Eco-Friendly Microbial Fungicide of *Bacillus* spp., *Trichoderma album*, and *Saccharomyces cerevisiae* on *Rhizoctonia solani*, the Causal Agent of Potato Black Scurf Disease

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ABSTRACT

Microbial biocontrol agents against *Rhizoctonia solani* (Rh), the causal agent of potato black scurf disease investigated. Endophyte *Bacillus subtilis* (Bac1 and Bac2), the fungal bioagents *Trichoderma album*, and *Saccharomyces cerevisiae* (yeast) were tested for their ability to inhibit the growth of *R. solani* using the dual culture technique. Four isolates of *R. solani* from infected potato tubers were collected from different areas in Alexandria and Behira governorates. All fungal isolates were identified by microscopy and confirmed with distinct morphologies. Molecular identification, DNA isolation, and ITS amplification were carried out on the tested *R. solani* isolates. The pathogenicity test of the *R. solani* isolates approved their ability to inhibit radish seeds germination on the water agar media compared to the control treatment in which the % of seed germination was 100%. Pathogenicity on potato plants also confirmed the isolates' potential. Isolate *R. solani* (Rh1) outperformed the others in terms of pathogenicity, severity on Cara and Spunta cultivars were 87.5% and 81.25%, respectively. As well, the Rh1 isolate exceeds the other isolates in increasing the colored area of potato leaves of the Cara cultivar. Co-culturing *B. subtilis* and *R. solani* isolate on PDA resulted in high inhibition rates for all isolates. *T. album* exhibited inhibition of all *R. solani* isolates tested on PDA at the rate of 2 according to the scale of Bell *et. al.,* 1982. Scanning electron micrographs showed that *T. album* hyphae aggregated, penetrated, and deformed the hyphae of *R. solani*. *S. cerevisiae* was demonstrated to inhibit all isolates of *R. solani* on PDA medium when compared to the control treatment, which had a rate of inhibition of 0.00%. Thus, the current study demonstrated the efficacy of endophytes *B. subtilis*, *T. album*, and *S. cerevisiae* in limiting *R. solani* growth *in vitro*.

Keywords: Eco-Friendly, Biocontrol, Bioagents, *Rhizoctonia solani*, endophytes, *Bacillus subtilis*, *Trichoderma album*, and *Saccharomyces cerevisiae*.

INTRODUCTION

Potato is one of the most important crops grown in Egypt for local consumption, export, and processing. Potatoes are the second most important vegetable after tomatoes, both in terms of cash value and total tonnage produced, due to of its high nutritional value, in addition to its importance as an export crop with an economic return according to the FAO, in Egypt (FAO, 2019).

Rhizoctonia solani, is the most economically important species of the genus *Rhizoctonia* (Sneh, 1996 and Sneh *et al.*, 2013), it causes potato black scurf disease, consequently, affecting the marketing value of the crop. The pathogen affects the plant parts below the soil surface, which is called stem canker. When the tubers are also infected sclerotia appear on the surface of the tuber completely, the distinguishable symptoms of the disease. Infection of tubers with this disease may lead to dry rot when stored (Yao *et al.*, 2002 and Tsror, 2010). Stems with cankers can become girdled, resulting in stunted plants. Leaves of infected plants develop a purplish and chlorotic coloration. In severe infections, green tubers develop above the ground. Affected tubers are deformed and can produce sclerotia on the surface (Tsror, 2010).

Biological control is the natural way to control plant diseases and improve crop yield through attributes that promote growth (Kiewnick and Sikora, 2006). Various researches have shown the importance of *Bacillus*, *Trichoderma* and Yeast for controlling black peel disease in potatoes (Patel and Singh, 2021). *T. harzianum* inhibited *R. solani* mycelial growth by up to 72.72% and *T. viride* inhibited *R. solani* mycelial growth by up to 56.80%. (Hussain *et al.*, 2014; Hussain and Khan, 2018). *T. harzianum* significantly inhibited

R. solani mycelial growth (Prasad and Gupta, 2002). Bacteria from the genus *Pseudomonas* and *Bacillus*, on the other hand, have been used in antagonism (Gasoni *et al.,* 1998 and Altindag *et al.,* 2006). To control fungal plant diseases, *Bacillus* (Sadfi, 2001; Hussain *et al.,* 2021 and Cui *et al.,* 2022) and Paecilomyces have been also used (Kiewnick and Sikora, 2006; Schreiter, 2018). The biological control is carried out by using the same organism or some of the antagonistic compounds produced by the biological agent.

The present study aimed to investigate the biocontrol effect of isolated endophyte B. *subtilis* and commercial product formulation of T. album isolate as well as S. *cerevisiae* potential on potato black scurf from different areas in Alexandria and Behira governorates.

MATERIALS AND METHODS

Samples collection

The potato tubers samples infected with black scurf disease were collected from Alexandria and Behira Governorate, the infected samples were taken to the laboratory after being placed in polyethylene bags and marked, and the samples were kept in the refrigerator until isolation was carried out from each site on the day following sampling.

Isolation of *Rhizoctonia solani* and culture conditions

The black scurf infected Potato tubers were gently washed with tap water for cleaning of suspended soil and cut into small pieces (0.5-1 cm). These tuber pieces were then sterilized with 1% sodium hypochlorite for 2 minutes and washed in sterile distilled water for 2 minutes to remove the effects of sterilization. Subsequently, the sterilized tubers pieces were dried using sterilized filter paper and transferred to 9 cm diameter Petri dishes containing potato dextrose agar (PDA) medium that was autoclaved and added with the antibiotic tetracycline (200 mg /L). After 3 days of incubation at 25±1°C, the fungal colonies were purified on PDA medium using the hyphal tip method. The fungi associated with symptomatic tubers were initially identified based on their morphological features.

The obtained isolates were kept in test tubes containing autoclaved PDA medium, the tubes were placed at an angle until solidification, and then inoculated by adding a 0.5 cm diameter disc taken from near the edges of the colonies of 5-day-old *R. solani* isolates. The tubes were placed in the incubator at a temperature of 25±1°C for 7 days, after which they were placed in the refrigerator at a temperature of 4 °C.

Identification of *R. solani* isolates

A- Morphological identification

The obtained fungal isolates were diagnosed based on the phenotypic characteristics of the colonies and microscopic examination after culturing them on PDA medium and using the taxonomic keys (Parmeter and Whitney, 1970; Moni *et al.,* 2016). The purified fungal isolates were kept in the refrigerator at 4°C.

B- Molecular identification

DNA isolation and ITS amplification of the *R. solani* isolats

R. solani genomic DNA was extracted using a rapid mini preparation procedure (Caligiorne *et al.,* 1999). Fungal isolates were grown for 5–10 days depending on the growth rate of the fungus on Potato Dextrose Agar (PDA) plates. 1 ml of lysis buffer (50 mM Tris-HCl (pH 7.5), 50 mM EDTA and 3% SDS) was added to the plate and the mycelium was scraped with a spatula. 500 μL of buffer mixed with mycelium was recovered in a microtube and mixed using a vortex shaker. The microtubes were incubated at 65°C for 10 min and centrifuged at 16000xg for 10 min at 4°C. The supernatants were transferred to new microtubes and DNA was precipitated by adding 0.5 volume of 3M sodium acetate and one volume of ice-cold isopropanol. Microtubes were gently inverted three times and centrifuged at 16000xg for 5 min at 4 °C. The supernatant was discarded, and the pellet was rinsed with 300 μL of 70% ethanol. After centrifugation at 16000xg for 5 min at 4°C, the ethanol was discarded. The DNA pellet was air-dried, dissolved in 100 μL of TE buffer (10 mM Tris-HCl, pH 8.0 and 1 mM EDTA) and stored at -20 °C until use (White *et al.,*1990).

Molecular identification through the Internal Transcribed Spacer (ITS) region of fungal cultures was carried out based on conserved ribosomal internal transcribed spacer (ITS) region (Moore *et al.,* 2011). It amplified the ITS regions between the small nuclear 18S rDNA and large nuclear 28S rDNA, including 5.8S rDNA using universal primer pairs ITS1 and ITS4 (Table 1). The PCR amplification was carried out in a total volume of 25 μL containing 3 μL of template DNA, 12.5 μL PCR Green Master Mix (Thermo Scientific™), 0.5 μL of forward and reverse primers (10 pmol) and 8.5 μL molecular grade water. The amplification cycle consists of an initial denaturation at 95°C for 1 min followed by 35 cycles at 94°C for 30s, 55°C for 2 min, and 72°C for 1 min and a final extension at 72°C for 10 min. Amplified PCR products were electrophoresed on 1.5% agarose gel, in 1X TAE buffer at 200 Volt for 15 min.

Table 1. Nucleotide sequence of primers ITS1 and ITS4

Primer	Nucleotide sequence, 5` to 3`
ITS1	TCCGTAGGTGAACCTGCGG
ITS4	TCCTCCGCTTATTGA TATGC

Sequencing of the amplified ITS region

The amplified fragment of ITS1-5.8s and ITS2 region (500-700 bp) of one selected isolate was sent for sequencing (lab Technology, Scientific Services Company). Identification of isolates were confirmed by applying Basic Local Alignment Search Tool (BLAST search) location on National Center for Biotechnology information (NCBI) site (http://www.ncbi.nlm.nih.gov) using the obtained sequences of the amplified regions.

Pathogenicity assessment

Pathogenicity assay using local radish seed

The pathogenicity of *R. solani* fungus was evaluated by plate method (Christensen *et al.,* 1988) that is briefly including petri dishes 9 cm diameter containing 15-20 ml of water agar medium. This water agar medium was prepared by mixing 20 g of agar in a liter of distilled water and autoclaved for 15-minute. After solidification of media, the center of dishes was inoculated with 0.5 cm disc in diameter that was picked from recently geveloped hyphae close to edges of the pure 5 days old colonies of the fungus. Control was represented as non inoculated media plates. All inoculated and non-inoculated dishes were incubated at 25 ± 1 °C for three days. Seeds of local radish were sterilized with 1% sodium hypochlorite for two minutes and washed with distilled water for three times and then placed circularly near the edge of the inoculated and non-inoculated dishes with 25 seed/plate. Four dishes were used for each fungal isolate in addition to comparison treatment. The dishes were incubated at 25±1°C. The percentage of germination and percentage inhibition according to the following equation:

$$\text{The percentage of germination} = \frac{\text{number of germinated seeds}}{\text{total number of seeds}} \times 100$$

$$\% \text{ of inhibition} = \frac{\text{number of germinated seeds in the control treatment - number of germinated seeds per treatment}}{\text{number of germinated seeds in the control treatment}}$$

Pthogenicity of the *R. solani* isolates on potato plants

To perform this test, the fungus isolates were cultured on local seeds of common millet (*Panicum miliaceum*), 50 g of seeds were placed in a 250 cm³ beaker 150 cm³ of water and soaked for 6 hours, the excess water was dicarded and sterilized with an autoclave for an hour. The seeds were inoculated with five discs with a diameter of 5 mm of fungus growing on PDA media (Dewan and Sivasithamparam, 1989). The above-prepared inoculum was added to the soil at a rate of 1% (weight/weight), each treatment was repeated

four times with the control treatment (sterile millet not inoculated with fungus). Potato piece tubers were planted three days after the fungal inoculation to in 2 piece tubers per pot, watered carefully and closed with perforated polyethylene bags and placed in the greenhouse at a temperature of 20-27 °C. After three days, the bags were lifted, and after 45 days of cultivation, cankered stems areas were obsereved and disease severity perecentage estimated by following the severity scale:

0- healthy plant
1-One spot on stem with a diameter of less than 25 mm
2- One spot on stem with a diameter within 26-50 mm
3- Presence of spots within 51-75 mm in diameter
4-The presence of spots more than 75 mm in diameter that completely surround the stem

The percentage of severity of damage was calculated according to the formula (McKinney, 1923):

$$\% \text{ of disease severity} = \frac{(\text{number of plants in degree } 0 \times 0) + (\text{number of plants in degree } 4 \times 4)}{\text{number of plants studied x 4}} \times 100$$

Pathogenicity of *R. solani* isolates on potato leaflets

The six obtained *R. solani* isolates were grown on tap water agar plates for three days at 22°C. Potato cultivar Cara leaflets were soaked in tap water for 6 hr, rinsed in sterile water and placed singly with the lower epidermis facing the culture of each isolate, then incubated for a further three days on the bench at about 16°C in diffuse day ligth. The virulence of each isolate was expressed as the percentage of discolored (invaded) leaflet tissue. The discoloration was measured using the computer software, Leaf area measurement, version 1.3, England, Sheffield University (Spencer and Fox, 1978).

The anatagonistic assay

Potato plants endophytic bacteria isolation and characterization

The endophytic bacteria were isolated from the internal tissues of tuber and stems of healthy potato plants. Potato tissues were washed with tap water to remove associated dust, tissues were cut into small segments then surface sterilized by immersing in 70% ethanol for 5 min and a solution of 5% sodium hypochloride for 10 min. The samples were rinsed three times in sterile distilled water to remove surface sterilizing agents and dried with sterilized filter paper. Five segments were placed on PDA petri dishes and the plates were incubated at 27°C±1 for tow days. To obtain pure colony, a single colony of the culture was transferred onto Nutrient Sucrose Agar (NSA) medium (peptone 5.0g, beef extract 3.0g, sucrose 5.0g, agar 15g, and distilled water 1000ml) using the streak plate technique (Singh *et al.*, 2010). The microscopic

characteristics of bacteria (Bac1, Bac2) were determined after staining with gram stain (Black, 1965).

Bacterial inoculum preparation

Bacterial endophytes were grown in 250 mL glass flasks with 100 mL Nutrient Broth (NB) medium. The culture media were individually inoculated with the bacterial inoculum, which was taken from the bacterial cultures at the age of one day and incubated at $28\pm1°$C for 1-2 days. Before inoculation, the numerical density of bacteria was estimated.

In vitro inhibitory effects of *Bacillus subtilis* on *R. solani*

Two full loops of bacterial suspension were streaked as two lines parallel to each other on nutrient agar medium for each bacterial strain and incubated for 24 hours at 28°C. The *R. solani* disc was placed in the middle of the two bacterial growth lines. For each *R. solani* isolate, five replicates were performed. The inoculated Petri dishes were incubated at 28°C for 5 days, and the fungal growth was monitored daily. Growth measurements were taken. In the streaking test, the suppressive effect of the bacteria on fungal growth was visually assessed by comparing the plates of each treatment with their control (without bacteria) (Matar *et al.*, 2009).

In vitro inhibitory effcets of *Trichoderma album* on *R. solani*

Dual culturing technique was applied to assess the antagonistic ability of *T. album* (Table 2) using PDA petri dishes taking a disk 0.5 in diameter cm from near the edges of the colony of the fungus *R. solani* placed in the center of one half of the dish while the center of the another half of dish was inoculated with 0.5 cm disc taken from near the edges of the colony of the biological agent *T.album*. The control treatment contained *R. solani* co-cultured with empty disc. Four dishes were used for each treatment that were placed in the incubator under $25\pm2°$C until the comparison fungal growth reached to the edge of the dish.

The antagonism of bioagent was estimated according to the five-standard criteria of (Bell *et al.,* 1982) as follow:

1- indicates the growth of biological agent covers the entire area of the dish without allowing *R. solani* fungus to grow

2- indicates the growth of biological agent covers two-thirds of the area of the dish and the growth of *R. solani* fungus covers the remaining one-third.

3- indicates the growth of biological agent covers half of the dish and the growth of *R. solani* fungus covering the other half with no buffer zone between the colonies.

4- the growth of biological agent covers one-thirds of the area of the dish and the growth of *R. solani* fungus covers the remaining two-thirds and

5- indicates non-growth of biological agent and the growth of *R. solani* covers the entire area of the dish.

The effective biological agent is considered when its antagonism is class 2 or less with the pathogenic fungus *R. solani*.

Interaction zones in dual cultures by Scanning Electron Microscope

A scanning electron microscope (SEM) was used to study the interaction zone between the *R.solani* hyphae and the biocontrol agents *T. album* using dual culture, according to (Nofal *et al.*, 2021). At the Electron Microscope Unit, Faculty of Science, Alexandria University, Alexandria, Egypt, samples were examined for mycoparasitism and photographed at different magnifications using the JSM-IT200 SEM series (JEOL Ltd., Tokyo, Japan).

In vitro inhibitory effects of *Saccharomyces cerevisiae* (yeast) on *R. solani*

R. Solani was cultured on autoclaved PDA Petri plates amended with baking yeast *S. cerevisiae* (Turkish origin). *S. cerevisiae* was added to the medium at a concentration of 10 g/liter. Four replicates were used for each treatment, the plates were inoculated with a disk with a diameter of 5 mm of *R. solani* and incubated Check the at a $25\pm2°$C (Saleh *et al.*, 2009). The results were recorded by calculating the average measurement of two perpendicular diameters from each colony after the growth in the comparison treatment reached the edge of the plate. The percentage of inhibition was calculated in the equation:

% of inhibition= $\dfrac{\text{The growth rate of the colony in control -The growth rate of the colony in the treatment}}{\text{The growth rate of the colony in control}} \times 100$

Table 2. *Trichoderma album* **isolate used in the study**

Commercial name and formulation	Common name (Active ingredients)	Recommended rate	Source (manufacture)
Bio Zeid®2.5% WP (Powder)	*Trichoderma album*	$25*10^6$ spores/g at 250 g/100 l.	Kafr-El-Zayat Organic Biotechnology

Statistical analysis

All data were statistically analyzed using analysis of variance (ANOVA) and differences among the means were determined for significance at $p \leq 0.05$ using revised LSD test using the statically system SAS (SAS Institute, 1988).

RESULTS AND DISCUSSION

Characteristics of *R. solani* isolates

The isolated fungus was identified based on its morphological characteristics such as the asexual phenotypic features, represented by the formation of the fungal mycelium divided by perforated septa, the color initiate as white and turns from light brown to dark in its later stages of growth. Branching and the sclerotia formation, begin to appear after a week of incubation and are in the form of irregular, cohesive and solid blocks, brown to black in color, with a length of 3-5 mm (Figure 1) (Ajayi-Oyetunde and Bradley, 2018). These results were consistent with many studies on isolating the fungus R. solani from infected potato tubers, considering this fungus is the main cause of black scurf disease around the world (Al-Abedy *et al.,* 2018; Malik *et al.,* 2014 and Siddique *et al.,* 2020). The fungal isolates were listed in Table (3), codded as Rh1, Rh2, Rh3, and Rh6 associated with the geographic origin of potato culture samples (Table 3 and Figure 1).

Molecular Identification

R. solani, the isolates molecular identification was carried out using ITS4 and ITS1 primers, where a 629 bp PCR product was amplified and electrophoresed (Fig. 2). The amplicon was sequenced, blasted, and deposited in the GenBank database at the National Center for Biotechnology Information (NCBI) with accession number ON514186 for Rh1, OP787992 for Rh2, ON514185 for Rh3, OP787993 for Rh6. The BLAST analysis showed >98–87% identity with several known sequences from *R. solani* species.

Table 3. Sources of black scurf disease isolates from naturally diseased potato tubers

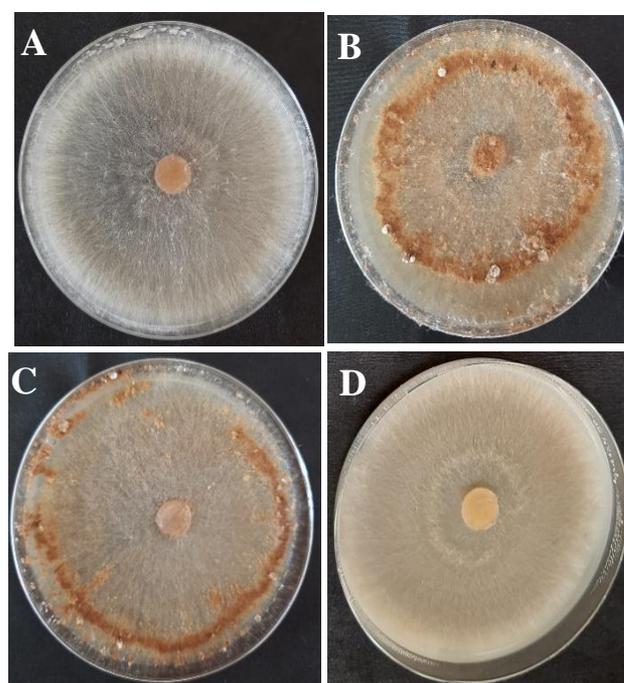| Isolates code	Locality	cultivars
Rh1	Alexandria desert way	Diamant
Rh2	Alexandria desert way	Spunta
Rh3	Behira	Cara
Rh6	Alexandria's wholesale market	Cara

Fig.1. Morphological characteristics of *Rhizoctonia solani* (Rh) isolates, A: Rh1 isolate, B: Rh3 isolate, C: Rh6 isolate, D: Rh2 isolate.
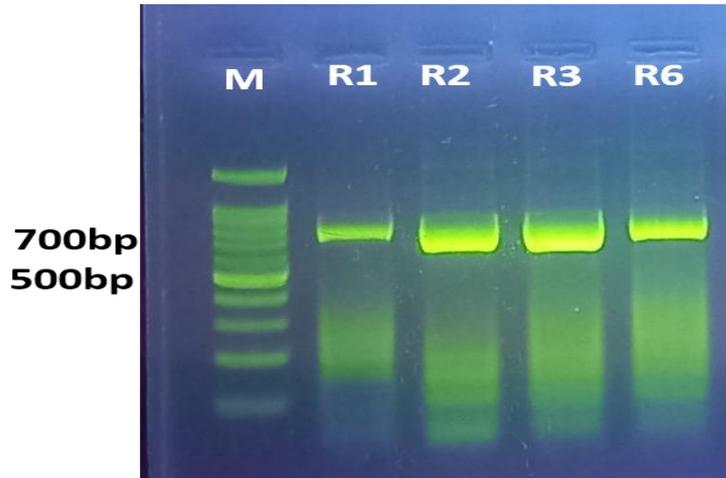
Figure 2. PCR product (629) banding for *Rhizoctonia solani* with ITS1 and ITS4 primers. Marker (M) at the left side of the gel is loaded with a 1500 bp DNA ladder

Pathogenicity of *R. solani* isolates using radish seeds

   R. solani isolates showed a significant reduction in germination percentage of radish seeds compared to control treatment in which the percentage of seed germination was 100.00% (Table 4 and figure 3). The isolate (Rh1) outperformed in reducing the percentage of germination than the rest of the isolates, as the average percentage of germination was 9%, while the percentage of germination of the rest of the isolates ranged between 12-32% . On paralell, the inhibition percentage of isolate Rh1 was the highest (91%) figure 2, for the rest of the isolates, the percentage of inhibition

reached 68-88% compared to the control treatment, in which the percentage of inhibition of seeds was 00.00%.

   The reason for the high pathogenicity of this fungus is due to its parasitic nature of the type necrotroph, which infects the tissues of the host by secreting decomposing enzymes such as Chitinase, Cellulase, Protease and Pectinase, which enables it to break down cell walls and penetrate into the various plant tissues, in addition to releasing some toxins such as oxalic, which kills cells and extracts nutrients from them (Wilson *et al.,* 2008; Ajayi-Oyetunde and Bradley, 2018).

Table 4. Pathogenicity test of *Rhizoctonia solani* (Rh) isolates on radish seeds

Isolate	Germination % of radish seeds	* Inhibition %
Rh1	9% e	91% a
Rh2	12% d	88% b
Rh3	32% b	68% d
Rh6	25% c	75% c
Control	100% a	0.0% e

* Data are mean of four replicates, data with the same letter, in column, are not significantly different at *P*≤0.05.
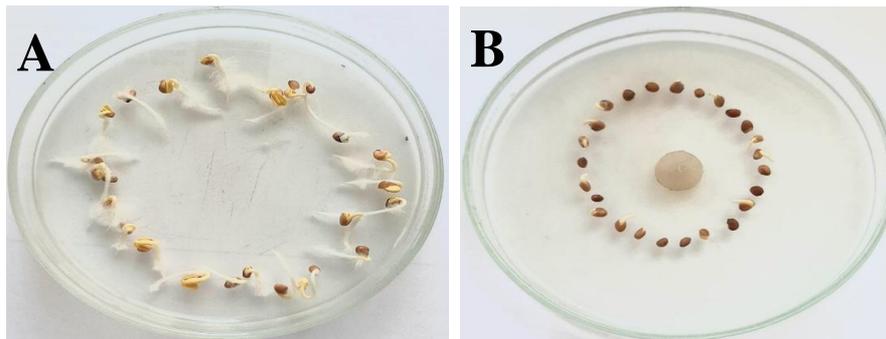

Figure 3. Pathogenicity test of the fungus *Rhizoctonia solani* (Rh) on radish seeds, A: control, B: radish seed + Rh1

Disease severity on potato plants

    R. solani isolates exhibited significant disease severity compared to control, Rh1 had the highest rate of infection severity with 87.5% for the Cara cultivar and 81.25% for the Spunta cultivar, followed by the Rh2 isolate, whose percentage of infection was 81.25 % for the Cara variety and 75% for the Spunta cultivar, as for the other isolates, the percentage of infection severity varied (Table 5 and Figure 4). These results are consistent with the results of detecting the pathogenicity of isolates of the fungus *R. solani* on radish seeds, which proved that isolates of fungus *R. solani* differ in

their pathogenicity to their families (Abdo *et al.,* 2012 and El-Shewy, 2019).

Potato detached leaflets coloration after inoculation

    The pathogenicity of *R. solani* isolates was tested on the leaves of the potato plant Cara cultivar. The results showed in (Table 6) indicate that isolate Rh1 was superior to the rest of the isolates in the coloration of the leaflets as the percentage of infected area was 37.89 followed by isolate Rh2 (31.31), Rh6 was 30.53, and finally isolate Rh3, which achieved a leaf infection percentage of 18.46.

Table 5. Effect of the fungus *Rhizoctonia solani* (Rh) on potato tubers of Cara and Spunta cultivar

Isolate	* the severity% Cara cultivar	*the severity% Spunta cultivar	Mean of isolate
Rh1	87.50% a	81.25% b	84.37% a
Rh2	81.25% b	75% c	78.12% b
Rh3	56.25% f	50% g	53.12% d
Rh6	68.75% d	62.50% e	65.62% c
Control	0.0%	0.0%	0.0% e

✱ Data are the mean of three replicates, data with the same letter, in column, are not significantly different at *P*≤0.05

Figure 4. Pathogenicity and disease syndrome of *Rhizoctonia solani* on potato plants, (A) Cankers on subterranean stem and stolons, (B) Developed canker on stem and sclerotia formation initiation on small baby tubers, (C) Sclerotia developed on potato tubers.

Table 6. Pathogenicity test of *Rhizoctonia solani* (Rh) isolates on leaf of the potato plant Cara cultivar

Isolate	coloration ratio ✱
Rh1	37.89 a
Rh2	31.31 b
Rh3	18.46 c
Rh6	30.53 b
Control	00.00 d

*Data are mean of four replicates, data with the same letter, in column, are not significantly different at *P*≤0.05.

Biological control of *Rhizoctonia solani* causing potato black scurf

In vitro* antagonism of *B. subtilis* on *R. solani

The results of Table (7) and Figure (5) indicated that the use of *B. subtilis* led to the inhibition of the growth of all isolates of *R. solani* on PSA culture media, and the bacteria also achieved a high rate of inhibition for all tested fungi isolates compared to the comparison treatment of pathogenic fungi only, in which the inhibition rate was 0.00%. These results are in agreement with the results of other studies that demonstrated the ability of bacteria to inhibit the growth of the *R. solani* on culture media (Elkahoui *et al.,* 2012 and Montealegre *et al.,* 2003).

***Trichoderma album* antagonistic effects on *R. solani* cultured on PDA medium**

The bioagent *T. album* showed high potentiality in limiting *R. solani* growth on PDA medium (Figure 6). The antagonistic ability of *T. album* represented by occupying two third of the culturing medium where *R. solani* colonized the remaining area, as a result the bioagent could be ranked (2) according to the Bell scale (Bell *et al.,* 1982).

The Scanning Electron Micrograph appears that the *T. album* has an anti-bioagent effect on *R. solani* (Rh1) (Fig 7). The electron micrograph revealed the hyphae of *T. album* grew abundantly, besides being intertwined with the *R. solani* hyphae. Micrographs exhibited that the hyphae of *T. album* combine, penetrate, and deform *R. solani* hyphae. The reason for *T. album's* high antagonism against pathogens is due to the direct parasitism of pathogenic fungi on the mycelium of pathogenic fungi. As well as wrapping around the pathogen and decomposition of cell walls by chitinase and B-1,3 glucanase enzymes. In addition to *T. album* having a competitive ability for food and the place (Mahmoud, 2017).

The inhibitory effects of *Saccharomyces cerevisiae* (yeast) on the growth of pathogenic fungus *R. solani* cultured on PDA

The additive *S. cerevisiae* (yeast) (Table 8 and Figure 8) to the growth medium at a concentration of 10 g/liter of PDA showed inhibition of all *R. solani* isolates compared to control. This could be as result of parasitism; in addition to its high competition for food and place in agreement with Matny and Eisa (2013); Raspor *et al.* (2010).

Table 7. The antagonistic inhibitory effect of *Bacillus subtilis* (Bac1, Bac2) on the pathogenic *Rhizoctonia solani* (Rh) isolates cultured on PDA medium.

Treatment	*R. solani* radial growth (cm)		*Inhibition ratio%	
	B. subtilis 1	*B. subtilis* 2	*B. subtilis* 1	*B. subtilis* 2
Rh1	3.21 b	3.11 b	64.33% d	65.44% d
Rh2	2.92 c	2.81 c	67.55% c	68.77% c
Rh3	2.17 e	2.20 d	75.88% a	75.55% a
Rh6	2.73 d	2.69 d	69.66% b	70.11% b
Control	9.0 a	9.0 a	0.0 e	0.0 e
LSD$_{0.05}$	0.01	0.01	0.38	0.18

*Data are mean of four replicates, data with the same letter, in column, are not significantly different at $P \leq 0.05$.

Control (Rh1)   Rh1+*B. subtilis1*   Rh1+*B. subtilis2*

Figure 5. Anti-bacterial effects of *Bacillus subtilis* strains on *Rhizoctonia solani* (Rh1)

Control (Rh1)   **Rh1+*T. album***

Figure 6. Parasitism of *Trichoderma album* on *Rhizoctonia solani* (Rh1) (right), Control *R. solani* only (left).

Fig. 7. The Scannig Electron Micrograph of *Trichoderma album* towards *Rhizoctonia solani (Rh1)*.

R. solani hyphae (➡), *T. album* hyphae (➡)

T. album hyphae attach on the *R. solani* hyphae (➡),

T. album hyphae penetrate the *R. solani* hyphae (➡),

The deformation of R. solani hyphae caused by T. album activity (➡)

Table 8. The radial growth (cm) and inhibition % of *Rhizoctonia solani* (Rh) isolates co-cultured with *Saccharomyces cerevisiae* (yeast) on PDA

Treatment	*****R. solani* radial growth (cm)**	*****Inhibition ratio%**
Rh1	3.02 b	66.44% d
Rh2	2.88 c	68.00% c
Rh3	2.11 e	76.55% a
Rh6	2.67 d	70.33% d
Control	9.0 a	0.0% e
LSD$_{0.05}$	0.01	0.22

*****Data are mean of four replicates, data with the same letter, in column, are not significantly different at *P*≤0.05.
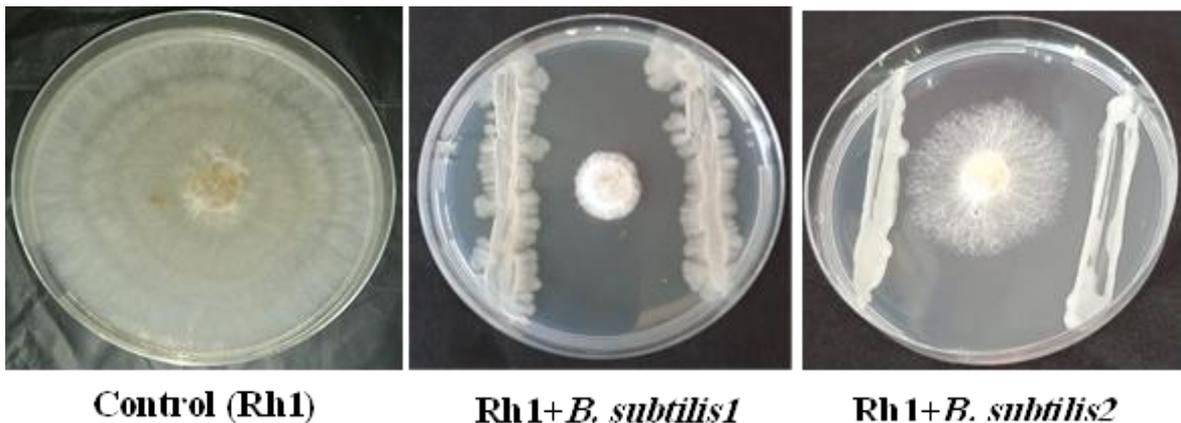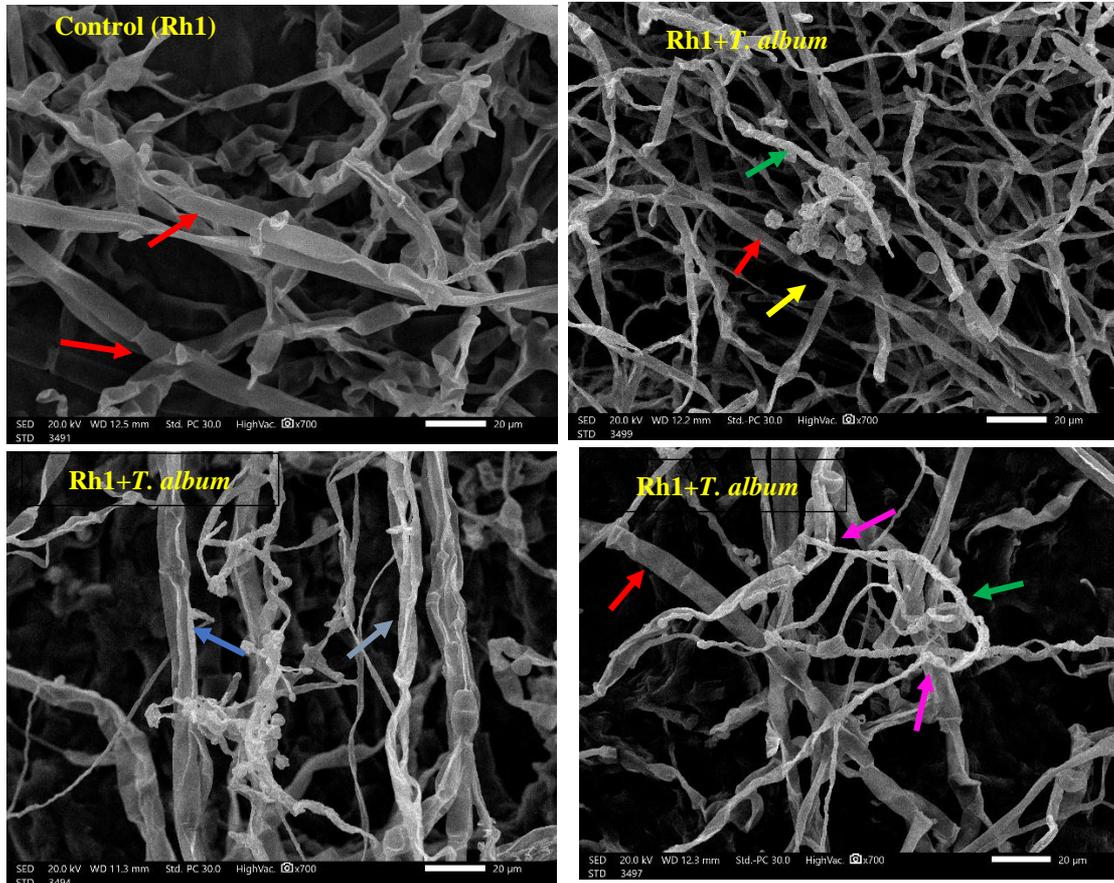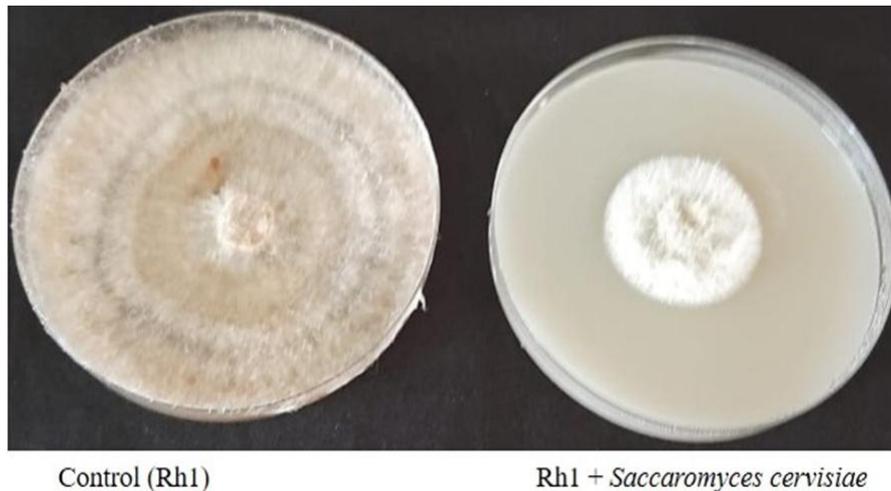

Control (Rh1)   Rh1 + *Saccaromyces cervisiae*

Figure 8. Effect of *Saccharomyces cerevisiae* (yeast) on the *Rhizoctonia solani* (Rh1) radial growth.

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الملخص العربي

المبيدات الميكروبية الصديقة للبيئة من .Bacillus spp ، *Trichoderma album*، *Saccharomyces cerevisiae* على فطر *Rhizoctonia solani* المسبب لمرض القشرة السوداء بالبطاطس

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تم دراسة بعض اجناس المكافحة الحيوية الميكروبية ضد فطر (Rh) *Rhizoctonia solani*، العـامل المسبب لمرض القشرة السوداء بالبطاطس. حيث تم اختبار سلالتين من البكتيرة (Bac1, Bac2) *Bacillus subtilis*، وفطرين المكافحة الحيويـة *Trichoderma album*، و *Saccharomyces cerevisiae* لقدرتها على تثبيط نمو الفطر *R. solani*، باستخدام تقنية الزراعة المزدوجة. جُمعت أربع عزلات من *R. solani* من درنات البطاطس المصابة من مناطق مختلفة بمحافظتي الإسكندرية والبحيرة. تم تعريف جميع العزلات الفطرية عن طريق الفحص المجهري. تم اجراء الاختبارات التعريفية الجزيئية عن طريق عزل الـ DNA وتضاعف منطقة ITS لعزلات *R. solani* المختبرة. أثبت اختبار الإمراضية للعزلات قدرتها على تثبيط إنبات بذور الفجل على وسط أجار الماء مقارنة بمعاملة المقارنة حيث كانت نسبة إنبات البذور ١٠٠٪. كما أكدت الإمراضية على نباتات البطاطس إمكانات العزلات. تفوقت العزلة (Rh1) *R. solani* على الآخرين من حيث الإمراضية، حيث أصابت الصنفين

Cara وSpunta بنسـبة ٨٧,٥٪ و ٨١,٢٥٪ علـى التـوالي. كـذلك، تفوقت عزلـة Rh1 على العزلات الأخرى في زيـادة المسـاحة المصـابة لأوراق البطـاطس للصـنف Cara. أدى الاستزراع المشترك لعزلات *B. subtilis* و *R. solani* على PDA إلى معـدلات تثبيط عاليـة لجميع العزلات الفطريـة الممرضة. أظهرالفطر *T. album* قُدرته على تثبيط جميع عزلات *R. solani* المختبرة علـى PDA بمعـدل ٢ وفقًـا لمقيـاس Bell. أظهـرت الصـور الالكتروميكروسكوبية أن خيوط التريكوديرما *T. album* تتجمع وتخترق وتشوه خيوط الفطر الممرض *R. solani*. ثَم إثبات قدرة الخميرة *S. cerevisiae* على تثبيط جميـع عزلات الفطر *R. solani* على وسط PDA عند مقارنتها بمعاملة المقارنة، والتـي كـان معـدل تثبيطها ٠,٠٠٪. وعلـى ذلـك، أوضحت الدراسـة الحاليـة فعاليـة كـل مـن ميكروبـات الـ endophytes المختبرة، *B. subtilis*، *T. album*، والخميرة في الحد من نمو *R. solani* المسـبب المرضـي للقشرة السـوداء بالبطـاطس معمليا.