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Systemic Resistance in Snap Bean (*Phaseolus vulgaris* L.) Elicited by Some Chemicals and Biotic Inducers Against White Mold Disease Caused by *Sclerotinia sclerotiorum*

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The effect of chemicals and biotic inducers *i.e.*, Bion [benzothiadiazole (BTH)], Salicylic acid (SA), *Bacillus subtilis* and *Trichoderma harzianum* as seed treatments of snap bean (*Phaseolus vulgaris* L. cv. Paulista.) compared to the fungicide Topsin M-70, were evaluated in the greenhouse and under field conditions during the two successive growing seasons 2016/2017 and 2017/2018 at Ashmoun, Menoufia Governorate, Egypt to control the white mold disease caused by *Sclerotinia sclerotiorum* (Lib.) de Bary. All the tested chemicals and biotic inducers treatments significantly reduced the percentages of pre- and post-emergence damping-off compared with the untreated control under greenhouse conditions. The highest percentage of survived plants was achieved by Topsin M-70 and Bion, followed by *B. subtilis*, Salicylic acid, and *T. harzianum*. Activities of defense-related enzymes peroxidase (PO), polyphenoloxidase (PPO) enzymes, and phenol content were determined. Bion treatment showed the highest increase in PO and PPO activity and total phenols followed by *B. subtilis*, Salicylic acid, and *T. harzianum* treatments in the presence of *S. sclerotiorum*. In the two successive growing seasons under field conditions, all the tested chemicals and biotic inducers treatments reduced the percentage of white mold incidence and severity of the treated snap bean plants compared with the untreated control. The highest reduction of disease incidence and severity was recorded by Topsin M-70 followed by Bion, *B. subtilis*, Salicylic acid, and *T. harzianum*, respectively. As well as, all treatments significantly increased vegetative characteristics *i.e.*, plant height, No. of branches/plant, plant fresh and dry weight, and yield parameters *i.e.*, average pod weight (g) and yield (ton/fed). Furthermore, chemical composition of green pods showed a significant increase in protein and carbohydrates content (%) beside all minerals Nitrogen (N), Phosphorus (P) and Potassium (K) content as compared with the untreated control in the two growing seasons.

Keywords: *Phaseolus vulgaris*, *Sclerotinia sclerotiorum*, Systemic resistance, .Bion, Salicylic acid, *Bacillus subtilis*, *Trichoderma harzianum*.

Phaseolus vulgaris L. the common bean is a herbaceous annual plant grown worldwide for its edible dry seeds or unripe fruit (both commonly called beans). The main categories of common beans, based on use, are dry beans (seeds harvested at complete maturity) and snap beans (tender pods with reduced fiber harvested before the seed development phase).

Phaseolus vulgaris is a highly nutritious grain legume crop, including a good source of carbohydrates and protein. It also helps in the improvement of soil fertility by biological N^2 fixation (Singh, 1999). In 2016, world production of green beans was 23.6 million metric tons as well as the world dried bean production in 2016 was 26.8 million metric tons, which are produced worldwide (FAOSTAT, 2017).

White mold, caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, is among the most devastating and widely distributed fungal diseases of common beans (*Phaseolus vulgaris* L.) worldwide (Schwartz and Singh, 2013). As well as, many investigators in Egypt reported it as a detrimental disease affecting the common bean plants during the fall-winter season (Amer *et al.,* 2009; Abdel-Syed, 2016 and Elsheshtawi *et al.,* 2017).

The fungus is favored by temperate climates, moderate temperatures, and high relative humidity (Sabaté *et al.,* 2018). Sclerotia of *S. sclerotiorum* can reside in the soil for several years and, under appropriate environmental conditions, germinate to form a mycelium, leading to infectious hyphae, or producing apothecia, which release millions of airborne ascospores (Coley-Smith and Cooke, 1971 & Bardin and Huang, 2001). *Sclerotinia sclerotiorum* primarily spreads by spores and usually in forms of sclerotia, which may infect stems, leaves, and flowers, and even spread to adjacent plants (Zhou and Boland, 1998). Considering its persistence in the soil or seeds, as well as its wide host range which reaching approximately to more than 400 species belonging to more than 200 genera of higher plants and further, the lack of resistant cultivars, this fungus can cause devastating economic losses in the crops. (Figueiredo *et al.,* 2010; Schwartz and Singh, 2013 and Sabaté *et al.,* 2018), therefore, the importance of its management is of a great value.

Plant diseases are conventionally controlled by chemical fungicides. However, due to their potential harmful effects on the environment induced resistance becomes important. Non-classical and ecologically friendly alternatives were developed to avoid the application of pesticides for plant diseases control that achieved remarkable success in controlling plant diseases and diminish the hazardous effects on ecosystem (Reddy *et al.,* 2014 and Ozkara *et al.,* 2016).

There are two forms of systemic resistance, systemic acquired resistance (SAR), and induced systemic resistance (ISR). Applications of biotic and abiotic inducers have a potential in controlling plant diseases (Simonetti *et al.,* 2012). SAR, against pathogens can be induced by several synthetic chemical agents, such as salicylic acid, methyl salicylate, benzothiadiazole (Bion), β-aminobutyric acid, isonicotinic acid, benzoic acid,

chitosan, saccharin and so forth which affect the production of phenolic compounds and activation of various defense-related enzymes in plants (Thakur and Sohal, 2013 and Walters *et al.*, 2013).

Plant Growth-Promoting Rhizobacteria (PGPR) has a vital role in agriculture. The positive effects of PGPR have a direct or indirect performance in plants, direct promotion of growth by production of metabolites that enhances plant growth, indirect growth promotion occurs via the removal of pathogens by the production of secondary metabolites (Compant *et al.*, 2005; Reddy *et al.*, 2014 and Sarhan and Shehata, 2014). Induced systemic defense reaction in plants using PGPR is considered one important means to suppress plant diseases as it can induce plant defense in the host plants in response to microbial infection including defense-related enzymes and pathogenesis-related proteins such as β-1,3-glucanase, peroxidase, polyphenoloxidase, phenylalanine ammonia-lyase, indoleacetic acid (IAA), lignin synthesis, accumulation of phenolic compounds and specific flavonoids, meantime, a promising approach to control diseases caused by soil-borne pathogens (Walters *et al.*, 2013; Pieterse *et al.*, 2014 and Prasannath, 2017).

The objective of the present study aimed to evaluate the efficacy of some biotic and abiotic inducers for inducing resistance of snap bean plants against white mold under greenhouse and field conditions. Moreover the activity of defense related-enzymes and the accumulation of phenolic compounds as well as the growth and yield parameters of snap bean plants were also studied.

Materials and Methods

1.1. Source of fungal pathogens

Naturally infected bean plants displaying typical symptoms of white mold disease collected from different locations in Menoufia Governorate, Egypt. Infected roots stems and pods were washed with running tap water to remove any soil remains, and then cut into small pieces (0.5-1.0 cm) before being dipped in sodium hypochlorite solution (2%) for two minutes for surface sterilization. These plant pieces were then passed through changes of distilled water, dried between sterilized filter paper, then placed on PDA medium in Petri-dishes supplemented with streptomycin sulfate (100µg/L). The plates were incubated at 22±1°C and scanned daily for fungal development. The isolated fungi were microscopically examined and identified according to their morphological features as described by Richard (1998). *Sclerotinia sclerotiorum* isolates were purified using the hyphal tip technique (Sinclair and Dhingra, 1995). Representative isolates were maintained on PDA slants for further studies.

1.2. Source of bean Seed

Bean cultivar Paulista seeds were used in these experiments. The seeds were kindly supplied by Vegetable Crop Research Institute, Horticultural Research Institute, ARC, Egypt.

1.3. Chemical inducers

Bion [benzothiadiazole (BTH)], wettable granule (WG 50%), Benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester, (Syngenta Crop Protection, Inc); and Salicylic acid (Sigma Aldrich, USA) were used in this study.

1.4. Source of biocontrol agents:

In this study, two bioagents *i.e., Bacillus subtilis* and *Trichoderma harzianum* were kindly obtained from Biofertilizers Production Unit.; Soils, Water & Environment Research Institute.; Agricultural Research Center, Giza, Egypt.

1.5. The fungicide Topsin M-70 (WP):

Common name: Thiophanate-methyl., Chemical name: dimethyl [1,2-phenylenebis (iminocarbonothioyl)] bis [carbamate]., Dose per kg seed: 3g/Kg Seed

2.1. Preparation of fungal inoculum

Sclerotinia sclerotiorum equal five disks (0.5 cm diameter) were taken from 7 days old culture representing the fungal isolates were used to inoculate glass bottles 500-ml containing 100 g sterilized sorghum grains medium and incubated at 25°C±1 for two weeks. Inoculated bottles were vigorously shaken daily to encourage more rapid colonization of the sorghum grains and ensure uniform distribution of the fungal growth. The colonized sorghum grains were removed from the bottles, air-dried at room temperature, and were grounded in a mill then sieved through 60 mesh (0.25 mm) sieve. The granulated culture was kept in a polythene bag and treated as the fungal inoculum within one week (Tewari and Bhanu, 2004).

2.2. Preparation of tested bioagents inocula:

2.2.1. Bacillus subtilis:

The culture of *B. subtilis* was activated on fresh slants and after 24 hrs was transferred to flasks with 50 ml of nutrient yeast dextrose broth (NYDB) medium (per liter: nutrient broth 8 g, yeast extract 5 g and dextrose 10 g). Flasks were placed on a rotary shaker to grow at 120 rpm for 3 days at 24±1°C. Bacterial concentration in the suspension was adjusted to a proximately 5×10^8 CFU/mL by measuring absorbance at 600 nm (A600) in a spectrophotometer and using standard curves for *B. subtilis* (Sinclair and Dhingra, 1995).

2.2.2. Trichoderma harzianum:

The formulation of *T. harzianum* was prepared by growing the fungus in glass bottles 500cc containing 100g sterilized sorghum grains medium (Rini and Sulochana, 2007). The bottles were inoculated with actively five growing 0.5 cm diameter mycelial disc of 7 days old *T. harzianum* culture for each bottle. The bottles were incubated at 27±1°C for 18 days and were vigorously shaken daily to encourage rapid and uniform colonization of the sorghum grains. At the end of the incubation period, the colonized sorghum grains by mycelium and conidia of *T. harzianum* were removed from the bottles and air-dried in shade at room temperature, then was fine ground in a mill and

sieved through 60 mesh (0.25 mm) sieve (Tewari and Bhanu, 2004). The ground sorghum grains were kept in sterilized polyethylene bags at room temperature until used. A formula of *T. harzianum* was adjusted to $3x10^7$ CFU/g. (Sallam *et al.*, 2008).

3. Seed treatments

Healthy uniformity seeds of 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 hours.

3.1. Chemical inducers treatments:

The disinfected bean seeds were soaked in aqueous solutions of the inducers (Bion and salicylic acid) for 3 hours at the rate of 5 mM for each, then the treated seeds were air-dried prior of sowing.

3.2. The B. subtilis treatment:

Before treatment with the bacterial biocontrol agent, *B. subtilis*, bean seeds were surface disinfected in 1% NaOCl for 2 min, washed three times in sterilized distilled water, and dried between sterilized filter paper layers. Seeds were treated at the time with a bacterial bioagent isolate (10 mL of bacterial biocontrol agent suspension in 0.1 M MgSO4 and 0.5% Carboxymethyl cellulose per 100 g of bean seeds) then the treated seeds were air-dried prior of sowing.

3.3. The T. harzianum treatment:

Air-dried fine ground sorghum grains which contained $3x10^7$ (CFU/g) of *T. harzianum* (formulated Trichoderma) were used before sowing to coat the disinfected bean seeds moistened with 1% methylcellulose in sterile distilled water as a sticker, then the coated seeds were air-dried prior of sowing.

3.4. Fungicide treatment:

Seed dressing was carried out to the disinfected bean seeds by applying the Topsin M-70 (WP) at the recommended dose (3 g/kg seeds) and methylcellulose 1% was used as sticker, moistened seeds in polyethylene bags and shaking well to ensure even distribution of the fungicide. The treated seeds were air-dried before sowing.

3.5. Control:

The disinfected bean seeds were soaked in sterilized water for 3 hours then air-dried before sowing.

4. Greenhouse trials

These trials were carried out in the greenhouse of the Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt to evaluate the efficiency of the biotic and abiotic inducers *i.e.*, Bion, Salicylic acid, *B. subtilis*, and *T. harzianum* in controlling white mold, caused by *S. sclerotiorum* in snap beans plants. Pots (30 cm in diameter) with a bottom drainage hole were sterilized by dipping in a 5% formalin

solution for 15 minutes and left for one week till complete formalin evaporation. Pots were filled with steam disinfested sandy clay soil 1:2 (V/V). Soil infestation was achieved by mixing the previously prepared *S. sclerotiorum* inoculum with the soil at the rate of 3% of soil weight. Sterilized uninoculated grounded sorghum grains were added to the disinfested soil at the same rate for healthy control treatment. 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 soil. Five seeds of treated 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 under greenhouse conditions. Other agricultural procedures were performed according to normal practice. The treatments were as follows: (1) Bion; (2) Salicylic acid; (3) *B. subtilis*; (4) *T. harzianum*; (5) Topsin M-70 and (6) seeds soaking in water served as untreated control for both infested and non-infested soil. The experiment was repeated for determining the activity of oxidative enzymes and phenol content.

Disease assessment

The disease incidence (DI) % was determined by recording pre-emergence and post-emergence damping-off 15 and 30 days after sowing, respectively according to the following formulas.

$$\text{Pre-emergence (\%)} = \frac{\text{No. of non-emerged seedlings}}{\text{Total No. of sown seeds}} \times 100$$

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

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

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

$$\text{Reduction or Increasing (\%)} = \frac{\text{DI of Control - DI of treatment}}{\text{DI of Control}} \times 100$$

5. Effect of bean seed treatments with different inducers on the activity of oxidative enzymes and phenol content

Peroxidase (PO), polyphenoloxidase (PPO) and phenols contents were studied in tissue extracts of surviving bean plants from the following treatments: (1) Bion; (2) Salicylic acid; (3) *B. subtilis*; (4) *T. harzianum*; (5) Control (infested soil) and (6) Control (non-infested soil). All treatments were grown in sterilized soil infested with *S. sclerotiorum*. Samples of bean seedlings (shoot) of each treatment were collected 12 days after sowing with the tested pathogenic fungus as well as untreated healthy and infected seedlings were used as control treatments. One gram of plant tissue was

homogenized in 10 mL of ice-cold 50 mM potassium phosphate buffer (pH 6.8) containing 1M NaCl, 1% polyvinylpyrrolidone, 1 mM EDTA, and 10 mM β-mercaptoethanol (Biles and Martyn, 1993). After filtration through cheesecloth, the homogenates were centrifuged at 8,000 rpm at 4°C for 25 min. The supernatants (crude enzyme extract) were stored at -20°C or immediately used for the determination of PO and PPO activities according to Soltis and Soltis (1990). For the determination of enzyme activities, each treatment consisted of three replicates (three plants/replicate), and two spectrophotometric readings were taken per replicate using a Milton Roy 1201 Spectrophotometer (PEMEDR, Denver, CO, USA).

5.1. Peroxidase (PO) assay

PO activity was determined directly using a spectrophotometrical method Hammerschmidt *et al.* (1982) using guaiacol as a common substrate. The reaction mixture consisted of 0.2 mL crude enzyme extract and 1.40 mL of a solution containing guaiacol, hydrogen peroxide (H_2O_2) and sodium phosphate buffer (0.2 mL 1% guaiacol+0.2 mL 1% H_2O_2 +1 mL 10 mM potassium phosphate buffer). The mixture was incubated at 25°C for 5 min and the initial rate of increase in absorbance was measured over 1 min at 470 nm. The activity was expressed as units of PO/mg protein (Urbanek *et al.*, 1991).

5.2. Polyphenoloxidase (PPO) assay

The activity of PPO was determined by adding 50 µL of the crude extract to 3 mL of a solution containing 100 mM potassium phosphate buffer, pH 6.5, and 25 mM pyrocatechol. The increase of absorbance at 410 nm during 10 min at 30°C, was measured according to Gauillard *et al.* (1993). One PPO unit was expressed as the variation of absorbance at 410 nm per mg soluble protein per min.

5.3. Determination of phenolic compounds

To assess phenolic content, 1 g fresh plant sample was homogenized in 10 mL 80% methanol and agitated for 15 min at 70°C. One milliliter of the extract was added to 5 mL of distilled water and 250 µL of 1 N Folin-Ciocalteau reagent and the solution was kept at 25°C. Total phenol assay was by the absorbance read at 750 nm in a UV spectrophotometer against a reagent blank, as well as free phenol assay was by the absorbance read at 765 nm in a UV spectrophotometer (Agbor *et al.*, 2014)

6. Field trials

Field experiments were carried out during the two successive seasons of 2016/2017 and 2017/2018, at Ashmoun, Menoufia Governorate, Egypt, in fields naturally infested, with white mold disease, to investigate the effect of the tested biotic and abiotic inducers for controlling white mold disease. Bean seeds cv. Paulista were treated in the same manner in the greenhouse experiment. In the control treatment, seeds were soaked in distilled water as mentioned before. The treated bean seeds were sown in the field on 25th October 2016 and 1th November 2017. The field trial (18 plots) was designed in complete randomized blocks with three replicates. The area of each plot was 10.5 m^2

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 the eastern side of the row ridge, with two seed per hill. 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; (2) Salicylic acid; (3) *B. subtilis*; (4) *T. harzianum*; (5) Topsin M-70 and (6) seeds soaking in water served as untreated control.

Plots were rated for disease severity index (DSI) and disease incidence (DI) (Kolkman and Kelly, 2002) using a "quarter scale" (Hall and Phillips, 1996) shortly before harvest when the plants had reached physiological maturity. Thirty plants per plot were rated from 0 to 4, where 0 = no disease present, 1 = 1 to 25% of the plant with white mold symptoms, 2 = 26 to 50% of the plant with white mold symptoms, 3 = 51 to 75% of the plant with white mold symptoms, and 4 = 76 to 100% of the plant with white mold symptoms. DSI was calculated for each plot on a percentage basis by the following formula:

$$\text{Disease severity index (DSI) \%} = \frac{\Sigma \text{ (rating of each plant)}}{4 \times \text{(number of plants rated)}} \times 100$$

Disease incidence DI was calculated as the number of plants out of the 30 individuals with white mold infection on a percentage basis. Reduction % over the infected control was also calculated according to the following formula:

$$\text{Reduction (\%)} = \frac{\text{DSI of Control - DSI of treatment}}{\text{DSI of Control}} \times 100$$

At the end of experiment, the following vegetative characteristics *i.e.,* plant height (cm) number of branches/plant, plant fresh weight (g) and plant dry weight (g) were recorded. As well as, at the proper maturity stage and in each harvest from each sub-plot, the following yield parameters *i.e.,* average pod weight (g) and marketable yield (ton/fed.) were estimated

7. Chemical composition analyses of green pods

Snap bean green pods were collected at the proper maturity, oven-dried at 70°C and digested with H_2SO_4 and H_2O_2 until constant weight according to the method of Bremner and Malvaney (1982). Total nitrogen was colorimetrically determined using a spectrophotometer according to Novozamsky *et al.* (1984). Phosphorus was colorimetrically determined using a spectrophotometer according to Wilde *et al.* (1985). Potassium content was determined using a flame photometer according to Black (1965). Some chemical composition of samples of green pods from each experimental plot was taken randomly to determine the crude protein (% N × 6.25), total carbohydrates, crude fibers, according to AOAC (2000). All determinations were performed in triplicates and the means were calculated.

8. Statistical Analysis

All recorded data were subjected to the analysis of variance procedures and treatment means were compared using the L.S.D. at P≤0.05 of confidence as described by Gomez

and Gomez (1984). The statistical analysis was done using the computer program MSTATC software version 4.

Results

1- Effect of some chemicals or biotic inducers on damping-off caused by S. sclerotiorum under greenhouse conditions

Results in Table (1) indicate that all the tested inducers reduced the percentages of pre- and post-emergence damping-off caused by *S. sclerotiorum* and significantly increased with the survived plants compared with untreated infected control. Topsin M-70 and Bion, treatments gave the highest values in reducing pre- emergence (87.5 and 75.0%, respectively) and post-emergence damping-off (75.0 and 75.0%) and increasing survived plants by 76.9 and 69.2% compared to untreated infested control, followed by *B. subtilis*, Salicylic, and *T. harzianum*, respectively.

Table 1: Effect of some chemicals or biotic inducers and the fungicide Topsin M-70 as seed treatments on white mold disease of snap bean (cv. Paulista) under greenhouse condition

Treatment	Damping- off				Survived plants %	Increasing %
	Pre-emergence		Post- emergence			
	Incidence %	Reduction%	Incidence %	Reduction%		
Bion	8	75.0	4	75.0	88	69.2
Salicylic acid	16	50.0	8	50.0	76	46.2
B. subtilis	12	62.5	4	75.0	84	61.5
T. harzianum	16	50.0	8	50.0	76	46.2
Topsin M-70	4	87.5	4	75.0	92	76.9
Control (infested soil)	32	-	16	-	52	-
Control (non-infested soil)	0	-	4	-	96	-
L.S.D. at 0.05	15.17		ns		17.52	

2-Effect of snap bean seed treatments with some chemicals or biotic inducers on the activity of oxidative enzymes and phenol content

2.1- Activity of oxidative enzymes

Activities of peroxidase (PO) and polyphenoloxidase (PPO) enzymes of snap bean plants were evaluated with different chemicals or biotic inducers in the presence of *S. sclerotiorum* (Table 2). Results show that all treatments were effective in increasing enzyme activities. The highest increase of PO and PPO activities as compared to the untreated control was achieved with Bion treatment (206.4 PO and 242.9% PPO) over untreated control, in the presence of *S. sclerotiorum*. Meantime, *B. subtilis* and Salicylic acid treatments showed a considerable increase in the two enzymes (149.6 and 141.9% PO and 228.6 and 221.4% PPO) over untreated control, as well the least increase activity

of the enzymes, being 106.8 PO and 164.3% PPO over untreated control, was obtained when *T. harzianum* was applied. However, results showed clear higher values of PO activity than PPO activity in all treatments in the presence of the pathogens. Also, the percentage of increase in enzyme activity by inducers treatments presented clear higher values of PPO than PO in all treatments. Meanwhile, it has to notice that infestation with the fungal pathogen in the absence of inducers increased the activity of both PPO and PO than that recorded in healthy untreated plants as blank of all treatments.

Table 2: Effect of some chemicals or biotic inducers as seed treatments on the peroxidase and polyphenoloxidase activity in snap bean plants (cv. Paulista) grown in artificially infested soil by *S. sclerotiorum* under greenhouse conditions

Treatments	Peroxidase activity (absorbance at 470 nm) (Enzyme unit/mg protein/min)		Polyphenoloxidase activity (absorbance at 410 nm) (Enzyme unit/mg protein/min)	
	Activity	Increasing over control %	Activity	Increasing over control %
Bion	0.362	206.4	0.072	242.9
Salicylic acid	0.286	141.9	0.068	221.4
B. subtilis	0.295	149.6	0.069	228.6
T. harzianum	0.244	106.8	0.056	164.3
Control (infested soil)	0.140	-	0.029	-
Control (non-infested soil)	0.118	-	0.021	-

2.2-Phenol content

Content of total phenols coincided with the trend of data of both PO and PPO enzymes, where it was highly enhanced in snap bean plants treated with different chemicals or biotic inducers compared with untreated plants in the presence of *S. sclerotiorum* (Table 3). The maximum increase in the free phenolic compounds was recorded with Bion treatment (184.1%) over untreated control, followed by *B. subtilis* and Salicylic acid (131.8 and 118.2%), whereas the least increase in free phenols content was recognized with *T. harzianum* treatment (106.8%) over untreated control. As for conjugated phenols, *B. subtilis* treatment gave the highest increase (160.0%) over untreated control, followed by Bion and Salicylic acid (126.7 and 120.0%), whereas the minimum increases were recognized in the conjugated phenols (93.3%) over untreated control when *T. harzianum* was applied. The maximum increase in the content of total phenolic compounds was recorded with Bion treatment (169.5%) over untreated control followed by *B. subtilis* and Salicylic acid (139.0 and 118.6%) over untreated control, whereas the fewer increases in total phenols content (103.4%) was recognized with *T. harzianum* treatment. Moreover, the least values in total, free and conjugated phenols were recorded in the healthy control treatment, indicating the role of pathogens in raising the phenol content of the host plant. On the other hand, free phenols represented the

highest figures of phenolic compounds than the conjugated phenols in all treatments as well as control untreated plants.

Table 3: Effect of some chemicals or biotic inducers as seed treatments on levels of phenolic compounds in snap bean plants (cv. Paulista) grown in artificially infested soil by *S. sclerotiorum* under greenhouse conditions

Treatments	Phenolic contents (mg/g fresh weight)					
	Free phenols	Increase over control %	Conjugated phenols	Increase over control %	Total phenols	Increase over control %
Bion	6.25	184.1	1.70	126.7	7.95	169.5
Salicylic acid	4.80	118.2	1.65	120.0	6.45	118.6
B. subtilis	5.10	131.8	1.95	160.0	7.05	139.0
T. harzianum	4.55	106.8	1.45	93.3	6.00	103.4
Control (infested soil)	3.65	-	1.30	-	4.95	-
Control (non-infested soil)	2.20	-	0.75	-	2.95	-

3- Field trials

3.1- Effect of some chemicals or biotic inducers on the incidence of white mold disease of snap bean under field conditions

Results in Table (4) show that all the tested chemicals or biotic inducers significantly reduced the white mold disease incidence and severity of snap bean plants treated with inducers as compared with untreated control in the two growing seasons 2016/2017 and 2017/2018. Topsin M-70 gave the highest value in reducing disease incidence (75.5%), as well as the highest values in decreasing the disease severity (76.9%) in the two growing seasons, respectively, followed by Bion, *B. subtilis* and Salicylic acid in reducing disease incidence being 52.8, 45.4 and 32.4%, and in decreasing the disease severity being 55.5, 52.0 and 35.8% in the two growing seasons, respectively. However, *T. harzianum* resulted in the lowest values even in decreasing disease incidence or severity, being 23.6 and 17.9% compared with untreated control.

3.2- Effect of some biotic or abiotic inducers on growth parameters of snap bean plants

Data in Table (5) show that all treatments significantly increased growth parameters performances *i.e.*, plant height, No. of branches/plant, plant fresh and dry weight as compared with untreated control in the two growing seasons 2016/2017 and 2017/2018. Bion and Topsin M-70 treatments recorded the maximum average values of plant height, No. of branches/plant, plant fresh and dry weight, (55.0 cm, 6.3, 96.7 g and 19.3 g) and (51.8 cm, 5.7, 94.5 g and 18.8g), respectively, in the two growing seasons 2016/2017 and 2017/2018, followed by *T. harzianum* (51.1 cm, 5.5, 92.0 g and 18.4g), Salicylic acid (48.8 cm, 5.5, 92.9 g and 18.5 g). Meanwhile, *B. subtilis* recorded the lowest values (48.4 cm, 4.9, 91.8 g, and 17.8 g) compared with untreated control. However, in the first growing season 2016/2017, all the growth parameters were significantly increased than

the second growing season 2017/2018, while in two growing seasons there were no significant differences between Salicylic acid, *T. harzianum* and *B. subtilis* treatments on the average.

Table 4: Effect of some chemicals or biotic inducers and the fungicide Topsin M-70 as seed treatments on white mold disease of snap bean (cv. Paulista) in two successive seasons 2016/2017 and 2017/2018

Treatments	Disease incidence %			Reduction %			Disease severity %			Reduction %		
	2016/2017	2017/2018	Mean	2016/2017	2017/2018	Mean	2016/2017	2017/2018	Mean	2016/2017	2017/2018	Mean
Bion	15.0	19.0	17.0	57.9	47.7	52.8	10.3	15.3	12.8	63.5	47.7	55.5
Salicylic acid	23.3	25.3	24.3	34.6	30.3	32.4	18.3	18.7	18.5	35.3	36.4	35.8
B. subtilis	19.3	20.0	19.7	45.8	45.0	45.4	12.3	15.3	13.8	56.5	47.7	52.0
T. harzianum	27.3	27.7	27.5	23.4	23.9	23.6	23.0	24.3	23.7	18.8	17.0	17.9
Topsin M-70	7.3	10.3	8.8	79.4	71.6	75.5	5.0	8.3	6.7	82.4	71.6	76.9
Control	35.7	36.3	36.0	-	-		28.3	29.3	28.8	-	-	
L.S.D. at 0.05												
Treatments (T)	1.502						1.538					
Seasons (S)	0.867						0.888					
T×S	2.124						2.175					

Table 5: Effect of some chemicals or biotic inducers and the fungicide Topsin M-70 as seed treatments on growth parameters *i.e.*, Plant height (cm), No. of branches/plant, Plant fresh and Plant dry weight (g) of snap bean (cv. Paulista) in two successive seasons 2016/2017 and 2017/2018

Treatments	Plant height (cm)			No. of branches / plant			Plant fresh weight (g)			Plant dry weight (g)		
	2016/2017	2017/2018	Mean	2016/2017	2017/2018	Mean	2016/2017	2017/2018	Mean	2016/2017	2017/2018	Mean
Bion	55.7	54.4	55.0	6.4	6.2	6.3	97.4	96.0	96.7	19.7	18.9	19.3
Salicylic acid	50.5	47.1	48.8	5.8	5.2	5.5	93.3	92.6	92.9	17.8	19.1	18.5
B. subtilis	49.4	47.4	48.4	5.0	4.8	4.9	91.6	92.0	91.8	18.0	17.7	17.8
T. harzianum	53.3	48.8	51.1	5.9	5.1	5.5	92.8	91.3	92.0	18.3	18.5	18.4
Topsin M-70	52.3	51.3	51.8	5.9	5.4	5.7	92.9	96.0	94.5	18.3	19.2	18.8
Control	44.7	39.7	42.2	4.3	3.6	4.0	88.9	77.6	83.2	16.8	15.5	16.2
L.S.D. at 0.05												
Treatments (T)	1.318			0.254			1.216			0.631		
Seasons (S)	0.761			0.147			0.702			0.364		
T×S	1.864			0.359			1.720			0.892		

3.3- Effect of some chemicals or biotic inducers on yield parameters of snap bean plants

Data in Table (6) show that all treatments significantly increased yield parameters *i.e.,* average pod weight (g) and yield (ton/fed) as compared with untreated control in the two growing seasons 2016/2017 and 2017/2018. Topsin M-70 and Bion treatments recorded the maximum values of average pod weight (5.2 and 5.1 g) and the yield (4.9 and 4.5 ton/fed), respectively, in the two growing seasons 2016/2017 and 2017/2018, followed by *B. subtilis*, Salicylic acid and *T. harzianum* (5.1, 4.9 and 4.8 g) average of pod weight (g), and (4.4, 4.1 and 4.1 ton/fed) yield, respectively, compared with untreated control (4.0 g) average pod weight (g) and (3.1 ton/fed) yield.

Table 6: Effect of some chemicals or biotic inducers and the fungicide Topsin M-70 as seed treatments on average pod weight/plant (kg) and yield (ton/fed) of snap beans (cv. Paulista) in two successive seasons 2016/2017 and 2017/2018

Treatments	Average Pod weight (g)			Yield (ton/fed)		
	2016/2017	2017/2018	Mean	2016/2017	2017/2018	Mean
Bion	5.1	5.0	5.1	4.5	4.4	4.5
Salicylic acid	5.0	4.9	4.9	4.2	4.1	4.1
B. subtilis	5.1	5.1	5.1	4.4	4.3	4.4
T. harzianum	4.7	4.9	4.8	4.1	4.0	4.1
Topsin M-70	5.3	5.2	5.2	5.1	4.7	4.9
Control	4.7	3.3	4.0	3.6	2.6	3.1
L.S.D. at 0.05						
Treatments (T)	0.304			0.388		
Seasons (S)	0.176			0.244		
T×S	0.430			0.549		

3.4- Effect of some chemicals or biotic inducers on protein and carbohydrate content in green pods of snap bean

Data in Table (7) show that all treatments significantly increased protein and carbohydrates content (%) in green pods of snap bean (as percentages of the dry weight) as compared with untreated control in the two growing seasons 2016/2017 and 2017/2018. Bion treatment recorded the maximum average values of protein content (4.6%) followed by *T. harzianum*, Salicylic acid, and Topsin M-70, being 3.9, 3.8 and 3.8%, respectively, whereas, *B. subtilis* recorded the lowest protein content (3.5%) compared with untreated control (2.7%) in the two growing seasons 2016/2017 and 2017/2018. Similarly, in regards to the carbohydrates content, Bion recorded the

maximum average of carbohydrates content (17.8%) followed by Salicylic acid, *T. harzianum*, and Topsin M-70, being 17.0, 16.9 and 16.9%, respectively, whereas, *B. subtilis* recorded the lowest protein carbohydrates (16.3%) compared with untreated control (14.8%) in the two growing seasons 2016/2017 and 2017/2018.

Table 7: Effect of some chemicals or biotic inducers and the fungicide Topsin M-70 as seed treatments on protein content (%) and carbohydrates content (%) in green pods of snap bean (cv. Paulista) in two successive seasons 2016/2017 and 2017/2018

Treatments	Protein content (%)			Carbohydrates content (%)		
	2016/2017	2017/2018	Mean	2016/2017	2017/2018	Mean
Bion	5.1	4.2	4.6	17.9	17.7	17.8
Salicylic acid	4.1	3.5	3.8	17.2	16.8	17.0
B. subtilis	3.5	3.4	3.5	16.5	16.1	16.3
T. harzianum	4.0	3.7	3.9	17.1	16.7	16.9
Topsin M-70	3.9	3.6	3.8	17.2	16.6	16.9
Control	3.1	2.3	2.7	15.7	13.8	14.8
L.S.D. at 0.05						
Treatments (T)	0.278			0.409		
Seasons (S)	0.160			0.236		
T×S	0.393			0.578		

3.5 - Effect of some chemicals or biotic inducers on Minerals (Nitrogen, Phosphorus and Potassium) content in green pods of snap beans

Data in Table (8) show that all minerals Nitrogen (N), Phosphorus (P), and Potassium (K) contents were significantly higher in green pods of the treated snap beans as compared with untreated control in the two growing seasons 2016/2017 and 2017/2018. Bion treatment recorded the maximum average values of minerals content 3.23% (N), 0.44% (P) and 2.38% (K)% (as percentages of the dry weight) followed by Topsin M-70, Salicylic acid and *B. subtilis* respectively, whereas, *T. harzianum* recorded the lowest minerals content of 2.53% (N), 0.32% (P) and 2.18% (K) compared with untreated control 1.82% (N), 0.25% (P) and 1.78% (K) in the two growing seasons 2016/2017 and 2017/2018.

Table 8: Effect of some chemicals or biotic inducers and the fungicide Topsin M-70 as seed treatments on the minerals, nitrogen (N), phosphorus (P) and potassium (K) content in green pods (%) of snap bean (cv. Paulista) in two successive seasons 2016/2017 and 2017/2018

Treatments	Nitrogen (N) content (%)			Phosphorus (P) content (%)			Potassium (K) content (%)		
	2016/ 2017	2017/ 2018	Mean	2016/ 2017	2017/ 2018	Mean	2016/ 2017	2017/ 2018	Mean
Bion	3.27	3.20	3.23	0.45	0.43	0.44	2.55	2.21	2.38
Salicylic acid	2.80	2.93	2.87	0.38	0.33	0.36	2.27	2.36	2.32
B. subtilis	2.70	2.50	2.60	0.39	0.35	0.37	2.43	2.33	2.38
T. harzianum	2.67	2.40	2.53	0.32	0.32	0.32	2.24	2.11	2.18
Topsin M-70	3.00	2.70	2.85	0.38	0.37	0.38	2.27	2.39	2.33
Control	2.03	1.60	1.82	0.28	0.23	0.25	1.89	1.66	1.78
L.S.D. at 0.05									
Treatments (T)	0.168			0.112			0.300		
Seasons (S)	0.097			0.064			0.173		
T×S	0.237			0.158			0.424		

Discussion

Systemic acquired resistance (SAR) is a broad spectrum resistance that can be induced in plants following a localized infection with a necrotizing pathogen or treatment with chemical or biotic elicitors (Sticher *et al.,* 1997).

Chemicals and biotic inducers have potential in agriculture concerning controlling plant diseases (Reddy *et al.,* 2014). Chemicals and biotic inducers are known to have eliciting activities leading to a variety of defense reactions in host plants in response to microbial infection, including the defense-related enzymes, pathogenesis-related proteins, and accumulation of phenolic compounds (Walters *et al.,* 2013).

The activity of defense-related enzymes, peroxidase, and polyphenoloxidase is known to be induced via systemic resistance of many infected plants with fungal pathogens (Prasannath, 2017). These enzymes act as elicitors of the phenylpropanoid pathway, resulting in the biosynthesis of a diverse array of plant metabolites such as phenolic compounds, flavonoids, tannins, and lignin. These products can provide defense in plants against pathogenic attack (Walters *et al.,* 2013 and Mayer, 2006). Many studies indicated a greater accumulation of phenolics as a result of increasing the activities of these oxidative enzymes which could offer protection against plant diseases (Reddy *et al.,* 2014 and Prasannath, 2017).

The main objective of the present study was to evaluate the efficacy of some chemicals and biotic inducers in management white mold disease of snap bean plants under greenhouse and field conditions, which was reliant to the induction of systemic

resistance as the main mechanism of action and as illustrated via reduction of the disease incidence and severity

In the present work, bean seed treatments with Bion [benzothiadiazole, (BTH)], and salicylic acid (SA) as chemical inducers resulted in a significant reduction of white mold disease, and highly increased vegetative characteristics as well as average pod weight and marketable yield. Such results are in harmony with many investigators who reported the use of Bion [benzothiadiazole, (BTH)] to induce plant resistance against a broad spectrum of pathogens in many plant species.

Early, Bion (BTH) was shown to induce the expression of systemic acquired resistance "SAR" genes (Görlach *et al.*, 1996). The mechanisms of BTH as inducer have been shown to involve in the activation of SAR mechanisms based on the SA pathway (Friedrich *et al.*, 1996), with consequent upregulation of defense genes (Bovie *et al.*, 2004) also, activating resistance by increasing the activity of peroxidase enzyme and the accumulation of pathogenesis-related proteins (PR), some of which have antimicrobial properties and accumulation of phenolic compounds (Iriti *et al.*, 2004).

Iriti and Faoro (2003) reported that Bion induces resistance in bean cultivars against rust caused by *Uromyces appendiculatus*, a single 0.3 mm BTH spray 7 days before inoculation was sufficient to fully control the disease in all the examined cultivars. Guo and Stotz (2007) stated that the systemic acquired resistance inducer benzothiadiazole reduced susceptibility in *Arabidopsis thalianato* to *S. sclerotiorum*. Azami-Sardooei *et al.* (2013) investigated the effects of foliar applications of different concentrations of BTH on resistance to *B. cinerea* on bean and cucumber, only concentrations of 250 mg/l and higher, strongly reduced susceptibility against *B. cinerea*. Recently, Bán *et al.* (2017) found that the application of Bion reduced sunflower white mold (*S. sclerotiorum*) disease symptoms; besides both localized and systemic inductions of resistance were observed.

Salicylic acid (SA) is a phenolic plant hormone which plays an important role in regulating defenses in plants against biotrophic and hemibiotrophic pathogens, however signaling pathways mediated by salicylic acid (SA) are widely studied in various host-pathogen interactions (Gaffney *et al.*, 1993 and Sticher *et al.*, 1997). Salicylic acid as a key to plant hormone plays an important role in the induction of plant defense against a variety of biotic and abiotic stresses through morphological, physiological, and biochemical mechanisms (Kumar, 2014).

These results are in harmony with those recorded by Reglinski, *et al.* (1997) found that Salicylic acid (2mM) was effective elicitor and induced a 10-fold rise in phenylalanine ammonia-lyase (PAL) activity after 2 days to control *S. sclerotiorum* on kiwifruit leaves, caused a reduction in the size of lesions arising from subsequent *S. sclerotiorum* infection and reduced disease levels, relative to controls, by up to 48% reduction. Reglinski *et al.* (2001) indicated that systemic acquired resistance (SAR) to

Sclerotinia sclerotiorum was induced in mature kiwifruit vines after localized treatment with 0.2 mM salicylic acid (SA) or previous inoculation with the same pathogen, however, phenylalanine ammonia-lyase (PAL) enzyme activity did not increase locally until 48h post-inoculation and remained unchanged throughout the experiment in adjacent leaves. Idrees *et al.* (2011) stated that salicylic acid improved antioxidant defense system via enhancing the activities of various enzymes such as, peroxidase (PO), polyphenoloxidase (PPO), phenylalanine ammonia-lyase (PAL), etc., which were the major components of induced plant defense against biotic and abiotic stresses. Wang *et al.* (2012) suggested that defense against *S. sclerotiorum* in oilseed rape (*Brassica napus*) is associated with sequential activation of salicylic acid (SA) signaling and jasmonic acid (JA) signaling, which provide important clues for designing strategies to curb diseases caused by *S. sclerotiorum*., also SA- and JA-mediated signaling pathway are essential for the regulation of plant growth, development, reproduction, and survival. Ahmed (2016) reported that, sowing soaked bean seeds in the tested antioxidant [salicylic acid (SA)] in soil artificially inoculated with *Rhizoctonia solani* significantly reduced the incidence of pre- and post-emergence damping-off with a significant increase in the fresh and dry weight of roots and shoots compared with control treatment as well as, caused a considerable increase in the activity of peroxidase and polyphenoloxidase enzymes that play an important role in plant defense mechanisms against pathogens infection.

Induced systemic defense reaction in plants using plant growth-promoting rhizobacteria (PGPR) is considered one important means to suppress plant disease symptoms. As well as several biocontrol agents such as *Bacillus* spp. and *Trichoderma* spp. can induce plant defense responses that are directly linked with induction of defense enzymes and pathogenesis-related proteins (PR) such as β-1,3-glucanase, peroxidase, polyphenoloxidase, phenylalanine ammonia-lyase and indoleacetic acid (IAA) and accumulation of phenolic compounds (Compant *et al.,* 2005; Walters, 2013 and Reddy *et al.,* 2014).

Results obtained here are in agreement with those reported by many investigators who found an antifungal activity of some bioagents which can play an important role in the resistance to soil-borne fungi and *S. sclerotiorum* infection and consequently, improved crop parameters. Abdullah *et al.* (2008) showed that *T. harzianum* and *B. amyloliquefaciens* inhibited the *S. sclerotiorum* growth and production of mycelia and sclerotia *in vitro*, as antagonists, these isolates protected over 80% of tomato, squash and eggplant seedlings inoculated with *S. sclerotiorum*. Khater (2010) showed that *B. subtilis* and *P. fluorescens* were effective to control Sclerotinia damping-off disease on common bean (*Phaseolus vulgaris* L.) and reduced the disease incidence; furthermore, there was a highly significant increase in the plant growth (shoot fresh weight (g) and shoot dry weight (g) compared with the control treatment. Hu *et al.* (2014) in field trials, strain *B. subtilis* provided control to Sclerotinia stem rot disease of oilseed rape, as reduced disease incidence and increase seed yield that was similar to the chemical control

treatment. Kowsari *et al.* (2014) reported that our results offered positive evidence that a transgenic approach to engineer *Trichoderma* strains transformed with chit42 can offer a much more antagonistic effect on *S. sclerotiorum* than the wild type. Kamal *et al.* (2015) showed that *Bacillus cereus* SC-1 can effectively suppress (*S. sclerotiorum*) Sclerotinia stem rot disease of canola both *in vitro* and *in vivo*. Ahmed (2016) recorded that under greenhouse conditions, treated bean seeds with *B. subtilis* and *T. harzianum* significantly reduced the incidence of *Rhizoctonia solani* damping-off, as well as caused a considerable increase in the activity of peroxidase and polyphenoloxidase enzymes. Marques *et al.* (2016) showed that *Trichoderma koningiopsis* and *T. brevicompactum* isolates with good antagonistic potential for *S. sclerotiorum* were considered to be promising for both biocontrol parameters (inhibition of mycelial growth and reduction of sclerotia). Zhang *et al.* (2016) found that *T. harzianum* increased resistance against *S. sclerotiorum* infection in soybean seedlings, also, primed pathogenesis-related proteins expression after *S. sclerotiorum* challenge, increased the activity of the antioxidant enzymes peroxidase PO, superoxide dismutase SOD, and catalase CAT and, improved the contents of chlorophyll and phenolic, indicating that these physiological and molecular mechanisms are involved in the induction of systemic resistance by *T. harzianum*. Sabaté *et al.* (2018) reported that *Bacillus* spp. and *B. amyloliquefaciens* treatments were effective in reducing the incidence of white mold in the common bean (*S. sclerotiorum*), furthermore had beneficial properties on the growth of common bean.

Conclusions

The present study indicated that application of chemicals and biotic inducers *i.e.*, Bion (BTH), Salicylic acid (SA), *Bacillus* subtilis, and *Trichoderma harzianum* could play a significant role in the protection against white mold disease (*Sclerotinia sclerotiorum*) of snap bean plants as reduced the disease incidence and severity under greenhouse and field conditions, mainly through the induction of systemic resistance via increasing the activities of the antioxidant enzymes peroxidase (PO), polyphenoloxidase (PPO), improving the phenolic contents. Besides, promote plant growth and increasing the marketable yield and improving the chemical composition of snap bean green pods. Our results provide a basis for better understanding of this interaction and the theoretical basis for such chemicals and biotic inducers on the field scale might provide a practical supplement to environmentally friendly disease management of the white mold of snap bean plants when they are combined with appropriate integrated disease management.

References

Abdel-Syed, M.H.F. 2016. Control of white mold of common bean caused by *Sclerotinia sclerotiorum* Using potassium sulfate and organic soil amendments under field conditions. *Middle East J. of Agric. Res.*, **5**(4): 925-935.

Abdullah, M.T.; Ali, N.Y and Suleman, P. 2008. Biological control of *Sclerotinia sclerotiorum* (Lib.) de Bary with *Trichoderma harzianum* and *Bacillus amyloliquefaciens*. *Crop Prot.,* **27**: 1354-1359.

Agbor, G.A.; Vinson, J.A and Donnelly, P.E. 2014. Folin-Ciocalteau reagent for polyphenolic assay. *Int J Food Sci Nutr Diet.,* **3**(8): 147-156.

Ahmed, G.A. 2016. Efficiency of some antioxidants and bioagents in controlling Rhizoctonia damping-off of snap bean. *Middle East J. of Applied Sci.,* **6**: 748-758.

Amer, M.A.; Abou-el-seoud, I.; Rasmy, M.R and Khater, M.M. 2009. White mould of common bean incited by *Sclerotinia sclerotiorum* Lib. de Bary in Egypt. *Common Agric. Appl. Bio. Sci.,* **74**(3):771-784.

AOAC, 2000. Official Methods of Analysis of the Association of Official Analytical Chemists, P. Cunniff (Ed.). Association of Official Analytical Chemists Inc., sixth edition, Arlington.

Azami-Sardooei, Z.; Seifi, H.S.; David De Vleesschauwer, D and Höfte, M. 2013. Benzothiadiazole (BTH) induced resistance against *Botrytis cinerea* is inversely correlated with vegetative and generative growth in bean and cucumber, but not in tomato. *Australasian Plant Pathol.,* **42**:485-490.

Bán R.; Baglyas G.; Virányi F.; Barna B.; Posta K.; Kiss J. and Körösi K. 2017. The chemical inducer, BTH (benzothiadiazole) and root colonization by mycorrhizal fungi (*Glomus* spp.) trigger resistance against white rot (*Sclerotinia Sclerotiorum*) in sunflower. *Acta. Biol. Hung.,* **68**: 50-59.

Bardin, S.D. and Huang, H.C. 2001. Research on biology and control of Sclerotinia diseases in Canada. *Can. J. Plant Pathol.,* **23**: 88-98.

Biles, C.L. and Martyn, R.D. 1993. Peroxidase, polyphenoloxidase, and shikimate dehydrogenase isozymes in relation to tissue type, maturity and pathogen induction of watermelon seedlings. *Plant Physiol. Biochem.,* **31**: 499-506.

Black, C.A. 1965. Methods of Soil Analysis. American Society Agronomy, first edition, Madison, WI., USA.

Bovie C.; Ongena M.; Thonart P. and Dommes J. 2004. Cloning and expression analysis of cDNAs corresponding to genes activated in cucumber showing systemic acquired resistance after BTH treatment. *BMC Plant Biology,* **26**: 4-15.

Bremner, J.M. and Mulvaney, C.S. 1982. Total nitrogen. In: A.L. Page, R.H. Miller and D.R. Keeny (Eds.). Methods of Soil Analysis, American Society of Agronomy and Soil Science Society of America, Madison, pp: 1119-1123.

Coley-Smith, J.R. and Cooke, R.C. 1971. Survival and germination of fungal sclerotia. *Annu. Rev. Phytopathol.,* **9**: 65-92.

Compant, S.; Duffy, B.; Nowak, J.; Clément, C. and Barka, E.A., 2005. Use of plant growth promoting bacteria for biocontrol of plant diseases: principles, mechanisms of action, and future prospects. *Appl. Environ. Microbiol.,* **71**: 4951-4959.

Elsheshtawi, M.; Elkhaky, M.T.; Sayed, S.R.; Bahkali, A.H.; Mohammed, A.A.; Gambhir, D.; Mansour, A.S and Elgorban, A.M. 2017. Integrated control of white rot disease on beans caused by *Sclerotinia sclerotiorum* using Contans® and reduced fungicides application. *Saudi J. Biol. Sci.,* **24**: 405-409.

FAOSTAT, 2017. Data base result 2016, Food and Agriculture Organization of United Nations, http://Fao.org

Figueiredo, G.; Figueiredo, L.; Cavalcanti, F.; Santos, A.; Costa, A. and Oliveira, N. 2010. Biological and chemical control of *Sclerotinia sclerotiorum* using *Trichoderma* spp. And *Ulocladium atrum* and pathogenicity to bean plants. *Braz. Arch. Biol. Technol.,* **53**(1): 1-9.

Friedrich L.; Lawton K.; Ruess W.; Masner P.; Specker N.; Gut Rella M.; Meier B.; Dincher S.; Staub T.; Uknes S.; Metraux J.P.; Kessmann H. and Ryals J. 1996. A benzothiadiazole derivative induces systemic acquired resistance in tobacco. *The Plant J.,* **10**: 61-70.

Gaffney, T.; Friedric1, L.; Vernooij, B.; Negrotto, D.; Nye, G.; Uknes, S.; Ward, E.; Kessmann, H. and Ryals, J. 1993. Requirement of salicylic acid for the induction of systemic acquired resistance. *Science,* **261**(5122):754-756.

Gauillard, F.; Richard-Forget, F and Nicolas, J. 1993. New spectrophotometric assay for polyphenol oxidase activity. *Anal. Biochem.,* **215**: 59-65.

Gomez, K. and Gomez, A. 1984. Statistical procedures for agricultural research, 2nd edn. Wiley, New York, p 680.

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.*

Guo, X. and Stotz, H.U. 2007. Defense against *Sclerotinia sclerotiorum* in Arabidopsis is dependent on Jasmonic acid, Salicylic acid, and Ethylene signaling. *Molecular Plant-Microbe Interactions,* **20**: 1384-1395.

Idrees, M.; Naeem, N.; Aftab, T.; Khan, M.M.A. and Moinuddin 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,* **33**: 987-999.

Hall, R. and Phillips, L.G. 1996. Evaluation of parameters to assess resistance of white bean to white mold. *Annu. Rep. Bean Improv. Coop.,* **39**:306-307.

Hammerschmidt, R.; Nuchles, E.M and Kuc, J. 1982. Association of enhanced peroxidase activity which induce systemic resistance of cucumber to *Colletotrichum lagenarium*. *Physiological Plant Pathology,* **20**(1): 73-82.

Hu, X.; Roberts, D.P.; Xie, L.; Maul, J.E.; Yu, C.; Li, Y.; Jiang, M.; Liao, X.; Che, Z. and Liao, X. 2014. Formulations of *Bacillus subtilis* BY-2 suppress *Sclerotinia sclerotiorum* on oilseed rape in the field. *Biol. Control,* **70**: 54-64.

Iriti, M and Faoro, F. 2003. Benzothiadiazole (BTH) Induces Cell-Death Independent Resistance in *Phaseolus vulgaris* against *Uromyces appendiculatus*. *J. Phytopathol.*, **151**: 171-180.

Iriti, M.; Rossoni, M.; Borgo, M. and Faoro, F. 2004. Benzothiadiazole enhances resveratrol and anthocyanin biosynthesis in grapevine, meanwhile improving resistance to *Botrytis cinerea*. *J. Agric. Food Chem.,* **52**: 4406-4413.

Kamal, M.M.; Lindbeck, K.D.; Savocchia, S. and Ash, G.J. 2015. Biological control of sclerotinia stem rot of canola using antagonistic bacteria. *Plant Pathology,* **64**(6): 1375-1384.

Kowsari, M.; Zamani, M.R, and Motallebi, M. 2014. Enhancement of *Trichoderma harzianum* activity against *Sclerotinia sclerotiorum* by over expression of Chit42. Biotech., **12**(2): e13869.

Khater, M.M.N. 2010. Biological control of *Sclerotinia sclerotiorum* the causal agent of white basal rot disease of beans (*Phaseolus vulgaris* L.). M.Sc. Fac. of Agric., Alexandria Univ.

Kolkman, J.M. and Kelly, J.D. 2002. Agronomic traits affecting resistance to white mold in Common bean. *Crop Sci.,* **42**: 693-699.

Kumar, D. 2014. Salicylic acid signaling in disease resistance. *Plant Sci.,* **228**: 127-134.

Mayer, A.M. 2006. Polyphenoloxidases in plants and fungi: going places? A review. *Phytochemistry,* **67**: 2318-2331.

Marques, E.; Martins, I.; Cunha, M.O.C.; Lima, M.A.; da Silva, J.B.T.; da Silva, J.P.; Inglis, P.W. and Mello, S.C.M. 2016. New isolates of Trichoderma antagonistic to *Sclerotinia sclerotiorum*. *Biota Neotropica,* **16**(3): e20160218

Novozamsky, I.; Houba, V.J.G.; Temminghoff, E. and Van Der Lee, J.J. 1984. Determination of total N and total P in a single soil digest. *Neth. J. Agric. Sci.,* **32**: 322-324.

Ozkara, A.; Akyıl, D. and Konuk, M. 2016. Pesticides, environmental pollution, and health. In: Larramendy, M.L., Soloneski, S. (Eds.), Environmental Health Risk - Hazardous Factors to Living Species, pp. 3-27.

Pieterse, C.M.J.; Zamioudis, C.; Berendsen, R.L.; Weller, D.M.; Van Wees, S.C.M. and Bakker, P.A.H.M. 2014. Induced systemic resistance by beneficial microbes. *Annu. Rev. Phytopathol.,* **52**: 347-375.

Prasannath, K. 2017. Plant defense-related enzymes against pathogens: a review. AGRIEAST: *J. of Agric. Sci.,* **11**(1): 38-48.

Reddy M.S.; Ilao, R.I.; Faylon, P.S.; Dar W.D.; Batchelor W.D.; Sayyed, R.; Sudini, H.; Kumar, V.K.K.; Armanda, A. and Gopalkrishnan, S. eds. 2014. Recent advances in biofertilizers and biofungicides (PGPR) for sustainable agriculture. Cambridge Scholars Publishing, p510.

Reglinski, T.; Poole, P.R.; Whitaker, G. and Hoyte, S.M. 1997. Induced resistance against *Sclerotinia sclerotiorum* in kiwifruit leaves. *Plant Pathology,* **46**: 716-721.

Reglinski, T.; Whitaker, G.; Cooney, J.M.; Taylor, J.T.; Poole, P.R.; Roberts, P.B. and Kim, K.K. 2001. Systemic acquired resistance to *Sclerotinia sclerotiorum* in kiwifruit vines. *Physiol. Mol. Plant Pathol.,* **58**(3): 111-118.

Richard, T.H. 1998. Combined Keys to Illustrated Genera of Ascomycetes volumes I & II. APS Press. St. Paul, MN.

Rini, C.R. and Sulochana, K.K. 2007. Substrate evaluation for multiplication of *Trichoderma* spp. *J. of Trop. Agric.,* **45**: 58-60.

Sabaté, D.C.; Brandan, C.P.; Petroselli, G.; Erra-Balsells, R. and Audisio, M.C. 2018. Biocontrol of *Sclerotinia sclerotiorum* (Lib.) de Bary on common bean by native lipopeptide-producer *Bacillus* strains. *Microbiol. Res.,* **211**: 21-30.

Sallam N.M.A.; Abo-Elyousr, K.A.M. and Hassan, M.A.E. 2008. Evaluation of *Trichoderma* species as biocontrol agent for damping-off and wilt diseases of *Phaseolus vulgaris* L. and efficacy of suggested formula. *Egypt J Phytopathol.,* **36**(1-2): 81-93.

Sarhan, E.A.D and Shehata, H.S. 2014. Potential plant growth-promoting activity of *Pseudomonas* spp. and *Bacillus* spp. as biocontrol agents against damping-off in Alfalfa. *Plant. Pathol. J.,* **13**: 8-17.

Schwartz, H.F and Singh, S.P. 2013. Breeding common bean for resistance to white mold: A Review. *Crop Sci.,* **53**: 1832-1844.

Simonetti, E.; Carmona, M.A.; Scandiani, M.M.; Garcı´a, A.F.; Luque, A.G.; Correa, O.S and Balestrasse, K.B. 2012. Evaluation of indigenous bacterial strains for biocontrol of the frogeye leaf spot of Soybean caused by *Cercospora sojina*. *Lett. in App. Microbiol.,* **55**(2): 170-173.

Sinclair, J. and Dhingra, O. 1995. Basic Plant Pathology Methods. 2nd Edition Boca Raton: CRC

Singh, S.P. 1999. Common Bean: Improvement in the Twenty-First Century. Kluwer Academic Publishers, London, pp: 2-7.

Soltis, D.E. and Soltis, P.S. 1990. Isozymes in Plant Biology. Dioscorides press; Portland; Oregon pp. 259.

Sticher, L.; Mauch-Mani, B. and MeÂtraux, J.P. 1997. Systemic acquired resistance. *Ann. Rev. of Phytopathol.*, **35**: 235-270.

Tewari, L. and Bhanu, C. 2004. Evaluation of agro-industrial wastes for conidia based inoculum production of biocontrol agent: *Trichoderma harzianum*. *J. Sci. Ind. Res.*, **63**: 807-812.

Thakur, M. and Sohal, B.S. 2013. Role of elicitors in inducing resistance in plants against pathogen infection: a review. *ISRN Biochemistry*: Vol. **2013**, Article ID 762412, 10 p.

Urbanek, H.; Kuzniak-Gebarowska, E and Herka, H. 1991. Elicitation of defense responses in bean leaves by *Botrytis cinerea* poly-galacturonase. *Acta Physiol. Plant.*, **13**: 43-50.

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.

Wang, Z.; Tan, X.; Zhang, Z.; Gu, S.; Li, G. and Shi, H. 2012. Defense to *Sclerotinia sclerotiorum* in oilseed rape is associated with the sequential activations of salicylic acid signaling and jasmonic acid signaling. *Plant Science*, **184**: 75-82.

Wilde, S.A.; Corey, R.B.; Lyer, J.G. and Voigt, G.K. 1985. Soil and Plant Analysis for Tree Culture. Mohan Primlani, Oxford, IBH Publishing Company. New Delhi, pp: 1-42.

Zhang, F.; Ge, H.; Zhang, F.; Guo, N.; Wang, Y.; Chen, L.; Ji, X. and Li, C. 2016. Biocontrol potential of *Trichoderma harzianum* isolate T-aloe against *Sclerotinia sclerotiorum* in soybean. *Plant Physiol. and Biochem.*, **100**: 64-74.

Zhou, T. and Boland, G.J. 1998. Biological control strategies for Sclerotinia diseases. In: Boland, G.J., Kuykendall, L.D. (Eds.), Plant-Microbe Interactions and Biological Control. Marcel Dekker, New York, pp. 127-156.

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المقاومه الجهازية في الفاصوليا الخضراء (*Phaseolus vulgaris* L.) المحفزه ببعض المستحثات الكميائية والحيوية ضد مرض العفن الابيض المتسبب عن *Sclerotinia sclerotiorum*

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تم دراسة تأثير معاملة بذور الفاصوليا (صنف بوليستا) بالمستحثات الكيمائية والحيوية ، البيون ،و حامض السلسيلك ،والبكتيريا *Bacillus subtilis* ،والفطر *Trichoderma harzianum* مقارنة بالمبيد الفطري توبسين م-70 تحت ظروف الصوبة والحقل خلال موسمي الزراعة المتعاقبين ٢٠١٦/٢٠١٧ ،و ٢٠١٧/٢٠١٨ في أشمون بمحافظة المنوفيه لمكافحة مرض العفن الأبيض المتسبب عن الفطر *Sclerotinia sclerotiorum* (Lib.) de Bary. وقد أدت كل المعاملات تحت ظروف الصوبة الي نقص 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 الحياة من المعاملة بالمبيد الفطري توبسين م-70 ومركب البيون يليها البكتيريا *B. subtilis* وحامض السلسيلك والفطر *T. harzianum*. كذلك تم تقدير نشاط انزيمي البيروكسيديز والبولي فينول اوكسيديز ومحتوي الفينولات في نباتات الفاصوليا المعاملة بالمستحثات الكميائية والحيوية المختبرة معمليا مقارنة بالنباتات الغير معاملة المنزرعة في كل من التربة المعداه والغير معداة بالفطر ،و أظهرت المعاملة بالبيون اعلي زيادة في نشاط انزيمي البيروكسيديز والبولي فينول اوكسيديز ،ومحتوي الفينولات الكليه يليها البكتيريا *B. subtilis* وحامض السلسيلك والفطر *T. harzianum*. أدت كل المعاملات تحت ظروف الحقل خلال موسمي الزراعة المتعاقبين إلي إختزال معنوي في نسبة حدوث مرض العفن الأبيض وشدة الأعراض المرضية علي نباتات الفاصوليا المعاملة مقارنة بالنباتات الغير معاملة. وقد تحققت اعلي نسبة في اختزال الحدوث والشدة المرضية بالمبيد الفطري توبسين م-70 ومركب البيون يليها البكتيريا *B. subtilis* وحامض السلسيلك والفطر *T. harzianum* علي التوالي. كذلك فان كل المستحثات أدت الي زيادة معنوية كبيره في اداء الصفات الخضرية مثل ارتفاع النباتات ،و عدد الفروع للنبات ووزن النبات الطازج والجاف ، كذلك الصفات الانتاجية مثل متوسط وزن القرون ،و الانتاج الكلي للمحصول ،و علاوة علي ذلك اظهر التركيب الكميائي للقرون الخضراء زيادة معنويه في محتوي البروتين والكربوهيدرات ، بجانب العناصر المعدنيه من النيتروجين ،و الفسفور ،و البوتاسيوم مقارنة بالنباتات الغير معاملة في كلا موسمي الزراعة.