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ORIGINAL PAPER

Efficiency of Some Bio-control Agents and Plant Extracts Against Beans (*Phaseolus vulgaris* L.) Damping-off and Root Rot Diseases under Greenhouse and Field Conditions

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ABSTRACT

Damping-off and root-rot diseases in bean caused by *Rhizoctonia solani* Kuhn are considered the most destructive diseases. *Rhizoctonia solani* isolates were pathogenic and varied in their virulence on bean plants (Nebraska cv.) *in vivo*. The isolate No. 7 of *R. solani* obtained from Sakha was the most aggressive one, which gave the highest percentages of pre- and post-emergence damping-off (66.7 and 26.7%), respectively. All Bio-agents *viz. Trichoderma harzianum*, *T. aureoviride*, *Bacillus subtilis* and *B. amyloliquefaciens*, plant extracts of garlic, onion and neem, biocides *viz.* Bio-Arc and Bio-Zeid in addition to chemical fungicide (Rizolex-T as positive control) were evaluated against bean damping-off and root-rot diseases *in vitro* and *in vivo*. All tested antagonistic fungal and bacterial bioagents and plant extracts at different concentrations significantly reduced the linear growth of *R. solani in vitro* and decreased the incidence of *R. solani* damping-off and root-rot diseases under greenhouse and field conditions. *Trichoderma harzianum*, *T. aureoviride* and *B. subtilis* were the most effective bio-agent, while garlic and onion at 5 and 7% concentration were the most effective plant extracts against damping-off and root-rot diseases meanwhile, *B. amyloliquefaciens* and neem extract were the lowest effective treatments compared to the control treatment. Under field conditions during the 2019 and 2020 growing seasons, all tested treatments significantly reduced damping-off, root-rot diseases and increased the percentage of survived plants as well as improved bean plant growth parameters (plant height, number of branches/plant), yield and yield components (No. of pods/plant, weight of pods/plant and 100 seed weight) compared to the control treatment. Also, the effects of antagonistic bioagents, plant extracts at 5%, biocides and chemical fungicide on the activity of lytic enzymes (β-1,3-glucanase and protease) and oxidative enzymes (peroxidase and polyphenoloxidase) were determined in the leaves of bean plant.

Key words: Bean, *Phaseolus vulgaris*, *R. solani*, damping-off, root-rot, bio-agents, plant extracts, defense-related enzymes.

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INTRODUCTION

Legumes are considered the main source of protein and oil in many African countries (Abd El Moity and Hanna, 1994). Bean (*Phaseolus vulgaris* L.) is considered the most important and economic legume crop grown in Egypt for local consumption, export, and its beneficial effects in improving soil fertility. Bean plays an important role in human nutrition as a cheap source of proteins, minerals, vitamins, and amino acids. The total cultivated area of bean in Egypt during season 2021 reached about 36719 feddan in the open field, which yielded about 39437 ton of seeds, respectively (FAOSTAT, 2021).

The bean crop is exposed under greenhouse and field conditions to infection with several root and foliar diseases at all stages of plant growth, causing a considerable reduction of either number of bean plants or yield (Harveson *et al.*, 2005; Abd El-Moneim *et al*., 2012 and Sarhan *et al*., 2018). Damping-off and root rot diseases are considered as a serious and persistent problem of bean plants during growing season (Broughton *et al*., 2003; Anonymous**,** 2005 and Abdellatif *et al.*, 2015). Rhizoctonia root rot is a common disease throughout the world. It is one of the most economically important root diseases of beans (Beebe and Pastor-Corrales, 1991). *Rhizoctonia solani* has a broad host range that includes most annual and many perennial plants and generally survives between crops as sclerotia or as fungal mycelia in the soil. Infection with root rot diseases reduces seed germination and seedling emergence and affects the bean yield and its components (Pedroza, 1994; Valentin, 2010; Yaquelyn *et al*., 2010 and Sarhan *et al*., 2018).

Synthetic fungicides affect the environment and human health due to their highly toxic

effect, leading to a great disturbance in biological balance and toxic substances in the food chain. Application of the fungicides is not economical for a long time due to polluting the environment, leaving harmful residues and leading to the development of resistant strains of the pathogen with repeated use (Vinale *et al*., 2008). Therefore, alternative control methods are needed for controlling soil-borne pathogens, including inducing resistance by using biotic and abiotic inducers, which produce a wide variety of antifungal compounds. These compounds play a major role in controlling several soil-borne fungal diseases and reducing the use of chemical fungicides in a system of integrated plant disease management (Barakat and Al-Masri, 2005 Abdellatif *et al.*, 2015 and Sarhan *et al*., 2018).

Trichoderma spp. were well documented as effective biological control agents for plant diseases caused by soil-borne fungi (Harman *et al*., 2004 and Khalifa, 2016). Application of biological and plant extracts as biotic and abiotic inducers stimulate some defense mechanisms against root-rot/wilt diseases such as increasing phenolic compounds, oxidative enzymes (peroxidase and polyphenoloxidase) and lytic enzymes (protease and β-1,3glucanase) which play an important role in plant defense mechanisms against pathogens infection (Anand and Kulothungan, 2010 and El-Mohamedy *et al*., 2013).

This work aimed to evaluate the efficacy of some different bioagents, plant extracts and biocides for controlling damping-off and root-rot diseases of bean under greenhouse and field conditions and their effect on enzymes activity.

MATERIALS AND METHODS

Plant material:

Bean seeds (*Phaseolus vulgaris* L.) Nebraska cv. were obtained from Vegetable Crops Res. Depart., Horticulture Research Institute, Agricultural Research Center, Giza, Egypt.

Isolation of fungi associated with bean root-rot:

Samples of root-rotted bean plants were collected from various locations at different governorates in Egypt. The infected bean roots were firstly washed under tap water, air dried and sterilized by immersing in 2% sodium hypochlorite for 2 minutes (Sinclair and Dhingra, 2019) and subsequently rinsed with sterile distilled water and dried between two sterilized filter papers. The sterilized fragments were placed on potato dextrose agar (PDA) medium in Petri dishes and incubated at 26±2°C, for five days. Each of the emerged fungi was picked up and purified using the hyphal tip technique (Brown, 1924) and identified according to their cultural and morphological features using the descriptions of Barnett and Hunter (1987) and Sneh *et al*. (1991) at the Unit of Identification of Microorganisms, Plant Pathology Res. Institute., Agricultural Research Center, Giza, Egypt.

Isolation and identification of the antagonistic microorganisms:

Different fungal and bacterial antagonistic microorganisms *i.e., Trichoderma harzianum, T. aureoviride, Bacillus subtilis,* and *B. amyloliquefaciens* were obtained from Identification of Microorganisms, Biological control of Plant Diseases and Evaluation of Biofungicides Unit. The obtained antagonistic microorganisms were previously identified and examined (Khalifa, 2016 and Khalifa *et al.,* 2016).

Pathogenicity test:

Pathogenicity of isolated *R. solani* toward bean plants (Nebraska cv.) was estimated in potted soils under greenhouse conditions. Inocula of *R. solani* isolates were prepared by growing each isolate on sorghum-sand medium for 15 days at 26±2°C. Sterilized pots (20 cm diameter) were filled with sterilized clay soil and infested individually with *R. solani* isolates, at the rate of 3% of soil weight (W/W) per pot (Abou-Zeid *et al*., 2002) and mixed with the soil, then irrigated and left 7 days. Control pots were treated with the same amount of fungal-free autoclaved sorghum sand medium without fungal inocula (Whitehead, 1957). Bean seeds were sown at the rate of five seeds/pot. Three replicates were used for each treatment. The pots were arranged in a complete randomized block design. The most aggressive isolate of *R. solani* was used *in vitro* and *in vivo*. Disease incidence of pre- and post-emergence damping-off diseases as well as survived plants was determined. In addition, infection with root-rot was taken into consideration.

Disease assessment:

In all greenhouse and field experiments, percentages of pre- and post-emergence damping-off and root-rot diseases at 15, 30, and 45 days after planting, respectively as well as survived plants were determined according to the method described by El-Helaly *et al*. (1970) and Ahmed (2013), using the following formulae:

$$\textbf{Pre-emergence \%} = \frac{\text{Number of non-germinated seeds}}{\text{Total number of planted seeds}} \times 100$$

$$\textbf{Post-emergence \%} = \frac{\text{Number of dead seedlings}}{\text{Total number of planted seeds}} \times 100$$

$$\textbf{Root rotted plants \%} = \frac{\text{Number root rotted plants}}{\text{Total number of planted seeds}} \times 100$$

$$\textbf{Survived plants \%} = \frac{\text{Number of survivals plants}}{\text{Total number of planted seeds}} \times 100$$

***In Vitro* studies:**

Evaluation of the antagonistic activity of isolated microorganisms against *R. solani in vitro*:

The antagonistic effect of fungal and bacterial isolates (*T. harzianum*, *T. aureoviride*, *B. subtilis* and *B. amyloliquefaciens*) against *R. solani* was evaluated using the dual culture technique (Coskuntuna and Özer, 2008). Each *Trichoderma* sp. and *R. solani* were cultured, separately, on PDA medium for 4 days at 26 ºC. Meanwhile, bacterial isolates were cultured on Luria-Bertani (LB) broth medium for 2 days at 28 ºC. Plates 9 cm, each contained 10 mL of PDA medium, were inoculated at one side with 5 mm agar disc of any of the antagonistic isolate of *Trichoderma* spp or streaked with a loop full of the antagonistic bacteria. A similar agar disc of *R. solani* was inoculated at an equal distance (1 cm from the plate edge) on the opposite side of the Petri dish. Petri dishes were inoculated with *R. solani* only as control. Three plates were used as replicates for each treatment. The inoculated plates were incubated at 26±2 ºC until the mycelial growth covered the medium surface in the control treatment. The plates were examined, and mycelial growth of each treatment was measured to determine the antagonistic activity of bio-agents as a reduction in linear growth according to Yeh and Sinclair (1980), using the following formula:

$$G = C\text{-}T/C \times 100$$

Where:

G= Percentage of fungal growth reduction.

C= Fungal growth of control (Pathogen alone)

T= Fungal growth of treatment (Pathogen against the antagonist).

Antifungal activity of plant extracts *in vitro*:

The plant extracts of neem (*Azadirachta indica*), garlic (*Allium sativum* L.) and onion (*Allium cepa*) were used at different concentrations as antifungal activity against *R. solani* the causal of damping-off and root-rot of beans *in vivo* and *in vitro*.

Aqueous plant extracts were obtained as described by Amadioha (2000) and Cherkupally *et al*., (2017). The plant materials of neem leaves, garlic cloves, and onion were washed thoroughly with distilled water. Ten g of fresh leaves/cloves of each plant species were taken and crushed in 100 mL sterilized distilled water and ground separately in an electric grinder. The plant extracts were filtrated through double layers of cheesecloth to remove plant debris. The filtrated extracts were centrifuged at 3000 rpm for 10 minutes and the clear supernatant was collected.

Assay of antifungal plant extracts:

Prepared plant extracts were sterilized by filtration through a centered glass filter (G5) and added individually to conical flasks 125 mL containing sterilized PDA before solidification to obtain concentrations of 1, 3, 5, and 7%, and mixed thoroughly (El-Mougy *et al.*, 2007 and El-Shahawy, 2009). Twenty mL of amended media were poured into 9 cm diameter Petri dishes and another set of untreated PDA plates was used as control. For each treatment, 3 replicate plates were used. All plates were inoculated individually with 0.5 cm diameter discs of the tested isolate of *R. solani* 5-days old cultures and then incubated in the dark at 26±2°C, until the control plates reached full growth. All plates were examined, and the percentage of reduction in linear growth of *R. solani* was calculated as mentioned before.

Greenhouse experiment:

Evaluation of different biocontrol agents for controlling *R. solani* under greenhouse conditions:

This experiment was carried out using bio-agents (*T. harzianum*, *T. aureoviride*, *B. subtilis*, and *B. amyloliquefaciens*), biocides (Bio-Arc and Bio-Zeid), and fungicide (Rizolex-T) to evaluate their ability to protect bean plants (Nebraska cv.) against infection by damping-off and root-rot diseases caused by *R. solani*. Active ingredients and rate of applications are shown in Table (1).

The experiment was designed as a complete randomized block design with four replicates. Pots (40 cm in diameter) were dipped in 5% formalin solution for 10 min and left to dry in open air. The inoculum of *R. solani* was grown on sorghum grain sand medium for 15 days at 26±2 °C and mixed with the soil at the rate of 3%, then the pots were planted after one week. The antagonistic fungi and bacteria were grown on PDA broth medium and Luria-Bertani (LB)

broth medium for 10 and 3 days, respectively. Each antagonistic fungus and bacterial strain was used as spore suspension (1×10^7 spore/mL) for *Trichoderma* spp., and. bacterial cell suspension (2.5×10^7 CFU cell/mL). Bean seeds were soaked in the tested suspension for two hours. Meanwhile, Bio-Arc, Bio-Zeid and Rizolex-T were used at the recommended dose as seed dressing (Table, 1). Four pots were used for each treatment as replicates; each pot was planted with 10 treated bean seeds. Disease incidence of pre- and post-emergence damping-off and root-rot diseases at 15, 30 and 45 days after planting, respectively as well as survived plants were determined as mentioned before.

Table 1: Active ingredients of the biocides, most active bioagents isolates, fungicide Rizolex-T.

Tested treatment	Name, concentration/active ingredients	Used rate
Bio-Arc	*Bacillus megaterium*, 6.0% (2.5×10^7 cells/g)	2.5 g/kg seeds
Bio-Zeid	*Trichoderma album*, 2.5 % (10×10^6spores/g)	2.5 g/kg seeds
T. aureoviride	*T. aureoviride*	1×10^7/mL
T. harzianum	*T. harzianum*	1×10^7/mL
B. subtilis	*B. subtilis*	2.5×10^7/mL
B. amyloliquefaciens	*B. amyloliquefaciens*	2.5×10^7/mL
Rizolex-T 50%	20% Rizolex: Tolclofos-(ethyluro-o-dimethyl)-o-(2,6-dichloro-4-methyl-phenyl)-o,o-dimethyl phosporothioate.; 30% Thiram: bis (dimethyl- thiocarbamoyl)	1.5 g/kg seeds

Evaluation of the antifungal activity of plant extracts under greenhouse conditions:

The antifungal activity of neem, garlic, and onion at 3 and 5% concentrations was evaluated under greenhouse against Rhizoctonia root-rot disease. Aqueous plant extract of each plant was prepared as mentioned before and transferred in a beaker and boiled at 80°C for twenty minutes in a hot water bath. From this standard/stock solution(s), required concentrations (3 and 5%) were prepared by adding sterile distilled water and applied as seed soaking. The tested bean seeds were soaked in the previously prepared plant extracts at 3 and 5% for 2 hrs. Pots (40 cm diam.) each contained 4 kg of sterile loamy soil were prepared. The pots were watered after infestation with the tested fungus at the rate of 3% of soil weight (W/W), then sown after one week. The control was treated with the same amount of fungal-free autoclaved sorghum sand medium without fungal inocula, and another control contained the same amount of fungal inoculum only. Bean seeds (Nebraska cv.) were sown at the rate of 10 seeds/pot. Three replicates were used for each treatment. The experiment was arranged in a completely randomized block design. Disease incidence of pre- and post-emergence damping-off and root-rot diseases as well as survived plants were determined as mentioned before.

Determination of some plant enzymes activity:

Related to plant defense, the effect of seed treatment with the most efficient plant extracts and bioagents on the activity of oxidative (polyphenoloxidase, peroxidase) and lytic-enzymes (β-1,3-glucanase, and protease) against the infection with Rhizoctonia damping-off and root-rot diseases was determined in leaves of bean plants (Nebraska cv.), raised from treated seeds at different plant ages *i.e*., 7, 14 and 28 days after application of the tested treatments.

Enzymes extraction and bioassay:

Bean leaves (1g) collected from each treatment were homogenized separately in a mortar with 0.1 M. sodium phosphate buffer at pH= 7.1 at the rate of 2 mL/g fresh weight leaves for 1 min. These triturated tissues were strained through four layers of cheesecloth and the filtrates were centrifuged at 3000 rpm for 15 minutes at 6°C. The clear supernatant was collected and considered a crude extract for enzymes assay. The supernatant was stored in the refrigerator at -20°C till the determination of enzymes activities.

Assay of polyphenoloxidase (PPO):

PPO activity was determined according to Matta and Dimond (1963). The reaction mixture contained 1.0 mL enzyme extract, 1.0 mL of 0.2 M sodium phosphate buffer at pH 7.0, 1.0 mL catechol. The reaction mixture was brought to a final volume of 6.0 mL with distilled water. Polyphenoloxidase activity was expressed as change in absorbance/min at 495 nm.

Assay of Peroxidase:

Peroxidase activity was determined according to Allam and Hollis (1972) by measuring the oxidation of pyrogallol to pyrogallin in the presence of H_2O_2 at 425 nm. The reaction mixture contained 0.5 mL of 0.1 M sodium phosphate buffer at pH 7.0, 0.5 mL enzyme extract, 0.3 mL pyrogallol, 0.1 mL 1.0

% H_2O_2 brought to final volume of 3.0 mL with distilled water. Peroxidase activity was expressed as the change in absorbance/min at 425 nm.

Assay of β-1, 3-glucanase:

The method described by Abeles and Forrence (1970) was used to determine β-1,3-glucanase activity. 0.5 mL of enzyme extract was added to 0.5 mL of 0.05 M of potassium acetate buffer (pH 5) containing 2% Laminarin. The mixture was incubated at 40°C for 60 minutes. The reaction was stopped by adding 1 mL of dinitro salicylic acid and heated for 5 min at 100°C in a boiling water bath. The tubes were cooled, and 3 mL of distilled water were added before assay. β-1,3-glucanase activity was expressed as the change in absorbance / min at 500 nm.

The enzyme activity was expressed as μmol equivalent of glucose/ released gram fresh weight tissues/min.

Assay of Protease assay:

The protease activity was assayed according to Kunitz (1947). The reaction was carried out in reaction mixture containing 450 μl of 1% (w/v) casein or hemoglobin with 50 mM Tris-HCl at pH 7.8 and 50 μl of the diluted enzyme for 20 min incubation at 35ºC. The reaction was stopped by adding 750 μl of Trichloroacetic acid (TCA) solution [5% (w/v) TCA, 9% (w/v) Na-acetate, 9% (v/v) acetic acid], followed by 30 min incubation at room temperature and centrifugation (15000 rpm, 15 min). The absorbance of the soluble peptide (supernatant) was measured at 280 nm. One unit of the enzyme activity was defined as the amount of enzyme which releases μmol of tyrosine per min under the assay conditions.

Field experiments:

This experiment was carried out at Giza Agricultural Research Station during the two successive seasons 2019 and 2020 to study the effects of the application of active bioagent (*T. harzianum, T. aureoviride*, *B. subtilis* and *B. amyloliquefaciens*) and also the effective concentration of plant extracts (neem, garlic, and onion) at 5 % as seed soaking compared to biocides (Bio-Arc and Bio-Zeid) and fungicide (Rizolex-T) as a seed dressing on controlling damping-off and root-rots, as well as their effects on some bean crop parameters. All tested treatments of bioagents, plant extracts, biocides and fungicide were used at the recommended dose in (Table, 1). The experiment was designed as a complete randomized block design with three replicates for each treatment. The field plot was 3.0 × 3.0 m^2 in three rows with about 15-20 cm distances between sowing holes (about 2 seed/hell). Disease assessments were measured as percentages of pre-and post-emergence damping-off and survived plants and crop parameters were also estimated. At harvest, ten plants were randomly taken from each plot to determine the following parameters: plant height (cm), number of branches/plant, number of pods/plants, weight of pods/plant and 100-seed weight (g), for each treatment.

Statistical analysis:

Data obtained were statistically analyzed by the analysis of variance (ANOVA) using Computer Statistical Package (Assistat V.7.6 beta) originated by Silva and Azevedo (2009). Mean comparisons were made using the least significant differences (LSD) at 0.05 % level of significance (Snedecor and Cochran, 1989).

RESULTS AND DISCUSSION

3.1. Isolation and identification of the pathogens of damping-off and root rot diseases of bean:

The isolated fungi, which were associated with bean plants and seedling showing root-rot and damping-off symptoms, were purified and identified as *Rhizoctonia solani* Kühn according to their cultural and morphological features.

3.2. Pathogenicity test:

Data in Table (2) reveal that the obtained nine *R. solani* isolates were tested for their ability to infect bean seedlings. The obtained results showed that they were pathogenic and significantly varied in virulence on damping-off and root-rot diseases on bean plants (Nebraska cv.) under greenhouse. Sakha-R7 isolate was the most aggressive, which gave the highest percentages of pre- and post-emergence damping-off and less survived plants, being 66.7, 26.7, and 6.7%, respectively. On the other hand, Toukh-R6 isolate was the least virulent isolate (20.0, 6.7, and 73.3%) at pre- and post-emergence damping-off and survived plants, respectively. Accordingly, Sakha-R7 isolate was chosen to conduct the greenhouse experiment.

These results are in agreement with those reported by Aly *et al*. (2010), Al-Abdalall (2010) and Sennoi *et al*. (2010), who mentioned that *R. solani*, *S. rolfsii*, and *F. solani* were the most important destructive soil-borne pathogenic fungi to roots of many plants, such effect might be due to the synergistic action between polygalacturonase and oxalic acid produced by these pathogenic fungi. Moreover, Beebe and Pastor-Corrales, 1991 mentioned that the main disease is the damping-off and root rot

of bean caused by *R. solani*. *Rhizoctonia solani* isolates were the highest virulent, significantly increased damping-off and reduced the survived plants (Mahmoud and Abo-Elyousr, 2014).

Table (2): Pathogenicity test of nine *R. solani* isolates on bean (Nebraska cv.) under greenhouse conditions.

Isolate No.	Source		Damping-off (%)		Survived plants (%)
	Governorate	Location	*Pre-	**Post-	
R1	Menoufia	Shbeen El Koom	13.3	20.0	66.7
R2		El-Bagour	46.7	20.0	33.3
R3		Serce-Alian	20.0	33.3	46.7
R4	Beheira	Kafr-El Dowwar	26.7	13.3	60.0
R5		El-Nubaria	53.3	26.7	20.0
R6	Qalubiya	Toukh	20.0	6.7	73.3
R7	Kafr-El Sheikh	Sakha	66.7	26.7	6.7
R8		Kleen	26.7	33.3	40.0
R9		Sidy-Salem	46.6	26.7	26.7
LSD at 0.05			16.7	15.84	10.58

* At 15 days after sowing.; ** At 30 days after sowing.

3.3. Laboratory studies:

3.3.1. Evaluation the antagonistic activity of biocontrol agents against *R. solani in vitro*.

Data presented in Table (3) indicate that all tested antagonistic fungal and bacterial isolates significantly reduced the linear growth of *R. solani in vitro*. *Trichoderma harzianum* and *T. aureoviride* were the most effective bio-agents, which gave the highest reduction in growth of *R. solani*, followed by *B. subtilis* and *B. amyloliquefaciens*, respectively. The antagonistic isolates were evaluated to control bean damping-off and root-rot diseases in greenhouse and field experiments.

Many investigators used bioagents as potential antagonists for controlling many plant pathogens (Deshmukh *et al.*, 2010). *Trichoderma* spp. and *B. subtilis* reduced linear growth of *R. solan* (Barari and Foroutan, 2016; Khalifa, 2016 and Khalifa *et al*., 2016), due to the production of lytic and oxidative enzymes which degrade the pathogen cell wall (Pieta and Pastucha, 2004; Mausam *et al.*, 2007), production of antibiotics (Bender *et al.*, 1999), volatile compounds and phytotoxic substances (Hoagland and Cutler, 2000 and Barakat *et al.*, 2014).

3.3.2. Antifungal activity of the plant extracts against *R. solani in vitro*:

Data presented in Table (4) reveal that all tested plant extracts and their different concentrations significantly increased the growth reduction of *R. solani* compared to the control treatment. This reduction was gradually increased by increasing the concentration of the tested plant extracts from 1, 3, and 5 to 7%. In this respect, garlic extract was the most effective in reducing the linear growth of *R. solani*, followed by onion and neem extracts, as they recorded averages of growth reduction, being 89.08, 38.52, and 28.97 %, respectively compared to control.

These results are in agreement with those obtained by Ahmed (2013) who showed that plant extracts resulted in a significant reduction in the linear growth of the pathogenic fungi. This effect might be due to that plant extract can synthesize the secondary metabolites and some of them as well as their derivatives have antimicrobial such as phenolic compounds, which may sensitize the phospholipids, that hinder the movement of fungi, as mentioned by EL-Mougy and Abdel-Kader (2007), Qari (2008) and Sarhan *et al*. (2018).

Table (3): Antagonistic effect of some biocontrol agents on the linear growth of *R. solani* (R7) *in vitro*.

Antagonistic isolates	Linear growth (cm)	Growth reduction (%)
T. aureoviride	3.5	60.77
T. harzianum	2.8	68.89
B. amyloliquefaciens	5.5	38.89
B. subtilis	5.2	42.22
Control	9.0	-
LSD at 0.05	1.14	1.21

Table (4): Antifungal activity of the plant extracts on the linear growth of *R. solani* (R7) *in vitro*.

Plant extracts	Reduction (%) in the linear growth of *R.solani* after 5 days of incubation				Mean
	Concentration (%)				
	1	3	5	7	
Garlic	76.67	88.15	91.48	100	89.08
Onion	20.74	31.11	48.89	53.33	38.52
Neem	14.81	25.56	35.55	39.96	28.97
Control	00.00	00.00	00.00	00.00	0.00
Mean	28.06	36.21	43.98	48.32	39.14
LSD at 0.05 for					
Plant extracts (P)			1.35		
Concentrations (C)			1.73		
Interaction (P × C)			2.98		

3.4. Greenhouse experiment:

3.4.1. Evaluation of different biocontrol agents for controlling *R. solani* under greenhouse conditions:

Data in Table (5) show that all tested treatments of biocontrol agents, biocides and fungicide significantly decreased bean damping-off and root-rot in the greenhouse compared to control. Rizolex-T was the most effective treatment for controlling *R. solani* under greenhouse conditions, followed by *T. harzianum* and *T. aureoviride* (2.5, 7.5, 5.0 and 85.0%), (10.0, 7.5, 7.5 and 75.0%) and (12.5,10, 10.0 and 67.5%) for pre- and post- damping-off, root-rot and plant survival, respectively. On the other hand, *B. amyloliquefaciens* was the lowest effective treatment compared to Bio-Arc and Bio-Zeid (25.0, 10, 15.0 and 50.0%), (25.0, 10, 10.0, and 65.0%) and (12.5, 5, 7.5 and 72.5%) for pre- and post- emergence damping-off, root-rot and plant survival, respectively.

These results are in agreement with those recorded by Abou-Zeid *et al*. (2003) and Pieta and Pastucha (2004) who reported that treated seeds with bioagents and bio-fungicides protected bean seedlings against *Fusarium* spp. and *R. solani* infection. *Trichoderma* spp. and *Bacillus* spp. significantly reduced damping-off disease incidence (Ahmed, 2013; Abdellatif *et al*., 2015 and Khalifa *et al*., 2016) due to activating soil microbes and decreasing the population of *R. solani* (Anand and Kulothungan, 2010), enhancing the growth of root system as evidenced by increased biomass may be positively acted in diseases control, activation defense mechanisms and accumulation of antimicrobial secondary metabolites (Castro and Fontes 2005; Abd-El-Khair *et al.*, 2011 and Atwa *et al*., 2014).

Table (5): Evaluation of different biocontrol agents for controlling bean damping-off and root-rot diseases caused by *R. solani* under greenhouse conditions.

Treatments	Damping-off (%)		Root-rot (%)	Survived Plant (%)
	Pre-	Post-		
T. aureoviride	12.5	10.0	10.0	67.5
T. harzianum	10.0	7.5	7.5	75.0
B. amyloliquefaciens	25.0	10.0	15.0	50.0
B. subtilis	10.0	17.5	12.5	60.0
Bio-Arc	15.0	10.0	10.0	65.0
Bio- Zeid	12.5	5.0	7.5	72.5
Rizolex-T	2.5	7.5	5.0	85.0
Control	30.0	20.0	17.5	32.5
Uninfected control	0.0	0.0	0.0	100.0
LSD at 0.05	8.57	9.16	10.24	10.49

3.4.2. Evaluation the antifungal activity of plant extracts on damping-off and root rot diseases caused by *R. solani* under greenhouse conditions:

The obtained results in Table (6) indicate that all plant extracts (garlic, onion, and neem) at different concentrations (3 and 5%) were effective in controlling damping-off and root-rot diseases *in vivo* compared to control. Garlic and

onion at 5% concentration were the highly effective extracts against *R. solani* (7.5, 7.5, 5.0, and 80.0%) and (12.5, 7.5, 7.5, and 72.5%) for pre- and post-emergence damping-off, root-rot, and plant survival, respectively. However, neem with 5% concentration was the lowest effective extract in this concern.

These results are in harmony with those obtained by El-Mougy and Abdel-Kader (2007) and El-Fiki *et al*. (2014) who reported that plant extracts as seed treatment led to reduction of damping-off and root-rot diseases of bean plants, increment the plant survival, improvement plant growth parameters (Morsy *et al*., 2009), due to the antioxidant material, volatile oil compounds which are found as a major content of these extracts and liberated from the carrier.

Table (6): Evaluation of plant extracts at different concentrations for controlling bean damping-off and root-rot diseases *in vivo*.

Treatments	Conc. (%)	Damping-off (%)		Root-rot (%)	Survived Plant (%)
		Pre-	Post-		
Garlic extract	3	12.5	7.5	5.0	75.0
	5	7.5	7.5	5.0	80.0
Onion extract	3	15.0	10.0	10.0	65.0
	5	12.5	7.5	7.5	72.5
Neem extract	3	20.0	15.0	12.5	52.5
	5	17.5	7.5	10.0	65.0
Rizolex-T		2.5	7.5	5.0	85.0
Infested control		30.0	20.0	17.5	32.5
Uninfested control		0.0	0.0	0.00	100.0
LSD at 0.05		9.98	9.98	10.49	11.45

3.4.3. Detection of some plant enzymes activity:

Effect of the enzyme activity associated with induction of resistance by treated bean plants with biocontrol agents, plant extracts, biocides and fungicide treatment against damping-off and root-rot diseases of bean caused by *R. solani* was also investigated.

A. Oxidative enzymes:

A.1. Peroxidase activity:

Data in Table (7) reveal that treated seeds of bean plants with biocontrol agents, plant extracts, biocides and fungicide treatment increased peroxidase activity compared to uninfected control. Peroxidase activity was more in infected plants than in the healthy ones. The maximum increase in enzyme activity was recorded after 7th days in all treatments then decreased gradually at 14th and 28th days after inoculation with *R. solani*.

The highest increase of peroxidase activity was recorded in bean plants treated with garlic extract, Rizolex-T, *T. harzianum*, and Bio-Zeid (1.474, 1.388, 1.333 and 1.288), respectively.

The highest peroxidase activity value was recorded using the garlic extract and Rizolex-T at 7th days after inoculation, being 1.993 and 1.837, respectively. In comparison, the lowest value of enzyme activity was recorded with *B. amyloliquefaciens* at 28th days after inoculation in treated plants, being 0.598. Our results are aligned with other reports documented by Shetty *et al*. (2007) and Houssien *et al*. (2010). The biotic and abiotic inducers stimulate some defense mechanisms such as phenolic compounds, peroxidase, and polyphenoloxidase which play an important role in plant defense mechanisms against pathogens infection by production phenolic compounds, oxidative enzymes and other metabolites (Abdel-Monaim *et al*., 2011 and El-Mohamedy *et al*., 2013). The enzymatic activity in treated bean plants was increased than in untreated one (Caruso *et al*., 2001; Nawar and Kuti, 2003; Abd El-Khair *et al*., 2011 and Sarhan *et al*., 2018).

A.2. Polyphenoloxidase:

Data in Table (8) indicate that all treatments resulted in an increase compared to untreated ones. The highest enzyme activity value was recorded at the 7th days after application in the infected plants then decreased gradually at 14th and 28th days after inoculation. The highest average of polyphenoloxidase activity was recorded in treated bean plants with Bio-Zeid, garlic extract and *T. harzianum*, being 0.690, 0.649, and 0.602, respectively. Meanwhile, the lowest values of polyphenoloxidase activity were recorded in both infected and uninfected bean plants, being 0.363 and 0.347 on the average without significant differences, respectively.

Therefore, results showed that antioxidant enzyme activity was increased in bean plants in

response to both *R. solani* and bioagents fungus (Hathout *et al*., 2010). Polyphenoloxidase is important in the defense mechanism against pathogens through its role in the oxidation of phenolic compounds to quinones, resulting in increased antimicrobial activity (Chranowski *et al*., 2003 and El-Khallal, 2007). Oxidation of phenolic compounds in the plant cell is responsible for initiating the Hypersensitive response (HR) used by the plant to prevent the spread of infection by microbial pathogens (Ragab *et al*., 2009 and Eid, 2014).

B. Lytic enzymes:

B.1. β-1,3-glucanase activity:

Data in Table (9) show that β-1,3-glucanase activity was increased more in infected plants treated with biocontrol agents, biocides, plant extracts and fungicide treatments than in the healthy ones. The maximum increase in enzyme activity was recorded after the 7th day in all treatments then gradually decreased at the 14th and 28th days. The highest enzyme activity was recorded in infected bean plants with *R. solani* and treated by Rizolex-T, *T. harzianum*, and garlic extract, being 3.420, 3.224 and 2.957 respectively. Meanwhile, the treatment with *B. amyloliquefaciens* recorded 1.378.

B.2. Protease activity:

Results in Table (10) indicate that protease activity was decreased considerably with increasing the period after application in comparison with the control. The enzyme activity was increased in infected plants more than in healthy ones. The highest enhancement of protease activity was also observed after 7th days in all treatments then decreased at the 14th and 28th days after application, being 0.402, 0.306, and 0.194, respectively. The maximum increase in enzyme activity was occurred in plants raised from bean seeds treated with *T. harzianum*, Rizolex-T, garlic extract, and Bio-Arc, being 0.406, 0.374, 0.365 and 0.345, respectively. Meanwhile, infected bean plants raised from treated seeds with *B. amyloliquefaciens*, recorded the lowest increase in enzyme activity, being 0.253.

Results obtained in the current study are in agreement with those reported by Jayalakshmi *et al*. (2009 and 2011) who showed that application of biological and chemical inducers induced some defense mechanisms against wilt/root-rot diseases and increased the activity of protease and β-1,3glucanase enzymes, Also, Anand and Kulothungan (2010) reported that biocontrol agents produce lytic enzymes and the antibiotics thus could act synergistically with other mechanisms (Vinale *et al*., 2006). Proteases cleavage of peptide bonds in proteins and play a significant role in cell wall lysis. Meanwhile, β-1,3-glucanase hydrolyses β-1,3-glucan, are the major components of cell wall of fungal pathogen (Chun-Yi and Lin, 2008 and El-Gammal, 2013).

Table (7): Effect of different biocontrol agents, biocides and plant extracts on peroxidase activity in bean plants at different periods after inoculation, greenhouse experiment.

Treatments	Peroxidase enzyme activity (min/g fresh weight of bean)			
	Days after treatment			Mean
	7	14	28	
T. aureoviride	1.608	1.003	0.751	1.121
T. harzianum	1.797	1.321	0.881	1.333
B. amyloliquefaciens	1.134	0.748	0.598	0.827
B. subtilis	1.522	0.889	0.629	1.013
Garlic extract	1.993	1.432	0.998	1.474
Onion extract	1.669	1.137	0.619	1.142
Neem extract	1.258	0.848	0.772	0.959
Bio-Arc	1.566	0.949	0.703	1.073
Bio- Zeid	1.759	1.288	0.818	1.288
Rizolex-T	1.837	1.395	0.933	1.388
Infected control	0.789	0.675	0.574	0.679
Uninfected control	0.538	0.566	0.537	0.547
Mean	1.456	1.021	0.734	1.070
LSD at 0.05				
Treatments (T)		0.010		
Days (D)		0.003		
Interaction (T × D)		0.013		

Table (8): Effect of different plant extracts and biocontrol agents on polyphenoloxidase activity in bean plants at different periods after inoculation, greenhouse experiment.

Treatments	Polyphenoloxidase enzyme activity (min/g fresh weight of bean)			
	Days after treatment			Mean
	7	14	28	
T. aureoviride	0.619	0.519	0.409	0.516
T. harzianum	0.685	0.636	0.485	0.602
B. amyloliquefaciens	0.526	0.436	0.375	0.446
B. subtilis	0.577	0.466	0.382	0.475
Garlic extract	0.789	0.646	0.511	0.649
Onion extract	0.643	0.537	0.449	0.543
Neem extract	0.448	0.408	0.358	0.405
Bio-Arc	0.599	0.481	0.388	0.489
Bio- Zeid	0.866	0.675	0.528	0.690
Rizolex-T	0.661	0.592	0.475	0.576
Infected Control	0.398	0.376	0.315	0.363
Uninfected Control	0.339	0.347	0.355	0.347
Mean	0.596	0.510	0.420	0.509
LSD at 0.05				
Treatments (T)		0.084		
Days (D)		0.030		
Interaction (T × D)		0.1224		

Table (9): Effect of different biocontrol agents, plant extracts and biocides on β-1,3-glucanase activity in bean plants at different periods after inoculation, greenhouse experiment.

Treatments	β 1, 3 Glucanase enzyme activity (min/g fresh weight of bean)			
	Days after treated application			Mean
	7	14	28	
T. aureoviride	2.958	2.115	1.475	2.183
T. harzianum	3.894	3.219	2.558	3.224
B. amyloliquefaciens	1.879	1.243	1.012	1.378
B. subtilis	2.347	1.591	1.083	1.674
Garlic extract	3.673	2.875	2.322	2.957
Onion extract	2.768	1.916	1.121	1.935
Neem extract	2.317	1.508	1.068	1.631
Bio-Arc	2.897	2.197	1.904	2.333
Bio-Zeid	3.244	2.358	2.167	2.590
Rizolex-T	4.436	3.233	2.591	3.420
Infected Control	1.604	1.395	0.834	1.278
Uninfected Control	0.851	0.895	0.931	0.892
Mean	2.739	2.045	1.589	2.125
LSD at 0.05				
Treatments (T)		0.0040		
Days (D)		0.0014		
Interaction (T × D)		0.0070		

Table (10): Effect of different biocontrol agents, plant extracts and biocides on protease activity in bean plants at different periods after inoculation, greenhouse experiment.

Treatments	Protease enzyme activity (min/g fresh weight of bean)			
	Days after treatment			Mean
	7	14	28	
T. aureoviride	0.389	0.297	0.193	0.293
T. harzianum	0.562	0.425	0.230	0.406
B. amyloliquefaciens	0.339	0.243	0.178	0.253
B. subtilis	0.369	0.291	0.189	0.283
Garlic extract	0.498	0.378	0.219	0.365
Onion extract	0.458	0.326	0.200	0.328
Neem extract	0.347	0.266	0.182	0.265
Bio-Arc	0.476	0.355	0.204	0.345
Bio-Zeid	0.431	0.309	0.194	0.311
Rizolex	0.506	0.395	0.221	0.374
Infected Control	0.294	0.221	0.160	0.225
Uninfected Control	0.153	0.165	0.162	0.160
Mean	0.402	0.306	0.194	0.301
LSD at 0.05				
Treatment		0.008		
Days		0.003		
Interaction (T × D)		0.015		

3.5. Field experiment:

This experiment was carried out to study the effect of treatment bean seeds with biocontrol agents, plant extracts, biocides and fungicide treatment on the incidence of damping-off and root rot diseases, yield and yield components of bean plants under field conditions during the 2019 and 2020 growing seasons.

Data in Tables (11 and 12) indicate that all tested treatments in both growing seasons significantly reduced damping-off, root-rot diseases and increased the percentage of survived plants as well as improved bean plant growth parameters, yield and yield components (plant height, No. of branches, No. of pods/plant, weight of pods/plant and 100 seed weight), compared with control treatment.

Obtained data in Table 11 indicate that Rizolex-T was the most effective treatment for controlling damping-off on Nebraska cv., followed by garlic extract, Bio-Zeid, and *T. harzianum* (3.8, 7.3, 12.5 and 13.2%) and (5.9, 9.5, 11.7 and 14.8%) for damping-off and (96.2, 92.7, 87.5 and 86.8%) and (94.1, 90.5, 88.3 and 85.2%) for survived plants during 2019 and 2020 growing seasons, respectively in comparison with control treatments. However, *B. amyloliquefaciens* and neem extract were the lowest effective treatments.

As illustrated in Table 12, Rizolex-T, garlic extract, Bio-Zeid, and *T. harzianum* were the most effective treatments for increasing plant height, No. of branches, No. of pods/plant, weight of pods/plant and 100 seed weight, while *B. amyloliquefaciens* and neem extract were the least effective treatments compared with control under field conditions during the two growing seasons.

These results are in agreement with those obtained by Abou-Zeid *et al.* (2002), Ahmed, (2013), and Khalifa *et al*. (2016) who reported that seed treatment with bioagents, plant extract and bio-fungicides decreased damping-off and root-rot diseases and improved bean plant growth parameters and yield components by enhancing the growth of root system, activation defense mechanisms as other previous repotrs (Wu *et al*., 2000; Agrios, 2005 and Abd-El-Khair *et al*., 2011). *Trichoderma* spp. and *Bacillus* spp. significantly reduced the incidence of damping off disease in bean under field conditions (El-Kafrawy, 2002 and Malik *et al*., 2005) by activation defense mechanisms and accumulation of antimicrobial compounds

(Castro and Fontes 2005 and Abd-El-Khair *et al.*, 2011), production of bacterial allelochemicals, including iron-chelating siderophores, antibiotics, biocidal volatiles, oxidative and lytic enzymes and detoxification enzymes (Compant *et. al*., 2005; Hass and Defago, 2005; Anand and Kulothungan, 2010 and Sarhan *et al*., 2018). Also, Morsy *et al*. (2009) and Ahmed, (2013) reported that seeds treatment with garlic, and neem extracts was the most effective treatments for controlling damping-off disease of bean due to activated soil microbes, decreased the population of *R. solani*.

Table (11): Effect of bioagents, plant extracts, biocides and the fungicide Rizolex-T on the incidence of damping-off of Nebraska bean cultivar under field conditions during 2019 and 2020 growing seasons.

Treatments	2019 growing season				2020 growing season			
	Damping-off (%)			Plant survival (%)	Damping-off (%)			Plant survival (%)
	Pre-	Post-	Total		Pre-	Post-	Total	
T. aureoviride	13.1	4.4	17.5	82.5	11.4	5.8	17.2	82.8
T. harzianum	9.1	4.1	13.2	86.8	10.3	4.5	14.8	85.2
B. amyloliquefaciens	19.1	7.5	26.6	73.4	21.9	9.9	31.8	68.2
B. subtilis	14.7	5.4	20.1	79.9	16.3	7.0	23.3	76.7
Garlic extract	5.4	1.9	7.3	92.7	7.4	2.1	9.5	90.5
Onion extract	9.9	4.2	14.1	85.9	11.8	4.8	16.6	83.4
Neem extract	16.6	7.1	23.7	76.3	19.0	8.4	27.4	72.6
Bio-Arc	12.4	3.9	16.3	83.7	14.9	4.2	19.1	80.9
Bio- Zeid	8.6	3.9	12.5	87.5	8.8	2.9	11.7	88.3
Rizolex	2.6	1.2	3.8	96.2	3.5	2.4	5.9	94.1
Control	25.4	11.4	36.8	63.2	27.3	11.6	38.9	61.1
LSD at 0.05	0.85	0.53	1.12	1.23	0.49	0.48	0.68	0.88

Table (12): Effect of bioagents, plant extracts, biocides and the fungicide Rizolex-T on plant height and some yield components of Nebraska bean cultivar under field conditions during 2019 and 2020 growing seasons.

Treatments	Plant height (cm)		No. of Branches/plant		Number of pods/plants		Weight of pods/plant (g)		Weight of 100 seeds (g)	
	2019	2020	2019	2020	2019	2020	2019	2020	2019	2020
T. aureoviride	53.0	55.1	4.64	4.45	20.48	19.25	77.82	67.33	42.87	45.93
T. harzianum	62.2	65.8	4.03	4.82	25.96	26.89	89.33	75.16	49.77	47.16
B. amyloliquefaciens	45.8	44.8	4.4	4.1	17.86	18.27	64.22	62.45	38.2	42.13
B. subtilis	47.6	46.3	4.9	4.3	19.63	18.39	74.54	64.65	39.99	43.73
Garlic extract	60.3	58.2	5.4	4.72	24.32	24.71	83.66	80.33	46.7	48.3
Onion extract	55.6	53.6	4.73	4.55	20.48	19.86	79.24	70.11	44.11	46.7
Neem extract	44.1	49.9	4.7	4.27	17.86	18.27	66.68	60.75	38.57	41.09
Bio-Arc	50.3	52.0	4.56	4.33	20.27	19.04	75.26	66.04	41.44	44.9
Bio- Zeid	59.1	60.7	5.1	4.91	22.07	21.60	98.67	92.11	53.11	56.33
Rizolex-T	57.1	57.6	4.92	4.64	23.43	22.66	91.46	86.45	51.44	50.17
Control	37.9	39.2	3.80	3.56	15.88	14.27	56.44	49.45	36.73	38.1
LSD at 0.05	4.02	2.11	0.89	0.51	0.92	0.57	0.69	0.68	0.59	0.66

CONCLUSION

The present work demonstrated that application of biocontrol agents (*T. harzianum*, *T. aureoviride*, *B. subtilis* and *B. amyloliquefaciens*), plant extracts (garlic, onion, and neem), biocides (Bio-Arc and Bio-Zeid) and chemical fungicide (Rizolex-T) significantly reduced bean damping-off, root-rot and increased the percentage of survived plants, improved bean plant growth parameter, yield, and yield components and affected the activity of lytic enzymes and oxidative reductive enzymes. It may be concluded that application with active bioagents is considered an applicable safe and cost-effective method for controlling damping-off and root rot diseases.

CONFLICTS OF INTEREST

The author(s) declare no conflict of interest.

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