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Effectiveness of Selected Biological Agents, Chemical Inducers, and Fungicides in Managing Cucumber Root Rot Disease caused by *Rhizoctonia solani*

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ABSTRACT

Cucumber is regrettably greatly impacted by a variety of diseases in the field, which results in significant yield losses. Cucumber root rot disease, which is brought on by the pathogenic soil-borne fungus *Fusarium oxysporum*, *Rhizoctonia solani*, *Fusarium solani*, and negatively impacts cucumber production in Egypt. The Experimental Farm of the Faculty of Agriculture at Benha University served as the site for this experiment. Five biological agents were examined for their antagonistic effects on the growth of R. solani (RS1) in vitro, including the fungi *Trichoderma harzianum*, *T. viride*, and *T. album*, and the bacteria *Pseudomonas fluorescens* and *Bacillus subtilis*. For preventing Rhizoctonia root rot disease, root dipping treatments including salicylic acid (SA), ascorbic acid (As), calcium chloride (CaCl2), and dipotassium hydrogen phosphate (K2HPO4) at an 8 mM concentration were tried. When compared with the control, *R. solani* (RS1)'s growth was inhibited by all the biocontrol agents in use. In terms of growth reduction percentage, *Trichoderma viride* was the most effective antagonistic fungus (50.33%), followed by *Trichoderma album* (40.77%). Every tested fungicide increased total, conjugated, and free phenols as well as peroxidase, polyphenoloxidase and chitinase. This study determines the effectiveness of bioagents, chemical inducers, and fungicides in controlling R. solani caused cucumber root rot disease.

Key words: Cucumber, root rot, Rhizoctonia, enzymes, phenols.

INTRODUCTION

One of the most significant vegetable crops in the world, cucumber (*Cucumis sativus* L.), is a member of the Cucurbitaceae family. Either in an open field or beneath covered structures, cucumbers are grown. Because they contain flavour and critical nutrients, including vitamins and minerals, particularly potassium and magnesium, fresh cucumber fruits are crucial for human nutrition and health. Additionally, according to Arul *et al*. (1994), it is a significant source of fibre, complex carbohydrates, antioxidants and anticarcinogenic compounds. One of the most significant vegetable crops in the world, cucumber, is regrettably plagued by numerous diseases in the field, which results in significant yield losses (Mohammed and Hasan 2018). In Egypt, cucumber production is severely affected by root rot disease caused by various pathogenic fungi present in the soil. Common cucumber root rot disease culprits include *Fusarium oxysporum*, *Fusarium solani*, *Rhizoctonia solani*, *Sclerotium rolfsii*, *Macrophomina phaseolina*, and *Sclerotinia* spp. (Elwakil *et al*., 2015). Multiple management strategies have been

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employed to combat the detrimental effects of Rhizoctonia root rot disease in cucumber. One of the most established methods is the use of fungicides. However, the application of fungicides poses environmental pollution risks, leaves harmful residues, and might aid in the emergence of resistant pathogens (Vinale *et al*., 2008). As a result, many strategies have been investigated, such as using resistant cucumber cultivars (Borrelli *et al*., 2018), plant extracts (Han *et al*., 2018) and chemical control techniques (Karim *et al*., 2018). In addition, microbial adversaries such *Trichoderma harzianum*, *Trichoderma koningi*, *Trichoderma viride*, *Gliocladium* species, *Streptomyces* species and *Bacillus* species have shown promise in controlling plant pathogens (Minuto *et al*., 2006 and khalifa *et al*.,2019).

In recent studies, microbial species such as *Pseudomonas fluorescens* and *Bacillus subtilis* have shown effective capabilities in managing plant pathogens (David *et al*., 2018). These biocontrol agents utilize various mechanisms, including fungistatic, antibiosis, hyperpara-sitism, induced systemic resistance and modification of the rhizosphere, to combat plant diseases (Elsharkawy *et al*., 2012). A promising disease control strategy involves inducing plant resistance to counteract pathogen infections. It is possible to achieve induced resistance by applying abiotic agents known as chemical inducers, such as salicylic acid, potassium salts, and sorbic acid (Akram and Anjum, 2011 and Awad,2016). This study aimed to assess the efficacy of specific bioagents, chemical inducers and fungicides in managing cucumber root rot disease caused by *R. solani*. Additionally, the study sought to examine the effects of these treatments on plant defense-related enzyme activities and phenol content.

Materials and Methods

1. Root-rot fungi infecting cucumber plants: isolation and identification

Three governorates in Egypt, El-Behaira, Kafr El-Sheikh, and Minufiya, collected infected cucumber plants exhibiting symptoms of root rot disease from open fields. For further examination, isolation tests were performed on the gathered samples. The diseased roots were carefully washed with tap water, dried by air drying, and then chopped into little pieces to begin the isolation procedure. For three minutes, a 0.5% sodium hypochlorite solution was used to surface sterilize these root portions. The sterilized root pieces were then dried between sterile filter sheets after being rinsed numerous times with sterilized distilled water. Four sections of each of the sterilized root segments were then placed on Petri dishes with Potato Dextrose Agar (PDA) medium. The Petri dishes were incubated for 5-7 days at a temperature of 25±2°C while being periodically inspected. After *R.*

solani's mycelial growth had matured, it was transferred to brand-new PDA plates using the hyphal tip method (Brown, 1924) and cultured there for seven days. The resulting pure cultures were kept at 4°C until additional examination. Following the recommendations provided by Barnett and Hunter (1987), the isolated fungi were identified using cultural, morphological, and microscopic characteristics.

2. Inoculate preparation and soil inoculation

Rhizoctonia solani inoculum was made with autoclaved sand barley medium at a 1:3 ratio (Abd-El-Moneem, 1996). In this regard, to prepare the inoculum, 25 g of clean sand, 75 g of barley, 2 g of sucrose, 0.1 g of yeast, and 100 mL of water were combined at a rate of 1:3 and placed in 500 mL glass bottles. The bottles were then autoclaved at 121°C for 20 minutes on two separate days. Before incubation at room temperature (25±2°C) for 14 days, prepared bottles of sand barley grain media were inoculated with fungal discs (5 mm) of the tested *Rhizoctonia* isolate. To inoculate the soil, formalin sterilized pots (20 cm) were filled with the sterilized soil (1:3 w/w sandy clay soil) and then placed in a greenhouse environment. Before transplanting the tomato transplants, the soil was 3% (w/w) infected with the *R. solani* inocula and then consistently watered for a week. The control treatment consisted of pots with simply sterilized soil and no inoculation of *Rhizoctonia* isolate. The inoculated and un-inoculated pots were kept in greenhouse settings at roughly 25–30°C and 70% relative humidity (RH) and were regularly watered twice a week. We used three pots as duplicates. Cucumber transplants four-week-old, cv. Beita alpha a total of three transplants per pot were made into the previously prepared pots. At the Plant Pathology Department, Moshtohor, Faculty of Agriculture, Benha University, Egypt, pathogenicity tests and Koch's postulates for isolated *R. solani* were successfully completed.

3. Varietal reaction:

In this study, cultivars (cv. Suit Kransh, Beit alpha) and two Hybrids (Hybrid Nada and Hybrid victor) of cucumber were evaluated for their susceptibility to root-rot infection caused by *Rhizoctonia solani* (RS1) under greenhouse conditions at 25±2^{o}C. The tested cultivars and hybrids were obtained kindly from commercial greenhouses belonging to Agriculture Ministry, Egypt.

The most virulent strain of *R. solani* (RS1) was used for soil infestation at a rate of 3.0 % of soil weight (w/w) individually. Inocula were thoroughly mixed with soil and regularly tap watered for a week before planting. Seedlings of each cultivar and hybrid (4 weeks old) were transplanted under greenhouse conditions at 25-30°C with 70% RH. Three pots were used as replicates for each cultivar or hybrid. The experiment was

designed in a completely randomized design.

4. Effects of certain tried treatments on the *in vitro* growth of *R. solani*

4.1 Evaluation of some Biocontrol agents

The antagonistic effects of five biological agents, including three fungi (*Trichoderma viride, T. harzianum* and *T. album*) and two bacteria (*Bacillus subtilis* and *Pseudomonas fluorescens*), on the *in vitro* growth of *R. solani* (RS1) were examined. The fungal collections bank of the Plant Pathology Department, Faculty of Agriculture, Benha University, Egypt, kindly provided the tested bioagents that were used in this investigation. The investigated bio control-agents were evaluated using the dual culture method (Zlata *et al.,* 2008).

In this case, *T. harzianum, T. viride*, and *T. album* were perpendicularly infected on one side of the Petri plate with sterile PDA, while mycelia discs (5 mm) of the *R. solani* (RS1) isolate were injected on the opposite side. In the meantime, using the inoculation loop, *B. subtilis* or *P. fluorescens* (bacterial antagonists) were streaked at one side, 1 cm from the plate's edge, and incubated for 24 hours at 22°C (Wang *et al.,* 2003). Each treatment received three replications. The plates were then incubated for seven days at 25 ± 2 °C. Three replications were assigned to each treatment. The plates were then incubated at 25 ±2 °C for seven days.

K=((N-V)/N)*100,where,K=Inhibition Over control percentage,N=Growth of the pathogen under test without an antagonist (mm) and V=Growth of the pathogen under test with an antagonist (mm).

4.2. Effect of some chemical inducers:

Salicylic acid, ascorbic acid, dipotassium hydrogen phosphate, and calcium chloride at doses of 2, 4 and 8 mM were tested against *R. solani in vitro* as resistance inducers. The modified method of Nene and Thapliyal (1993) was employed during this experiment to apply the poisoned food technology to PDA media. As previously noted, the growth inhibition percentage (GI%) of the examined fungus was determined.

4.3. Effect of some fungicides:

Using the approach described by Nene and Thapliyal (1993), the fungicidal activities of 3 fungicides with 3 concentrations, namely Premium 39.1% SC (3-(3,5-Dichlorophenyl)-N-isopropyl2,4dioxo-imidazolidine-1-carboxamide) at concentrations of 0.017, 0.33, and 0.66 mL/L, Flumid 24% (2',6'-Dibromo-2-methyl-4'trifluoromethoxy-4-trifluoromethyl-1,3- thiazole- 5-carboxanilide) at concentrations 20, 40, and 80 mg/L, and Celest FS 10% (4-(2,2-Difluorobenzo[d][1,3]dioxol-4-yl)-1H-pyrrole-3-) at concentrations 37, 75, and 150 mg/L, were assessed against the growth of *R. solani* onto PDA medium. As previously noted, the growth inhibition percentage (GI%) of the examined fungus was determined.

5. Control of cucumber root rot caused by *Rhizoctonia* in a greenhouse.

During the growing season of 2021, at the Experimental Station, Moshtohor, Faculty of Agriculture, Benha University, Egypt, under greenhouse circumstances, the current experiment was totally conducted on cucumber plants that were 4 weeks old (cv. Beit alpha) in pots that had been inoculated with *Rhizoctonia*. According to Abd-El-moneem (1996), the *Rhizoctonia* inoculum was replicated on the sand barley medium as previously indicated. Three replicates of the experimental treatments—each represented by a single pot with three plants inside it—were set up using a randomized full-block design.

5.1 Preparation of the inocula of the tested bio-agents

The antagonistic fungi that were being tested (*T. harzianum, T. viride,* and *T. album*) were cultivated on a PDA medium before having their spore suspensions adjusted with the help of a hemocytometer slide to a concentration of roughly 10^7 spores per milliliter.

Bacillus subtilis and *P. fluorescens*, the studied antagonistic bacteria, were cultured on a nutrient broth medium (Abd-Alla *et al.,* 2007). According to Abdel-Kader *et al.,* (2012), the concentration of each examined bacterial isolate was utilizing a turbidity meter up to roughly 10^8 cfu/mL to ensure even distribution of the inoculum, the pots (20 cm) were filled with 2 kg of sterilized soil before the cucumbers were planted. The soil was then inoculated at a rate of 3% of the soil weight, mixed, and watered daily for a week.

5.2. Chemical inducers preparation

Salicylic acid (SA), ascorbic acid (As), calcium chloride ($CaCl_2$), and dipotassium hydrogen phosphate (K_2HPO_4) at an 8mM concentration were investigated for their effectiveness in reducing Rhizoctonia root rot illnesses when used as a root dipping treatment. Before planting, these substances were applied as seedling root dipping for two hours (Abdel-Monaim *et al.,* 2012). The chemical inducers that were investigated were used as previously mentioned.

5.3. Preparation of the tested fungicides:

Three fungicides were applied to the roots as a root dipping treatment: Celest FS 10% (1.5 mL/L water), Flumid 24% (0.8 mL/L water), and Premium 39.1% SC (0.66 mL/L water) as in table 1. Before planting, healthy cucumber seedlings were elevated, dipped for 5 minutes in each fungicide solution, and then left to air dry.The fungicides under test were applied as previously stated.

Table 1: The concentrations of fungicides, chemical inducers, and bioagents that were tested in greenhouse settings against the Rhizoctonia root rot disease on cucumber plants

Treatment				Used concentration
1	Rhizoctonia-inoculated +	Biological agents	*T. harzianum*	10^7
2			*T. viride*	10^7
3			*T. album*	10^7
4			*P. fluorescens*	10^8
5			*B. subtilis*	10^8
6		Chemical inducers	Ascorbic acid	8 mM
7			Salicylic acid	8 mM
8			Di potassium hydrogen phosphate	8 mM
9			Calcium chloride (cacl2)	8 mM
10		Fungicides	Premium 39.1% SC	0.66ml /L (recommended dose)
11			Flumid 24%	0.8ml/L (recommended dose)
12			Celest FS 10%	(1.5 mL /L (recommended dose)
13	Rhizoctonia-inoculated only		Control	Water-dipped

6. Disease evaluations and vegetative parameters of plants.

At 60 days after transplanting under greenhouse settings, Rhizoctonia root rot of cucumber disease incidence and disease severity percentages were noted, respectively. The disease incidence percentage (DI%) was calculated using the formula below and shown using a percentage scale: DI% is calculated as (D/T) X 100, where (D) is the total number of plants that were observed and (T) is the number of diseased plants.

The disease scale was used to determine the disease severity percentage (DS%), where 0 = no symptoms, 1 = a few lesions (covering 10% of the root), mild secondary root rot, 2 = rot of secondary roots or lesions covering approximately 25% of the root, and 3 denotes lesions covering at least 50% of the root and dead secondary roots (Aegerter *et al.,* 2000). Also, fresh weight/plant and dry weight/plant were determined.

7. Determination of some bio-constituents

7.1. Total phenol contents:

Using the **Simons and Ross (1971)** approach leaves from cucumber plants (CV) Beta alpha were collected separately 30 days after transplantation. The Folin-Ciocalteu reagent method, as modified by Bary and Thorpe (1954), was used to determine the amount of total phenol in the extracts.

$$\textbf{Efficacy}\,\% = \frac{\boldsymbol{Treatment - Control}}{\boldsymbol{Control}}\ \boldsymbol{x100}$$

7.2. Plant defense-related Enzyme activities:

Cucumber plant samples in the form of leaves. Thirty days after the transplant, beta-alpha was taken. To assess the enzyme activity associated with plant defense, leaves samples from each specific potted treatment were collected in a greenhouse environment. Tuzun *et al*., (1989) instructions were followed to prepare the crude leaf enzyme extract.

7.3. Enzyme actions associated with plant defense:

According to Allam and Hollis (1972), the method was used to measure the peroxidase (PO) activity. The method outlined by Matta and Dimond (1963) was used to measure polyphenoloxidase (PPO) activity.

7.4. Determination of chitinase enzyme

The activity of chitinase was determined according to the method of **Boller and Mauch, (1988).**

8. Field Experiments:

From February to May 2023, this experiment was carried out in the Experimental Farm of Benha University's Faculty of Agriculture. As a result of the integrated management of cucumber root rot, a field with fine texture soil that was severely naturally infected with *R. solani* was used to assess disease incidence, disease severity, and fruit weight.

Treatments applied in greenhouses demonstrated effectiveness. The trial consisted of three greenhouse experiments with 16 repetitions and 16 treatments.: *Trichoderma viride,* Salicylic acid, *Bacillus subtilis*, Premium, *Trichoderma viride* + *Bacillus subtilis*, *Trichoderma viride* + Premium, *Trichoderma viride* + Salicylic acid, *Bacillus subtilis* + Premium, *Bacillus subtilis* + Salicylic acid, Premium + Salicylic acid, *Trichoderma viride* + *Bacillus subtilis*+ Premium, *Trichoderma viride* + *Bacillus subtilis* +Salicylic acid, *Trichoderma viride* + Premium + Salicylic acid, *Bacillus subtilis* + Premium + Salicylic acid, *Trichoderma viride* + *Bacillus subtilis* + Premium + Salicylic acid and control.

9. Statistical analyses:

According to the methods (ANOVA) described by Snedecor and Cochran (1989), statistical analyses of all the previously designed experiments have been conducted. It was done using the least significant difference test

(LSD) compare treatment means at a 5% level of probability.

Results

1. Isolation of Rhizoctonia root rot pathogenic fungi:

As shown in Table 2, samples of cucumber plants with symptoms of Rhizoctonia root rot were collected from Minufiya (Shebin El-Kom), Behaira (El-bostan), and Kafr El-sheikh (Kafr El-sheikh) governorates in Egypt.

Table 2: Isolation of Rhizoctonia root rot pathogenic fungi of different Egyptian governorates:

Governorate	Locality	Isolate No.
Minufiya	Shebin El-Kom	RS1
Behaira	El-Bostan	RS2
Kafr El-Sheikh	Kafr El-Sheikh	RS3

2. Pathogenicity test:

2.2. Pathogenicity test of the three *Rhizoctonia solani* isolates:

Three *R. solani* isolates were tested for pathogenicity on cucumber plants of the cv. Beta alpha variety in a greenhouse environment. Table 3 shows that every isolate of *R. solani* was pathogenic to the plants that were tested and produced signs of root rot. RS1, which was isolated from Behaira samples and caused the highest rates of disease incidence (DI%) and disease severity (DS%) to be 100.0 and 86.5%, respectively, was the most pathogenic strain. It was followed by the isolate from Minufiya (91.66 and 60.41%) with no significant difference. On the other hand, Kafr El-Sheikh isolate had the lowest DI% and DS% ever observed (66.66 and 35.41%, respectively).

Table 3: Pathogenicity test of the three *Rhizoctonia solani* isolates:

Governorate	Locality	Isolate No.	*DI %	**DS %
Behaira	**El-bostan**	RS1	100	86.5
Minufiya	**Shebin El-Kom**	RS2	91.66	60.41
Kafr El-Sheikh	**Kafr El-Sheikh**	RS3	66.66	35.41
Un-inoculated soil		------	0.0	0.0
LSD 0.05				

*DI=% disease incidence, ** DS =% disease severity (DS%)

3. Varietal reactions of some Cucumber cultivars and hybrids to Rhizoctonia root-rot infection:

Under greenhouse circumstances, the ability of four cucumber cultivars and hybrids to withstand or be vulnerable to the most virulent strain of *R. solani* (RS1) was studied. Data in Table 4 reveal that, all tested cucumber cultivars and hybrids were infected with Rhizoctonia root- rot

disease caused by RS-1 (Behaira isolate) and exhibiting different degrees of DI% and DS%. In this respect, cv. Beta alpha followed by Suit Kransh Cultivar were the most susceptible since they recorded the percentages of DI% and DS% of 100.0 and 87.5 and 83.33 and 77.08 %, respectively.

Also, it is clear from the obtained results that, the victor hybrid was the least DI% and DS% where it recorded 66.66 and 53.47 % of DI% and DS% respectively.

Table 4: Varietal reactions of some Cucumber cultivars and hybrids to Rhizoctonia root rot infection:

Cultivar & Hybrid	DI%	DS%
Beta alpha*	100	87.5
Victor**	66.66	53.47
Suit Kransh*	83.33	77.08
Nada**	75	43.75
LSD at 5%	**30.38**	**11.77**

***=Cultivar, ** =Hybrid**

4. *In vitro* evaluation of some biotic and abiotic factors against the growth of *R. solani* (RS1):

4.1. Evaluation of some biocontrol agents:

Data in Table 5 demonstrate that, as compared to controls, all biocontrol agents utilized slowed the growth of *R. solani* (RS1). *Trichoderma viride*, the tested species, was the most effective antagonistic fungus, causing a growth reduction of 50%, followed by *Trichoderma album*, which recorded a growth reduction of 47%. Regarding the two examined bacteria used as biocontrol agents, *Bacillus subtilis* proved to be more successful, as it reduced *R. solani* growth by 31.55%.

Table 5: Effect of some biological agents on the growth of *Rhizoctonia solani in vitro*:

Treatment	Growth (mm)	% reduction
Trichoderma album	47.0	47.77
Trichoderma harzianum	50.0	44.44
Trichoderma viride	44.7	50.33
B. subtilis	61.6	31.55
Ps. fluorescens	62.3	30.77
Control	90.0	0.0
LSD at 0.05	**4.03**	

4.2. Evaluation of some chemical inducers:

Results in Table 6 show that, when compared to the control, the investigated chemical inducers considerably reduced the linear development of the *R. solani* isolate. The acquired data show that, in various ways compared to the control, the growth of *R solani* was reduced by each of the chemical inducers under investigation. Salicylic acid (SA) at concentrations of 4 and 8 mM, ascorbic acid at concentrations of 4 mM, and ascorbic acid, calcium chloride

(Cacl2) at concentrations of 8 mM completely inhibited the mycelial growth of *R solani*, while K_2HPO_4at concentrations of 8 mM and 4 mM reduced the growth of *R. solani* by 70.37 and 69.77%, respectively. K2HPO4, on the other hand, was least effective in this regard at a concentration of 2 mM.

Table 6: Evaluation of some chemical inducers on the growth of *R solani in vitro*:

Treatment	Concentration (mM)	Mycelial Growth (mm)	% Efficacy
Salicylic acid	**2**	33.08	63.24
	4	0.0	100.0
	8	0.0	100.0
Ascorbic acid	**2**	57.0	36.66
	4	26.66	70.37
	8	0.0	100.0
K_2HPO_4	**2**	67.5	25
	4	63.33	29.63
	8	27.2	69.77
Calcium chloride	**2**	63.33	29.63
	4	32.08	64.35
	8	0.0	100.0
Control		90	0.0
LSD at 0.05	**Chemical**	**5.63**	
	Conc.	**4.47**	
	Interaction	**17.30**	

4.3. Evaluation of some fungicides:

Results in Table 7 reveal that *R. solani* mycelial growth was greatly slowed down by the tested fungicides when compared with the control. With an increase in fungicide concentration, the studied fungicides' inhibitory effects became more pronounced. In this regard, the high concentration of the fungicides, namely Flumid and Celest, prevented the growth of *R. solani* while Premium fungicide, at a concentration of 0.66 mL/L, fully inhibited the fungus.

5. *In vivo* evaluation of some biotic and abiotic factors for controlling cucumber root-rot infection under greenhouse conditions:

5.1. Evaluation of tested biocontrol agents:

The Data in Table 8 demonstrate that all antagonistic fungi and bacteria examined were successful in lowering the incidence and severity of disease, as well as in increasing the fresh and dry weight

of shoots and roots in comparison to the control. *T. viride*, which reduced disease incidence and severity by 11.11 and 2.77%, respectively, was the most effective isolate in this regard, followed by *T. harzianum*. *T. album*, on the other hand, was the least successful in lowering illness incidence and severity. As for, the tested antagonistic bacteria, *B. subtilis* was the most effective isolate where it reduced disease incidence and disease severity by 11.11 and 2.77%, respectively and increased fresh and dry weights of shoots and roots. Also, the highest increase in fresh and dry weights of shoots and roots was scored with *T. viride* followed by *T. harzianum*. Meanwhile, *T. album* was the least effective biocontrol agent in this respect.

Table 7: Effect of fungicides on the growth of *R. solani in vitro*:

Treatment	Conc.	Mycelial Growth (mm)	% Efficacy
Premium39.1 % SC	0.17 mL/L	25.66	71.48
	0.33 mL/L	9.6	89.33
	0.66 mL/L	0.0	100.0
Flumid 24 %	0.2 mL /L	69.0	23.33
	0.4 mL /L	35.0	61.11
	0.8 mL /L	16.16	82.04
Celest FS 10%	0.37 mL/L	55.66	38.15
	0.75 mLl/L	46.16	48.71
	1.5 mL/L	19.7	78.11
Control		90.0	0.0
LSD at 0.05	**Fungicide**	**7.81**	
	Conc.	**8.04**	
	Interaction	**27.86**	

Table 8: Evaluation of some tested biocontrol agents for controlling Rhizoctonia root rot diseases in the greenhouse:

Treatment	*DI%	**DS%	FW(g)		DW(g)	
			Shoot	Root	Shoot	Root
T. album	55.55	16.66	27.33	4.67	5.3	1
T. viride	11.11	2.76	35.33	5.33	6.3	1.1
T. harzianum	55.55	11.11	29.66	4.33	5.0	1
B. subtilis	11.11	2.77	34	4.67	6.0	1
Ps. fluorescens	55.55	16.67	27.33	4.33	4.67	1
Control	100	80.55	7.67	1.77	1.23	0.42
LSD at 0.05	**30.65**	**7.65**	**3.89**	**1.15**	**1.09**	**0.05**

***DI=% disease incidence, ** DS =% disease severity, Fw=Fresh weight, DW=Dry Wehgt.**

6. Evaluation of some chemical inducers:

The results shown in Table 9 indicate that all applied treatments considerably decreased the incidence and severity of the root rot disease as well as the fresh and dry weights of the shoots and roots when compared with the control. Calcium chloride ($Cacl_2$), which recorded 11.11 and 2.77%, respectively, of DI and DS%, was the next best chemical compound in reducing disease incidence and disease severity to 0.0 and 0.0%, respectively. However, K_2HPO_4 was the least successful in this regard. Salicylic acid treatments produced the biggest increases in the fresh and dry weights of the shoots and roots, followed by treatments with calcium chloride. K_2HPO_4 was, however, the least successful in this regard.

7. Evaluation of some fungicides:

Results in Table 10 demonstrate that all tested fungicide treatments that were administered both considerably decreased the incidence and severity of the root rot disease and raised the fresh and dry weights of the shoots and roots in comparison to the control. In this regard, Premium fungicide, which lowered disease incidence to 33.33% and disease severity to be 11.11%, was followed by Flumid fungicide in terms of the largest reduction percentages of disease incidence and disease severity. The Celest fungicide, on the other hand, was the least effective and decreased disease incidence and severity to 66.66 and 25.0%, respectively.

Additionally, Premium fungicide produced the biggest increases in the fresh and dry weights of the shoots and roots, plant height, and root length. On the other hand, Celest fungicide was the least effective fungicide in this respect.

Table 9: Evaluation of some chemical inducers for controlling Rhizoctonia root rot disease on cucumber plants *in vivo*:

Treatment	DI%	DS%	FW(g)		DW(g)	
			Shoot	Root	Shoot	Root
Salicylic acid	0.0	0.0	53.66	5.67	9.33	0.96
Ascorbic acid	33.33	11.08	48.33	4.3	5.67	1.0
K_2HPO4	66.66	19.44	34.66	3.67	4.67	0.93
$CaCl_2$	11.11	2.77	50.33	5.67	5.67	1.0
Control	100	80.55	7.67	1.77	1.23	0.42
LSD at 0.05	**12.20**	**8.83**	**9.11**	**1.08**	**1.93**	**0.11**

***DI=% disease incidence, ** DS =% disease severity ,Fw=Fresh weight,DW=Dry Wehgt.**

Table 10: Evaluation of some fungicides for controlling Rhizoctonia root rot disease on cucumber plants *in vivo*:

Treatment	DI%	DS%	FW(g)		DW(g)	
			Shoot	Root	Shoot	Root
Premium 39.1 %SC	33.33	11.11	36.66	4.3	7.3	0.96
Flumid 24 %	66.66	22.22	20.33	3.16	5.6	0.86
Celest FS 10%	66.66	25.0	18.33	2.9	4.6	0.8
Control	100	80.55	7.67	1.77	1.23	0.41
LSD at 0.05		**9.21**	**3.07**	**0.73**	**2.66**	**0.06**

DI= disease incidence, DS= disease severity, FW= Fresh weight, DW= Dry weight

8. Determination of defense-related enzyme activities in infected cucumber plants with root rot pathogens in the greenhouse:

8.1.Effect of some tested biocontrol-agents

Data in Table 11 show that, when compared to the control treatment, all evaluated biocontrol agents significantly raised the activity of the enzymes involved in external plant defense, namely peroxidase (PO), polyphenol oxidase, and chitinase, in the leaves of cucumber plants. The most effective biocontrol agent among the tested treatments was *T. viride*, which was followed by *T. harzianum. T. viride* raised the activities of peroxidase, polyphenol oxidase, and chitinase enzymes with reported efficacy% of 154.52, 179.24, and 124.82%, respectively. In terms of antagonistic bacteria, *B. subtilis* had the greatest documented effectiveness% of PO, PPO, and chitinase enzyme activity (243.89, 111.32, and 107.96%, respectively).

Table 11: Effect of some tested biocontrol agents on defense-related enzyme activities in infected cucumber plants with Rhizoctonia root rot pathogen under greenhouse conditions:

Treatment	PO	PPO	Chitinase	Efficacy %		
				PO	PPO	Chitinase
T. album	28.86	12.42	16.56	117.64	160.37	93..91
T .viride	33.75	13.32	19.2	154.52	179.24	124.82
T. harzianum	30.45	9.72	18.3	129.63	103.77	114.28
B. subtilis	45.6	10.08	17.76	243.89	111.32	107.96
Ps. fluorescens	40.2	7.38	15.3	203.16	54.71	79.15
Control	13.26	4.77	8.54	0.00	0.00	0.00

*Peroxidase activity was expressed as the increase in absorbance at 425 nm/gram fresh weight/15 minutes.

* Polyphenol oxidase activity was expressed as the increase in absorbance at 420nm/g fresh weigh/ minutes.

* Chitinase activity was expressed as mM N-acetylglucose / gram fresh weigh tissue / 60 minutes.

Data in Table 12 reveals that all chemical inducers examined significantly raised the activity of the enzymes peroxidase, polyphenol oxidase, and chitinase when compared to the control treatment. The most effective chemical inducers were calcium chloride and salicylic acid (SA), which enhanced the activity of the enzymes peroxidase, polyphenol oxidase, and chitinase while recording efficacy percentages of 200.90, 343.39, and 163.70%, respectively. However, K_2HPO_4 had the least impact on the activity of the chitinase, PPO, and PO enzymes in this regard.

8.3. Effect of some fungicides:

Results in Table 13 **reveal** that all tested fungicides significantly increased the activities of peroxidase, polyphenol oxidase, and chitinase enzymes compared with control treatments. Premium 39.1 % was the best fungicide treatment among the other tested ones, where it increased the activities of peroxidase, polyphenol oxidase (PPO) and chitinase enzymes and recorded efficacy % of 133.78, 122.22, and 104.44%, respectively, followed by Celest FS 10%. Whereas Flumid was the least effective treatment in this respect.

Table 12: Effect of some tested chemical inducers on defense-related enzyme activities in infected cucumber plants with Rhizoctonia root rot pathogen under greenhouse conditions:

Treatment	PO	PPO	Chitinase	Efficacy %		
				PO	PPO	Chitinase
Salicylic acid	39.9	21.15	22.52	200.90	343.39	163.70
Ascorbic acid	37.5	14.04	15.9	182.80	194.33	86.18
K_2HPO_4	19.89	7.12	13.92	50.0	49.26	62.99
$Cacl_2$	36.0	13.32	21.3	171.49	179.24	149.41
Control	13.26	4.77	8.54	0.00	0.00	0.00

*Peroxidase activity was expressed as the increase in absorbance at 425 nm/gram fresh weight/15 minutes.

* Polyphenol oxidase activity was expressed as the increase in absorbance at 420nm/g fresh weigh/ minutes.

Table 13: Effect of some tested some fungicide on defense-related enzyme activities in infected cucumber plants with Rhizoctonia root rot pathogen under greenhouse conditions:

Treatment	PO	PPO	Chitinase	Efficacy %		
				PO	PPO	Chitinase
Premium 39.1 % SC	31	10.6	17.46	133.78	122.22	104.44
Flumid 24 %	16.82	7.2	16.86	26.84	50.94	97.42
Celest FS 10%	22.26	7.56	12.3	67.87	58.49	44.02
Control	13.26	4.77	8.54	0.00	0.00	0.00

*Peroxidase activity was expressed as the increase in absorbance at 425 nm/gram fresh weight/15 minutes.

* Polyphenol oxidase activity was expressed as the increase in absorbance at 420nm/g fresh weigh/ minutes.

* Chitinase activity was expressed as mM N-acetylglucose / gram fresh weigh tissue / 60 minutes.

9. Determination of phenol contents in infected cucumber plants with root rot pathogens *in vivo*:

9.1. Effect of some tested biocontrol agents:

Data in Table 14 show that each studied biocontrol agent had a significant impact on total, conjugated, and free phenol levels. When compared to the control, all antagonistic fungi increased the free and total phenols. *T. viride* caused the greatest increases in free and total phenols, which recorded efficacy% of 335.29, 662.45, and 420.42%, respectively. On the other hand, *T. album* was the least efficient, which recorded efficacy percentages of the free and total phenols of 230.49, 445.392, and 286.41%.

Data from the same table show that *B. subtilis* raised the free and total phenols with an effectiveness% of 288.23, 261.09, and 281.17%, respectively, as compared to the antagonistic bacteria.

9.2. Effect of some chemical inducers:

Results in Table 15 indicate that all tested chemical compounds increased the free and total phenols. The highest increase in the free and total phenols was induced by salicylic acid which recorded efficacy % as 262.30 and 455.23% followed by ascorbic acid (187.75 and 411.72 %). However, K_2HPO_4 was the least effective treatment in increasing the free and total phenols with efficacy % as 151.74 and 223.71%.

Also, all treatments increased the conjugated phenols compared with control. The highest increase was induced by ascorbic acid (1048.46 %) followed by salicylic acid (1003.75%). However, K_2HPO_4 was the least effective treatment in this respect.

Table 14: Effect of some tested biocontrol agents on phenol content (mg/1g fresh weight) in infected cucumber plants with Rhizoctonia root rot pathogen under greenhouse conditions:

Treatment	Free Phenol	Conj. Phenol	Total Phenol	Efficacy %		
				Free Phenol	Conj. Phenol	Total Phenol
T. album	27.53	15.98	43.51	230.49	445.392	286.41
T .viride	36.26	22.34	58.60	335.29	662.45	420.42
T. harzianum	29.89	19.69	49.58	258.82	572.01	340.31
B. subtilis	32.34	10.58	42.92	288.23	261.09	281.17
Ps. fluorescens	26.85	10.39	37.24	222.32	254.60	230.72
Control	8.33	2.93	11.26	0.00	0.00	0.00

Table 15: Effect of some tested some chemical inducers on phenol content (mg/1g fresh weight) in infected cucumber plants with Rhizoctonia root rot pathogen under greenhouse conditions:

Treatment	Free Phenol	Conj. Phenol	Total Phenol	Efficacy %		
				Free Phenol	Conj. Phenol	Total Phenol
Salicylic acid	30.18	32.34	62.52	262.30	1003.75	455.23
Ascorbic acid	23.97	33.65	57.62	187.75	1048.46	411.72
K_2HPO_4	20.97	15.48	36.45	151.74	428.32	223.71
CaCl2	23.03	28.71	51.74	176.47	879.86	359.50
Control	8.33	2.93	11.26	0.00	0.00	0.00

9.3. Effect of some fungicides:

Results in Table 16 show that, when compared to the control, all tested fungicides raised the levels of free, conjugated, and total phenols. The amount of free, conjugated, and total phenols increased the most when treated with Premium 39.1% SC, which had efficacy percentages of 230.49, 1188, and 479.66%. However, Flumid 24 percent was the medication that increased free and total phenols the least effectively.

10. Effect of interaction between selected treatments on cucumber root rot disease caused by *Rhizoctonia solani*:

Data in Table 17 show that the tested treatments considerably decreased disease incidence and severity while also boosting fruit yields per plant. In comparison to the individual treatments, interactions between multiple treatments often reduced the incidence and severity of *Rhizoctonia* root rot disease and increased the amount of fruit produced per plant.

The most successful treatment, which decreased disease incidence and disease severity and recorded 5.55 and 2.78%, respectively, compared with control, which recorded 100.0 and 76.39%, was integration between *T. viride* + *B. subtilis* + Premium + SA.

Additionally, compared to the individual treatments, there was a 4.62 kg/plant increase in fruit weight per plant. Premium, on the other hand, was the most successful individual treatment, reducing both the incidence and severity of the disease and recorded 16.67 and 5.55%, respectively, compared to control.

Other individual or combined treatments decreased moderate disease incidence and severity and increased fruit weight per plant compared with the control.

Table 16: Effect of some tested some fungicide on phenol content (mg/1g fresh weight) in infected cucumber plants with Rhizoctonia root rot pathogen under greenhouse conditions:

Treatment	Free Phenol	Conj. Phenol	Total Phenol	Efficacy %		
				Free Phenol	Conj. Phenol	Total Phenol
Premium 39.1 %SC	27.53	37.74	65.27	230.49	1188	479.66
Flumid 24 %	16.85	12.74	29.59	102.28	334.81	162.78
Celest FS 10%	18.81	15.49	34.30	125.81	428.66	204.61
Control	8.33	2.93	11.26	0.00	0.00	0.00

Table 17: Effect of interaction between treatments on cucumber root rot disease incidence and disease severity and weight fruits yield kg/plant under field conditions during 2023 season.

Treatment	% Disease incidence	% Disease severity	Fruits weight /plant with kg
Trichoderma viride	33.33	13.89	3.15
B. subtilis	38.89	16.67	3.08
Salicylic acid (SA)	33.33	8.33	3.67
Premium 39.1 % SC	16.67	5.55	3.73
T. viride* + *B. subtilis	27.78	9.72	3.36
***T. viride* +Premium 39.1 % SC**	22.22	8.33	3.81
***T. viride* + SA**	22.22	6.94	3.74
***B. subtilis* + Premium 39.1 % SC**	27.78	9.72	3.80
***B. subtilis* + SA**	27.78	11.11	3.73
Premium 39.1 % SC + SA	11.11	6.94	3.91
***T. viride* + *B. subtilis* + Premium**	16.67	8.33	4.00
***T. viride* + *B. subtilis* + SA**	22.22	6.94	4.03
T. viride + Premium 39.1 % + SA	11.11	4.16	4.37
***B. subtilis* + Premium 39.1 % + SA**	16.67	5.55	4.35
***T. viride* + *B. subtilis* + Premium + SA**	5.55	2.78	4.62
Control	100.00	76.39	1.85
LSD 0.05 =	**15.36**	**6.97**	**0.49**

DISCUSSION

Several studies have highlighted the pathogenic nature of *R. solani* on different plant species, resulting in symptoms of root rot (Smith *et al.,* 2017). These investigations have emphasized the ability of *R. solani* to infect and harm plant roots, leading to decrease plant health and productivity.

present study reaffirms these previous findings by demonstrating the pathogenicity of *R. solani* isolates on the plants tested, with all isolates causing symptoms of root rot. Additionally, the study observes that the RS1 isolate from Behaira exhibited the highest level of pathogenicity, aligning with prior research indicating that the pathogenic potential of *R. solani* isolates may vary depending on their geographic origin (Li *et al.,* 2018; Wang *et al.,* 2020). These findings support the idea that *R. solani* is a

widespread pathogen capable of inflicting significant damage to agricultural crops.

Similar studies investigating the pathogenicity of *R. solani* isolates have reported varying degrees of disease incidence and severity. To address plant diseases caused by pathogens such as *R. solani*, the use of biocontrol agents offers a sustainable and environmentally friendly approach. Among the tested *Trichoderma* species, *Trichoderma viride* demonstrated the highest antagonistic activity against *R. solani*, resulting in a 50.33% reduction in growth. *T. viride* emerged as the most effective biocontrol agent among the treatments, enhancing the activities of peroxidase, polyphenol oxidase, and chitinase enzymes. This finding is consistent with previous studies that have showcased the efficacy of *T. viride* as a biocontrol agent against various plant pathogens (El-Katatny *et al.*, 2001; Vinale *et al.*, 2008). *T. viride* is renowned for its capacity to produce antifungal compounds, including chitinases and β-1,3-glucanases, which inhibit the growth of fungal pathogens (Hermosa *et al.*, 2012). Similar findings have been documented in previous studies, highlighting the antagonistic potential of *T. album* against various plant pathogens (Hermosa *et al.*, 2012; Vinale *et al.*, 2008).

Trichoderma species produce a range of antifungal metabolites and enzymes that impede the growth and development of fungal pathogens. In addition to *Trichoderma* species, this study also evaluated two bacterial biocontrol agents. *Bacillus subtilis* exhibited greater effectiveness in reducing the growth of *R. solani*. *Bacillus subtilis* is known for its ability to produce various antifungal compounds, such as antibiotics, enzymes, and volatile organic compounds, which contribute to its antagonistic activity against plant pathogens (Compant *et al*., 2019).

The efficacy of biocontrol agents in reducing the growth of *R. solani* can be attributed to their diverse mechanisms of action, including competition for nutrients and space, production of antifungal compounds, induction of plant defense responses, and mycoparasitism (Harman *et al.*, 2004; Vinale *et al.*, 2008). Ahmed *et al.* (2017) conducted a study where they investigated the effects of bioagents on tomato plants. They found that treating the tomato plants with bioagents resulted in a positive increase in the activities of peroxidase (PO), polyphenol oxidase (PPO), chitinase, and β-1,3 glucanase enzymes in the leaves, as compared to the control treatment. Similarly, Ahmed (2016) conducted a study on treated bean seeds with bioagents and observed significant increases in the activity of peroxidase, polyphenol oxidase, chitinase, and β-1,3-glucanase enzymes. These enzymes play a crucial role in plant defense mechanisms against pathogen infections.

The results also indicated that the enzymatic activity in treated snap bean plants was higher than that in the untreated ones. Oxidative enzymes like peroxidase and polyphenol oxidase contribute to the formation of lignin and other oxidative phenols that reinforce the cell structure and form defense barriers (Avdiushko *et al.,* 1993). Chitinase and β-1,3 glucanase enzymes play a significant role in plant defense against fungi by hydrolyzing their cell walls (Barilli *et al.,* 2010). The current study aimed to explore the effects of various chemical inducers on the growth of *R. solani*.

The results showed that all the tested chemical inducers significantly reduced the linear growth of the *R. solani* isolate compared to the control, indicating their

potential in inhibiting the growth of *R. solani* and their potential application in managing diseases caused by this pathogen. Among the chemical inducers tested, salicylic acid (SA) at concentrations of 4mM and 8mM, as well as ascorbic acid and calcium chloride (CaCl2) at a concentration of 8mM, completely inhibited the mycelial growth of *R. solani*. Salicylic acid (SA) treatment was particularly effective, as it increased the activities of peroxidase, polyphenol oxidase, chitinase enzymes, and phenol contents. These findings are consistent with previous research that has demonstrated the inhibitory effects of salicylic acid, ascorbic acid, and calcium chloride on the growth and development of various plant pathogens (Lurie *et al.,* 2004; Zhou *et al.,* 2012). Salicylic acid and ascorbic acid are known to play important roles in plant defense mechanisms by regulating various defense responses against pathogens (Klessig *et al.,* 2018; Loake and Grant, 2007). Calcium chloride, on the other hand, has been reported to enhance plant resistance by strengthening cell walls and activating defense-related signaling pathways (Zhou *et al.*, 2012). The effectiveness of chemical inducers in inhibiting the growth of *R. solani* can be attributed to their ability to disrupt the physiological and biochemical processes that are essential for the pathogen's growth and development. Furthermore, a premium fungicide was evaluated in the study, and it completely inhibited the growth of the fungus at a concentration of 0.66 mL/L. It was also found to be the most effective fungicide in reducing disease severity and increasing cucumber fruit yield. Additionally, the fungicide treatment increased the activities of peroxidase, polyphenol oxidase, chitinase enzymes, and phenol contents.

Similar findings have been reported in previous studies by Khalifa (1997), who found that Benlate and Rizolex T were effective fungicides for controlling infections caused by *M. phaseolina* and *F. oxysporum* and increasing the health of sesame plants in a greenhouse. Haggag and El-Gamal (2012) investigated that, the fungicide Tachigaren 30%, followed by Monceren 25%, Aracur 72.2%, Topsin M 70%, Hymexate 30%, and Moncut 25% at tested doses, showed the greatest inhibition of *F. solani* and *R. solani* on mycelial growth.

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