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BIOLOGICAL CONTROL OF WHITE MOULD DISEASE ON DRY BEAN CAUSED BY *SCLEROTINIA SCLEROTIORUM* IN EGYPT

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ABSTRACT: In this study *Sclerotinia sclerotiorum* (Lib.) de Bary on dry bean (*Phaseolus vulgaris* L.) and how to be controlling the disease in Egypt. Novel antifungal substances such as biocontrol agents and commercial biocides were used instead of fungicides which have a dangerous effect on human health or ecology.

Twelve isolates of *S. sclerotiorum* were selected from 315 isolates from the diseased bean grew in many governorates in Egypt.These isolates were identified and coded while it was used in pathogenicitytest and other experiments. In the pathogenicity isolate coding (SMZ11) was very aggressive than the other isolates.

Eleven Isolates from rhizosphere bean i.e. (eight *Trichoderma* spp, two of *Bacillus subtilis,* and one of *Pseudomonas fluorescens*) were isolated from healthy bean rhizosphere. These isolates were tested on *S. sclerotiorum* (SMZ11) growth in vitro. Generally, all tested isolates reduced the mycelial growth and sclerotia formation of the pathogen but it showed a contrast between treatments whereas *T. harzianum* (TH1) gave highly reduction of mycelial growth followed by *T. viride* (Tv1) and *B. subtilis* (B1). The same treatments were studied on the disease in a greenhouse at the Faculty of Agriculture, Al-Azhar University, Nasr City, Cairo, Egypt. All treatments significantly reduced the percentage of pre- and post-emergence damping off compared to the untreated control.Results in the greenhouse experiment were different from than laboratory test, where *T. harzianum* (TH1and TH3) and *T. viride* (Tv1) gave highly effect on disease incidence and survival plants which recorded 90, 85 and 85% survival plants respectively. Some commercial biocides were experimented on bean white mould disease in a greenhouse. The biocide (Blight stop) was highly effective on disease incidence which recorded 80% survival plants.

In applied experiments commercial biocides and biocontrol agents isolates which give highly effect on bean white mould disease under the greenhouse were used to control the disease in the open field during the season (2021) from natural infestation and epidemically fields at Zawyet Razin, Menouf, Menoufia governorate, Egypt. *Trichoderma harzianum* (TH1) recorded highly reduction of disease incidence and high yield of bean crop while Rhizo-N was given low effect on the pathogen and smallest yield. Generally, all the tested biocontrol agents and commercial biocide treatments reduced the percentage of white mold incidence and severity of the treated dry bean plants compared with the untreated control.

Key words: *Phaseolus vulgaris*, White mould, *S. sclerotiorum*, biocontrol agents, commercial biocides.

INTRODUCTION

Bean (*Phaseolus vulgaris* L.) is considered one of Egypt's most important leguminous crops. Their seeds and pods are rich in proteins, calcium, mineral salts, vitamins, and amino acids. Its well-growth characterizes it in moderate regions. It’s cultivated for use as green pods or dried seeds. Recently, it’s been highly demanded to export to the European market (Heia, 2003). The cultivated area of beans was 122155 feddan in the season 2016/2017, while in the season 2019/2020 was decreased to 101011 feddan (Statistics Dept. Ministry of Agriculture, Egypt). Beans are a primary protein source for billions of people globally, yet they are suspected of harboring a variety of illnesses.

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White mould, caused by *Sclerotiorum sclerotiorum*, is the most prevalent disease that infects green and dried common beans (*Phaseolus vulgaris* L.) across the world's producing areas, causing severe yield losses of up to 100% under ideal climatic conditions for pathogen growth. (Schwartz and Singh, 2013). The devastating soil-borne pathogen *S. sclerotiorum* causes white rot in over 600 plant species, including practically all dicotyledonous and some monocotyledonous plants, and may live in the soil for up to 10 years in the form of sclerotia (Liang and Rollins, 2018 and Mohamed and Atallah, 2020). Plants of both dry and green beans (*Ph. vulgaris* L.) are often cultivated in Egypt in very damp and chilly circumstances. Depending on the suitable circumstances, the pathogen produces white mould illness, which results in losses ranging from 30% to 100% (Mohamed and Atallah, 2020).

The pathogen severely affected bean plants from December till early March, causing significant economic losses in bean fields. After the beans harvest, *S. sclerotiorum* survives in soil and crop debris as sclerotia, a dormant and resistant stage, on infected plant tissue typically incorporated into the soil and can be endured fertile for up to 10 years (Lopes *et al.,* 2010). Because of its distinct life cycle, the pathogen infects host plants by ascospores that may be grudgingly expelled upwards from apothecia into the air or via mycelium arising from infected tissue and germinated sclerotia (Zheng *et al.,* 2019). The fungus causes cell death in plant tissue and shows on the crop as soft rot or white mould. The disease reduces agricultural productivity and quality, which may cost millions of dollars every year (Saharan and Mehta, 2008). Under favorable climatic circumstances for *S. sclerotiorum* growth, such as high humidity and moderate temperatures ranging from 15 to 20°C, the common bean may sustain losses of 30% or more, up to 100% during rainy seasons if preventative measures are not implemented (Singh and Schwartz, 2010). Many global obstacles face *S.sclerotiorum* management efforts, including long-term persistence and tolerance of reproductive structures (sclerotia), a diverse host range, and the unpredictability of infection (Arfaoui *et al.,* 2018). These circumstances necessitate the use of fungicides, which are known to have negative effects on non-target species. Furthermore, the growing costs of soil fumigation, a lack of adequate substitutes for hazardous fumigants, and public concerns about recurrent fungicide usage for disease management have prompted producers to consider alternate control approaches, such as biological control. Microbial agents for plant disease control may be an environmentally benign and cost-effective component of an integrated pest management program (Mao *et al.,* 1997). Biological management is seen to be the most promising alternative to chemical fungicides for reducing white mould in common beans. *Epicoccum purpurascens* is one of many biocontrol agents reported for disease control (Zhou and Reeleder, 1989). Biocontrol Increased the number of pods per plant and yield by up to 40% as compared to controls, even under greater disease burden (2010) Geraldine *et al.,* (2013). Furthermore, *Trichoderma* and *Bacillus* species seemed to be efficient anti-*S. sclerotiorum* bioagents (Fernando *et al.*, 2007). *Trichoderma* spp., namely *T. harzianum and T. virens*, have also been demonstrated to attack *Sclerotinia* spp. sclerotia and mycelium. Field experiments against sclerotia have been found to provide some disease control (Wenting *et al.,* 2012). Under laboratory circumstances, *T. harzianum* killed the sclerotia of *S. sclerotiorum* and converted them to *T. harzianum* spores in 12 days. *T. viride* isolated from degraded sclerotia accelerated decay in *S. sclerotiorum* (Mohamed andGomma 2001).

T. harzianum parasitized the pathogen and hindered mycelial development, including mycoparasitism 41 processes such as coiling round and attachment to host hyphae, microconidia and penetration into the hyphae, or breaking the septa of hyphae and conidia. *T. viride* generated nonvolatile antibiotics that inhibited pathogenic fungus growth. However, its antagonistic action in vitro was comparatively poor. Some bacterial strains, such as *Erwinia herbicola*, *Bacillus* spp., and *Pseudomonas* spp., display antifungal activity against *S.*

sclerotiorum (Fernando *et al.,* 2007). Liquid extracts of fermented agricultural waste increased sclerotia colonization by the mycoparasitic fungus *Trichoderma* spp (Huang *et al.,* 1997).

The aim work of this study was

1- Isolation and identification of the pathogen from infected bean tissues collected at selected locations in Egypt.
2- Isolation and identification of microorganisms from rhizosphere bean plants collected from diseased fields in Egypt.
3- Evaluated these microorganisms as biological control compared to commercial biocides and their effect on dry bean yield.

MATERIALS AND METHODS

1. Collection of infected plant materials

Bean plants showing identical Sclerotinia rot symptoms on the stem, pods, and leaves were collected from different fields in many governorates of Egypt, i.e., Al-Menoufia, Alexandria, Qalubeiah, El-Behira, Al-Gharbia, Kafr Elsheikh, Ismailia, and Al Sharqiya governorate, during the winter 2019 growing season.

2. Isolation, purification, and identification of the causal organism

Isolation experiments were performed on collected material. The infected tissues (lesions) were cut into small pieces and surface sterilized for 3 minutes with sodium hypochlorite (0.5 percent), then washed several times with sterilized distilled water and dried between sterilized filter papers before being transferred directly to the PDA medium in 9 cm-Petri dishes. The plates were incubated at 25±2°C for three days. Fungi were cultivated on potato dextrose agar (PDA) slants from cultured pieces. Fungal isolates were purified using the hyphal tip procedure (Brown, 1924) and stored at 5°C on PDA slants. Plant Pathology Laboratory, Agricultural Botany Department, Faculty of Agriculture, Al-Azhar University, Nasr City, Cairo, Egypt, identified the isolated fungus. According to the morphological properties of mycelium and sclerotia (size-count-color-germination) as described by Singh (1982).

3- Molecular identification of the pathogen

Total genomic DNA extraction was carried out as Toda *et al.* (1999) described. Polymerase chain reaction Gebily *et al.* Egyptian Journal of Biological Pest Control (2021) 31:33 Page 2 of 15 (PCR) was conducted by the universal primers, i.e., forward primer ITS-1(5′-TCCGTAGGT GAACCTGCGG-3′) and reverse primer ITS-4 (5′-TCCTCCGCTTATTGATA TGC-3′) as described by White *et al.* (1990). The sequence divergence within the fungal ribosomal DNA internal transcribed spacer (rDNA-ITS) regions is targeted for amplification. In GATC Biotech German Company, PCR was performed using the technique published by Hayakawa *et al.* (2006). (Under License of Sigma). 1.5 percent agarose gel electrophoresis was used to separate PCR products. The DNA ladder (100 bp) was also injected into the gels and imaged to estimate the correct band size of amplified products. The PCR product was cleaned using an EZ-10 spin column PCR products purification Kit (BIO BASIC). Finally, the PCR product was sequenced using an ABI PRISM 3730XL DNA sequencer with forward and reverse primers. The species identification of the sequenced PCR result was validated using the NCBI (National Center for Biotechnology Information) mega blast. The species name was discovered based on the proportion of homogeneity between our isolate and the recognized isolates. The isolate was identified and deposited to the National Center for Biotechnology Information (NCBI) under the GenBank accession number (OP431391). The nucleotide sequences of the matching 16S rRNA gene were compared to 10 sequences from different fungal isolates stored in GenBank. The alignment tool and phylogenetic tree were used to calculate evolutionary distances between the isolated fungus's nucleotide sequences.

4. Pathogenicity test

Giza bean cultivar 6 cv. In these studies, seeds were employed. The seeds were generously provided by the Vegetable Crop Research Institute, Agricultural Research Center, Giza, Egypt. *S. sclerotiorum* isolates were evaluated for pathogenicity on apparently healthy seeds.

Preparation of greenhouse experiment

By extensively mixing with a 5% commercial formalin solution, loamy sand soil (3 clay:1 sand w/w) was sterilized. Similarly, plastic pots (25 cm in diameter) were sterilized by soaking them in a 5% commercial formalin solution for 15 minutes, allowing them to dry over 24 hours before filling them with previously sterilized soil. An inoculum of *S. sclerotiorum* isolates was generated for soil infestation by growing the pathogen for two weeks in 500 ml glass bottles containing sterilized 100 g of natural medium with a combination of sorgam (100g); cleaned sand (50g); and 100 ml water (Abdel Kader *et al.,* 2012). Each isolate was applied individually to the potting soil at a rate of 3 percent w/w, mixed completely with the soil surface of each pot, then watered and left for one week to ensure uniform dispersion of the inoculum. The pots were then put in a dew plastic chamber with 100% relative humidity at 25±2°C for 48 hours in the greenhouse. Giza 6 cv. Healthy bean seeds were surface sterilized by soaking them in a 2.5 percent sodium hypo-chloride solution for 3 minutes, then rinsing them three times in sterilized water and drying them. Five seeds were sowed in each pot, with four duplicates used for each pathogen isolate and four pots used without inoculum as a control.

Data of disease estimation was recorded as pre-emergence damping-off (15 days after sowing), post-emergence damping-off (45 days after planting), and the average of the survival plant (at the end of the season).

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

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

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

5- Isolation of antagonistic microorganisms from bean plants' rhizosphere

Roots and stems samples were collected from the rhizosphere of healthy bean plants grown in naturally infected fields at Al- Menoufia Governorate. Five samples were collected randomly from each location and mixed. Samples were cut into small fragments, and an isolation process was carried out to isolate the different antagonistic fungi and bacteria using PDA and NA media. The growing fungi were microscopically examined and pure cultures of the different isolates were obtained using the hyphal tip and single spore technique. Bacterial cultures were purified using NA plates. Different isolated microorganisms (fungi and bacteria) were stored on PDA and NA media for further studies. Identification of rhizosphere microorganisms was carried out using the morphological and microscopic characteristics according to Barnett and Hunter, (1972). Identification of bacterial isolates was conducted according to Bergey's Manual of determinative bacteriology (Breed *et al.,* 1957).

Antagonistic fungi were applied at the rate of 3×10^{6} CFU/ml, and bacterial antagonists were amended at the rate of 2×10^{6} CFU/ml.

6- Effect of Microorganisms on mycelial growth and sclerotia formation of *S. sclerotiorum in vitro*

In this experiment, eight fungi isolates (four of *T. harzianum*, two of *T.viride*, one of *T. hamatum,* and one of *T. koningii*), and three isolates of bacteria (two of *B. subtilis* and one of *P. fluorescens*) were isolated from the rhizosphere of bean plants. *S. sclerotiorum* (SMZ11) was grown on PDA plates for 7 days. Disc 7 mm in diameter taken from the edge of the fungal culture growth was placed 1 cm away from the edge of the 9 cm diameter of PDA plates. The opposite edge of the plates was individually inoculated with the isolated

microorganisms from bean plants, i.e., bacteria and fungi (Dennis and Webster, 1971). Four plates were used for each treatment. The inoculated plates were incubated at 25±2°C for 6-7 days, and the bean white rot pathogen development was assessed as a percentage of Inhibition of Radial Growth (%IRG) or % reduction as described by Fokkema, (1973), as follows:-

% IRG = (R1-R2) / R1 x100 = % reduction

Where:-

R1= length of radial growth of pathogenic fungus far away from the effect of antagonistic microorganism.

R2= length of radial growth of pathogenic fungus affected by the antagonistic microorganism.

7- Greenhouse Experiments

These experiments were carried out at the Faculty of Agriculture, Al-Azhar University, Nasr City, Cairo, Egypt. The individual effects of Biocontrol agent and biocide under artificial soil infestation conditions.

8- Preparation of fungal inoculum

As mentioned before, the inoculum of *S. sclerotiorum* (SMZ11) was prepared for the pathogenicity test. The same method was used to prepare antagonistic fungi (*Trichoderma* spp.) While antagonistic bacteria (*P. fluorescens* and *B. subtills*) were prepared by dilution plate assay, bacterial suspensions were (2×10^6 CFU/ml) as described by Callan *et al.,* (1990).

9- Application of biological agents for controlling Sclerotinia rot

Inocula of each Trichoderma isolate was added to the potting soil at a rate of 3 percent w/w, properly mixed with the soil surface of each pot (25 cm), then watered and allowed for one week to ensure uniform dispersion of the inocula. According to Park *et al.,* (1991), bean seeds were dipped into the cell suspensions of each studied antagonistic bacteria for 15 minutes at a rate of 5 ml/L. Healthy bean cv. Giza 6 was grown in pots. Each treatment included five seeds per container and four duplicates. Seeds were sown in pots altered with a combination of soil containing antagonistic fungus and untreated pots for the untreated control. As previously stated, soil infestation was carried out. The severity of white rot disease was determined using the disease scale indicated previously in this section.

As previously stated, disease estimate data was collected as pre- and post-emergence damping-off.

10- Application of commercial biocides for controlling Sclerotinia rot

Eight commercial biocides were compared with antagonistic isolates isolated from the healthy bean. These biocides were: Plant guard (*T. harzianum*), Blight Stop (Mixture of *Trichoderma* spp.), Biocontrol T34 12% W.P. (*T. asperellum*), Bio Z (*T. album*) Rhizo-N (*B. subtilis*), Bio Arc (*B. megaterium*), New Acteno (A Mixture of *Actenomycetes* spp.), and (Mixture of *Bacillus* spp.) these compounds were used at the recommended rate and dipping bean seeds before planting.

11- The *Trichoderma* spp. treatment

Air-dried fine ground sorghum grains containing 3×10^6 (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. The coated seeds were air-dried prior to sowing.

12. The *B. subtilis* and *P. fluorescens* 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 $MgSO_4$ and 0.5% Carboxymethyl Cellulose per 100g of bean seeds). Then the treated seeds were air-dried before sowing.

13. Control

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

14. Field experiments

Field experiments were carried out during season 2021 at Zawyet Razin, Menouf, AL-Menoufia Governorate, Egypt, in epidemic fields (naturally infested) with white mould disease to investigate the effect of the tested antagonistic microorganisms and biocide to controlling white mould disease. Bean seeds cv. Giza6 was 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 1st September 2021. The field was designed in complete randomized blocks with three replicates. The area of each plot was 21 m^2 and consisted of five rows; each row was 4.20 m in length and 5 m in width. All treatments were sown in hills 10 cm apart on the eastern side of the row ridge, with two seeds per hill. All other recommended agricultural practices were followed according to the Egyptian Ministry of Agriculture and Land Reclamation recommendations. The treatments were as follows biocontrol agent and biocide seeds soaking in water served as untreated control. Different control method treatments were applied by seed treatment before planting and spraying the plants five times. These were 15 days after seeding, 30 days after seeding, 45 days after seeding, 55 days after seeding, and 65 days after seeding.

Data recorded

Pre- and post-emergence damping-off (20 days after planting).

1. Percentage of infection.
2. The severity of infection.

A scale of 0 – 5 was used to estimate the severity of infection: (0: healthy, 1: 20%, 2: 40%, 3: 60%, 4: 80%, and 5: 100%).

Using the following formula (Soliman *et al.,* 1988):

At the end of the experiment, the average weight of the dry bean (kg per plot) was recorded.

The data were statistically analyzed, and significance among means was assessed by least significant difference (LSD) at a 5% probability level using SAS Anova program v. 9 (Anonymous, 2014).

RESULTS

1. Isolation and identification of the causal organism

Twelve isolates were selected from 315 isolates of *S. sclerotiorum* from the diseased bean plants which were isolated from eight locations of different governorates from the winter 2019 season. These isolates were identified by morphological characteristics and sclerotia formation (count and size of sclerotia per plate). Fig (1) indicates that many different characters between isolates. Isolate that code (SMZ11) which was isolated from Zawyet Razin,Menouf, Al Menoufia governorate, gives a count of sclerotia more than other isolates. One sclerotia of this isolate were used to produce pure culture to identify by PCR. PCR amplification with the primers (ITS-1/ITS-4) produced a 600-bp product inside the fungal isolate's internal transcribed spacer (rDNA-ITS) region (Fig. 2a). The isolate's sequencing was submitted to NCBI Gen-Bank and given the accession number OP431391. Based on the nuclear ribosomal internal transcribed spacer, the ITS rDNA, a phylogenetic tree of the Egyptian isolate *S. sclerotiorum,* and ten additional isolates were built (amplicons from primers ITS-1 and ITS-4). The results indicated that the nucleotide sequence similarity of the ITS rDNA gene between *S. sclerotiorum* isolates varied from 98 to 100 percent, as illustrated in Figure 1. (Fig. 2b). PCR amplification of the ITS gene using a precisely designed primer pair (ITS-1 and ITS-4), followed by sequencing and phylogenetic analysis, identified the isolate as *S. sclerotiorum.*

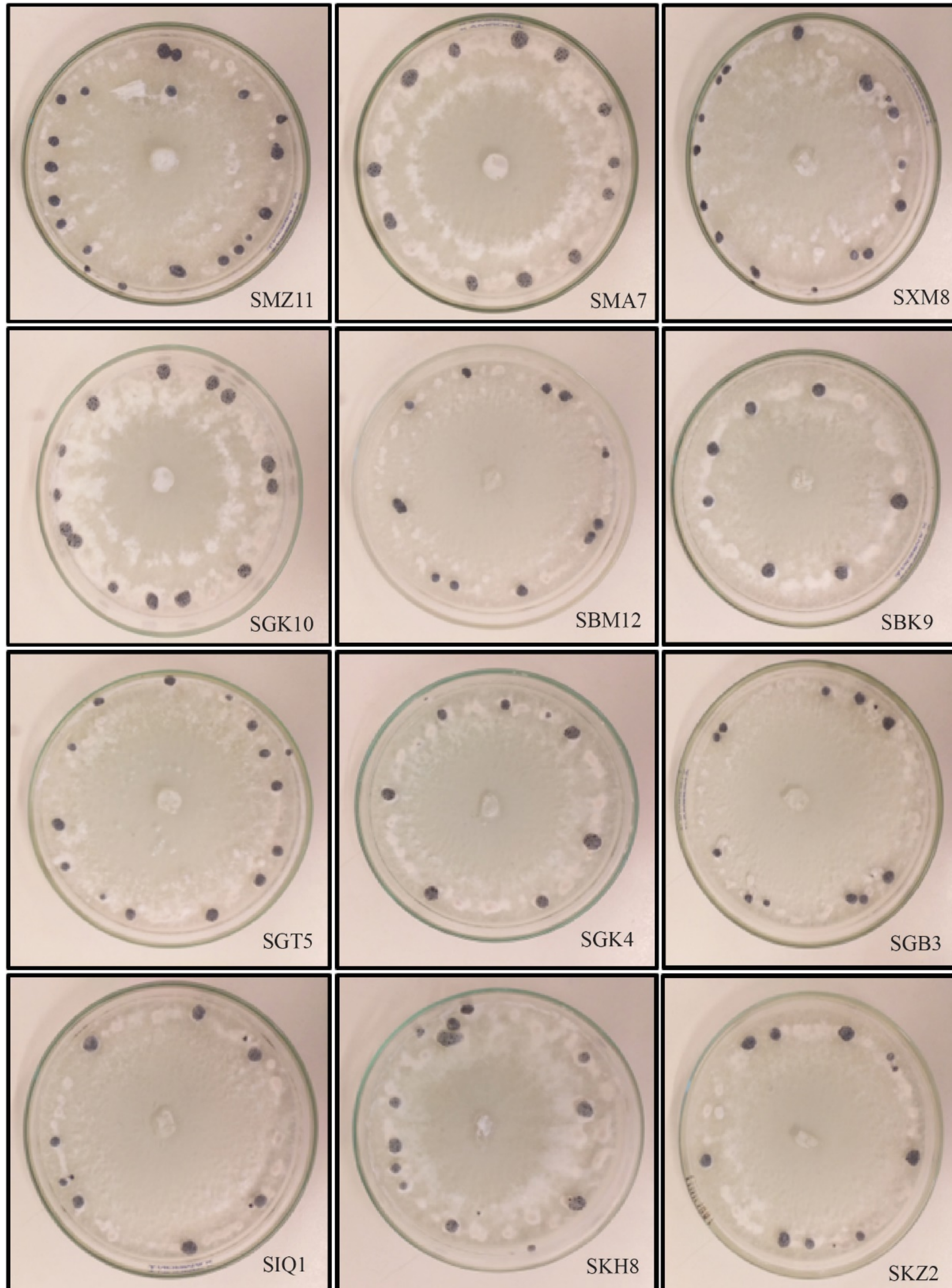

Fig (1): Differentiation between twelve isolates of *S. sclerotiorum*.

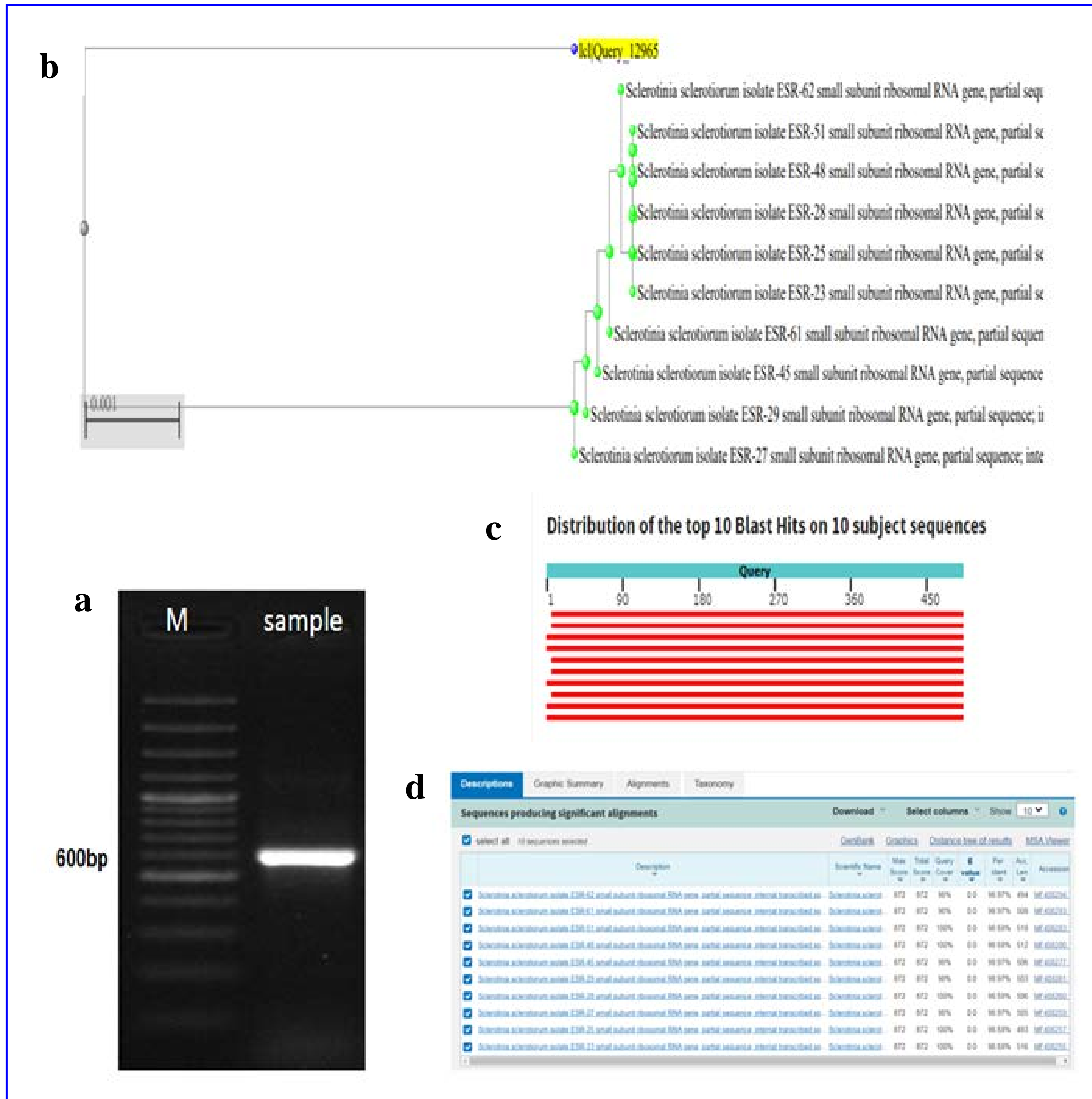

Fig. 2a. Products of polymerase chain reaction amplification isolate using the universal primers ITS-1 and ITS-1. A product (amplicon) of 553 bp length was amplified in Egyptian isolate (Is). M = Leader and Is = isolated fungus. Fig. 2b, c, d. Phylogenetic tree obtained from the alignment of nucleotide sequences of the Egyptian isolate and the high similarity of ten sequences from GenBank. All 10 sequences show their GenBank codes and sequences hits.

2. Pathogenicity results: (variability of isolates)

Data presented in Table (1) indicate that all the tested isolates of *S. sclerotiorum* were pathogenic to Giza 6 bean cultivar plants. Isolate (SMZ11) was the most aggressive one, where all the seeded plants were lost (40% pre-emergence and 45 post-emergence damping off), and 85% of plants dead. On the other hand, flowed by (SPK4) isolate, which gives 80% dead plants, and isolates (SMA7, SGT5, and SKZ2) which recorded 75% dead plants for everyone. Whereas isolate (SIQ1) that was isolated from the Ismailia was the least virulent, it only gives 45% dead plants.

Table 1. Results of pathogenicity test of *S. sclerotiorum* isolates on Giza 6 bean cultivar.

District	code of isolate	Pre-emergence damping-off	Post-emergence damping-off	Survival plants (%)
Menouf	SMZ11	40.00	45.00	15.00
Ashmoun	SMA7	35.00	40.00	25.00
Alexandria	SXM8	25.00	35.00	40.00
Tookh	SGK10	20.00	45.00	35.00
Abu al-Matamir	SBM12	30.00	40.00	30.00
Kafr-El-Dawar	SBK4	40.00	40.00	20.00
Tanta	SGT5	40.00	35.00	25.00
Kafr-El Zayat	SGK4	20.00	35.00	45.00
Kafr Elsheikh	SGB3	30.00	30.00	40.00
Al Qassaseen	SIQ1	20.00	25.00	55.00
Abu Hammad	SKH8	35.00	30.00	35.00
Zagazig	SKZ2	35.00	40.00	25.00
Control		0.00	0.00	100.00
Mean		27.30	31.53	41.15
LSD 0.05		24.56	19.70	23.91

3. Laboratory experiments

Isolate (SMZ11) was used in the following experiments because it was the most aggressive one on Infected plants.

Effect of biocontrol agents on fungal growth

Microorganisms isolated from healthy beans were used to determine the effect or antagonistic to the mycelial growth and sclerotia formation of *S. sclerotiorum* isolate (SMZ11). Data in Table (2) show that all antagonistic fungi reduced the growth of *S. sclerotiorum,* while *T. harzianum* isolate (TH1) was the best antagonistic fungus which give 80.28% reduction of mycelial growth, followed by *T. viride* (TV1) with 77.22% growth reduction compared to control. In the other respect, *B. subtilis* (B2) was more effective as an antagonistic bacteria and reduced the growth of *S. sclerotiorum* by 76.11%, followed by *P. fluorescens,* causing a 68.88% growth reduction. *T. harzianum* and *T. viride* showed over the growth of the pathogen. The same results indicated that, highly antagonistic microorganisms reduced sclerotia formation in plates, which *T. harzianum* (TH1)*, T. viride* (TV1), and *B. subtilis* (B2) give a low count of sclerotia (2, 2.75 and 3.25 sclerotia respectively). In the meantime, all tested biocontrol agents significantly reduced the pathogen's growth. Inhibition zones were noticed when either *P. fluorescens* or *B. subtilis* were tested.

4. Greenhouse experiment

Microorganisms isolated from the healthy bean and tested to be antagonistic to *S. sclerotiorum* in the laboratory were used with some biocide compounds under the greenhouse to evaluate the effect on disease incidence in the greenhouse.

Table (2). Effect of a biocontrol agent on the growth and sclerotia formation of *S. sclerotiorum* under laboratory conditions.

Biocontrol agent	Linear growth (cm)	Growth reduction (%)	Sclerotia formation count
T. harzianum TH1	1.78	80.28	2.00
T. harzianum TH2	2.33	74.11	2.75
T. harzianum TH3	3.50	61.11	4.00
T. harzianum TH4	3.18	64.72	6.75
T. viride TV1	2.05	77.22	3.50
T. viride TV2	2.68	70.28	7.50
T. koningii	2.91	67.64	5.50
T. hamatum	3.59	60.14	6.50
B. subtilis B1	2.15	76.11	3.25
B. subtilis B2	3.01	66.53	4.75
P. florescence	2.80	68.88	7.75
Control	9.00	00.00	20.25
Mean	3.29	63.47	6.21
LSD 0.05	0.50	5.56	1.10

Results in Table (3) indicate that all the tested Microorganisms and biocide compounds reduced the percentages of pre- and post-emergence damping-off caused by *S. sclerotiorum* and significantly increased with the survival plants compared with control (untreated infested soil). *T. harzianum* (TH1) was highly treated, which gave survival plants (90 %), followed by *T. harzianum* (TH3), *T. viride* (TV1), *T. harzianum* (TH2), and *B. subtilis* (B1) 85%, 85%, 80%, 80% respectively, while *P. fluorescens* have the lowest effect on disease control. At the same time (Blight Stop) gives pest control treatment from biocide compounds, which recorded 80% survival plants in the greenhouse. While Bio zeid and root guards affected 75% and 75% of survival plants, Rhizo-N treatment was the least effective in reducing pre- and post-emergence damping-off and survival plants (55%) compared to untreated infected control. Generally, all treatments affected *S. sclerotiorum* on beans in the greenhouse, while TH1 was more highly affected by white rot disease than other treatments.

Plant height was different with treatments and did not correlate between survival plants and plant height which (THI) gave high survival plants and high plant height. In contrast, New Acteno gave plant height more than Bio N, although Bio N gave survival plants more than New Acteno, so (TH4) gives the same result as (TH3)

5. Field experiments

5.1 Effect of some biocontrol agents and biocides on the incidence of white mould disease of dry bean under field conditions

The bio-control agent (isolated microorganisms from the healthy bean), which gives the best greenhouse results, was used with

some commercial biocides to manage the white mold disease on dry beans in open field conditions from the growing season 2021. Results in Table (4) recorded that all treatments reduced the disease incidence and severity of dry bean plants treated with inducers compared to untreated plants. *T. harzianum* (TH1) gave the highest value in reducing disease incidence (80%), followed by *B. subtilis,* Blight Stop, Bio Zeid, (TV1), And New Acteno which give survival plants 77,75 %, 76.75%, 75%,74,5%, and 73,88% respectively. However, Rhizo-N resulted in the lowest values even in decreasing disease incidence, which recorded 65.63% survival plants compared with untreated plants (control) 43,25%.

Table (3). Effect of a biocontrol agent and commercial biocides on the disease incidence and plant height under greenhouse and artificial soil infestation.

Biocontrol agent	Pre-emergence damping-off	Post-emergence damping-off	Survival plants (%)	Plant height (cm)
T. harzianum (TH1)	0.00	10.00	90.00	38.50
T. harzianum (TH2)	5.00	15.00	80.00	34.58
T. harzianum (TH3)	10.00	5.00	85.00	36.23
T. harzianum (TH4)	15.00	15.00	70.00	36.45
T. viride (TV1)	5.00	10.00	85.00	36.68
T. viride (TV2)	15.00	10.00	75.00	33.48
T. koningii	10.00	20.00	70.00	32.03
T. hamatum	25.00	10.00	65.00	31.20
B. subtilis (B1)	0.00	20.00	80.00	34.65
B. subtilis (B2)	10.00	25.00	65.00	31.25
P. florescence	20.00	20.00	60.00	30.00
Bio Arc	20.00	15.00	65.00	27.80
Bio Zeid	15.00	10.00	75.00	32.18
Plant Guard	5.00	25.00	70.00	29.35
Blight Stop	15.00	5.00	80.00	32.98
New Acteno	20.00	15.00	65.00	28.08
Root Guard	5.00	20.00	75.00	29.88
Bio-N	15.00	15.00	70.00	27.08
Rhizo-N	25.00	20.00	55.00	26.00
Control (infested soil)	50.00	40.00	10.00	18.70
Control (non-infested soil)	0.00	0.00	100.00	21.10
Mean	13.57	15.48	70.95	30.86
LSD 0.05	18.07	20.22	29.11	1.06

Table (4). Effect of a biocontrol agent and commercial biocide on white mould disease incidence and yield production of Giza 6 cultivar under field and natural soil infestation conditions.

Biocontrol agent	Pre-emergence damping-off	Percentage of infection	Severity of infection	Survival plants (%)	Yield Kg/plot
T. harzianum (TH1)	8.83	20.00	11.25	80.00	5.620
T. viride (TV1)	11.04	25.50	14.13	74.50	5.420
T. koningii	13.00	28.13	16.69	71.88	4.950
T. hamatum	14.50	30.75	19.63	69.25	4.640
B. subtilis (B1)	10.00	22.25	15.56	77.75	5.540
P. fluorescens	16.67	32.38	21.13	67.63	4.580
Bio Arc	20.25	31.50	21.13	68.50	4.495
Bio Zeid	14.75	25.00	14.75	75.00	5.108
Plant Guard	17.00	27.88	17.13	72.13	4.995
Blight Stop	12.25	23.25	12.25	76.75	5.260
New Acteno	15.00	26.13	15.63	73.88	4.745
Root Guard	17.58	29.75	17.38	70.25	4.950
Bio-N	19.75	32.00	21.88	68.00	4.668
Rhizo-N	21.00	34.38	23.88	65.63	4.240
Control	25.33	56.75	45.88	43.25	1.850
Mean	15.80	29.71	19.22	70.29	4.74
LSD 0.05	3.64	4.61	2.96	4.6	0.38

Data in the histogram (Fig 3) indicated that all treated plants were gave yielded more than untreated plants. Results recorded that treated plants by (TH1) gave a higher yield of dry bean seeds, which recorded 5,620 Kg/plot, exactly 1,124 tone/feddan, followed by (B1) 5,54 Kg/plot, exactly 1,108 tone/feddan. Treated plants by TH1, TV1, B1, Bio Zeid, and Blight stop separately were given high yield than ton/feddan, while other treatments gave a yield of approximately between 0,848 to 0,999 ton/feddan. Untreated plants give a low yield by the plot, which gives 1,850 Kg/plot exactly 0.370 ton/feddan.

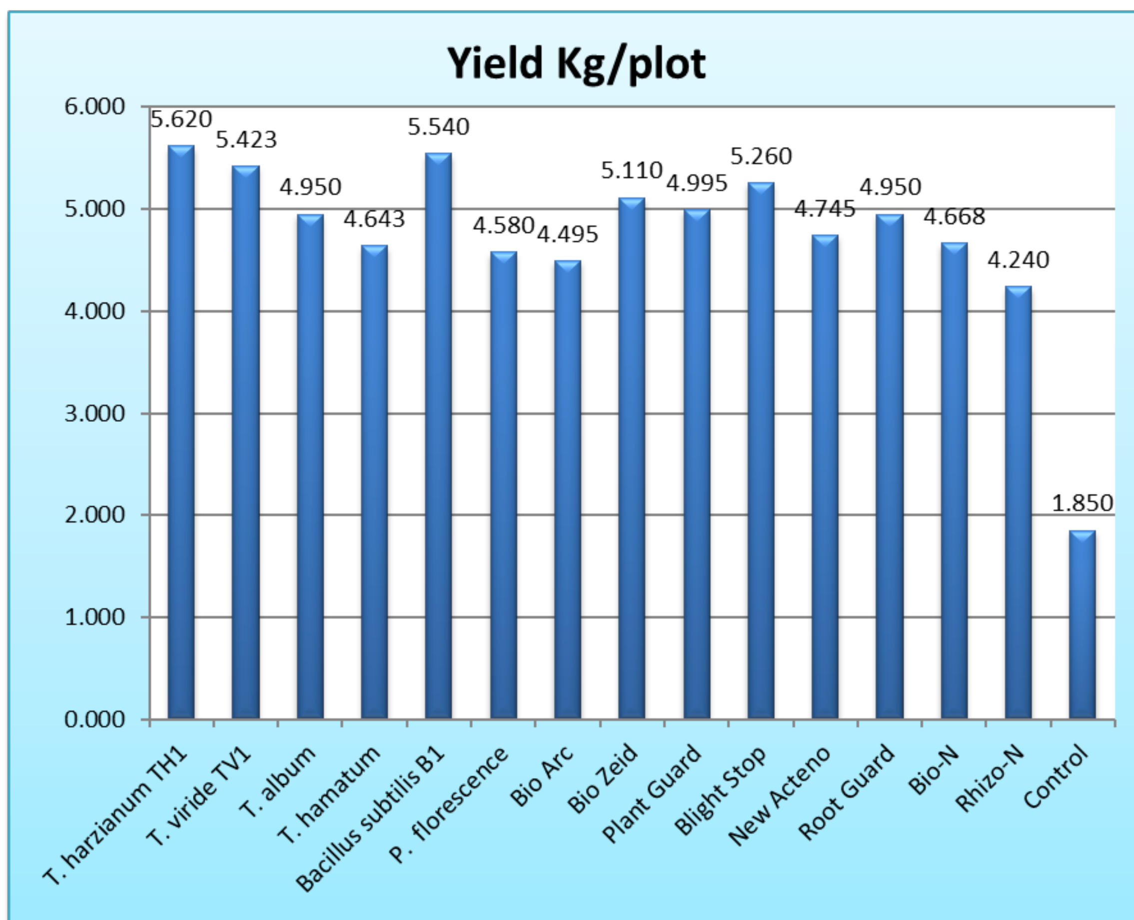

Figure 3: Effect of some biological agents and commercial biocide on yield production of Giza 6 cultivar under naturally infested fields by white mould pathogen.

Discussion

White rot disease caused by *S. sclerotiorum* was difficult to control because it has a long life in the soil as sclerotia and production ascospores, so it has over 600 plant hosts. Many investigators were using different strategies for disease management. Chemical fungicides were used and available in Egypt, but the doses of these fungicides increased from year to year. These compounds were causing environmental pollution and affected biological balance. The residual products threaten human health. In this study, one of the environment-friendly methods was used to decrease the infection by *S. sclerotiorum*. Biological control is one of these methods, it's very important to export crops to other countries and maintain human health.

S. sclerotiorum was isolated from diseased bean plants that grew in many Egyptian governorates, i.e., Al-Menoufia, Alexandria, Al-Qalubeiah, El-Behira, Al-Gharbia, Kafr El-sheikh, Ismailia, and Al-Sharqiya. The isolates were identified and tested for pathogenicity on susceptible bean cultivar Giza 6. Pathogenicity test experiments were conducted under greenhouse and artificial soil infestation conditions using Giza 6 bean cultivar. All tested isolates were pathogenic, but isolate (SMZ11) obtained from Zawyet Razin, Menouf, Menoufia was the most aggressive than other isolates. This variation between these isolates may be due to sexual reproduction, and ascospore formation from apothecia or sclerotia was acclimation to

environmental factors and chemical compounds in the soil, such as fungicides, pesticides, and herbicides. All parts of the plant upper the soil can be infected by ascospores, which makes it more difficult to prevent past the presence of apothecia on sclerotia in the soil or plant steam. Such results make clear that *S. sclerotiorum* is a very dangerous necrotrophic fungus that attacks bean plants and can survive in different soil types. While Pascual, (2010) found that obtained isolates of *S. sclerotiorum* in time work were pathogenic to the Giza 6 bean cultivar with different rates of aggressiveness. Those caused by 70% dead plants or more were considered highly pathogenic. The remaining six isolates were moderately pathogenic, resulting in 65% or fewer dead plants. While (Mohamed and Atallah, 2020) reported that white mould disease caused by *S. sclerotiorum* leads to losses ranging from 30 to 100% of beans in favorable conditions.

S. sclerotiorum (SMZ11) has a larger proportion of dead plants and a higher sclerotia count than others and has been totally identified by PCR. In terms of pathogen characterization, the Egyptian isolate *S. sclerotiorum* shared the findings of PCR amplification (600-bp product), sequencing, and a phylogenetic tree with the other ten isolates. as they revealed that the PCR produced a 564 bp product in the rDNA-ITS region of the fungus *S. sclerotiorum* isolated from Hyacinth Bean (Lablab purpureus). Furthermore, multiple studies have shown that the nuclear ribosomal internal transcribed spacer (ITS) region sequences found between the nuclear small- and large-subunit rRNA genes are useful in species identification (Prova *et al.* 2018).

To study, some eco-friend methods were followed to control this disease instead of chemical fungicides. These methods included biocontrol agents, and biocides were tested under laboratory, greenhouse, and field conditions. The rhizosphere of healthy bean plants in the same fields was used to isolate microorganisms to evaluate here effect on fungal growth and disease control. Microorganisms (fungi and bacteria) were used to determine mycelial growth and sclerotia formation in vitro. All the tested biocontrol agents reduced the mycelial growth and sclerotia formation of *S. sclerotiorum*. *T. harzianum*, *T. viride* and gave the best growth reduction and overgrown the pathogen, while *B. subtilis* has a high effect on mycelial reduction from bacterial isolates under study *in vitro*. These results may be due to one or more metabolic action between the pathogen and antagonistic fungi and bacteria as well as competition for place or food or inhibitory compounds formed by microorganisms. This reason agreement with Dennis and Webster 1971, Elad *et al*. 1982 which reported that the effect of *Trichoderma* spp. on pathogens by cell wall degradation with extracellular enzymes as lytic enzymes (chitinase and 1,3-glucanase) with microparasites includes coiling round and attachment to hyphae, and conidia can penetration the hyphae.

The experiment results in the greenhouse and artificial soil infestation conditions indicate that all treatments reduced the percentages of pre- and post-emergence damping-off caused by *S. sclerotiorum* and significantly increased with the survival plants. Antagonistic microorganisms isolated from healthy beans give highly effect on disease incidence than biocide compounds. *T. harzianum* (TH1) was a highly affected treatment which gave survival plants (90%) followed by (TH3), (TV1), (TH2), and *B. subtilis*, while *P. fluorescens* give the lowest affect on disease control. At the same time (Blight Stop) gives pest control treatment from biocide compounds, which recorded 80% survival plants followed by Bio zeid and root guard. In contrast, Rhizo-N treatment was the least effective in reducing pre- and post-emergence damping-off. Plant height was different with treatments and did not correlation between survival plants and plant height which (THI) gave high survival plants and high plant height, while New Acteno gave plant height more than Bio N although Bio N gave survival plants more than New Acteno, so (TH4) give the same result with (TH3). These results agree with Levy *et al.,* (2004), and Mohamed *et al.,* (2010), which recorded that *Trichoderma* spp. was parasitism to the pathogen and attachment to host hyphae, or micro-conidia was

penetration into the hyphae and producing antibiotics. They also mentioned that the percentage of infected bean plants with *S. sclerotiorum* had been reduced by inoculating seeds before planting with biocontrol agents.

Antagonistic fungi and bacteria with commercial biocide compounds were used in open epidemic field conditions to determine the efficacy of these microorganisms on disease incidence and crop yield of bean. *T. harzianum*, *B. subtilis* and *T. viride* were also the best control for disease reduction and yield production. The yield Kg \ plot, or ton \ feddan. These may be due to some metabolites production such as antibiotics, phyto-hormones, plant growth promoting and enzymes or hyper-parasitism on sclerotia and apothecia or inhibited ascospore formation. Mohamed *et al.,* (2010), Geraldine *et al.,* (2013), and Carvalho *et al.* (2015 reported that complete control of bean white mould using *T. viride* and *Trichoderma* spp. increased the number of pods per plant and yield up to 40%, even under higher disease pressure in 2010. *T. harzianum* Rifai has been explored as an eco-friendly option for controlling soil-borne pathogens and increased growth parameters. They indicated that *T. harzianum* decreased the number of *S. sclerotiorum* apothecia per square meter compared to control in the field, while (Sharma et al., 2012) indicated that *T. harzianum* was found to restrain enzymes of foliar pathogens, the activities of exo and endopolygalacturonase, pectin methyl esterase, pectatelyase, chitinase, and cutinase, which are thought to be involved in mycoparasitism process in leaves infested with fungi. Furthermore, *Trichoderma* spp. Could produce cyanamide hydratase, rhodanese, and ß–cyanoalnine synthases, which are known to play an important function in reducing the growth of plant pathogenic fungi.

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المكافحة الحيوية لمرض العفن الابيض في الفاصوليا الجافة المتسبب عن اسكليروتينيا اسكليروتيورم في مصر

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الملخص العربى

تعتمد هذه الدراسة على الفطر اسكليروتينيا اسكليروتيورم المسبب لمرض العفن الأبيض في الفاصوليا الجافة وكيفية مكافحته في مصر. بعض المضادات الجديدة للفطريات مثل الكائنات الحية الدقيقة والمبيدات الحيوية التجارية تم إستخدامها للحد من آثار المبيدات الكيماوية التي تضر بصحة الإنسان والبيئة. تم إنتخاب ١٢ عزلة من ٣١٥ عزلة للفطر الممرض اسكليروتنا اسكليروتيورم والتي تم عزلهم من نباتات مصابة نامية في عدد من المحافظات المصرية فى موسم شتاء ٢٠١٩م . تم تنقية الفطريات وتعريفها ووضع كود لكل منها. وبإجراء العدوى للفاصوليا صنف جيزة ٦ وُجِد أن العزلة SMZ11 المتحصل عليها هي أشرس العزلات وأقواها في القدرة المرضية. تم دراسة تأثير ١١ عزلة من الكائنات المعزولة من ريزوسفير النباتات السليمة في الحقول الموبوءة بالمرض وكانت كالآتى: عزلات مختلفة من الترايكودرما مع عزلات من البكتريا باسلس ساتلس وسيدوموناس فلورسنس وذلك على النمو الميسيليومى وتكوين الأجسام الحجرية في المعمل. وأثبتت الدراسة أن جميع الكائنات المعزولة قللت من النمو الميسيليومى للفطر الممرض كما قللت من إنتاجيته من الأجسام الحجرية بصورة معنوية. كان الفطر ترايكودرما هارزيانم الأقوى تأثيراً متبوعا بالترايكودرما فيريدى ثم بكتيريا باسيلس بينما البكتيريا سيدوموناس كانت أقلهم تأثيرا على النمو وتكوين الأجسام الحجرية. تم إستخدام نفس الكائنات بالإضافة إلى بعض المبيدات الحيوية لدراسة تأثيرها على الإصابة في الصوبة. وأثبتت الدراسة أن الفطر ترايكودرما هارزيانم كان أفضل المعاملات في مكافحة المرض متبوعا بالترايكودرما فيريدى ثم بكتيريا باسيلس بينما كان المبيد الحيوي (Blight stop) كان الأفضل في مكافحة المرض . وعامة كانت الفطريات المضادة المعزولة أفضل في المكافحة من المركبات التجارية . تم إختيار حقل موبوء بالمرض بقرية زاوية رزين – منوف – المنوفية في موسم ٢٠٢١م وأثبتت الدراسة فاعلية المكافحة بالفطريات والبكتيريا المعزولة من ريزوسفير نباتات الفاصوليا عن المركبات الحيوية في تقليل نسبة الإصابة وزيادة الإنتاجية في المحصول الجاف.