Fayoum Journal of Agricultural Research and Development
ISSN:1110- 7790

Biological control of damping-off and root rot disease caused by *Rhizoctonia solani* on cucumber plants

[1*]Mohamed A. A. Hassan, [1]Hoda M. H. Ahmed, [2]Said M. Kamel, [2]Wallaa F. M. Abd El-Hamed [1]Heba M. A. Yousef

[1]Botany Department, Faculty of Agriculture, Fayoum University, Fayoum, Egypt.
[2]Plant Pathology Research Institute, Agriculture Research Center, Giza, Egypt.

Abstract

Damping-off and root rot is a serious disease infecting cucumber on both seedling and adult stages under protected cultivation causes severe losses in the cucumber yield. Thirteen biocontrol agents i.e. six *Trichoderma* spp., five *Bacillus* spp. and two species of actinomyces were tested for their ability to control of cucumber damping-off and root rot disease caused by *Rhizoctonia solani in vitro* and *in vivo* and their ability to promoting growth of cucumber plants. *Trichoderma asperellum***,** *Bacillus subtilis1*, *Pseudomonas fluorescens* and *Streptomyces spp1* act to be highly efficient bioagnts to inhibit mycelial growth of *Rhizoctonia solaniin in vitro*. All tested bio agents of *T. asperellum*, *B. subtilis1*, *P. fluorescens* and *Streptomyces spp1* significantly reduced pre- and post-emergence damping off and root rot disease incidence causing by *R. solani* in both Fayoum and Giza governorates experiments under greenhouse conditions as seed and seedling treatment. Also, these treatments significantly increase growth parameters and yield components compared with the check treatment. All treatments had considerable increase in the peroxidase, polyphenol oxidase, catalase enzymes and total phenol activities that play an important role in plant defense mechanisms against pathogens infection. These results proved that bioagent i.e. *Pseudomonas flourecens* is an efficient method as to control the causal pathogen of damping-off and root rot of cucumber.

Key words: bioagents, cucumber, damping off, *Rhizoctonia solani*, enzymatic activity, root rot.

Introduction

Cucumber (Cucumis sativus L.) is one of the most important vegetable grown all over the world. And it is one of the oldest cultivated vegetables dating back to 5,000 years. Cucumber is being attacked by many fungal diseases. **Sabbagh et al. (2017)** Damping-off and root rot is a serious disease infecting cucumber on both seedling and adult stages under protected cultivation causes severe losses in the cucumber yield. Seeds, seedlings and young plants may be affected, resulting in greenhouses, and commercial fields. Losses due to damping-off can be severe, especially when cool, wet weather prevails at seeding seedling or seed emergence **Aljawasim et al. 2020.**

*Corresponding author: Email:maa22@fayoum.edu.eg
Received: 3/10/ 2021
Accepted:15/11/ 2021

Many fungal pathogens such as *Rhizoctonia solani*, *Fusarium oxysporum*, *F. solani*, *Sclerortium rolfsi*, *Macrophomina phaseolina* and *Phytopathora sp.* which cause damping-off and root rot disease in cucumber plants and causing serious losses in seed germination and plant stand. **Kazerooni et al. 2019** and **AL-fadhal et al. 2019.**

Several attempts to control root rot and wilt diseases could be accepted. However, fungicides are considered one of several factors involving in environmental pollution, in spite of their satisfactory results in the control of plant diseases. In addition, control of disease with fungicides has proven very difficult, and almost all fungicides are effective only at phytotoxic levels. Recently, the growing concern over the use of pesticides to human health and environment has brought increasing interest in the use of alternatives characterized with negative impact on the environment **Aljawasim et al. 2020.** The application of biological control using antagonistic microorganisms proved to be successful for controlling various plant diseases in many countries. Biological control is proposed to be an effective and non-hazardous strategy to reduce crop damage caused by plant pathogens **Fasusi et al., 2021** and **wang et al., 2018**. In recent years the Trichoderma spp., Bacillus spp. and actinomyces spp. have been extensively used for plant growth promotion and disease control **Awad and Fayyadh. 2018** and **Mahmoud 2015.**

Thus, the aim of this study is to screen biocontrol agents a capable of inhibiting the growth of *R. solani* and determine the biocontrol efficiency of *Trichoderma* spp., *Bacillus* spp. and actionmyces amendment against damping-off and root rot diseases of cucumber under greenhouseAlso determine their effect of promoting growth and yield components of cucumber.

Materials and Methods

Sources of seeds

- Seeds of cucumber cv. Hayel that used in this study were obtained kindly from the Horticultural Research Institute, Agricultural Research Center (ARC). Giza, Egypt.

Isolation and identification of *Rhizoctonia solani*.

Rhizoctonia solani was isolated from naturally infected root of cucumber plants showing typical symptoms of damping off and root rot which collected from different fields in different counts of Fayoum governorate. . The infected roots were thoroughly washed with running tap water, cut into small fragments (0.5 cm), surface disinfected with 5% sodium hypochlorite for 2 min, rinsed with sterile distilled water, dried between sterile filter paper, cultured on Potato Dextrose Agar medium (PDA) and incubated for 5-7 days at 25 ± 2 ^{o}C. Plates were examined daily for fungal growth. Pure cultures of the pathogen were obtained by using hyphal tip technique **Mahmoud and Abdallah. 2020.** Obtained isolate were maintained on PDA slants and kept at 5 ^{o}C for further study **(Cooke *et al.*, 2006)**. Their pathogenicity were previously confirmed and identified on the basis of cultural properties and microscopic morphological characters according to **Desvani et al., 2018,** and **Moni et al., 2016.**

Preparation of *Rhizoctonia solani* inocula:

Rhizoctonia solani was prepared by growing in 500 mL glass bottles contained (50 g washed sand, 50 g corn and enough tap water to cover the mixture). Autoclaved bottles, containing the medium, were inoculated with equal disks (0.5 cm) of seven days old *R. solani* culture and incubated for 15 days at 25 ± 2°C; during this period the bottles were vigorously shaken daily to encourage more rapid and ensure uniform distribution of the fungal growth thin added to soil within one week. **Atwa, 2016**.

Isolation and identification of bio-agents

Antagonistic bio-agents were isolated from the soil and rhizosphere of healthy cucumber plants. Isolation was carried out by serial dilution technique on Potato Dextrose Agar (PDA) medium. After an incubation period, fungal, bacterial and actinomyceties colonies were purified and identified according to their cultural, morphological and physiological characters. Fungal isolates were subjected to identification tests according to the methods stated by **(Domsch *et al.*, 1980)**. On the other hand, bacterial and actinomycetes isolates were identified based on **Bergey's Manual of Systematic Bacteriology 1984** using the methods recommended by (**Parry *et al.*, 1983**. Identification was confirmed through both of the Mycological Research and Disease Survey Department and Bacterial Disease Department, Plant Pathology Research Institute, ARC, Giza, Egypt as shown in Table, 1.

Table (1). Fungal, bacterial and actinomycetes isolates used as bioagents

Name of Isolates	Cods
Trichoderma .asperellum	(TA1)
Trichoderma .viride	(TV)
Trichoderma .harzianum	(TH1)
Trichoderma .hamatum	(TH2)
Trichoderma.album	(TA2)
Trichoderma harzianum	(TH3)
Bacillus subtilis	(B1)
Bacillus subtilis	(B2)
Bacillus subtilis	(B3)
Bacillus subtilis	(B4)
Pseudomonas fluorescens	(PF)
Streptomyces spp.	(1)
Streptomyces spp.	(2)

Antagonistic activity of Trichoderma isolates against growth of *Rhizoctonia solani in vitro*:

The dual culture technique was used to evaluate the six *Trichoderma* spp. (Table, 1) for their antagonistic activity against *R. solani* was grown on PDA plates for 7 days. the disks of mycelia (5 mm-diameter) from the antagonist bio agent and pathogen isolates put on plates containing 10 ml PDA medium interval 7 cm apart from each other and 1 cm from the edge of the plate. In control treatment, the plates were inoculated only with pathogenic fungi. These plates were incubated at 25±2 ^{o}C for five days. Three plates were used as replicates for each treatment. Measures were carried out daily until the meeting of the two mycelia and/or until one of the two fungi were overlaid by the other or mycelium growth covers the entire medium surface in control plates (untreated). The linear growth area of *R. solani* was measured to determine the most effective antagonistic isolate among the tested bio-agents for further studies in greenhouse. Percentages of the fungal growth reductions (R) were calculated using the following formula: **Fokkema 1973 and Singh et al., 2021.**

R = (G1- G2 / G1) x 100

Where: R = reduction of fungal growth (%).

G1= linear growth of the pathogen grown in control plate (cm).

G2= linear growth of the pathogen towards the tested bio-agent (cm)**.**

Antagonistic activity of bacteria isolates against growth of *R. solani in vitro*:

The antagonistic effect of five Bacteria isolates (*Bacillus subtilis* (B1, B2, B3 and B4), and *Pseudomonas fluorescens* (Pf1)) (table, 1) against *R. solani in vitro* was evaluated using the dual culture technique. Bacillus isolates was grown on nutrient broth agar medium for 2 days at 28 ± 2°C. Loop growth of each antagonistic bacterium was streaked individually in one side 1 cm apart from the plates edge contained PDA medium and incubated for 24 hrs. at 28 ^{o}C, thereafter the same plates were inoculated at the opposite side 1 cm apart of the plate edge with 9 mm disc from *R. solani* (7 day old). Petri dishes were inoculated with pathogen fungi only as control. Plates were incubated at 28±2 ^{o}C for 48-72 h and observed daily for the inhibition of the pathogen. Three plates used as replicates for each treatment. The organisms that showed the high antagonistic reaction were selected for further studies. Percentages of the fungal growth reductions (R) were calculated **Fokkema 1973** and **Dunlap et al., 2017.**

Antagonistic activity of Streptomyces isolates against growth of *R. solani in vitro*:

The antagonistic effect of two Streptomyces isolates (1 and 2) (table, 1) were tested against *R. solani*. Streptomyces isolates were grown on nutrient broth medium for two days at 28 ± 2°C. Loop growth of each antagonistic Streptomyces was streaked individually in the opposite side of inoculated *R. solani* isolate (disc, 5mm) on PDA plates. A disc (5 mm - diameter) of *R. solani*, separately, was inoculated at equal distance of the opposite side of Petri dish. Petri dishes were inoculated with pathogen fungi only as control. Three Petri dishes for each bio-agent, as well as the control, were used as replicates. The inoculated Petri dishes were incubated at 25 °C for 7 days. The inoculated plates were examined daily and then the linear growth area of *R. solani* was measured to determine the most effective antagonistic isolate among the tested bio-agents. Percentages of the fungal growth reductions (R) were calculated **Fokkema1973** and **Awad and Fayyadh. 2018.**

Green house experiments:

These experiments were conducted twice in experimental pots at two different locations vegetable diseases research dep. Plant Pathology Research institute, Agriculture Research center (ARC), Giza, Egypt and plant pathology Department, faculty of agriculture, Fayoum university at march, April, and may during 2020 growing seasons under greenhouse conditions. Cucumber cv. Hayel was used.

Effect of bioagent microorganisms on damping off and root rot disease incidence of cucumber under greenhouse conditions:

In this experiment, the most effective bioagents were chosen Based on the previous laboratory experiments to evaluate their efficiency in controlling damping off and root rot disease incidence. Bio efficacy of *T. asperellum*, *B. subtilis* and *P. fluorescens*, and Streptomyces (1) were evaluated against *R. solani* under greenhouse conditions as seed and seedling treatment. The tested antagonistic Trichoderma fungus was grown on potato dextrose broth medium in 500 ml flask and incubated at 25℃, for 11 days. Bacterial isolates were grown on nutrient broth medium and incubated at 30℃ for 3-4 days. Streptomyces isolate was grown on starch casein broth medium and incubated at 30℃ for 8 days. Different bio-agents were prepared as suspension. Suspensions were prepared by putting (transferring into) different bio-agents on a rotary shaker at 250 rpm and adjust using sterilized distilled water(SDW), to be containing (2.5×10^4 spore/ml) of Trichoderma,(2×10^6 cfu/ml) of bacteria, and (2×10^5 cfu/ml) of Streptomyces by using a haemocytometer slide. Fungicide Rhizolex using at a

recommended dose of 3g/l**. Ketta et al., 2021** and **Yao et al., 2021.**

Preparation of soil, pots and infestation:

Soil was sterilized using formalin solution (5%) and covered with a polyethylene sheet for ten days. It was then removed and left it exposed to the air for 5 days to remove the traces of formaldehyde fumes. Soil infestation was carried out by adding the fungal inoculum to the sowing media peat moss + vermiculite (3:1, w: w); at the rate of 5% of sowing media weight. Plastic pots (30 cm diameter) were sterilized by immersing in 5% formalin for 15 minutes and then air dried for 5 days. The sterilized soil was filled into pots at rate of 3 kg/pot. Sterilized seeds of cucumber (cv. Hayel) were immersed in 1.0% Arabic gum as sticker for 2 minutes, and then soaked in the spore suspension of *Trichoderma* spp., *Streptomyces* spp., *Bacillus subtilis* and *Pseudomonas fluorescens* for 3 hrs. (Seed coat treatments), then left to air dry before sowing. Coated seeds were sown in potted soil (30 cm- diam.) infested with *R. solani* at five seeds in each pot for 60 day of sowing. Inoculated pots with pathogen and untreated with bioagents served as check (1) (positive control) and inoculum free treatment was used as a check (2) (Negative control). Three replicates were used for each particular treatment. The seedlings were irrigated by bioagents as a concentration/treatment of spore suspension of Trichoderma, Streptomyces and bacteria, as seedlings treatment. Pots were regularly irrigated and received the recommended dose of N, P and K fertilizers. The percentage of pre-post-emergence damping off and root rot were calculated 15, 45 and 60 days after sowing respectively, according to **Qiu *et al.*, 2012.**

Disease assessment:

The pre-emergence damping-off and post-emergence damping off was determined by recording 15 and 45 days after sowing, then the percentage of survived plants were counted according to the following formula: (**Ketta *et al*., 2021).**

$$\textbf{Pre-emergence damping off (\%)} = \left(\frac{\textbf{Number of non germinated seeds}}{\textbf{Number of sown seeds}}\right) \textbf{x 100}$$

$$\textbf{Post-emergence damping off (\%)} = \left(\frac{\textbf{Number of dead seedlings}}{\textbf{Number of sown seeds}}\right) \textbf{x 100}$$

$$\textbf{Survival seedlings (\%)} = \left(\frac{\textbf{Number of survived seedling}}{\textbf{Number of sown seeds}}\right) \textbf{x 100}$$

Disease incidence (DI %) percentages for cucumber root rotted plants were recorded after 60 days of sowing for each individual location and calculated as described by **Mohammed *et al.*, (2020),** using the following formula:

$$\textbf{Disease incidence (DI) \%} = \left(\frac{\textbf{Number of infected plants}}{\textbf{Total number of examined plants}}\right) \textbf{x 100}$$

- Morphological parameters:

At both cultivation seasons, three plants were randomly taken from each treatment after (60 days) from transplanting. The plants were carefully uprooted from pots and roots/suckers were washed in running tap water to remove the adhering soil particles. Excess water was removed with blotting paper. The following vegetative growth and fruit yield parameters for all treatments and controls were receded: **Kamel et al., 2017**.

1. Average plant shoot and root lengths (cm) were measured from the cotyledonary node to the terminal bud of the main stem by using a ruler.
2. Average fresh weight/plant (g) was determined using a digital balance.
3. Average dry weight/plant (g). Samples were wrapped in a butter paper and dried in an electric oven at 65– 75 °C for 48-72 h or till constant weight and weighted using the digital balance.

4. Average plant product (kg) were measured by harvesting fruits at marketable size and weighted.

- Chemical analysis:

Estimation of total chlorophyll:

Total chlorophyll was quantified using the SPAD-501 portable leaf chlorophyll meter (Minolta Corp) for greenness measurements in the 5th apical fully expanded leaf. Samples from leaves at the ages of 40 and 50 days. Three plants from each treatment as replicates. **Akhtar and Azam., 2014** and **Kamel et al., 2017.**

Determination the activity of enzymes and total phenol:

Leaves of treated cucumber plants were taken 30 days after sowing. Leaf samples were ground with 0.2 M Tris HCl buffer (pH 7.8) containing 14 mM β-mercaptoethanol at the rate 1/3 w/v. The extracts were centrifuged at 10,000 rpm for 20 min at 4°C. The supernatant was used to determine enzyme activities. **jyothi et al., 2018.**

Determination of peroxidase (PO):

Peroxidase activity was determined according to the method described by **Hammerschmidt et al., 1982.** Peroxidase activity was expressed as the increase in absorbance at 425 nm/g fresh weight/minutes.

Determination of polyphenoloxidase (PPO):

The polyphenoloxidase activity was determined according to the method described by **Matta and Dimond., 1963**. Polyphenoloxidase activity was expressed as the increase in absorbance at 420nm/g fresh weigh/min.

Determination of Catalase (CAT):

Catalase enzyme activity was determined according to the procedure of **Aebi 1984**. A total reaction mixture of 3 ml, consisting 2400 µl phosphate buffer (50 mM), 100 µl of the enzyme extract and 500 µl H_2O_2 (10 mM) was used to measure enzyme activity. The reaction mixture absorption was recorded at 240 nm with a spectrophotometer twice with an interval of 2 min and the enzyme activity was calculated using an extinction coefficient of 0.28 mM^{-1} cm^{-1}).

Estimation of total phenols:

Changes in total phenols were determined after 40 days from planting. In seedling stage, Cucumber treated plants leaves were used. Total phenolic content (TPC) was determined as per **Bozarth and Diener., 1963 and Mofidnakhaei et al., 2016**. Samples of 2 g were homogenized in 80% aqueous ethanol at room temperature and centrifuged at 10000 rpm for 15 min. under cooling and the supernatants were saved. The residues were extracted in 80% ethanol. The supernatants were taken and evaporated to dryness at room temperature. Residues were dissolved in 5 mL distilled water. One hundred microliters of each extract was water diluted to 3 mL. The 0.5 mL of Folin-Ciocalteau reagent was added. After 3 min, 2 mL of 20% of sodium carbonate was mixed thoroughly to the extract. The developed color was spectrophotometrically measured at 650 nm. After 60 min, while catechol was used as a standard. The results were expressed as mg catechol/100 g fresh weight.

Statistical analyses:

Statistical analyses of the obtained data have been carried out according to the procedures (ANOVA) reported by **Snedecor and Cochran., 1967**. Treatment means were compared by the least significant difference test "L.S.D" at 5% level of probability.

Result and Discussion:

***In vitro* Antagonistic effect of *Trichoderma* spp. isolates on mycelial growth of *Rhioctonia solani* causing damping off and root rot of cucumber plants:**

The antagonistic effect of six *Trichoderma* spp. isolates against the growth of *R. solani*

isolate has been studied using dual-culture technique. The results are presented in Table (2) show that *T. asperellum* (TA1) was the best isolate where recording the lowest linear growth of rate (about 1.4 cm) and caused significantly increased the percentage of mycelium growth reduction (about 84.44%) followed by *T. harzianum* (TH1 and TH3) reduced the percentage of *R. solani* growth recording reduction reached (81.89 %) and have been over lapping growth covering the pathogenic growth.

Table (2): *In vitro* antagonistic effect of *Trichoderma* spp. isolates on mycelial growth of *R. solani*.

***Trichoderma* spp.**	**Linear growth (L.G)**	**Growth reduction G.R (%)**
T .asperellum(TA1)	1.40	84.44
T .viride(TV)	2.17	75.89
T .harzianum(TH1)	1.63	81.89
T .hamatum(TH2)	3.37	62.56
T. album(TA2)	2.87	68.11
T .harzianum(TH3)	1.63	81.89
control	9.00	0
$LSD_{at\ 0.05}$	0.57	------

While *T. hamatum (TH2)* and *T. album(TA2)* recording the high linear growth of rate (about 3.37 and 2.87 cm) and caused significantly increased the percentage of mycelium growth reduction (about 62.56 and 68.11%). There was no inhibition zone with hyphae contact in this dual-culture technique study, but the pathogenic growth was overlapping the growth of the *Trichoderma* spp. isolates. These results are in harmony with those reported by many researchers. **Mahmoud 2015** studied *in vitro* the antagonistic effect of different bioagents against *Fusarium solani* and *Rhizoctonia solani*. All bioagents significantly reduced mycelial growth of the pathogenic fungi. *T. harzianum* gave the most reduction effect on the pathogenic fungi. All bioagents were destructive the mycelial growth of the pathogenic fungi.

***In vitro* antagonistic effect of bacterial isolates on mycelial growth of *Rhioctonia solani*:**

The antagonistic potentiality of these bacterial (Four *Bacillus subtilis* and one *Pseudomonas fluorescens*)were tested for their ability to inhibit the growth of *R. solani* the causal agents of damping off and root rot diseases in cucumber plants using a dual-culture technique. Data presented in Table (3) show that all five bacterial isolates significantly decreased in the linear growth and increased the percentage of growth reduction for *R. solani* comparing with the control. *Bacillus subtilis* (B1) was the best isolate where recording the lowest linear growth of rate (about 2.50 cm) and caused significantly increased the percentage of mycelium growth reduction of *R. solani* by 72.22% and 0.73cm. Followed by *P. fluorescens* was more effective after *Bacillus subtilis* (B1) on the reduction of radial growth and increasing the inhibitory effect on pathogen than other isolates. While *Bacillus subtilis* (B4) recording the high linear growth of rate (about 3.50 cm) and caused significantly increased the percentage of mycelium growth reduction of *R. solani* by 61.11% and 0.43cm. Our five bacterial strains (B1, B2, P3, B4 and B5) which isolated from the rhizosphere of cucumber plants showed decreasing radial growth and increasing inhibitory effects against *R. solani in vitro*, this may be due to the production of anti-fungal compounds and/or secondary metabolites. These results are agreed with those obtained by **Ma et al., 2021** reported that the antifungal activity of

Bacillus subtilis v26 significantly inhibited *R. solani* growth compared to the untreated control. **Ruiz et al., 2014** reported that *Bacillus subtilis* cbck 36 and cbrf 24 were the most effective inhibitors of *Macrophomina phaseolina* where caused more than 60% inhibition of colony growth. **Santoyo et al., 2012 and Awais et al., 2010** found that *P. fluorescens* and *Bacillus subtilis* isolates inhibited hyphal growth most effectively for suppressing damping-off and root rot casual agents *in vitro*. There was a suggestion made that their biocontrol effect was associated with the production of enzymes, phenazines, pyrrole type antibiotics, pyo-compound indole derivatives peptide anti-biotic moenomycins, difficidin, and bacillaenes.

Table (3): *In vitro* antagonistic effect of bacterial isolates on mycelial growth of *R. solani*.

Bacteria	Linear growth (L.G.)	Growth reduction G.R.(%)
B.subtilis(B1)	2.50	72.22
B.subtilis(B2)	3.17	64.78
P. fluorescens(PF)	2.97	67.0
B.subtilis(B3)	3.00	66.67
B.subtilis(B4)	3.50	61.11
Control	9.00	0.00
LSD $_{\text{at 0.05}}$	0.25	--------

***In vitro* antagonistic effect of *Streptomyces* spp. strains on mycelial growth of *Rhioctonia solani*.**

The antagonistic potentiality of these ***Streptomyces* spp.** isolates were tested for their ability to inhibit the growth of *R. solani*, the causal agents of damping off and root rot diseases using a dual-culture technique. The data presented in Table (4) show that all ***Streptomyces* spp.** strains significantly decreased in the linear growth and the percentage of growth reduction for *R. solani* comparing with the control. ***Streptomyces* sp**. strain (St1) was the best recording the lowest linear growth of rate (about 3.67 cm) with *R. solani*. Two *Streptomyces* strains (St1 and St2) from the rhizosphere of cucumber plants showed decreasing radial growth and increasing inhibitory effects against *R. solani in vitro*. This may be due to the production of anti-fungal compounds and/or secondary metabolites secreted. According to several studies, Actinomycete strains can be used for biocontrol of damping off and root rot fungi, **Patil et al., 2011** stated that Actinomycetes have the ability to synthesise a wide variety of antagonistic active secondary metabolites and they have different modes of action against pathogenic fungi, including the production of secondary metabolites, antibiotics, pesticides, anti-parasitic compounds and enzymes like cellulose, xylanase, proteinase, and chitinase. **Wang et al., 2016** reported that *Streptomyces albospinus* CT205 show an inhibitory effect on the mycelial growth of *Fusarium oxysporum in vitro*. **Li et al., 2017** reported that five actinomycetes isolates (*Streptomyces globisporus* sub sp. *globisporus*, *S. globisporus*, *S. flavotricini*, *S. pactum* and *S. senoensis*) showed significant inhibitory effects on the mycelial growth of *Scelrotium rolfsii in vitro*. **Sadeghi et al., 2017** isolated 717 isolates of *Streptomyces* from rhizosphere of cucumber plants, out of which two isolates showed more than 70% inhibitory effect against *Phytophthora drechsleri* causing damping-off disease in cucumber. **Awad and Fayyadh., 2018** actinomyces isolate showed high antagonistic activity against *R. solani* where the inhibition zone reached 1.7 cm, whereas the inhibition zone for actinomyces isolates 24 and *S. griseus* was 1.2 cm against *Pythium* sp. for both isolates.

Table (4): *In vitro* antagonistic effect of *Streptomyces* isolates on mycelial growth of *R. solani*

Streptomyces	Linear growth L.G	Growth reduction G.R (%)
***Streptomyces* sp. (St1)**	3.67	59.22
***Streptomyces* sp. (St1)**	3.87	57.00
Control	9.00	0.00
LSD $_{\text{at 0.05}}$	0.52	--------

Greenhouse experiments:

1. Suppressive efficacy of bio-agents isolates on cucumber damping off and root rot disease incidence under greenhouse conditions during autumn growing season at (Fayoum and Giza):

The selected antagonistic bio-agents isolates, *i.e.* (*T. asperellum (Ta), Bacillus subtilis* (B1), *P. fluorescens* (Pf), *Streptomyces* sp. (St1)) which exhibited better antagonism *in vitro* screening experiment, were evaluated for their performance in plants against *R.solani*, as cucumber cv. hayel seed and seedling treatments under greenhouse conditions during autumn growing seasons at two different places (Fayoum and Giza) Governorates. The disease assessments were estimated as pre- at 15 days, post-emergence damping-off and the survival seedlings at 45 days after seed sowing, the root rot incidence percentage was determined 60 days after sowing. The data presented in **Table (5)** and illustrated in **Fig.(1)** Clearly demonstrate that all tested bio agents significantly reduced the percentage of cucumber damping off and root rot and increased plant survival, comparing with infested pots with pathogens (check1). In general, *Pseudomonas fluorescens* (Pf) treatment is considered the best treatment compared to other treatments. *P.fluorescens* recorded the lowest percentage of pre- and post emergence damping-off (20.00 and 13.33 %), respectively with *R. solani* in Fayoum and (53.33 and 27.78%), respectively in Giza experiment. Meanwhile, *P.fluorescens* recorded the high percentage of plant survival(66.67 and 33.33 %)in Fayoum and Giza. with root rot percentage recording (38.33 and 33.33%) in Fayoum and Giza experiment. Pseudomonas and Bacillus strains were a strong antagonistic effect that makes them excellent bio agents. It is possible that their active metabolites such as siderophore, hydrogen cyanide, indole acetic acid, and salicylic acid play an important role in the prevention of plant diseases. Besides their ability to promote plant growth, they also have a number of other benefits. These results are agreement with those obtained by **El-Mougy et al., 2012** studied the efficacy of *Bacillus subtilis* and *Pseudomonas fluorescens* as bioagents against some root rot fungi, including *Fusarium solani*, *F. oxysporium*, *R. solani*, *Sclerotium rolfsii*,. Cucumber, cantaloupe, tomato, and pepper are just a few of the vegetables that grow in plastic houses under protected cultivation. Also, Trichoderma may play a role in this study as an opportunistic secondary invader, spore producer, cell wall degrading enzyme, and antibiotic producer, Similar results were reported by **Muriungi et al., 2014** reported that the post emergence seedling damping off on seeds coated with *T. asperellum* was 24.07% while the control (non-coated) had 65.89% seedling mortality. The disease decreased by 41.82%. In addition, **Otadoh et al., 2011** and **El-Mohamedy et al., 2015** reported that *Rhizoctonia solani* causing root rot was reduced by 70.2, 68.4, and 63.6 %, respectively, and Fusarium root rot was reduced by 68.2, 65.8, and 70.4%, respectively, when seeds were coated with *T. harzianum* treatments. It is possible that the tested Streptomyces strain is a good bio check for seedling damping off and root rot

diseases of plants, due to their ability to synthesise antimicrobial substance. These results are agreement with those obtained by **Chaurasia et al., 2018**, **Zhang et al., 2020** and **Chen et al., 2021** reported that Plant pathogens can be controlled by actinomycetes, a well-known source of antibiotics, in addition to acting as a growth promoter. Moreover, **Yao et al., 2021** reported that *Streptomyces albidoflavus* strain was effective at controlling Rhizoctonia rot of cucumber in a pot experiment. Candidicidin isomers are synthesised by *S. albidoflavus*, indicating that they are critical for antifungal and biocontrol activity.

Table (5): Efficacy of antagonistic bio agents isolates against damping off and root rot disease incidence on cucumber cv. Hayel under greenhouse conditions at Fayoum and Giza Governorates.

Bioagents	Fayoum experiment				Giza experiment			
	Damping off (%)		Survival seedlings (%)	Root rot (%)	Damping off (%)		Survival seedlings (%)	Root rot (%)
	Pre-emergence	Post-emergence			Pre-emergence	Post-emergence		
T.asperellum (Ta)	80.00	20.00	0.00	0.00	80.00	33.33	13.33	66.67
***B. subtilis* (B1)**	80.00	20.00	0.00	0.00	86.67	33.33	6.67	33.33
P. fluorescens (Pf)	20.00	13.33	66.67	38.33	53.33	27.78	33.33	33.33
***Streptomyces sp.* (St1)**	60.00	33.33	6.67	91.67	73.33	33.33	13.33	66.67
Rhizolex- T	6.67	6.67	86.67	33.33	46.67	16.67	40.00	16.67
Control (without infection)	0.00	0.00	100.00	0.00	0.00	0.00	100.00	0.00
Control(infested)	86.67	13.33	0.00	0.00	100.00	0.00	0.00	0.00
LSD $_{at\ 0.05}$	27.63	29.57	13.45	39.65	26.52	57.87	20.54	81.59

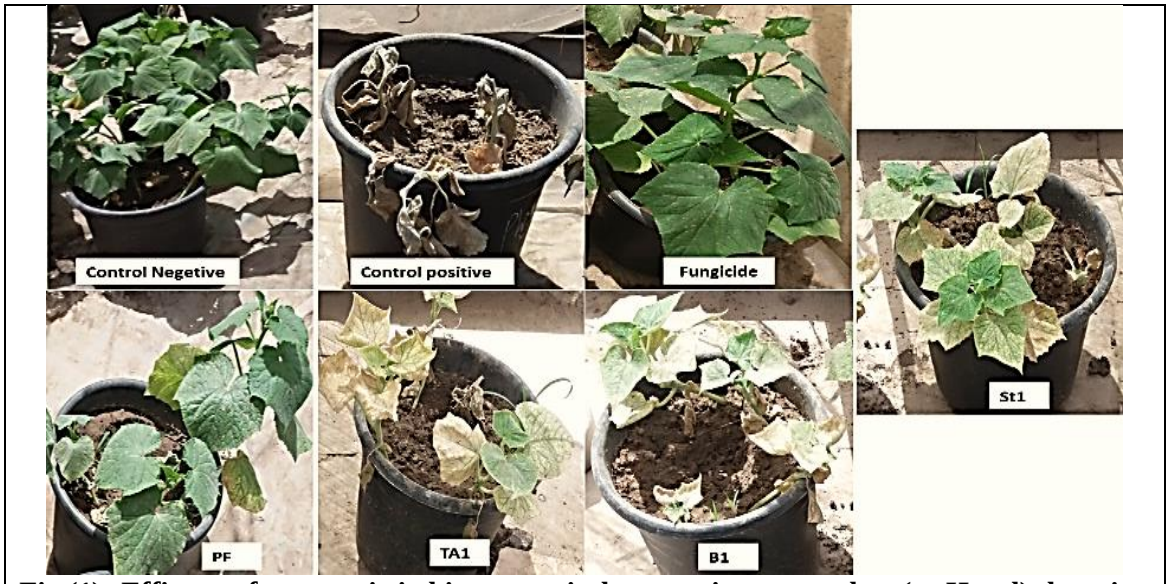

Fig.(1): Efficacy of antagonistic bio agents isolates against cucumber (cv.Hayel) damping off and root rot disease incidence, under greenhouse conditions.

Morphological parameters and total chlorophyll:

Table (6): Effect of cucumber seed soaking and as soil drench in bioagent on some growth parameter in cucumber plants under greenhouse conditions at Fayoum and Giza governorates.

Treatment	Fayoum experiment					Giza experiment					
	Plant high (cm)		Fresh weight (gm)	Dry weight (gm)	Plant product (kg)	Plant high (cm)		Fresh weight (gm)	Dry weight (gm)	Plant product (kg)	Total chlorophyll
	root	shoot				root	shoot				
T.asperellum (Ta)	0.0	0.00	0.00	0.0	0.00	1.00	2.67	1.00	0.20	0.02	33.40
***B. subtilis* (B1)**	0.0	0.00	0.00	0.00	0.00	6.50	43.00	1.50	0.50	0.08	33.20
P. fluorescens (Pf)	8.30	9.50	64.0	16.30	0.60	3.20	15.0	8.00	2.67	0.08	34.30
***Streptomyces sp.*(St1)**	5.00	2.50	2.00	0.40	0.09	4.30	9.3	1.50	0.33	0.07	31.40
Rhizolex	10.5	7.20	20.0	5.00	0.27	3.8 0	6.67	3.00	0.67	0.21	31.40
Control Negetive	8.30	9.00	63.3	15.3	0.51	8.5	9.00	63.30	16.67	0.51	22.40
Control positive	3.00	1.50	1.5	0.3	0.07	1.0	6.50	1.20	0.33	0.07	22.50
LSD $_{at\ 0.05}$	7.22	12.51	11.48	3.18	0.13	10.75	36.24	7.012	2.296	0.182	3.33

The results in Table (6) reveal that, all treatments significantly increase in plant high compared with positive control treatment in the individual experiments.

The highest value in plant hight (root and shoot) was obtained in plants treated with *P. fluorescens* 8.33 and 9.50, respectively, in Fayoum experiment. While, *B. subtillis* treatment recorded the highest value in plant high (root and shoot) 6.50 and 43.0%, respectively, in Giza experiment. All treatments significantly increase in Fresh, dry weight and plant product compared with positive control treatment. The highest value in fresh, dry weight and plant product were obtained in plants treated with *P. fluorescens* 64.00, 16.33 gm and 0.6 kg and 8.00, 2.67 gm and 0.08kg, respectively, in Fayoum and Giza experiment. Also, All treatments significantly increase in total chlorophyll (SPAD) compared with positive control treatment. The highest value in total chlorophyll was obtained in plants treated with *P. fluorescens* 34.30 SPAD, respectively, in Fayoum experiment. The results showed the positive effect of different treatments on controlling damping off and root rot disease in cucumber and significant increases in growth parameters and morphological characteristics of cucumber plants. The results showed the positive effect of different treatments on controlling damping off and root rot disease in cucumber and increasing the various growth processes such as chlorophyll formation. These results are in full agreement with those obtained by **Akhtar and Azam., 2014** studied under greenhouse conditions, the effects of plant growth-promoting rhizobacteria (PGPR) and antagonistic fungi on the growth and chlorophyll content of Fusarium root-rot of pea caused by Fusarium solani f. sp. Pisi were studied. The use of PGPR and antagonistic fungi resulted in a significant increase in both root-rot fungus inoculated and un-inoculated pea plant growth and chlorophyll content. When *B.*

pumilus was combined with *P. putida*, it resulted in the largest increase in growth and chlorophyll of root-rot fungus-inoculated plants and the least degree of disease.

Determination of enzymes activity and total phenol:

The results in **Table (7)** reveal that, all treatments significantly increased enzymes activity compared with positive control treatment. The highest activity of peroxidase and catalase was induced after 40 days by *P. fluorescens* 48.54 and 39.91%, respectively, in Fayoum experiment. While, *Trichoderma asperellum* recorded the highest activity of polyphenoloxidase about 81.25 %. Whereas, the highest increase in the total phenols was induced after 40 days by *S. scabies* (st1) about 15.76%. These results show that the increase in enzyme activity plays a major role in the resistance of plants to diseases and has certainly given plants the capacity to protect themselves against infection and the development of diseases by: increasing host resistance by stimulating host defence mechanisms; preventing the extent of fungal growth in plant tissues; penetrating microorganisms; and causing significant damage to cell metabolisms. In this respect, the present results concerning the increase in peroxidase, catalase, polyphenoloxidase enzyme activity and total phenol are in agreement with results reported by researchers. **(Jyothi *et al.*, 2018)** reported that the maximum level of activity was recorded as comparison to the susceptible genotype GNG 2228 and extremely susceptible genotype L550 with moderate chickpea's resistance viz., Phule G 12107, NDG 13-21 and IPC 2010-112. The most highly enzyme activity in moderately chickpea-resistant genotyls was compared to the sensitive genotype and highly susceptible genotype.

Table (7): Effect of cucumber seed soaking and as soil drench in bioagents on enzymes and phenol activities in cucumber plants under greenhouse at Fayoum.

Treatment	Enzyme activity peroxidase activity		catalase activity		Poly phenol oxidase activity		Total phenol	
	Activity	% Efficacy	Activity	%Efficacy	Activity	% Efficacy	Activity	% Efficacy
T.asperellum (Ta)	0.901	43.17	21.9	37.44	0.112	81.25	0.183	15.30
***B. subtilis* (B1)**	0.943	45.71	22.4	38.84	0.103	79.61	0.182	14.84
P. fluorescens (Pf)	0.995	48.54	22.8	39.91	0.100	79	0.182	14.84
***Streptomyces sp.* (St1)**	0.987	48.13	21.4	35.98	0.098	78.57	0.184	15.76
Rhizolex	0.752	31.91	22.2	38.29	0.043	51.16	0.166	6.63
Control Negetive	0.512	0.00	13.7	0.00	0.021	0.00	0.155	0.00
Control positive	0.478	-7.11	13.7	0.00	0.024	12.5	0.114	-35.96
LSD $_{at\ 0.05}$	0.03	2.80	0.00	1.74	0.00	1.34	0.00	2.80

Akhtar and Azam., 2014 studied effects on Catalase and Peroxidase activity on the Fusarium root of Pea induced by *Fusarium solani* f. sp. *Pisi*, under greenhouse conditions, of plant growth-promoting Rhizobacteria (PGPR) (*Bacillus pumilus* *and Pseudomonas putida*) and Antagonistic Fungi (*Aspergillus awamori*, *Aspergillus niger* and *Trichoderma harzianum*), Increased catalase and peroxidase in the inoculate and uninoculated pea plants were the results of PGPR and antagonistic fungi.

When used as compared to other tested combinations, *A. awamori* or *B. pumilus* with *P. putida* were achieved in the largest increase in catalases and peroxidase activity for the root-rot fungus-inoculated plants and the reduction of disease severity. **Mofidnakhaei et al., 2016** reported that higher antioxidant induction (2.2, 2.8 and 4 fold increase respectively in superoxide dismutase, catalase and peroxidase) could have reduced symptoms of damping in cucumber plants, leading to increased plant growth and yield. **Akbari-Moghadam et al., 2015** Studied the effect of various *Pseudomonas fluorescence* strains on cucumber-root rot disease causing *Pythium aphanidermatum*. After nine days of inoculation showed the most increasing in peroxidase activity, total phenol and polyphenoloxidase and then declining. In combination with bacteria and fungi treatments, the rate of increasing in peroxidase activity, phenolic content and polyphenol oxidase was significant compared to the control treatment. **Adhilakshmi et al., 2014** reported that for their biocontrol activity, three strains of *Streptomyces* sp. (CBE, MDU, and PDK) were tested, which showed higher levels of inhibition of growth of *M. phaseolina* in dual culture assay and plant growth-promoting activity against root rot disease of mung bean (Vigna radiata L.) under greenhouse and field conditions. Isozyme analysis of *Streptomyces* sp. treated plants shows that seed treatment with soil application substantially induces peroxidase (PO-1 and PO-2) and polyphenol oxidase (PPO-2 and PPO-3) activity in mung bean. Among the three strains examined, *Streptomyces* sp. strain MDU-treated plants had greater levels of PO and PPO activity. **Yousef et al., 2013** reported that cucumber induced resistance was related with an increase in total phenol content. As a result, the incidence of damping-off and root rot disease caused by *R. solani* is reduced. **Chen et al., 2010** reported that Plants treated with B 579 have considerably enhanced the activity of a plant defence-related enzyme, peroxidase, polyphenoloxidase (PPO) and phenylalanine ammonia-lyase (PAL). In B579 treated plants it was interestingly found that the increased IAA, an important plant growth regulator, was found. In addition, seed-soaking with B579 showed enhanced biological control effects and promoting ability for plant growth.

Conclusions: results suggest that using the application bio-agents for controlling the diseases in greenhouse can be an attractive alternative for pesticides in organic agriculture with addition improved plant growth and increased yield components.

References:

Adhilakshmi, M., Paranidharan, V., Balachandar, D., Ganesamurthy, K., and Velazhahan, R., 2014. Suppression of root rot of mung bean (*Vigna radiata* L.) by *Streptomyces* sp. is associated with induction of peroxidase and polyphenoloxidase. *Archives of Phytopathology and Plant Protection*, *47*(5):571-583.

Aebi, H. 1984. Catalase *in vitro*. Methods in enzymology, 105: 121-126.

Akbari-Moghadam, E., Saberi-Riseh, R., Khodaygan, P., and Alaei, H., 2015. Evaluate the control ability of Pseudomonas fluorescens strains on cucumber root rot disease. Biological Control Of Pests And Plant Diseases, 4(2):77-88.

Akhtar, M. S., and Azam, T., 2014. Effects of PGPR and antagonistic fungi on the growth, enzyme activity and fusarium root-rot of pea. Archives of

Phytopathology and Plant Protection, 47(2):138-148.

Al-Fadhal, F. A., AL-Abedy, A. N., and Alkhafije, D. A., 2019. Isolation and molecular identification of *Rhizoctonia solani* and *Fusarium solani* isolated from cucumber (*Cucumis sativus* L.) and their control feasibility by *Pseudomonas fluorescens* and *Bacillus subtilis*. Egyptian Journal of Biological Pest Control, 29(1):1-11.

Aljawasim, D. B., Khaeim, M. H., and Manshood, A. M., 2020. Assessment of arbuscular mycorrhizal fungi (*Glomus* spp.) as potential biocontrol agents against damping-off disease *Rhizoctonia solani* on cucumber. Journal of Crop Protection, 9(1):141-147.

Atwa, M. A. 2016. Induction of Resistance Against Damping-off and Root Rot Diseases In Faba Bean. Arab Universities Journal of Agricultural Sciences, 24(2), 555-578**.**

Awad, L. K., and Fayyadh, M. A., 2018. The activity of some Actinomycetes isolates in control of cucumber damping off disease caused by *Rhizoctiona solani* and *Pythium* sp. Basrah journal of Agricultural sciences, 31(2):11-23.

Awais, M., Pervez, A., Yaqub, A., and Shah, M. M., 2010. Production of antimicrobial metabolites by *Bacillus subtilis* immobilized in polyacrylamide gel. Pakistan Journal of Zoology, 42(3): 2-267

Bergeys Manual of systematic Bacteriology 1994. williams and wilkins, baltimore, USA. Vol. 1 krieg, N. NMR(ED.): Ordinary gram negative bacteria. Vol.2. Smeath, PHA (Ed.): Ordinary gram positive bacteria.

Bozarth, R. F., and Diener, T. O., 1963. Changes in concentration of free amino acids and amides induced in tobacco plants by potato virus X and potato virus Y. Virology, 21(2): 188-193.

Chaurasia, A., Meena, B. R., Tripathi, A. N., Pandey, K. K., Rai, A. B., and Singh, B., 2018. Actinomycetes: an unexplored microorganisms for plant growth promotion and biocontrol in vegetable crops. World Journal of Microbiology and Biotechnology, 34(9):1-16.

Chen, F., Wang, M., Zheng, Y., Luo, J., Yang, X., and Wang, X., 2010. Quantitative changes of plant defense enzymes and phytohormone in biocontrol of cucumber Fusarium wilt by *Bacillus subtilis* B579. World Journal of Microbiology and Biotechnology, 26(4): 675-684.

Chen, Q., Bai, S., Zhang, T., Duan, C., Zhao, J., Xue, Q., & Li, Y., (2021). Effects of Seed-Coating Preparations of Living Streptomyces globisporus on Plant Growth Promotion and Disease Control against Verticillium Wilt in Cotton. *Sustainability*, *13*(11):10-60

Cooke, B.M., D.G. Jones and B. Kaye 2006. The Epidemiology of Plant Diseases. Second Edition. Published by Springer, P.O. Box 17, 3300 AA Dordrecht, The Netherlands.

Desvani, S. D., Lestari, I. B., Wibowo, H. R., Supyani, Poromarto, S. H., and Hadiwiyono. 2018. Morphological characteristics and virulence of *Rhizoctonia solani* isolates collected from some rice production areas in some districts of Central Java. In AIP Conference Proceedings. AIP Publishing LLC, 2014(1):20-68.

Domsch, K. H., Gams, W., & Anderson, T. H., 1980. Compendium of soil fungi. Volume 1. Academic Press (London) Ltd.

Dunlap, C. A., Lueschow, S., Carrillo, D., and Rooney, A. P., 2017. Screening of bacteria for antagonistic activity against phytopathogens of avocados. Plant Gene, 11: 17-22.

El-Mohamedy, R. S., Shafeek, M. R., and Fatma, A. R., 2015. Management of root rot diseases and improvement growth and yield of green bean plants using plant resistance inducers and biological seed

treatments. Journal of Agricultural Technology, 11(5):1219-1234.

El-Mougy, N. S., Abdel-Kader, M. M., Aly, M. D. E., and Lashin, S. M., 2012. Application of fungicides alternatives as seed treatment for controlling root rot of some vegetables in pot experiments. Advances in Life Sciences, 2(3):57-64.

Fasusi, O. A., Cruz, C., and Babalola, O. O., 2021. Agricultural sustainability: Microbial biofertilizers in rhizosphere management. Agriculture, 11(2):163.

Fokkema, N. J., 1973. The role of saprophytic fungi in antagonism against *Drechslera sorokiniana* (*Helminthosporium sativum*) on agar plates and on rye leaves with pollen. Physiological Plant Pathology, 3(2): 195-205.

Hammerschmidt, R.; Nuckles, E. and Kuć, J., 1982. Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*. Physiol. Plant Pathol. 20: 73-82.

Jyothi, V., Saifulla, M., and Teli, K., 2018. Biochemical analysis of chickpea genotypes against dry root rots (*Macrophomina phaseolina* (Tassi) Goid). Journal of Pharmacognosy and Phytochemistry, 1:1895-1901.

Kamel, S. M., Ismail, A., Omara, R., and Ahmed, M., 2017. Influence of Humate Substances and Fungicides on the Control of Onion Downy Mildew. Egyptian Journal of Phytopathology, 45(1): 31-44.

Kazerooni, E. A., Rethinasamy, V., and Al-Sadi, A. M., 2019. *Talaromyces pinophilus* inhibits *Pythium* and *Rhizoctonia*-induced damping-off of cucumber. Journal of Plant Pathology, 101(2): 377-383.

Ketta, H. A., Elkhateeb, N. M., Saleh, M. M., and Kamel, S. M., 2021. Efficiency assessment of combinations between *Rhizobium leguminosarum* and *Trichoderma* spp. for controlling of pea (*Pisum sativum* L.) damping-off disease. Egyptian Journal of Phytopathology, 49(1): 1-14.

Li, Y., He, F., Lai, H., and Xue, Q., 2017. Mechanism of *in vitro* antagonism of phytopathogenic *Scelrotium rolfsii* by actinomycetes. European Journal of Plant Pathology, 149(2): 299-311.

Ma, Q., Cong, Y., Feng, L., Liu, C., Yang, W., Xin, Y., and Chen, K., 2021. Effects of mixed culture fermentation of *Bacillus amyloliquefaciens* and *Trichoderma longibrachiatum* on its constituent strains and the biocontrol of tomato Fusarium wilt. Journal of Applied Microbiology.

Mahmoud, A. F., and Abdalla, O. A., 2020. Biological control of fungi associated with damping-off and root rot disease of cucumber (*Cucumis sativus* L.). Archives of Phytopathology and Plant Protection, 1-16.

Mahmoud, M. A., 2015. Efficiency of some Bioagents and Nemastop compound in controlling damping off and root rot diseases on peanut plants. Int. J. of Adv. Res. in Biological Sciences, 2(11):77-86.

Matta, A., and Dimond, A. E., 1963. Symptoms of Fusarium wilt in relation to quantity of fungus and enzyme activity in tomato stems. Phytopathology, 53(5):574.

Mofidnakhaei, M., Abdossi, V., Dehestani, A., Pirdashti, H., and Babaeizad, V., 2016. Potassium phosphite affects growth, antioxidant enzymes activity and alleviates disease damage in cucumber plants inoculated with *Pythium ultimum*. Archives of Phytopathology and Plant Protection, 49(9-10):207-221.

Mohammed, A. F., Oloyede, A. R., and Odeseye, A. O., 2020. Biological control of bacterial wilt of tomato caused by *Ralstonia solanacearum* using *Pseudomonas* species isolated from the rhizosphere of tomato plants. Archives of Phytopathology and Plant Protection, 53(1-2):1-16.

Moni, Z. R., Ali, M. A., Alam, M. S., Rahman, M. A., Bhuiyan, M. R., Mian,

M. S., and Khan, M. A. I., 2016. Morphological and genetical variability among *Rhizoctonia solani* isolates causing sheath blight disease of rice. Rice science, 23(1): 42-50.

Muriungi, S. J., Mutitu, E. W., and Muthomi, J. W., 2014. Efficacy of cultural methods in the control of Rhizoctonia solani strains causing tomato damping off in Kenya. African Journal of Food, Agriculture, Nutrition and Development, 14(2):124-147.

Otadoh, J. A., Okoth, S. A., Ochanda, J., and Kahindi, J. P., 2011. Assessment of Trichoderma isolates for virulence efficacy on *Fusarium oxysporum* f. sp. *phaseoli*. Tropical and subtropical agroecosystems, 13(1): 99-107.

Parry, J. M., Turnbull, P. C. B., and Gibson, J. R., 1983. *A colour atlas of Bacillus species*. Wolfe Medical Publications Ltd.

Patil, H. J., Srivastava, A. K., Singh, D. P., Chaudhari, B. L., and Arora, D. K., 2011. Actinomycetes mediated biochemical responses in tomato (*Solanum lycopersicum*) enhances bioprotection against *Rhizoctonia solani*. Crop protection, 30(10):1269-1273.

Qiu, M., Zhang, R., Xue, C., Zhang, S., Li, S., Zhang, N., and Shen, Q., 2012. Application of bio-organic fertilizer can control Fusarium wilt of cucumber plants by regulating microbial community of rhizosphere soil. Biology and Fertility of Soils, 48(7):807-816.

Ruiz, S. E., Cristóbal, A. J., Reyes, R. A., Tun, S. J., García, R. A., and Pacheco, A. J., 2014. *In vitro* antagonistic activity of *Bacillus subtilis* strains isolated from soils of the Yucatan Peninsula against *Macrophomina phaseolina* and *Meloidogyne incognita*. Phyton, International Journal of Experimental Botany, 83(1): 45-47.

Sabbagh, S. K., Roudini, M., and Panjehkeh, N., 2017. Systemic resistance induced by *Trichoderma harzianum* and *Glomus mossea* on cucumber damping-off disease caused by *Phytophthora melonis*. Archives of Phytopathology and Plant Protection, 50(7-8): 375-388.

Sadeghi, A., Koobaz, P., Azimi, H., Karimi, E., and Akbari, A. R., 2017. Plant growth promotion and suppression of *Phytophthora drechsleri* damping-off in cucumber by cellulase-producing Streptomyces. BioControl, 62(6):805-819.

Santoyo, G., Orozco-Mosqueda, M. D. C., and Govindappa, M., 2012. Mechanisms of biocontrol and plant growth-promoting activity in soil bacterial species of *Bacillus* and *Pseudomonas*: a review. Biocontrol Science and Technology, 22(8):855-872.

Singh, S., and Balodi, R., 2021. Bio-management of soil borne pathogens infesting cucumber (*Cucumis sativus* L.) under protected cultivation system. Biological Control, 157(10):45-69.

Singh, S., Balodi, R., Meena, P. N., and Singhal, S. 2021. Biocontrol activity of *Trichoderma harzianum, Bacillus subtilis* and *Pseudomonas fluorescens* against *Meloidogyne incognita, Fusarium oxysporum* and *Rhizoctonia solani*. Indian Phytopathology, 1-12.

Snedecor, G. W., and Cochran, W. G., 1967. Statistical methods, 6th Ed. Ames, lowa: Iowa state university press.

Wang, S., Liang, Y., Shen, T., Yang, H., and Shen, B., 2016. Biological characteristics of Streptomyces albospinus CT205 and its biocontrol potential against cucumber Fusarium wilt. Biocontrol Science and Technology, 26(7): 951-963.

Wang, Z., Zhang, J., Wu, F., and Zhou, X., 2018. Changes in rhizosphere microbial communities in potted cucumber seedlings treated with syringic acid. PLoS One, 13(6): 7-20.

Yao, X., Zhang, Z., Huang, J., Wei, S., Sun, X., Chen, Y., and Li, S., 2021. Production of Candicidin Isomers Is Essential for Biocontrol of Cucumber Rhizoctonia rot By *Streptomyces*

albidoflavus W68. Applied and Environmental Microbiology.

Yousef, G. G., Brown, A. F., Funakoshi, Y., Mbeunkui, F., Grace, M. H., Ballington, J. R., and Lila, M. A., 2013. Efficient quantification of the health-relevant anthocyanin and phenolic acid profiles in commercial cultivars and breeding selections of blueberries (Vaccinium spp.). *Journal of Agricultural and Food Chemistry*, *61*(20): 4806-4815.

Zhang, D., Lu, Y., Chen, H., Wu, C., Zhang, H., Chen, L., and Chen, X., 2020. Antifungal peptides produced by actinomycetes and their biological activities against plant diseases. The Journal of antibiotics, 73(5): 265-282.

الملخص العربى

المكافحه الحيويه لمرض موت البادرات واعفان الجذور المتسبب بواسطه فطر ريزوكتونيا سولانى على نباتات الخيار

محمد احمد على حسن[١]، هدى محمد حسين احمد[١]، سعيد محمد كامل[٢]، ولاء فتحى مصطفى عبدالحميد[٢]، هبه محمد احمد يوسف[١]

١- قسم النبات - كليه الزراعه - جامعه الفيوم – مصر

٢ معهد بحوث امراض النبات – مركز البحوث الزراعيه – جيزة - مصر

يعتبر موت البادرات واعفان الجذور من الأمراض الخطيرة التي تصيب الخيار في كل من مرحله البادرات والنباتات الكبيرة تحت الزراعة المحمية ، مما يؤدي إلى خسائر فادحة في محصول الخيار. تم اختبارسته انواع من الترايكودرما وخمسه انواع من البكتريا ونوعان من الاكتينوميسس في المكافحة الحيويه لمرض اعفان الجذور وموت البادرات المتسبب عن فطرالريزوكتونيا سولانى فى الخيار وتشجيع نمو نباتات الخيار وذلك فى تجارب على مستوى المعمل وفى البيوت المحميه.

على مستوى الصوبه تم اختبار افضل المعاملات الحيويه الناتجه من تجارب المعمل وهى (*T. asperellum*, *B. subtilis*, *P. fluorescens* and *Streptomyces spp1*) على نسبه ما قبل الانبات ومابعد الظهور ونسبه النباتات المتبقيه السليمه وكانت النتائج ان كل المعاملات خفضت بشكل كبير من شدة المرض وموت البادرات فى كلا من تجربتى الجيزة والفيوم تحت ظروف الصوبه. كما أدت ايضا هذه المعاملات إلى زيادة معنوية في معاملات النمو ومكونات المحصول (الوزن الرطب والجاف للجذور والمجموع الخضرى بالإضافة إلى ارتفاع النبات والمنتج النباتي) مقارنة بمعاملة المقارنة. كما سببت زيادة كبيرة في نشاط انزيم البيروكسيديز ، بوليفينول أوكسيديز ، إنزيم الكاتاليز ومحتوى النباتات المعامله من الفينول الكلي والتي تلعب دورًا مهمًا في آليات دفاع النبات ضد العدوى بمسببات الأمراض. أثبتت هذه النتائج أن العامل الحيوى *Pseudomonas fluorescens* هى الافضل فى مقاومه مرض موت البادرات واعفان الجذور وفى تحسين النمو الخضرى وزياده محصول الخيار ويمكن استخدامها كبديل للمبيدات.

الكلمات المفتاحية : العوامل الحيوية، الخيار، موت البادرات واعفان الجذور، فطر الريزوكتونيا، النشاط الأنزيمي.