. solani* was prepared by growing the fungus in glass bottles 500cc containing sterilized sorghum medium (100g sorghum grains and 90ml water). The bottles were inoculated with equal disks (0.5 cm) of 4-day-old *R. solani* cultures and incubated at 24±1°C for 21 days, during this period the incubated bottles were shaken for 3 min. every three days to ensure uniform distribution of the fungal growth. After incubation period, the inoculum then air dried for 3 days and ground in a mill to pass through a 3-mm sieve. The ground inoculum was added to soil within one week (Gaskill, 1968).

Chemical inducers:

Bion® wettable granule (WG) 50%, Benzothiadiazole, [benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester] (Syngenta Crop Protection, Inc.); salicylic acid (Sigma Aldrich, USA) and saccharin (MP Biomedicals, LLC) were used in this study.

Growing of biotic inducers:

The cultures of the bacteria *Paenibacillus polymyxa* (isolate 9D14) and *Pseudomonas fluorescens* previously isolated by Shehata *et al*. (2006) were activated on fresh slants and, after 24 hrs were transferred to many 250 ml Erlenmeyer flasks with 50 ml of nutrient yeast dextrose broth (NYDB) medium (8g nutrient broth, 5g yeast extract and 10g dextrose per litre) for *P. polymyxa* and to many 250 ml Erlenmeyer flasks with 50 ml of King's medium B (KMB) broth (Protease peptone 20g, Glycerol 10ml, K_2HPO_4 1.5g and $MgSO_4$ 1.5g) for *P. fluorescens*. The flasks were shaked using a rotary shaker to grow at 120 rpm for 66 hrs at 24±1°C.

Seed and soil treatments:

Apparently healthy uniformity seeds of soybean 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 2 hrs.

A) Chemical inducers treatments:

The disinfected soybean seeds were soaked in aqueous solutions of the inducers (Bion, salicylic acid and saccharin) for 20 min just before sowing at the rate of 3 mM, 5 mM and 3 mM, respectively.

B) Biotic inducer treatments:

After growth of *P. polymyxa*, and *P. fluorescens*, the liquid cultures media were then centrifuged under cooling (4°C) at 10000 rpm for 10 min. Then, the disinfected soybean seeds were soaked in supernatant for 20 min. Cells of *P. polymyxa* and *P. fluorescens* were collected separately in 20 cm Petri dish and bacterial slurry was obtained by adding 1-1.5 ml of 1% methyl cellulose (Sigma-Aldrich, Milwaukee, WI, USA) in sterile distilled water to bacterial cells harvested from each Erlenmeyer flask. Healthy seeds of soybean 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 air-dried for 19 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) Fungicide treatment:

Seed dressing was carried out to the disinfected soybean seeds by applying the Rizolex-T 50% WP (Tolclofos-methyl-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, then the dressed seeds were left to dry before sowing.

D) Root-nodule bacteria treatment:

Formulation of *Bradyrhizobium japonicum* (*soybean*), 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 Bradyrhizobium formulation were mixed in each pot during sowing and 800 g of Bradyrhizobium 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 800g Bradyrhizobium formulation/feddan.

E) Check treatment:

The disinfected soybean seeds were soaked in sterilized water for 20 min just before sowing.

Greenhouse experiment:

The trials were carried out in the greenhouse of Plant Pathol. Res. Inst., Agric. Res. Centre, Giza. Plastic pots (25-cm-diam.) were sterilized by dipping in 5% formalin solution for 15 min, 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* with the soil at the rate of 2% of soil weight (Papavizas and Davey, 1962). Sterilized uninoculated grounded sorghum grains were sown in the disinfested soil at the same rate and used as healthy check. 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 pre-treated soybean seeds, as mentioned before, were sown in each pot and irrigated directly. Ten 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 and kept in a greenhouse under natural conditions. The treatments were as follows: (1) Bion 3 mM; (2) Saccharin 3mM; (3) Salicylic acid 5 mM; (4) *P. polymyxa*; (5) *P. fluorescens;* (6) Rizolex-T. Soaking in water served as a chick for both infested and disinfested soil. Twelve plants (three replicates each of four plants) were uprooted 60 days after sowing, shoots length were measured and 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:

Disease incidence (DI%) was determined by calculating pre- and post-emergence damping-off as well as the survived plants (%), 15, 30 and 45 days after sowing, respectively, according to the following formulas:

$$\text{Pre-emergence (\%)} = \frac{\text{Total No. of un-germinated seeds}}{\text{Total No. of planted seeds}} \times 100$$

$$\text{Post-emergence (\%)} = \frac{\text{Total No. of rotted seedlings}}{\text{Total No. of planted seeds}} \times 100$$

$$\text{Survived plants (\%)} = \frac{\text{Total No. of survived plants}}{\text{Total No. of planted seeds}} \times 100$$

Reduction or increasing (%) over the infected check (A) was also calculated according to the following formula:

$$\text{Reduction or increasing (\%)} = \frac{\text{DI of check A - DI of treatment}}{\text{DI of check A}} \times 100$$

Field experiments:

The field experiments were carried out during the two successive summer growing seasons of 2013 and 2014 at Etay El-Baroud Agric. Res. Station, Behira Governorate, Egypt, in a field known to have Rhizoctonia diseases history, in order to investigate the effect of chemical and biotic inducers for controlling damping-off disease. The disinfected soybean seeds were treated by the same manner in a greenhouse experiment. In the check treatment, seeds were soaked in distilled water as mentioned before. The disinfected soybean seeds were sown in the field on May 27, 2013 and 2014 seasons. The field trial (28 plots) was designed in complete randomized block with four replicates. The area of each plot was 9 m^2 consisted of five rows; each row was 3 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_2O_5) at 150 kg /feddan was added on rows during the soil preparation. Potassium sulphate (48% K_2O) at 50 Kg/feddan was applied as soil application at the second irrigation. Ammonium sulphate (20.5% N) at rate of 100 kg/feddan was added at the first irrigation as a starter dose of nitrogen. First irrigation was done 10 days after sowing and ten irrigations (approximately 10 days for each) were applied in each season. All other recommended agricultural practices were followed according to the recommendations of the Egyptian Ministry of Agriculture and Land Reclamation. The treatments were conducted as follows: (1) Bion 3 mM; (2) Saccharin 3mM; (3) Salicylic acid 5 mM; (4) *P. polymyxa*;(5) *P. fluorescens*; (6) Rizolex-T, (7) Water (check). The disease incidence (DI) % was determined as mentioned before. Random samples of ten soybean plants were collected (from the inner rows) at harvest stage from each plot. Plant growth parameters of plant height, number of branches, number of pods per plant, weight of one hundred seed and seed yield per plant were recorded as well as seed yield (Kg)/feddan were calculated.

Effect of soybean 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. Soybean plants were grown as mentioned before in greenhouse experiment. Activity of peroxidase (PO), polyphenoloxidase (PPO) and phenol contents was determined 15 days after sowing in tissue extracts of soybean plants surviving from the following treatments: (1) Bion 3 mM; (2) Saccharin 3mM; (3) Salicylic acid 5 mM; (4) *P. polymyxa*; (5) *P. fluorescens*; (6) Water in infested soil (check, infected) and (7) Water in disinfested soil (check, 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, 4.0g 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 min. 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 0.5M 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_2O_2 solution was added to it and inverting the tube mixed the content. The reaction was initiated by the addition of H_2O_2. Enzyme activity was recorded as the change in absorbance per minute at 430 nm immediately after the addition of substrate using a spectrophotometer (Milton Roy 601 UV-Vis). Similarly, check of non-enzymatic oxidation was maintained at different times by heating the extract at 100°C for 10 min. The activity was always measured zero indicating its complete inactivation by the heat treatment.

B) Assay of polyphenoloxidase (PPO):

Extraction and assay of polyphenoloxidase enzyme (PPO) were carried out according to Sadasivam and Manickam (1996). For extraction of the polyphenoloxidase enzyme, 2.0g 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 min. 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 495nm using a Milton Roy 601 UV-Vis spectrophotometer. One 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. In similar manner, check was maintained at different times by heating the extract at 100°C for 10 min. 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 min then left to cool. One ml of the Folin ciocalteu's reagent and 6 ml of Na_2CO_3 20% were added. The mixture was diluted to 10 ml with warm distilled water (30-35°C). After 30 min standing in dark, the optical density of the developed blue colour was measured at 520 nm using a spectrophotometer (Milton Roy 601 UV-Vis) against a reagent blank.

Free phenols content was determined by adding 1 ml of the reagent and 3 ml of 20% Na_2 CO_3 solution to 0.5 ml of the sample diluted to 10 ml with warm distilled water, let to stand for 30 min in dark and the optical density of the developed blue colour was measured at 520nm using a spectrophotometer (Milton Roy 601 UV-Vis) 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. Obtained data were subjected to computer statistical software (ASSISTAT) originated by Silva and 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 $P \leq 0.05$.

Results

1- Enumeration of bacteria coated on seeds:

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, after 21 hrs from treatment. Population densities of *P. polymyxa* were $1.9x10^5$; $1.6x10^5$; $1.5x10^5$ and $1.8x10^5$ CFU per seed of soybean and population densities of *P. fluorescens* were $2.8x10^5$; $2.7x10^5$; $2.5x10^5$ and $2.7x10^5$ in greenhouse experiment and field experiments (during 2013 and 2014), respectively.

2- Greenhouse experiment:

a) Effect of some inducers on damping-off disease resistance:

In this experiment, soybean seeds were soaked in aqueous solutions of Bion, salicylic acid and saccharin for 20 min as chemical inducers, or soaked in culture filtrate of *P. polymyxa* or *P. fluorescens* for 20 min and coated with bacterial cells

slurry as biotic inducers to study their effect on the incidence of Rhizoctonia damping-off disease in pots. Results in Table (1) indicate that all treatments induced significant reduction in the percentages of pre- and post-emergence damping-off caused by *R. solani* compared to the check treatment. Bion and salicylic acid treatments significantly gave the highest reduction effect for pre-emergence damping-off. Meantime, they were slightly less than the fungicide Rizolex-T treatment with no significant difference. While, Rizolex-T and Bion treatments significantly gave the highest reduction effect for post emergence damping-off and highest increase effect for survived plants followed by salicylic acid. However, the chemical inducer saccharin and the biotic inducer *P. polymyxa* and *P. fluorescens* treatments significantly were less effect for reduction of damping-off and for increasing of survived soybean plants.

Table 1. Effect of some chemical[1] and biotic[2] inducers as well as Rizolex-T[3] as seed treatments on the percentage of damping-off disease of soybean plants grown in artificial infested soil[4] by *Rhizoctonia solani* under greenhouse conditions

Treatment	Damping-off				Survived plants (%)	Increasing (%)
	Pre-emergence		Post-emergence			
	Incidence (%)	Reduction (%)	Incidence (%)	Reduction (%)		
Bion 3mM	8.0 ab	78.95	6.0 bc	62.5	84.0 bc	90.9
Saccharin (3mM)	16.0 c	57.89	12.0 d	25.0	72.0 d	63.6
Salicylic acid (5mM)	8.0 ab	78.95	8.0 c	50.0	82.0 c	86.4
P. polymyxa	12.0 bc	68.42	10.0 cd	37.5	74.0 d	68.2
P. fluorescens	16.0 c	57.89	12.0 d	25.0	72.0 d	63.6
Rizolex-T[3]	4.0 a	89.47	4.0 b	75.0	90.0 ab	104.5
Check[5] (*R. solani*)	38.0 d	0.00	16.0 e	0.0	44.0 e	0.0
Check[5] (healthy) (disinfested soil)	4.0 a	-	0.0 a	-	96.0 a	-

1- Soybean seeds (cv. Giza 22) were soaked in aqueous solutions of Bion, saccharin and salicylic acid for 20 min just before sowing.

2- Soybean seeds were soaked in culture filtrate for 20 min, and then seeds were coated with bacterial cells slurry of *Paenibacillus polymyxa* or *Pseudomonas fluorescens*.

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

4- Soil infestation was achieved by mixing the inoculum of *R. solani* with the soil at the rate of 2% of soil weight.

5- For check treatments, soybean seeds were soaked in sterilized water for 20 min just before sowing.

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

b) Effect of some inducers on some growth parameters of soybean plants infected with Rhizoctonia solani under greenhouse conditions:

Results presented in Table (2) exhibit that the different inducers improved plant growth as shown by the significant increases in shoot height, shoot and root dry weight of soybean plants. Number of nodules/plant and dry weight of nodules mg/plant were also improved compared with untreated check. Significant differences among treatments were observed for growth parameters. Plant height, shoot dry weight significantly increased with Bion inducer and the fungicide treatments followed by the inducers salicylic acid and *P. polymyxa* treatments. Meanwhile, there was no significant difference among the treatments with Bion, fungicide (Rizolex-T), salicylic acid, *P. polymyxa*, and *P. fluorescens* regarding to root dry weight. As for variability in nodular frequency among treatments Bion treatment resulted in significant increase in nodular frequency compared with untreated check followed by Rizolex-T and *P. polymyxa* treatments. Moreover, the maximum increase in dry weight of nodules were recorded in Bion treatment followed by *P. polymyxa*, Rizolex-T, salicylic acid and *P. fluorescens*, respectively, with no significant difference between Bion and *P. polymyxa* treatment.

Table 2. Effect of some chemical[1] and biotic[2] inducers as well as Rizolex-T[3] as seed treatments on some growth parameters of soybean grown in artificial infested soil[4] by *Rhizoctonia solani* under greenhouse conditions

Treatment	Plant height (cm)	Shoot dry weight (g/plant)	Root dry weight (g/plant)	No. of nodules /plant	Nodules dry weight mg/plant
Bion (3mM)	61.1 ab	3.9 ab	1.4 b	140.0 b	404.0ab
Saccharin (3mM)	47.5 f	2.6 d	0.98 c	107.5 e	368.2 e
Salicylic acid (5mM)	56.7 d	3.8 b	1.3 b	121.5 d	379.1 de
P. polymyxa	58.3 cd	3.8 b	1.34 b	128.5 cd	396.5 bc
P. fluorescens	54.3 e	3.7 c	1.30 b	125.5 d	374.5 de
Rizolex-T[3]	59.4 bc	3.8 bc	1.34 b	134.0 bc	386.5 cd
Check[5] (*R. solani*)	32.5 g	2.1 e	0.65 d	21.0 f	60.0 f
Check[5] healthy (disinfested soil)	63.1 a	4.0 a	1.5 a	153.5 a	417.0 a

1; 2; 3; 4; 5 and 6- As described in footnote of Table (1).

3- Field experiments:

a) Effect of some inducers on the incidence of soybean damping-off disease:

Effect of the biotic and chemical inducers on damping-off disease under field conditions at Etay El-Baroud, Agric. Res. Station, Behira Governorate, was studied in two successive seasons. Results in Table (3, I & II) showed that all treatments significantly reduced pre and post emergence damping-off and increased the percentage of survived plants compared with the untreated check in the two tested seasons. In 2013 growing season, the highest reduction of pre-emergence damping-off over the check treatment was obtained from treatments with fungicide and Bion.

Table 3. Effect of some chemical[1] and biotic[2] inducers as well as Rizolex-T[3] as seed treatments on the percentage of soybean damping-off under field conditions (natural infection) during summer seasons 2013 (Table 3 I) and 2014 (Table 3 II)

Table 3 I. Season 2013:

Treatment	Damping-off				Survived plants (%)	Increasing (%)
	Pre-emergence		Post-emergence			
	Incidence (%)	Reduction (%)	Incidence (%)	Reduction (%)		
Bion (3mM)	9.3 a	59.9	4.5 ab	53.4	82.2 a	30.9
Saccharin (3mM)	20.5 c	11.6	5.0 ab	48.5	71.8 c	14.3
Salicylic acid (5mM)	16.2 b	30.2	5.0 ab	48.5	76.2 b	21.3
P. polymyxa	14.8 b	36.2	4.7 ab	51.5	76.5 b	21.8
P. fluorescens	21.2 c	8.6	6.0 b	38.1	68.0 c	8.3
Rizolex-T[3]	8.5 a	63.4	3.5 a	63.9	84.0 a	33.8
Check[4]	23.2 d	0.0	9.7 c	0.0	62.8 d	0.0

Table 3 II. Season 2014:

Treatment	Damping-off				Survived plants (%)	Increasing (%)
	Pre-emergence		Post-emergence			
	Incidence (%)	Reduction (%)	Incidence (%)	Reduction (%)		
Bion 3mM	8.2 a	63.9	3.7 a	58.9	84.8 a	34.6
Saccharin 3mM	19.8 c	12.8	5.3 ab	41.1	70.0 c	11.1
Salicylic acid 5mM	14.5 b	36.1	4.8 ab	46.7	77.8 b	23.5
P. polymyxa	15.2 b	33.0	4.3 ab	52.2	75.8 b	20.3
P. fluorescens	18.5 c	18.5	6.0 b	33.3	70.8 c	12.4
Rizolex-T[3]	7.2 a	68.3	4.2 ab	53.3	85.2 a	35.2
Check[4]	22.7 d	0.0	9.0 c	0.0	63.0 d	0.0

1; 2; 3; 4; and 5: As (1; 2; 3; 5 and 6, respectively), that described in footnote of Table (1).

Meantime, there were no significant differences among the treatments regarding post-emergence damping-off except with *P. fluorescens* treatment. However, the highest percentage of survived plants was recorded from Rizolex-T and Bion treatments followed by salicylic acid and *P. polymyxa* treatments. Meanwhile, the lowest percentages of survived plants were obtained with *P. fluorescens* and saccharin treatments. Results recorded in 2014 growing season were nearly similar to those of 2013 growing season.

b) Effect of some inducers on growth parameters and yield of soybean plants:

Under field condition, most of inducer treatments significantly improved growth parameters and yield compared to the untreated check treatment in the two seasons (Table 4, I & II). Recorded results indicate that:

Plant height:

The stem length was affected by seed treatments with different inducers. In 2013 growing season, all treatments significantly increased plant height as compared with the untreated check, except in case of *P. fluorescens.* Maximum plant height was recorded with Bion treatment, followed by Rizolex-T, salicylic acid and *P. polymyxa* treatments. In 2014 growing season, all treatments significantly increased plant height except with saccharin treatment. No significant differences among treatments of Bion 3mM, Rizolex-T, salicylic acid 5mM, *P. polymyxa* and *P. fluorescens.*

Number of branches per plant:

Number of branches per plant was affected by seed treatments with some different inducers. All treatments significantly showed an increase in number of branches except the treatment with *P. fluorescens* in the two growing seasons 2013 and 2014 and the treatment with saccharin in the growing seasons 2014. Highest significant increase in the number of branches per plant was recorded with Bion, Rizolex-T and *P. polymyxa* treatments followed by salicylic acid treatment as compared with the untreated check in the two growing seasons.

Number of pods per plant:

All treatments significantly increased number of pods per plant as compared with the untreated check. The two growing seasons showed nearly similar results which indicated that maximum number of pods per plant was recorded with Bion and Rizolex-T treatments which significantly differed from other treatments, followed by *P. polymyxa* and salicylic acid. While the minimum figures of pods per plant were recorded in saccharin and *P. fluorescens* treatments.

Seed weight per plant:

All treatments significantly increased seed weight per plant as compared with the untreated check, except with the treatments of saccharin and *P. fluorescens* in the two growing seasons. Highest significant increase in the seed weight per plant was recorded with Bion and Rizolex-T treatments, followed by salicylic acid and *P. polymyxa* treatments in the two growing seasons. However, the least effect regarding seed yield/plant caused by *P. fluorescens* and saccharin treatments which had not significant difference with the untreated check in the two growing seasons.

The weight of one hundred seed:

The seed treatments with Bion and Rizolex-T resulted in 2013 growing season significantly increased the weight of one hundred seed which have contributed for higher seed yield/plant compared to the untreated check, followed by salicylic acid and *P. polymyxa*. While in 2014 growing season the Bion treatment significantly increased the weight of one hundred seed, followed by Rizolex-T and salicylic acid treatments. However, the least effect was recorded in *P. fluorescens* treatments in the two growing seasons.

Seed yield:

The maximum seed yield was recorded in case of Bion treatment in the two growing seasons (88.9% over the untreated check as means of the two seasons), followed by Rizolex-T and salicylic acid treatments. Whereas, minimum seed yield was recorded with *P. fluorescens* treatment in the two growing seasons.

Table 4. Effect of some chemical[1] and biotic[2] inducers as well as Rizolex-T[3] as seed treatments on some growth parameters of soybean under field conditions (natural infection) during summer seasons of 2013 (I) and 2014 (II)

Table 4 I. Season 2013:

Treatment	Plant height (cm)	No. of branches /plant	No. of pods/ plant	Seed weight /plant (g)	100 seed weight (g)	Seed yield (ton/fed)
Bion (3mM)	83.9 a	4.5 a	75.3 a	35.4 a	19.5 a	1.631 a
Saccharin (3mM)	72.3 c	3.7 bc	57.8 c	25.5 c	17.6c	1.282 c
Salicylic acid (5mM)	80.1 b	3.8 b	67.8 b	29.5 b	18.4 b	1.523 ab
P. polymyxa	79.6 b	4.1 ab	66.5 b	29.5 b	18.5 b	1.414 b
P. fluorescens	68.9 d	2.9 d	52.5 c	24.8 c	16.6 d	1.121 d
Rizolex-T	81.8 b	4.3 a	73.8 a	34.8 a	19.1 a	1.579 a
Check[4]	67.1 d	2.8 d	43.0 d	24.0 c	15.5 e	0.881 e

Table 4 II. Season 2014:

Treatment	Plant height (cm)	No. of branches /plant	No. of pods/ plant	Seed weight /plant (g)	100 seed weight (g)	Seed yield (ton/fed)
Bion (3mM)	81.5 a	4.0 a	70.3 a	31.3 a	19.2 a	1.629 a
Saccharin (3mM)	70.3 b	2.8 bc	49.7 d	25.3 d	17.8 c	1.152 e
Salicylic acid (5mM)	80.0 a	3.3 b	58.6 c	29.4 bc	18.7 b	1.405 c
P. polymyxa	79.5 a	4.3 a	60.4 c	28.4 c	18.1 c	1.318 d
P. fluorescens	78.0 a	2.8 bc	50.3 d	24.8 d	16.9 d	1.030 f
Rizolex-T	80.5 a	4.0 a	65.7 b	30.1 ab	18.9 b	1.551 b
Check[4]	69.3 b	2.5 c	39.5 e	24.8 d	15.8 e	0.844 g

1; 2; 3; 4 and 5: As (1; 2; 3; 5 and 6, respectively), that described in footnote of Table (1).

4- Effect of soybean seed treatment with inducers on activity of oxidative enzymes and phenol content:

a) Activity of oxidative enzymes:

Activities of peroxidase (PO) and polyphenoloxidase (PPO) enzymes of the soybean plants were determined with the different inducer treatments (Table 5). Results indicate that Bion treatment showed remarkable increase in both PO and PPO activities as compared to the untreated check. Salicylic acid treatment showed a considerable increase in PPO activity meantime, it had less effect on PO activity. On the other hand the biotic inducers *P. polymyxa* and *P. fluorescens* and chemical inducer saccharin, in decreasing order had comparatively slight effect.

Table 5. Effect of some chemical[1] and biotic[2] inducers as seed treatments on the peroxidase and polyphenoloxidase activity in soybean plants grown in artificial infested soil[3] by *R. solani* under greenhouse conditions

Treatment	Peroxidase activity[4] (absorbance at 430 nm)		Polyphenoloxidase activity[4] (absorbance at 495 nm)	
	Activity	Increasing over check	Activity	Increasing over check
Bion (3mM)	2.507	64.1	0.297	118.4
Saccharin (3mM)	1.814	18.7	0.161	18.4
Salicylic acid (5mM)	2.012	31.6	0.231	69.9
P. polymyxa	1.939	26.7	0.199	46.3
P. fluorescens	1.895	24.1	0.159	16.9
Check[5] (*R. solani*)	1.528	0.0	0.136	0.0
Check[5] healthy (disinfested soil)	1.213		0.117	

1; 2; 3; 4 and 5: As described in footnote of Table (4).

b) Phenol content:

The content of total phenols was greatly increased in plants treated with different inducers, compared with untreated plants (Table 6). Maximum increase in phenolic compound contents was recorded with Bion treatment (141.2%) over the untreated check, followed by salicylic acid and *P. polymyxa* treatments (50and 49.6% over the untreated check, respectively). On the other hand, *P. fluorescens* gave the lowest increase (9.5, 5.9and 34%, respectively), for total, free and conjugated phenols. However, the maximum increase in free phenols was obtained with Bion treatment followed with salicylic acid (79 and 33%, respectively, over the untreated check). As for conjugated phenols, Bion and *P. polymyxa* treatments gave the highest increase over the untreated check (596.1and 216.2%, respectively), followed by salicylic acid and saccharin (166.7and 147.15% over the untreated check).

Table 6. Effect of some chemical[1] and biotic[2] inducers as seed treatments on levels of phenolic compounds in soybean plants grown in artificial infested soil[3] by *R. solani* under greenhouse conditions

Treatment	Phenolic contents (mg/g fresh weight)					
	Total phenols	Increase over check	Free phenols	Increase over check	Conjugated phenols	Increase over check
Bion (3mM)	4.925	141.2	3.192	79.0	1.733	569.1
Saccharin (3mM)	2.552	24.9	1.912	7.2	0.640	147.1
Salicylic acid (5mM)	3.063	50.0	2.372	33.0	0.691	166.7
P. polymyxa	3.055	49.6	2.236	25.4	0.819	216.2
P. fluorescens	2.236	9.5	1.889	5.9	0.347	34.0
Check[4] (*R. solani*)	2.042	0.0	1.783	0.0	0.259	0.0
Check[4] healthy (disinfested soil)	1.495		1.352		0.143	

1; 2; 3 and 4: As 1; 2; 4 and 5, respectively, that described in footnote of Table (1).

Discussion

Root-rot and damping-off diseases, caused by *Rhizoctonia solani* Kühn, are considered as the most serious and economically important diseases affecting soybean production in most areas of the world (Yang, 1999). Control of these diseases is traditionally depending upon chemical control. So, research priorities call for novel protection methods that are compatible with sustainable agriculture. Acquired resistance that increases plant resistance to subsequent pathogen attack seems 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 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 and Hammerschmidt, 2005; Walters *et al.*, 2005 and Lyon, 2007).

In this study, seed treatment with Bion (BTH), salicylic acid (SA) and saccharin as chemical inducers induced systemic resistance in susceptible soybean cultivar and enhanced resistance against pre-emergence and post-emergence damping-off caused by *R. solani*. These results were obtained from greenhouse experiment and confirmed by field experiments. Consequently, growth parameters, yield and yield components of treated soybean plants were increased.

The exogenous application of salicylic acid or benzothiadiazole (BTH) commercialized as Bion, or Actigard trigger the SAR signal transduction pathway in several plant species by production of peroxidases, phytoalexins and the accumulation of pathogenesis-related (PR) proteins, some of which possess antimicrobial properties, (Kuc, 1995; Van Loon, 1997; Sarma *et al.*, 2007 and Thakur and Sohal, 2013) when applied to seeds (Latunde-Dada and Lucas, 2001). 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, application of salicylic acid had a significant effect on damping-off reduction. Moreover, salicylic acid is an important endogenous plant growth regulator that generates a wide range of metabolic and physiological responses in plants involved in plant defence in addition to their impact on plant growth and development (Lu, 2009 and Vicent and Plasencia, 2011). It regulates the activities of various enzymes such as, peroxidase (PO), polyphenoloxidase (PPO), phenylalanine ammonia lyase (PAL) *etc*., which are the major components of induced plant defence against biotic and abiotic stresses (Idresse *et al*., 2011).

Saccharin has been found to induce systemic resistance against several diseases as foliar application or root drench, tobacco mosaic virus (TMV) in tobacco, *Colletotrichum lagenarium* in cucumber, *Uromyces appendiculatus* in runner bean and *Blumeria graminis* in barley (Siegrist *et al*., 1998). Additionally, probenazole, of which saccharin is a metabolite, is effective in controlling bacterial blight caused by *Xanthomonas oryzae* and rice blast caused by *Magnaporthe grisea* (Oostendorp *et al*., 2001 and Siegrist *et al.*, 1997). Srivastava *et al*. (2011) found that saccharin applied as a root drench to soybean plants was usually more effective than the leaf

treatment at inducing protection. Similar response has been observed by other researchers in other plant species (Siegrist *et al*., 1997 and Boyle and Walters, 2005 and 2006). Results of the presented study cleared that saccharin as seed soaking treatment gave the least reduction of the disease among the chemical inducers. However, method of saccharin application was a significant factor influencing the severity of infection, when saccharin applied as a root drench was more effective than the foliar spray treatment at inducing SAR (Srivastava *et al*., 2011). So, additional study is required to establish the proper mode of application of saccharin to control Rhizoctonia diseases.

As for biotic inducers, in the presented study, plant growth promoting rhizobacteria (PGPR), *i.e. Paenibacillus polymyxa* and *Pseudomonas fluorescens*, were used in the presented study for controlling damping-off disease of soybean and results indicated that *P. fluorescence* was effective in reducing damping-off disease under greenhouse conditions in comparison with *P. polymyxa* which was effective under both of greenhouse and field conditions. The antagonistic mechanisms of *Paenibacillus* spp. include the production of hydrolytic enzymes, siderophore and antibiotics. It is also known to induce systemic resistance in host plant and competing out the plant pathogens (Sturz *et al*., 1997 and Choong-Min *et al.,* 2006). *Paenibacillus polymyxa* is also known to have important roles in the rhizospheres of different crops, with the ability of many strains to secrete plant growth-enhancing substances such as cytokinins and auxins (Lebuhn *et al.,* 1997; Timmusk *et al.*, 1999; da Mota *et al.*, 2008). 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).

However, fluorescent Pseudomonads are well known to suppress fungal root diseases of agronomic crops. They produce the siderophore, pyrrolnitrin, phenazine and lytic enzymes against fungal root and seedling pathogens on a variety of crops (Vanitha and Ramjegathesh, 2014). However, greenhouse conditions offer good opportunities for the application of biological control agents, mostly due to their more uniform and controlled environment (Albajes *et al*., 1999). Meantime, the *P. polymyxa* has other interesting properties for biocontrol applications mainly because of the formation of endospores which are resistant against chemicals, mechanical damage, heat, desiccation, UV and organic solvents (Tupinamba´*et al.*, 2008). So, *P. polymyxa* is more convenient to use in the greenhouse and the fields as it is easier to handle and apply providing commercial benefits (Kloepper *et al.*, 2004). Generally, the durability of resistance by PGPR differs from crop to crop and also due to different bacterial strains (Nayar, 1996 and Viswanathan, 1999).

On the other side, higher increase of phenolic compounds was obtained in plants treated with Bion, salicylic acid and *P. polymyxa*, respectively, compared with the untreated check. It is well known that synthesis of phenols occurs as an early response of plants to attempted infection by pathogens, as antimicrobial compounds (Kruger *et al.,* 2002). In the presented study, the activity of peroxides and polyphenoloxidase enzymes were obviously higher in plants grown from treated seed with Bion and salicylic acid, respectively, compared with untreated check. In this respect, increased peroxidase activity has been obtained in a number of

resistant interactions involving plant pathogenic fungal and bacterial interactions and their increase has been associated with decreases in the rate of multiplication and spread of the pathogen (Benhamou and Bélanger, 1998; Brisset *et al.*, 2000 and Trognitz *et al.*, 2002). Also, the expression of resistance is often accompanied by the activation of phenol-oxidizing enzymes such as PO, PPO (Goodmann and Novacky, 1994). Increased PO and PPO activity may contribute to defence through the production of oxidized forms of quinones, which can inactivate pectinolytic enzymes produced by pathogens. Moreover, peroxides and polyphenoloxidase enzymes are involved in several plant defence mechanisms, such as lignin biosynthesis, oxidative crosslinking of plant cell walls, and generation of active oxygen species (Faize *et al.*, 2004).

References

Ahemad, M. and Kibret, M. 2014. Mechanisms and applications of plant growth promoting rhizobacteria: Current perspective. *J. King Saud Univ. Sci.*, **26**: 1-20.

Albajes, R.; Gullino, M.L.; Van Lenteren, J.C. and Elad, Y. 1999. Integrated Pest and Diseases Management in Greenhouse Crops. Kluwer Academic Publishers, Dordrecht, Netherlands.

Anderson, N.A. 1982. The genetics and pathology of *Rhizoctonia solani*. *Annu. Rev. Phytopathol*., **20**: 329-347.

Anonymous. 2014. Food and Agricultural Organization (FAO) of the United Nations, http://fao.org.

Ash, C.; Priest, F.G. and Collins, M.D. 1993. Molecular identification of rRNA group 3 bacilli (Ash, Farrow, Wallbanks and Collins) using a PCR probe test. *Antonie Van Leeuwenhoek*, **64**: 253-260.

Benhamou, N. 1996. Elicitor-induced plant defence pathways. *Trends Plant Sci.*, **1**: 233-240.

Benhamou, N. and Bélanger R.R. 1998. Induction of systemic resistance to Pythium damping-off in cucumber plants by benzothiadiazole: Ultrastructure and cytochemistry of the host response. *Plant J.*, **14**(1): 13-21.

Birt, D.F.; Hendrick, S. and Alekel, D. L. 2004. Soybean and the prevention of chronic human disease. Pages: 1047-1117. In: *Soybeans: Improvement, Production, and uses Agronomy Monographs*. 3rd Ed. Boerma, H.R. and Specht, J.E. (eds.). Madison, WI, USA.

Boeswinkel, H.J. 1976. Storage of fungal cultures in water. *Trans. Br. Mycol. Soc*., **66**: 183-185.

Boyle, C. and Walters, D. 2005. Induction of systemic protection against rust infection in broad bean by saccharin: effects on plant growth and development. *New Phytol*., **167**:607-612.

Boyle, C. and Walters, D.R. 2006. Saccharin-induced protection against powdery mildew in barley: Effects on growth and phenylpropanoid metabolism. *Plant Pathol*., **55**: 82-91.

Bradley, C.A.; Hartman, G.L.; Nelson, R.L.; Mueller, D.S. and Pedersen, W.L. 2001. Response of ancestral soybean lines and commercial cultivars to Rhizoctonia root and hypocotyls rot. *Plant Dis*., **85**: 1091-1095.

Brisset, M.N.; Cesborn, S.; Thomson, S.V. and Paulin, J.P. 2000. Acibenzolar-S-methyl induces the accumulation of defence related enzymes in apple and protects from fire blight. *Eur. J. Plant Pathol*., **106**: 529-536.

Chakraborty, M.R. and Chatterjee, N.C. 2007. Interaction of *Trichoderma harzianum* with *Fusarium solani* during its pathogenesis and the associated resistance of the host. *Asian J. Exp. Sci.,* **21**: 51-355.

Choong-Min, R.; Jinwoo, K.; Okhee, C.; Seuk, H.K. and Chang, S.P. 2006. Improvement of biological control capacity of *Paenibacillus polymyxa* E681 by seed pelleting on sesame. *Biol. Control*, **39**: 282-289.

da Mota, F.F.; Gomes, E.A. and Seldin, L. 2008. Auxin production and detection of the gene coding for the auxin efflux carrier (AEC) protein in *Paenibacillus polymyxa*. *J. Microbiol*., **56**: 275-264.

da Rocha, A.B. and Hammerschmidt, R. 2005. History and perspectives on the use of disease resistance inducers in horticultural crops. *Hort. Technol.*, **15**: 518-529.

Edreva, A. 2004. A novel strategy for plant protection: Induced resistance. *J. Cell Molecular Biol.*, **3**: 61-69.

Faize, M.; Faize, L.; Koike, N.; Ishizaka, M. and Ishii, H. 2004. Acibenzolar-*S*-methyl-induced resistance to Japanese pear scab is associated with potentiation of multiple defence responses. *Phytopathology*, **94**: 604-612.

Gaskill, J.O. 1968. Breeding for Rhizoctonia resistance in sugar beet. *J. Am. Soc. Sugar Beet Technol*., **15**: 105-119.

Goodmann, R.N. and Novacky, A.J. 1994. The bacteria-induced hypersensitive reaction. Page: 244. In: *The hypersensitive Reaction in Plants to Pathogens.* Goodman, R.N. and Novacky, A.J. (eds.). St. Paul: Amer. Phytopathol. Soc.

Görlach, J.; Volrath, S.; Knauf-Beiter, G.; Hengy, G.; Beckhove, U.; Koge, K. H. l.; Oostendorp, M.; Staub, T.; Ward, E.; Kessmann, H. and Ryals, J. 1996. Benzothiadiazole, a novel class of inducers of systemic acquired resistance, activates gene expression and disease resistance in wheat. *Plant Cell*, **8:** 629-643.

Idresse, M.; Naeem, N.; Aftab, T.; Khan, M.M. and Moinuddin, A. 2011. Salicylic acid mitigates salinity stress by improving antioxidant defence system and enhances vincristine and vinblastine alkaloids production in periwinkle [*Catharanthus roseus* (L.) G. Don]. *Acta Physiol. Plant.*, **1**(33): 987-999.

Kajimura, Y. and Kaneda, M. 1997. Fusaricidins B, C and D, new depsipeptide antibiotics produced by *Bacillus polymyxa* KT-8: isolation, structure elucidation and biological activity. *J. Antibiot*., **50**: 220-228.

Kessmann, H.; Staub, T.; Hofmann, C.; Maetzke, T.; Herzog, J.; Ward, E.; Uknes, S. and Ryals, J. 1994. Induction of systemic acquired resistance in plants by chemicals. *Annu. Rev. Phytopathol.*, **32**: 439-459.

Kloepper, J. W.; Ryu, C.M. and Zhang, S. 2004. Induced systemic resistance and promotion of plant growth by *Bacillus* spp. *Phytopathology*, **94**: 1259-1266.

Kruger, W.M.; Carver, T.W. and Zeyen, R.J. 2002. Phenolic inhibition of penetration resistance to *Blumeria graminis* f.sp. *hordei* in barley near isogenic lines containing seven independent resistance genes or alleles. *Physiol. Mole. Plant Pathol*., **61**: 41-51.

Kuc, J. 1995. Phytoalexins, stress metabolism, and disease resistance in plants. *Annu. Rev. Phytopathol*., **33**: 275-297.

Kuc, J. 2001. Concepts and direction of induced systemic resistance in plants and its application. *Eur. J. Plant Pathol*., **107**: 7-12.

Latunde-Dada, A.O. and Lucas, J.A. 2001. The plant defence activator acidbenzolar-*S*-methyl primes cowpea [*Vigna unguiculata* (L.) Walp.] seedlings for rapid induction of resistance. *Physiol. Mol. Plant Pathol.*, **58**: 199-208.

Lebuhn, M.; Heulin, T. and Hartmann, A. 1997. Production of auxin and other indolic and phenolic compounds by *Paenibacillus polymyxa* strains isolated from different proximity to plant roots. *FEMS Microbiol. Ecol*., **22**: 325-334.

Lee, G.J.; Wu, X.; Shannon, J.G.; Sleper, D.A. and Nguyen, H. T. 2007. Soybean. Pages: 1-53. In: *Genome Mapping and Molecular Breeding in Plants.* Vol. 2. Kole, C. (ed.). Oilseeds. Springer-Verlag, Berlin Heidelberg.

Lu, H. 2009. Dissection of salicylic acid-mediated defence signalling networks. *Plant Signal Behav*., **4**: 713-717.

Lyon, G. 2007. Agents that can elicit induced resistance. Pages: 9-29. In: *Induced Resistance for Plant Disease Control: a Sustainable Approach to Crop Protection*. Walters, D.; Newton, A. and Lyon, G. (eds.). Blackwell Publishing, Oxford.

Mageshwaran, V.; Gupta, A.; Kumar, U.; Padaria, J. and Annapurna, K. 2010. Effect of seed treatment with *Paenibacillus polymyxa* HKA-15 on suppression of charcoal rot disease and growth promotion of soybean. *Pusa Agric. Sci*., **33**: 14-19.

Nayar, K. 1996. Development and evaluation of a biopesticide formulation for control of foliar disease of rice. Ph.D. Thesis, TNAU, Coimbatore, India, 223 pp.

Oostendorp, M.; Kunz, W.; Dietrich, B. and Staub, T. 2001. Induced disease resistance in plants by chemicals. *Eur. J. Plant Pathol*., **107**: 19-28.

Papavizas, G.C. and Davey, C.B. 1962. Isolation and pathogenicity of Rhizoctonia saprophytically existing in soil. *Phytopathology*, **52**: 834-840.

Ryu, C.M.; Kim, J.W.; Choi, O.H.; Kim, S.H. and Park, C.S. 2006. Improvement of biological control capacity of *Paenibacillus polymyxa* E681 by seed pelleting on sesame. *Biological Control*, **39**: 282-289.

Sadasivam, S. and Manickam, A. 1996. *Biochemical Methods*. 2nd Ed. New Age Int. Pvt. Ltd. Pub. and T.N. Agric. Univ. Coimbatore, Tamil Nadu, India.

Sarma, B.K.; Ameer Basha, S.; Singh, D.P.; Singh, U.P. 2007 Use of nonconventional chemicals as an alternative approach to protect chickpea (*Cicer arietinum*) from Sclerotinia stem rot. *Crop Prot*., **26**: 1042-1048.

Senthilkumar, M.; Govindasamy, V. and Annapurna, K. 2007. Role of antibiosis in suppression of charcoal rot disease by soybean endophyte *Paenibacillus* sp. HKA-15. *Curr. Microbiol*., **55**: 25-29.

Shehata, S.T.; Mosbah, M.M. and Hegazi, M.F. 2006. Selection of biocontrol agents for control of onion white rot disease. *J. Agric. Sci. Mansoura Univ.*, **31**: 2621-2637.

Siegrist, J.; Glenewinkel, D. and Schmidke, M. 1997. Chemically induced resistance in green bean against bacterial and fungal pathogens. *Zeitschriftfür Pflanzenkrankheiten und Pflanzenschutz*, **104**: 599-610.

Siegrist, J.; Mühlenbeck, S. and Buchenauer H. 1998. Cultured parsley cells, a model system for the rapid testing of abiotic and natural substances as inducers of systemic acquired resistance. *Physiol. Mol. Plant Pathol.*, **53**: 223-238.

Silva, F.; and Azevedo, C.A.V. 2009. Principal Components Analysis in the Software Assistat-Statistical Attendance. World Cong. on Computers in Agric., American Society of Agricultural and Biological Engineers.

Sneh, B.; Burpee, L. and Ogoshi, A. 1991. Identification of *Rhizoctonia* species. American Phytopathological Society Press, Saint Paul, USA. 133pp.

Snell, F.D. and Snell, C.T. 1953. Calorimetric methods of analysis, including some turbidimetric and nephelometric methods. 3rd Ed., Vol. III (Organic I). D. Van Nostrand CO. Inc., Princeton, NJ, USA. 606pp.

Srivastava, P.; George, S.; Marois, J.J.; Wright, D.L. and Walker, D.R. 2011. Saccharin-induced systemic acquired resistance against rust (*Phakopsora pachyrhizi*) infection in soybean: Effects on growth and development. *Crop Protec.*, **30**: 726-732.

Sturz, A. V.; Christie, B. R.; Matheson, B. G. and Nowak, J. 1997. Biodiversity of endophytic bacteria which colonize red clover nodules, roots, stems and foliage and their influence on host growth. *Biol. Fertil. Soils*, **25**: 13-19.

Sutha, R.; Ramiah, M. and Rajappan, K. 1998. Changes in protein and amino acid composition of tomato due to a tospovirus infection. *Indian Phytopathol*., **51**: 136-139.

Tachibana, H.; Jowett, D. and Fehr, W.R. 1971. Determination of losses in soybeans caused by *Rhizoctonia solani. Phytopathology*, **61**: 1444-1446.

Thakur, M. and Sohal, B.S. 2013. Role of elicitors in inducing resistance in plants against pathogen infection: A Review. ISRN Biochemistry, Vol. 2013, 10 pp.

Timmusk, S.; Nicander, B.; Granhall, U. and Tillberg, E., 1999. Cytokinin production by *Paenibacillus polymyxa*. *Soil Biol. Biochem*., **31**: 1847-1852.

Trognitz, F.; Manosalva, P.; Gysin, R.; Nino-Liu, D.; Simon, R.; Herrera, M.D.; Trognitz, B.; Ghislain, M. and Nelson, R. 2002. Plant defence genes associated with 78 quantitative resistances to potato late blight in *Solanum phureja* x dihaploid *S. tuberosum* hybrids. *Mol. Plant-Microbe Interact*., **l5**: 587-597.

Tupinamba, G.; da Silva, A.J.R.; Alviano, C.S.; Souto-Padron, T.; Seldin, L. and Alviano, D.S. 2008. Antimicrobial activity of *Paenibacillus polymyxa* SCE2 against some mycotoxin-producing fungi. *J. Appl. Microbiol.*, **105**: 1044-1053.

Van Loon, L.C. 1997. Induced resistance in plants and the role of pathogenesis-related proteins. *Eur. J. Plant Pathol*., **103**: 753-765.

Vanitha, S. And Ramjegathesh, R. 2014. Bio Control potential of *Pseudomonas fluorescens* against coleus root rot disease. *J. Plant Pathol. Microbiol.*, **5**: 216.

Vicent, M.R.S. and Plasencia, J. 2011. Salicylic acid beyond defence: its role in plant growth and development. *J. Exp. Bot.*, **62**: 3321-3338.

Viswanathan, R., 1999. Induction of systemic resistance against red rot disease in sugarcane by plant growth promoting rhizobacteria. Ph.D. Thesis, TNAU, Coimbatore, India, 175pp.

Walters, D.R.; Ratsep, J. and Havis, N.D. 2013. Controlling crop diseases using induced resistance: Challenges for the future. *J. Exp. Bot*., **64**: 1263-1280.

Walters, D.; Walsh, D.; Newton, A. and Lyon, G. 2005. Induced resistance for plant disease control: Maximizing the efficacy of resistance elicitors. *Phytopathology*, **95**: 1368-1373.

Yang, X.B. 1999. Rhizoctonia damping-off and root rot. Pages: 45-46. In: *Compendium of Soybean Diseases.* Hartmann, G.L.; Sinclair, J.B. and Rupe, J.C. (eds.). APS, St Paul, MN, USA.

Yang, G. and Li, C. 2012. General description of *Rhizoctonia* species complex. Pages: 41-52. In: *Plant Pathology*. C.J. Cumagun (ed.). Tech, Rijeka, Croatia.

Zhao, S. and Guo, J. 2003. Systemic acquired resistance and signal transduction. *Agric. Sci. in China*, **2**: 539-548.

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الحث علي مقاومة مرض موت البادرات في فول الصويا المتسبب عن فطر *Rhizoctonia solani*

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يهدف هذا البحث إلي دراسة تأثير معاملة بذور فول الصويا من الصنف جيزة ٢٢ بمواد البيون، حامض السلسيليك والسكارين كمستحثات كيماوية بالإضافة إلي بكتيريا *Paenibacillus polymyxa* و *Pseudomonas fluorescens* كمستحثات حيوية ضد الاصابة بفطر *Rhizoctonia solani* تحت ظروف الصوبة والحقل. وقد أدت كل المعاملات تحت ظروف الصوبة الي إختزال معنوي في نسبة موت البادرات قبل وبعد الظهور فوق سطح التربة مقارنة بالبذور الغير معاملة المنزرعة في التربة المعداه بالفطر. وقد تحققت أعلي نسبة للنباتات الباقية علي قيد الحياه من المعاملة بمركب البيون بتركيز ٣ ملي مول/لتر وكانت ٨٤% والمعاملة بحامض السلسليك بتركيز ٥ ملي مول/لتر وكانت ٨٢% مقارنة بمعاملة المبيد رايزولكس- تي بمعدل ٣ جم / كجم تقاوي وكانت ٩٠%. وقد سجلت زيادة معنوية في أطوال النباتات و الوزن الجاف للمجموع الخضري لنباتات فول الصويا مع المعاملة بمركب البيون والمعاملة بالمبيد يليها معاملة حامض السلسليك وبكتيريا *P. polymyxa* وذلك مقارنة بالنباتات الناتجة من البذور غير المعاملة.

تحت ظروف الحقل في محطة بحوث ايتاي البارود ـ مركز البحوث الزراعية ـ محافظة البحيرة خلال موسمي ٢٠١٣ و ٢٠١٤ ، أدت كل المعاملات إلي إختزال معنوي في نسبة موت النباتات قبل وبعد الظهور فوق سطح التربة وزيادة نسبة النباتات الباقية مقارنة بالنباتات الناتجة من بذور غير معاملة في الموسمين .وكانت نسبة النباتات الباقية علي قيد الحياه من معاملة المبيد رايزولكس تي ٨٤,٦% يليها المعاملة بحامض السلسليك ٧٧% وبكتيريا *P. polymyxa* ٧٦,٢% كمتوسط للموسمين مقارنة بالنباتات الناتجة من بذور غير معاملة وكانت ٦٢,٩%. كذلك فإن المعاملة بالمستحثات البيون ، حامض السلسيليك و بكتيريا *P. polymyxa* قد حسنت معنويا مقاييس النمو المدروسة في الموسمين مثل طول الساق ، عدد الافرع/ نبات ، عدد القرون/نبات ، ووزن البذور/ نبات وكذلك تم الحصول علي أعلي زيادة في المحصول (كجم /فدان) من المعاملة بمركب البيون و المبيد بنسب زيادة مئوية (٨٨,٩% و ٨١,٤% علي التوالي) يليها معاملة حامض السلسليك وبكتيريا *P. polymyxa* بنسب زيادة مئوية (٦٩,٧% و ٥٨,٤% علي التوالي) مقارنة بالنباتات الناتجة من بذور غير معاملة.

كذلك تم تقدير نشاط إنزيمي البيروكسيديز والبولي فينول أوكسيديز في نباتات فول الصويا معمليا ، وقد ازداد نشاط إنزيم البيروكسيديز في المعاملة بمركب البيون بنسبة ٦٤,١% وفى المعاملة بحامض السلسليك بنسبة ٣١,٦% وكذلك أظهرت المعاملة بمركب البيون زيادة في نشاط إنزيم البولي فينول أوكسيديز بنسبة ١١٨% عن النباتات الناتجة من بذور غير معاملة. من ناحية اخري قد أدت المعاملة بالمستحثات المختلفة إلي زيادة المحتوي الكلي للفينولات وسجلت الزيادة القصوي في المحتوي الكلي للفينولات في المعاملة بمركب البيون بزيادة قدرها ١٤١,٢% عن النباتات الناتجة من بذور غير معاملة يليها المعاملة بحامض السلسليك وبكتيريا *P. polymyxa* بنسبة زيادة مئوية قدرها ٥٠% و ٤٩,٦% علي التوالي.