The Journal of Animal & Plant Sciences, 27(2): 2017, Page: 542-549
ISSN: 1018-7081

MANAGEMENT OF SCLEROTINIA WHITE ROT OF BEANS WITH ANTAGONISTIC MICROORGANISMS

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ABSTRACT

In the present study, antagonistic activity of locally isolated bio agents including five fungi and three bacteria was evaluated against *Sclerotinia sclerotiorum* (Lib.) de Bary casual of white rot of snap beans. All tested biocontrol agents were able to inhibit radial growth and *sclerotia* viability in dual culture assay. *Trichoderma hamatum* (Bonorden) Bainier was the most effective agent in suppressing the mycelial growth of *S. sclerotiorum* by 93% compared to control. Whereas, tested isolates of *Trichoderma viride* Pers and *Coniothyrium minitans* Campbell were able to completely deactivating all treated *sclerotia*. In field trial, same isolates were tested in comparison of other commercial bio products. Naturally infested soil with *S. sclerotiorum* treated with local isolated bio agents as well as some commercial bio agents. Local isolate of *C. minitans* was the most effective in reducing disease incidence and the disease severity by 94.6% living plants (5.4 % mortality) and 13.0, respectively. *Trichoderma hamatum* and Contans® (commercial product *C. minitans*) also minimized disease severity by 14 and 16.2%, respectively when compared to untreated control. Among tested bacterial bicontrol agents, *Pseudomonas fluoroscens* was the best in reducing disease severity by 21.3% compared to controls. Yield data showed that *Trichoderma hamatum* increased total yield (10.485 ton/ha) Conversely, *C. minitans* was the best in increasing quality of yield in terms of exportable yield that giving 9.729 ton/ha.

Keywords: *Coniothyrium minitans;* white rot; low tunnel; beans.

INTRODUCTION

Sclerotinia sclerotiorum (Lib.) de Bary, considers one of the most destructive soil borne pathogens. It has been reported that this pathogen affects a wide range of wild and cultivated cops. It can infect over 408 species and 42 subspecies of plants at all stages of growth in field, moreover the infection could be developed during transit and storage of the product during postharvest stages (Barari *et al.,* 2010). The resulted disease is commonly known as white mold, sclerotinia wilt or stalk rot. White rot considers one of the most important limitation factor in producing green beans in Egypt. *S. sclerotiorum* has been isolated from soil samples obtained from greenhouses and protected agricultural areas. These areas - where bean plants are grown- usually known to be very moist and cool. Such conditions seemed to be subsidizing factors to incidence of white rot disease. One of the major problems in controlling this disease, that the pathogen produces large numbers of *sclerotia* which could stay viable for a long time in the soil. During the growing season, depending on various diverse environmental factors, sclerotia start to germinate and produce either mycelium or ascospores by developing an apothecium (Elgorban *et al.,* 2013). Ascospores are the primary inoculum for epidemics in many crops. They can move for a long distance to neighboring fields and infect plants in adhering fields. The fungus is capable of infecting flowers, leaves, fruits or stems. Wide host range of *S. sclerotiorum* make the control process more difficult. According to FAO STAT database Egypt exported about 37597 thousand ton of green beans in 2013. Most of this yield were exported to European Union fresh market. Beside known problems of using chemical control of white rot in beans such human health concerns, environmental pollution, and development of resistant isolates, most exporting regulation restrict using chemical pesticides for controlling white rot of snap beans. Biological control as a disease management strategy in protected agricultural areas could be economical and durable. It helps in reducing potential inoculum, which will lead to decreasing amount of disease produced by the pathogenic fungus. Several biocontrol agents have been screened for the control of *S. sclerotiorum*. For instance, *Ulocladium atrum* found to be a successful bio agent to management of *S. sclerotiorum* (Fernando *et al.,* 2007). Furthermore, *Trichoderma* and *Bacillus* species seemed to be effective bio agents against *S. sclerotiorum* (Zhang *et al.,* 2004;

Fernando *et al*., 2007;). The aim of this study was to evaluate the effectiveness of some local isolates of antagonistic fungi and bacteria in controlling white rot of snap beans and compare their controlling level to other available commercial bio and chemical pesticides taking in consideration the impact of that control level on the quantity and quality of green beans yield.

MATERIALS AND METHODS

Pathogenic fungus: *Sclerotinia sclerotiorum* used in this study was retrieved from sclerotia collected from diseased bean plants (*Phaseolus vulgaris* L.). Infected plants samples showing typical symptoms of white rot were collected from Ismailia governorate, Egypt. Collected sclerotia were surface sterilized with NaOCl solution then plated on PDA and incubated at 25 °C for 7 days. The purified fungal isolates were identified by Department of Plant Pathology, College of Agriculture, Mansoura University according to Kora *et al*., 2005. PDA slants from isolated fungus were kept at 4 ºC for further studies.

Isolation, purification and identification of antagonistic fungi: Soils from 20 different fields of Ismailia, Egypt were collected in sterile polyethylene bags. Standard serial dilution method was used for isolation of antagonistic soil fungi. The soil suspensions were done by suspending 1 g of each soil sample in 9 mL of 0.1 % peptone solution. Serial dilution has been done by transferring 1mL of the previous suspension to 9 mL of 0.1 % peptone to be diluted to 1/10. 0.1 mL from each dilution was plated onto PDA supplemented with 300 mg/L of chloramphenicol. Petri plates were incubated at 25±2ºC for 7 days until sporulation was observed. Individual colonies with typical *Trichoderma* characters such as green, velvety mycelia were transferred separately onto new PDA plates, incubated at 25±2 ºC for 3–5 days. The isolates that grew rapidly and formed greenish to white concentric circles were transferred to the *Trichoderma*-selective medium Rose Bengal agar (Williams, *et al*., 2003) (Magnesium sulphate heptahydrate 0.2 gm/L; Dipotassium hydrogen phosphate 0.9 gm/L; Ammonium nitrate 1.0 gm/L; Potassium chloride 0.15 gm/L; Glucose 3.0 gm/L; Rose Bengal 0.15 gm/L; and Agar 20 gm/L). The isolates were confirmed as having the same morphotype as on PDA and then stored as purified isolates in 50 % (v/v) glycerol at -80 ºC. *Trichoderma* spp. and *Clonostachys rose* were identified by microscopic observations according to identification keys of Bissett (Bissett, 1991a; Bissett, 1991b; Rifai, 1969). *Conothyrium minitans* Campbell, Isolate, the commercials product Trifender® (*Trichoderma asperellum* Samuels, Lieckf. & Nirenberg) and Contans® (*C. minitans* Campbell) were obtained from Plant Pathology Department, Plant Protection Research Institute, Budapest, Hungary. Spore suspensions of antagonistic fungi were prepared by subculture each fungus on PDA then incubated for 15 days at 25±2 ºC in the dark then adding 5 ml of sterilized distilled water and 2 drops of tween 20 to each plate and scrap the surface with sterilized spatula to harvest the spores. The resulted suspension has been transferred to sterilized baker through two layers of sterilized cheese cloth to get rid of mycelial fragments. Concentration of spore suspension was adjusted to $1x10^6$ using hemocytometer slide to count spores and sterilized distilled water to dilute the suspension

Isolation and Identification of antagonistic bacteria: *Pseudomonas fluorescens* was isolated from the rhizosphere of healthy green beans obtained from farmland in Ismailia, Egypt, using King's B medium. The identification of *P. fluorescens* was based on morphology, Gram staining, physiological and biochemical tests (Krieg & Holt, 1984). Locally isolated *Bacillus subtilis* was obtained from Center Laboratory Organic Agriculture, Agricultural Research Center, Egypt, where the commercial product Mycostop® (lyophilized spores of *Streptomyces griseoviridis*) obtained as a kind gift from the Kemira OY® of Finland. *B. subtilis* and *P. fluorescens* were grown on Nutrient Agar medium (NA), while *Streptomyces griseoviridis* was used as spore suspension from the commercial product Mycostop®.

Effect of antagonistic fungi on mycelial growth of *Sclerotinia sclerotiorum in vitro*: One mycelial discs 5 mm in diameter from 7 days old culture of antagonistic fungi (*Trichoderma harzianum*, *Trichoderma viride*, *Trichoderma hamatum*, *Clonostachys rosea*, and *Coniothyrium minitans*) were placed in facing 5 mm disc of *S. sclerotiorum* on in 90 mm Petri dish containing PDA, with four replicates. Control treatments conducted same as in treatments but without antagonistic fungi disc. All plates were incubated at 25±2 ºC for 15 days. Percentage of mycelial growth inhibition was calculated after 3 and 15 days by comparing the radial growth in treatments plates to control.

Effect of antagonistic bacteria on *Sclerotinia sclerotiorum* (Lib.) de Bary: One disc, 5 mm in diameter of mycelial growth of *S. sclerotiorum* was placed in side of Petri dish and antagonistic bacteria were spot inoculated at 3 cm distance from pathogen's disc on a PDA. The inhibition zone was observed after 3 and 15 days of incubation at 25±2 ºC.

Viability of *Sclerotinia sclerotiorum-sclerotia* treated with antagonistic microorganisms: Sclerotia were collected from 21 day old culture of S. *sclerotiorum* grown on PDA and incubated at 25±2 ˚C then dipped into a spore suspension $1x10^6$ cfu/ml in case of antagonistic fungi or bacterial cell suspension $1x10^3$ cfu/ml in case of

antagonistic bacteria for 5 min. After that, all sclerotia were dried on sterilized filter paper in an air current for two hours under laminar flow hood. Untreated *sclerotia* used as the control were dipped in sterilized distilled water. The *sclerotia* were placed on the bottom of Petri plates, incubated at 20±2 °C in a sterile humidity chamber (100% Rh). After 30 days, the viability of *S. sclerotiorum-sclerotia* was estimated by placing them on WA for 48 h at 25ºC and counting the number of emerging hyphae with phase contrast microscopy (100×). The *sclerotia* viability was assessed on a scale from 0 to 4, and *sclerotia* viability index (VI) was calculated according to Jager & Velvis, 1988. One hundred *sclerotia* were used for each treatment.

Low tunnels experiments:

Soil preparation: The experiment was performed on loamy sand soil (pH 7.16) at the protected agricultural area of exportable green bean var. Paulista under low tunnel conditions. This experiment was established at Gamal Ahmed Farm, Faid city, Ismailia governorate, where fields were naturally infested with *S. sclerotiorum*. Fertilizers were applied according to the recommendation of Agricultural extension department in that area, in amount per hectare were as follows , 168 kg agriculture sulfur, 480 kg calcium phosphate $Ca(H_2PO_4)_2$, 240kg Ammonium sulfate $(NH_4)_2SO_4$, and 120kg K_2SO_4, while the organic amendments were 24m^3 chicken manure and 12 m^3 livestock manure. All fertilizers were applied at the beginning of the first season. The experiment had randomized complete block design. The plots were 0.75×5.0 meter, 10 cm distance between plants, with three replicates per treatment.

Effect of antagonistic microorganisms on disease incidence and disease severity of white rot in beans: This test was done to evaluating antagonistic ability of five fungal antagonists and two bacterial antagonists in addition to three commercial bio-fungicides products; Contans® (*C. minitns* Campbell, 1x10^9 cfu/mL) and Trifender® (*T. asperllium* 1x10^6 cfu/mL) and Mycostop® (*S. griseoviridis*, 1x10^6 cfu/gm) in controlling whit rot of green beans under natural infestation conditions. Antagonistic fungi were applied as spore suspensions (1x 10^6 cfu/ml) through drenching soil at 5 days after sowing (DAS) at rate 50 ml spore suspension per plant. The control treatments were naturally infested soil without any treatment. Three chemical pesticides were used as chemical check at recommended dose. Chemical pesticides were also applied with same method (soil drenching 50 ml/ plant). Used pesticides were Topsin M-70® (2 gm/L), Rizolex® and Captan® (3 gm/L). The disease incidence calculated as average of dead plants numbers. The number of surviving plants after 15, 45 and 60 DAS were recorded. Disease severity (DS) was assessed on 0 to 4 scale. At the end of the season, plants were removed and washed to be free of soil then roots were visually assessed for percentage of affected root area that was due to Sclerotinia rot and each plant was assigned a root disease score 0-4 as follows:- 0=lesions and necrosis absent from roots; 1= 1-25 % of total root area necrotic; 2 = 26-50 % of total root area necrotic; 3 = 51-75 % of total root area necrotic; 4 = 76-100 % of total root area necrotic. The DS was calculated with the following formula:

$$Disease\ severity = \frac{Sum\ of\ all\ ratigs}{Total\ number\ of\ plants \times Maximum\ score} \times 100$$

In addition, number of branches, plant height, total yield and exportable yield were recorded as indicators for non-direct impact on controlling the pathogen.

Statistical analysis: Collected data were statistically analyzed using the Statistic Analysis System Package (SAS institute, Cary, NC, USA). Differences between treatments were studied using Fisher's Least Significant Difference (LSD) test and Duncan's Multiple Range lest (Duncan, 1955). All analysis was performed at P: 5 % level.

RESULTS

Effect of the antagonistic fungi on *Sclerotinia sclerotiorum:* After 3 days, *C. minitans* Campbell was the most effective against *S. sclerotiorum* with 74.4% reduction in the mycelial growth. This was followed by *T. hamatum* (Bonorden) Bainier that cause 60.0% inhibition in the mycelial growth (Table 1). Conversely, *T. hamatum* (Bonorden) Bainier was the most effective against the radial growth of *S. sclerotiorum* after 15 days, with 93.0%, followed by *T. viride* Pers, *T. harzianum* Rifai and *C. minitans* Campbell that causing 91.9, 91.7 and 91.1% reduction in the mycelia growth, respectively.

Effect of the antagonistic bacteria on *Sclerotinia sclerotiorum:* Data in Table 2 show the tested antagonistic bacterial strains significantly reduced pathogens growth in comparison to the control. *P. fluoroscens* was the most effective against *S. sclerotiorum* that giving 40.2 and 61.9% inhibition at 3 and 15 days, respectively.

Viability of *Sclerotinia sclerotiorum-sclerotia* inoculated with antagonistic fungi: Germination of *S. sclerotiorum -sclerotia* was extremely reduced by antagonistic fungi (Table 3). *S. sclerotiorum* -sclerotia inoculated with *T. viride* Pers and *C. minitans* Campbell were completely deactivated, while non-inoculated sclerotia showed 85.8% viability index.

Viability of *Sclerotinia sclerotiorum* sclerotia inoculated with antagonistic bacteria: The effect of antagonistic bacteria on *sclerotia* viability is presented in Table 4. Treatment of *sclerotia* by antagonistic bacteria

caused comparable results in VI of the sclerotia ranging from 19.5% VI in case of *P. fluorescens* to 20.5% in case of Mycostop®, while the control was 85.8% VI.

Low tunnel investigations:

Effect of soil application with antagonistic microorganisms on disease incidence and disease severity of white rot disease in beans

Disease incidence: Data in Table 5 clarify that there were significant differences between all treatments and control in suppressing the DI caused by *S. sclerotiorum*. *C. minitans* Campbell and *T. hamatum* (Bonorden) Bainier were the most effective in decreasing the DI of white rot disease by 94.6 and 94.0% living plants, respectively while untreated control gave 61.4% and topsin M -70 that giving 93.4%. These were followed by Contans® and Mycostop® which produced 90.6 and 89.4% living plants, respectively.

Disease severity: *Coniothyrium minitans* Campbell was the best antagonistic fungi of *S. sclerotiorum* that reduced the DS with 13.0% when compared with untreated controls (61.0%) and chemicals control (11.2% in case of Topsin M-70). Alternatively, *T. hamatum* and Contans® produced 14 and 16.2% DS, respectively (Table 5). Conversely, all treatment with antagonistic bacteria gave a moderate effective in the DS ranging from 21% (Mycostop®) to 23.2% (*B. subtilis*).

Effect of antagonistic microorganisms on growth characteristics

Number of branches: Data presented in Table 6 showed that *T. hamatum* (Bonorden) Bainier, *C. minitans* Campbell and *P. fluorescens* were the best treatments for affecting in number of branches that giving 17.33, 17 and 17 branch/plant, respectively when compared to untreated controls. There were followed by *B. subtilis* and Mycostop® which produced 16 and 15.62 branch/plant, respectively.

Plant height: Results in Table 6 illustrate that the best treatments which affected the plant height were *P. fluorescens* and *B. subtilis* that provided 91.28 and 90 centimeters, respectively when compared with untreated control (55.17 cm.) and chemicals control (86.68 cm. in case of topsin M-70). While, *C. minitans* Campbell and *T. hamatum* (Bonorden) Bainier produced 89.66 and 87cm, respectively.

Yield per hectare: *Trichoderma hamatum*, *C. minitans* and Mycostop® were the best antagonistic microorganisms affecting total yield per hectare that produced 10.485, 10.325 and 10.080ton/hectare., respectively when compared to untreated control (8.537 ton/ha.) and Topsin M-70 (10.494 ton/ha.). Also, Contans® produced 9.877 ton/ha. Furthermore, the least effective treatment in the total yield was *Clonostachys rosea* provided 8.863 ton/hectare (Table 6).

Exportable yield per hectare: Data in Table (6) explained that there were significant differences between all treatments and the untreated control. The best treatments affecting exportable yield were *C. minitans* Campbell and *T. hamatum* that increased the productivity by 9.729 and 9.449 ton/ha., respectively when compared to untreated control (6.233 ton/ha.) and chemicals control (9.734 ton/ha. in case of Topsin M-70). While, *T. viride*, Mycostop® and Contans® produced 9.103, 9.133 and 9.160 ton/ha., respectively.

Table 1. Effect of antagonistic fungi on radial growth of *Sclerotinia sclerotiorum.*

Fungi	After 3 days		After 15 days	
	R.G.	Inh.%	R.G.	Inh.%
Trichoderma harzianum	43.8^{c}	51.3	7.5^{cd}	91.7
Trichoderma viride	44.0^{c}	51.1	7.3^{cd}	91.9
Trichoderma hamatum	35.8^{d}	60.0	6.3^{d}	93.0
Clonostachys rosea	54.8^{b}	39.1	45.3^{b}	49.7
Coniothyrium minitans	23.0^{e}	74.4	8.0^{c}	91.1
Control	90.0^{a}	0.0	90.0^{a}	0.0
LSD	5.5		1.96	

values within a column followed by the same letter are not significantly different according to Duncun's multiple range test (p=0.05

Table 2. Effect of antagonistic bacteria on radial growth of *Sclerotinia sclerotiorum.*

Fungi	After 3 days		After 15 days	
	R.G.	Inh. %	R.G.	Inh. %
Pseudomonas fluorescens	53.8^{c}	40.2	34.3^{c}	61.9
Bacillus subtilis	55.5^{bc}	38.3	41.0^{b}	54.4
Mycostop® (*S. griseoviridis*)	58.8^{b}	34.7	36.3^{c}	59.7
Control	86.8^{a}	0.0	90.00^{a}	0.0
L.S.D	4.09		4.02	

Values within a column followed by the same letter are not significantly different according to Duncun's multiple range test (P=0.05)

Table 3. Viability of *Sclerotinia sclerotiorum-sclerotia* inoculated with antagonistic fungi.

Treatments	Percentage of *Sclerotia* according to the number of emerging hyphae					viability index
	0	1-5	6-10	11-25	>25	
Trichoderma harzianum	86	13	1	0	0	3.8
Trichoderma viride	100	0	0	0	0	0.0
Trichoderma hamatum	93	3	4	0	0	2.8
Clonostachys rosea	50	31	10	7	2	20.0
Coniothyrium minitans Campbell	100	0	0	0	0	0.0
Control	3	6	4	19	68	85.8

Table 4. Viability of *Sclerotinia sclerotiorum-sclerotia* inoculated with antagonistic bacteria.

Treatments	Percentage of *sclerotia* according to the number of emerging hyphae					Viability index
	0	1-5	6-10	11-25	>25	
Pseudomonas fluorescensns WCS365	50	30	15	2	3	19.5
Bacillus subtilis	57	22	10	7	4	19.8
Mycostop® (*S. griseoviridis*)	52	29	11	1	7	20.5
Control	3	6	4	19	68	85.8

Table 5. Effect of soil application with biocontrol agents on disease incidence and disease severity of white rot disease in beans.

Treatments	After 15 days			After 45 days			After60			DS%
	No.	Mo.%	Sur.%	No.	Mo.%	Sur.%	No.	Mo.%	Sur.%	
Non-treated	33.0^{i}	34.0	66.0	31.7^{h}	2.6	63.4	30.7^{j}	2.0	61.4	61.0
T. harzianum	40.7^{hg}	18.6	81.4	40.0^{fg}	1.4	80.0	40.0^{hi}	0.0	80.0	20.2
C. minitans Campbell	47.3^{a}	5.4	94.6	47.3^{a}	0.0	94.6	47.3^{ab}	0.0	94.6	13.0
Contans®	46.0^{ab}	8.0	92.0	45.3^{abc}	1.4	90.6	45.3^{abc}	0.0	90.6	16.2
Trifender®	43.7^{bcde}	12.6	87.4	43.0^{cde}	1.4	86.0	43.0^{def}	0.0	86.0	21.7
T. viride	44.7^{bcd}	10.6	89.4	44.3^{bcd}	0.8	88.6	44.3^{cde}	0.0	88.6	21.9
T. hamatum	47.7^{a}	4.6	95.4	47.0^{a}	1.4	94.0	47.0^{ab}	0.0	94.0	14.0
C. rosea	39.3^{h}	21.4	78.6	38.7^{g}	1.2	77.4	38.7^{i}	0.0	77.4	31.7
Mycostop®	45.7^{abc}	8.6	91.4	45.0^{abc}	1.4	90.0	44.7^{bcd}	0.6	89.4	21.0
P. fluoroscens	41.7^{efg}	16.6	83.4	40.7^{efg}	2.0	81.4	40.7^{fghi}	0.0	81.4	21.3
B. subtilis	43.3^{cdef}	13.4	86.6	43.0^{cde}	0.6	86.0	43.0^{defg}	0.0	86.0	23.2
Captan 50-WP	41.0^{fgh}	18.0	82.0	40.3^{fg}	1.4	80.6	40.3^{ghi}	0.0	80.6	33.7
Rizolex™	42.3^{defg}	15.4	84.6	42.0^{def}	0.6	84.0	42.0^{efgh}	0.0	84.0	25.5
Topsin M 70WP	47.3^{a}	5.4	94.6	46.7^{ab}	1.2	93.4	46.7^{abc}	0.0	93.4	11.2
LSD	2.4			2.6			2.5			

No. = number of living plant Mo. % = Mortality percentage

Sur.% = living plant percentage DS%=Disease severity percentage

Values within a column followed by the same letter are not significantly different according to Duncun's multiple range test (P=0.05)

Table 6. Effect of soil application with biocontrol agents on beans growth and yield.

Treatments	No. of branches	Plant Height (cm)	Total Yield (Ton/hectare)	Exportable yield (Ton/hectare)
Non-treated	10.00^{f}	55.17^{e}	8.537^{e}	6.233^{e}
T. harzianum	14.55^{cd}	70.83^{cd}	9.083^{de}	7.533^{d}
C. minitans	17.00^{ab}	89.66^{a}	10.325^{a}	9.729^{a}
Contans®	15.00b^{cd}	86.00^{ab}	9.877^{abc}	9.160^{abc}
Trifender®	14.87^{cd}	79.47^{abcd}	9.548^{bcd}	8.579^{bc}
T. viride	15.58^{abcd}	85.78^{abc}	9.806^{abc}	9.103^{abc}
T. hamatum	17.33^{a}	87.00^{ab}	10.485^{a}	9.449^{ab}
C. rosea	12.00^{ef}	69.33^{de}	8.863^{de}	7.493^{d}
Mycostop®	15.62^{abcd}	72.93^{bcd}	10.082^{ab}	9.133^{abc}
P. fluorescens	17.00^{ab}	91.28^{a}	9.258^{cd}	8.272^{cd}
B. subtilis	16.00^{bc}	90.00^{a}	9.380^{bcd}	8.614^{bc}
Captan 50-WP	11.53^{f}	71.64^{cd}	9.490^{bcd}	8.844^{ab}c
Rizolex™	14.00^{de}	81.60^{abcd}	9.929^{abc}	8.878^{abc}
Topsin M 70 WP	16.68^{abc}	86.68^{ab}	10.494^{a}	9.734^{a}
LSD	2.25	15.02	0.52	0.67

Values within a column followed by the same letter are not significantly different according to Duncun's multiple range test (P=0.05)

DISCUSSION

Beans white rot disease is a serious disease under the favored agro-climatic conditions in Ismailia Governorate, Egypt. One of the restrictions to fulfilling biological control against plant diseases is the lack of knowledge on the use of *in vitro* tests for selection of BCAs. Furthermore, the disease is usually overlooked by beans growers because it appears by the end of the growing season at the lower parts of the stem.

In the present study, it was noticed that *Trichoderma* species were highly efficient against *S. sclerotiorum* in the sequence *T. hamatum*, *T. harzianum* and *T. viride*. This high antifungal activity of *Trichoderma* spp. mostly dependent on some lytic enzymes, which act as fungal cell-well-degrading agents such as N-acetyl- ß-D-glucosedeaminidase, chitinase, ß–1, 3 gluconase, chitobiosidase and protease (Harsukh *et al.*, 2013). *T. harzianum* was found to restrain enzymes of foliar pathogens, the activities of exo-endopolygalacturonase, pectin methyl esterase, pectatelyase, chitinase and cutinase, which are thought to be involved in mycoparasitism process in leaves infested with fungi (Sharma *et al.*, 2012). Furthermore, *Trichoderma* spp. could produce cynamidehydratase, rhodanese and ß–cyanoalnine synthases, which known to play an important function in reducing the growth of plant pathogenic fungi (Wilmari, 2010). Under low tunnels studies, it was observed that *T. hamatum* was the greatest antagonistic fungi against *S. sclerotiorum* that produced 94% living plants and 14% DS when compared with controls. The results can be understood by synergistic involvement of a number of mechanisms, which may include activation of plant defense system (Manoj *et al.*, 2011). The synthesis of pathogenesis-linked proteins is one of the most ordinary defense mechanisms triggered in plants following infection with inducing agents (Markus & Susanne, 2010). Induced resistance is recognized as an important mode of biocontrol in vegetative tissue (Aidemark *et al.*, 2010). Induced systemic resistance caused by *Trichoderma* spp. and various microorganisms can protect plants against soil or foliar pathogens (Chowdappa *et al.*, 2013). Salicylic acid produced by *Trichoderma* spp. induced resistance to *B. cinerea* in bean (Martínez-Medina *et al.*, 2013). Besides, root colonization with *Trichoderma* induced increased peroxidase and chitinase activities in many plants (de Santiago *et al.*, 2011).

In the current study, we observed that *C. minitans* one of the best antagonistic fungi against *S. sclerotiorum*. This high antifungal activity of *C. minitans* Campbell may be attributed to causing destruction of hyphae and sclerotia of *S. sclerotiorum* (Whipps *et al.*, 2008). The extra-cellular enzyme ß-1, 3-glucanase (EC 3.2.1.39) appears to be an important enzyme involved in the mycoparasitism of *S. sclerotiorum* by *C. minitans* Campbell, as the expression of the gene cmg1 encoding ß-1,3-glucanase increases during infection of *sclerotia* of *S. sclerotiorum* by *C. minitans*.

Under low tunnel studies, *C. minitans* significantly reduced disease levels with 94.6% living plants and 13.0% DS when compared with untreated controls and establishment of the BCA in the rhizosphere protects the bean roots from infection with the mycelium of *S. sclerotiorum* growing into this region (Khokhar *et al.*, 2012).

From all tested antagonistic bacteria, it was observed that *P. fluorescens* gave a high influence on the mycelial growth and the VI of *S. sclerotiorum -sclerotia* with 61.9% and 19.5%, respectively. Also, it had highly

reduced the DI and the DS caused by *S. sclerotiorum* with 81.4% living plants and 21.3% DS. This high efficiency of *P. fluorescens* against *S. sclerotiorum* is probably related to the degradation of chitin in hyphal and *sclerotia* cell by several hydrolyzing enzymes (Sebastian *et al.,* 2010) such as endochitinase or, exochitinase and chitobiosidase. In addition, *P. fluorescens* produces three antibiotics which are possibly involved in its biocontrol activity: 2, 4-diacetylphloroglucinol, pyrrolnitrin and pyoluteerin (Sebastian *et al.,* 2010). *P. fluorescens* has been reported to succeed in decreasing plant diseases and promoting plant growth by inducing systemic resistance (Phrueksa, 2010). These mechanisms include competition for nutrients and place, and producing chitinase, lytic enzyme, siderophores besides many antibiotics viz, 2,4-diacetylphloroglucinol, pyoluteorin, pyrrolnitrin, pyocyanin and oomycin A (Vanitha & Umesha, 2011). Biocontrol bacteria may stimulate plants to secrete more chitinase when colonizing the rhizosphere, and the plant chitinase may play a more effective role in plant defense (Fgaier & Eberl, 2011). Hui *et al.* (2011) confirmed that *P. fluorescens* produced volatile hydrogen cyanide, which stopped the growth of *S. sclerotiorum* for a long period. In addition, Sharma *et al.* (2011) have discovered that *P. fluorescens* P13 could excrete siderophores that might inhibit *S. sclerotiorum*.

In thus study, it was indicated that Mycostop® was effective in controlling the fungus *S. sclerotiorum.* The effect of Mycostop® was similar to the previous reports above. It seems obvious that *S. griseoviridis* produces extracellular enzymes with lytic characteristics. *S. griseoviridis* is well known as a root colonizer and stimulates root growth during rhizosphere colonization (Doumbou *et al.,* 2001). In some cases, stimulation of plant growth could explain the enhanced yield results when the antagonist was combined with soil solarization. The effectiveness of Mycostop® against *Fusarium* wilt of tomato was satisfactory when applied to artificially infested soil. On the other hand, higher yields of healthy crops after Mycostop® application indicate that a growth promoting factor may also be involved. Another hypothesis for vigorous growth and yield increases is the control of minor pathogens. Sutthinan *et al.* (2010) had shown that *S. griseoviridis* produced indole-3-acetic acid which induces several effects in plant, among which the stimulation of growth is.

Conclusion: Finding harmless and ecological alternatives to chemical control is an urgent need to face increasing demand for safe, sustainable and effective management plan to white rot of beans counting on biocontrol agents, and other best disease management practices. This study demonstrated that the antagonistic fungi could be considered better biocontrol agents against *S. sclerotiorum* than the antagonistic bacteria in vitro and under the field conditions. The current study suggests that all tested biocontrol agents could be used in an integrated control program against *S. sclerotiorum*. Fungal biocontrol agents such *Trichoderma* spp. and *C. minitans* Campbell and bacterial biocontrol agents such *P. fluorescens* seemed to be efficient control elements if it is used in integration with other management practices, including cultural practices, using resistant varieties, and reduced chemical control.

Acknowledgments: This project was supported by King Saud University, Deanship of Scientific Research, College of Science Research Center. I would like to express my sincere gratitude and deep gratefulness to all our colleagues of the Department of Botany and Microbiology, King Saud University for their valuable criticism, advice and the authors have declared no conflict of interest.

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