Eco-friendly approaches to control wheat root rot and seedlings death caused by *Rhizoctonia solani*

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Abstract

Root rot and seedlings death pathogens are important diseases in wheat-growing regions of Iraq, particularly *Rhizoctonia solani* as it considered the main causative agent. Therefore, biological control and genotypes with low susceptibility are considered an effective and environmentally friendly way to reduce the impact of this soil pathogen. Current study aimed to evaluate the effect of *Trichoderma* fungi in reducing the effect of three isolates of the pathogenic fungus *Rhizoctonia solani* isolates (Rh1, Rh2, Rh3) on three wheat varieties including Abaa, Adna, and Barcelona. Experiments were carried out in postgraduate laboratories of the Department of Plant Protection, Faculty of Agriculture, University of Kufa. Results showed that Rh3 isolate was the most effective in reducing seed germination and seedling death. The use of bio-fungi led to an increase in germination, a decrease in seedling death, and a reduction in the severity and percentage of infection, which positively impacted plant vegetative growth. Abaa variety appeared to be slight resistant to the studied isolates of the pathogen compare to other varieties. Pots experiment showed that *Trichoderma* isolates significantly reduced the growth of the pathogenic fungus in double culture dishes with inhibition rates ranging from 55.97% to 69.85%. The outcomes indicate that *Trichoderma* fungi represent a promising option for the biological control of wheat root rot and seedlings death diseases.

Keywords: seed rot, *Rhizoctonia solani*, *Trichoderma* spp, biocontrol, fungal antogonsim.

Introduction

Wheat (*Triticum aestivum* L.) is valuable member of Poaceae family, rich of multiple nutrients including carbohydrates, vegetable proteins, minerals and dietary fiber that make it consumed as a main food globally (15, 28). currently grown in more than 70 countries around the world, with an estimated cultivated area of about 3,864.4032 hectares, with a production capacity of 3,052.939 million tons of harvested grain (10). Iraq is among these countries with a production of 1,518.471 tons on an area of 633.1116 hectares (6).

Non-biotic stress factors, pathogens and the use of biological control of plant pests, and bioremediation of plants have gained considerable importance in recent years due to climate change and pollution of soil and natural resources, which increase crop stress and limit or reduce the production (5). Fungal diseases on wheat become major challenge face the cultivation of crop specially at the early stages of growth, hence, *Rhizoctonia solani* the causal agent of wheat damping-off is one of aggressive soil pathogens that causing significant loose in grain yield 20, 18). Bud basal rot and main root rot by the pathogenic fungus are usually signs of disease, leading to poor crop establishment (31). Once present in the soil, *R. solani* survives on crop residues as solid bodies or fungi, causing root rot and damping-off diseases in subsequent crops in the field rotation (16). Chemical disease control techniques are nearing their limits and are unsustainable from both an environmental and human safety perspective (2). Therefore, new tools and methodologies must be developed. In addition, there is a growing demand for higher quality and safety standards across the food chain and its final products, starting from the agricultural production stage (27).

One way to reduce the use of chemicals, and consequently their associated side effects, is to use beneficial biological agents to protect crops (25). Biocontrol organisms are part of the natural ecosystem of healthy plants, residing primarily in the root zone, leaf surfaces, and within plant tissues (14). For biological control of plant diseases, the interaction between antagonistic organisms, host plants, and pathogens is a key area to explore (26). *Trichoderma* spp. have been shown an ability to be a grate biocontrol agents to reduce or eliminate many plant diseases including damping-off as well as enhance plant growth by different approaches (1). Furthermore, many studies have conducted to find wheat varieties as eco-friendly factors with low susceptibility or resistance to the damping-off to avoid the economic damages that it cause (17). Thus, current study aims to isolate and identify the pathogenic fungi of root rot and seedlings death disease from wheat fields, evaluate the antagonistic activity of these fungi with some *Trichoderma* isolates and study its effect on reducing the incidence and severity of infection and improving plant growth as well as examine the susceptibility some commercial varieties of wheat to this disease .

Material and Methods

Plant Materials and seed used in the study

All experiments of this study were done in postgraduate laboratories of the Department of Plant Protection, Faculty of Agriculture, University of Kufa. Samples were collected from infected wheat seedlings exposing symptoms of yellowing, stunting and wilting from some fields in three provinces including Najaf, Babylon and Kerbala during the 2024-2025 season. The samples were placed in paper bags labeled with the date and location of collection and stored pending diagnosis. Three varieties of wheat (Adna, Abaa, Barcelona) which were obtained from the Seed Testing and Certification Department in Najaf province and cultivated widely in the study area, used to determine the susceptibility of these varieties against root rot and seedlings death disease after confirming their identification and viability.

Isolation of *Rhizoctonia* and biocontrol agents

The collected seedlings of wheat were taken, then its roots were cut into 0.5-1 cm pieces, washed well under running tap water and sterilized by immersing them in a 2% sodium hypochlorite solution for 2 minutes, after which they were washed with sterile distilled water to remove any residues of sterilization solution. Roots pieces were dried using sterile filter paper and cultured in 9 cm diameter Petri plates prepared with P.D.A. culture medium treated with the antibiotic Ceftriaxone. Plates were incubated at 25°C, and the growing colonies of fungi were morphologically identified at the genus level based on the morphological characteristics on P.D.A. medium with the help of properties mentioned in (3). Two isolates of *Trichoderma* diagnosed as *T. longibrachiatum* (TH1) and *T. longibrachiatum* (TH3) were used, both obtained from the Plant Pathology Laboratory/Postgraduate Studies, Department of Plant Protection/Faculty of Agriculture/University of Kufa. These isolates were previously identified and registered under the accession numbers MZ021580 (TH1) (23) and PQ764160 (TH3) (4).

Molecular identification of *Rhizoctonia* isolate

Molecular diagnoses of one *Rhzoctonia* isolate that showed aggressiveness on wheat seedlings selected to represent the collected isolates from studied fields. DNA of the above isolate was extracted following Narayanasamy (22) method using suitable kits. Two primers forward ITS4 `5-TCCTCCGCTTATTGATATGC-3`and the reverse ITS5 5`-GGAAGTAAAAGTCGTAACAAGG-3` were used to apply polymerase chain reaction (30) following the instructions of the supplier company. The products of PCR injected into a wholes made in agarose gel 1.5% (w/v) containing 5 mg/ml RedSafe dye. At 50 V, electrophoresis was done for 1 h, then the products were sent to Korea (Macrogene Company) to make the sequencing of double stranded nucleic acids.

The antagonism between *Rhizoctonia* isolates and the biocontrol agents

The double culture technique was used in Petri plates containing sterile P.D.A. culture medium to test the antagonistic activity of the two isolates of *Trichoderma* against the pathogenic fungus *R. solani*. Petri plate was divided into two equal sections and the center of the first section was inoculated with a 0.5 cm disc of the pathogenic fungus *R. solani* using a sterile cork piercer from the edges of a 7-day-old colony grown on P.D.A. medium. The center of the second section of the Petri plate was inoculated with a 0.5 cm disc from the edges of a 7-day-old colony of *Trichoderma*. Each treatment was repeated three times, and a control treatment was also carried out by inoculating the center of the first section of the plate with either the pathogenic fungus or the bio-control agent only, each separately (9). All plates were incubated in an incubator at a temperature of (25±2)ºC. The diameter of the pathogenic and the biocontrol fungus was then measured after 7 days of double inoculation, and the percentage of inhibition was calculated according to the Abbott equation:

$$\% \text{ Inhibition} = \frac{\text{D of C} - \text{D of T}}{\text{D of C}} \times 100$$

Where D of C = Diameter of fungal growth in control, D of T= Diameter of fungal growth in treatment.

Pots experiment

In this experiment, loamy soil was used sterilized using commercial formalin (40%). A 1/50 solution of formalin was prepared and used at a rate of 3 liters/1m3. After ten days of sterilization, it was placed in plastic pots (diameter 20 cm and height 25 cm) in equal quantities and then distributed to four treatments in three replicates (three pots) for three varieties of wheat (Adna, Abaa, Barcelona). Three treatments was contaminated with 1% (w/w) *R. solani* fungal inoculum and sterile millet seeds were added to the other treatments at the same rate. The potting soil was moistened and left for three days after being covered with plastic bags. All seeds used were surface-sterilized with a 2% sodium hypochlorite solution for 2-3 minutes, then rinsed with sterile water.

Pots were watered carefully, and after seed germination and seedling emergence the following indicators were calculated:

1. Germination and seedling death rates.

2. Severity Index: Calculated according to Wheeler's 4-point scale described in Madhi et al. (19).

3. Average plant height (cm): Measured from soil surface to plant tip.

4. Fresh and dry weight of vegetative and root systems (g): Measured after drying the plants in an electric oven.

Experiment design and data analysis

Laboratory experiment was arrange using Randomize Complete Design, while pot experiment prepared using Randomize Complete Block Design. Data were analyzed using the computing software (Genstat 12th). Means among treatments were compared using the least significant difference (L.S.D.) test at the probability level of 0.05.

Results and Discussion

The effect of pathogen isolates on wheat germination rate

The results showed a significant decrease in germination percentage of wheat seeds compared to the control treatment (100%). *R. solani* isolates caused a significant decrease in germination percentage and an increase in the percentage and severity of infection, reflecting their high pathogenicity especially isolate Rh3 which recorded the lowest germination percentage (54.61%). Table 1 indicates a variation in virulence among the isolates, which is common in *Rhizoctonia* groups due to genetic differences and the ability to secrete cell wall-degrading enzymes such as cellulase and pectinase, which lead to the breakdown of seed and seedling tissues before germination is complete. Williamson-Benavides and Dhingra (31) indicated regarding the pivotal role of soil pathogens in causing early losses in the early growth stages. Furthermore, the persistence of the fungus in the soil as sclerotia or mycelium explains the continuity and recurrence of infection within the crop rotation. This aligns with the findings of Khangura et al. (16) regarding the fungus's ability to survive for extended periods in crop residues, thus increasing the density of pathogenic inoculum in the soil. There was also a significant effect on the germination rate of the varieties, with the Adna variety exhibiting the lowest germination rate at 66.78% compared to the other varieties.

Table 1. Effect of *Rhizoctonia* isolates on the germination rate of seeds wheat varieties

Treatment	**Variety**		**Average**	
	Abaa		100	
Control	Barcelona		100	
	Adna		100	
	Abaa		75	
Rhizoctonia 1	Barcelona		70	
	Adna		67.5	
	Abaa		65	
Rhizoctonia 2	Barcelona		60	
	Adna		50	
	Abaa		57.5	
Rhizoctonia 3	Barcelona		55	
	Adna		50	
Average of treatments	Control=100	*Rhizoctonia* 1 = 70.8	*Rhizoctonia* 2 = 61.7	*Rhizoctonia* 3 = 54.61
Average of varieties	Abaa=74.37	Barcelona=71.25	Adna=66.87	

L.S.D 0.05	Treatments=6.55	Varieties=5.67	Interaction=11.34

Each number in the table represent the average of four replicates.

The antagonistic ability of bio-control isolates

The outcomes of the double culture test showed high antagonistic activity of *Trichoderma* isolates with inhibition rates exceeding 66% in some treatments (Table 2). This effect is attributed to several integrated mechanisms including competition for food and space, direct parasitism of the pathogenic fungus (mycoparasitism), secretion of antibiotic compounds such as gliotoxin and viridin, and production of hydrolytic enzymes such as chitinase and β-1,3-glucanase, which lead to the breakdown of the fungal cell wall. Bamisile et al. (5), indicated that *Trichoderma* fungi are among the most efficient biological control agents due to their possession of a wide range of antagonistic mechanisms. The results of this study are consistent with those reported by Chopra et al. (8), which indicate that the use of biological control agents reduces pathogen density in the soil by modifying the microbial balance in favor of beneficial organisms. Furthermore, Guzmán-Guzmán et al. (13) demonstrated that *Trichoderma* not only directly inhibit pathogens but also induce systemic resistance (ISR), thus enhancing the plant's ability to resist infection.

Table 2. The effect of bio-control fungi in inhibiting the growth of pathogenic fungi

Bio-control agent	Pathogen isolate	Average	
	Rhizoctonia 1	68.14	
TH1	*Rhizoctonia* 2	66.44	
	Rhizoctonia 3	55.97	
	Rhizoctonia 1	68.03	
TH 2	*Rhizoctonia* 2	69.85	
	Rhizoctonia 3	62.29	
Average of bio-control agent	TH1= 63.52	TH2= 66.52	
Average of pathogen	*Rhizoctonia*1=68.08	*Rhizoctonia* 2=67.99	*Rhizoctonia* 3=59.13
L.S.D 0.05	Bio-control=2.849	Pathogen=3.489	Interaction=4.934

Effect of biological control on vegetative characteristics

The addition of the bio-control fungi resulted in an increase in plant height from

17.18 to 19.33 cm, an increase in root length from 5.92 to 7.55 cm (Tables 3 and 4) and a significant increase in both fresh and dry weight (Tables 5, 6, 7, and 8). The use of the bio-control led to a significant improvement in shoot and root length as well as fresh and dry weight compared to the treatment with the pathogenic fungus alone. This improvement is not only explained by a reduction in infection severity, but is also likely due to *Trichoderma's* role as a plant growth-promoting fungus. Studies indicate that this fungus stimulates auxin production, enhances mineral uptake and activates induced systemic resistance (ISR) pathways leading to improved photosynthetic efficiency and increased biomass (7).

Table 3. Effect of *Rhizoctonia* on the vegetative growth length (cm) of wheat varieties

Treatments	Without bio-control agent			With bio-control agent			Average
	Abaa	Barcelona	Adna	Abaa	Barcelona	Adna	
Control	20.57	19.95	19.35	23.00	20.95	21.85	20.95
Rhizoctonia 1	13.80	15.13	14.95	16.30	17.38	17.00	15.76
Rhizoctonia 2	19.70	17.75	14.05	21.75	18.50	17.55	18.20
Rhizoctonia 3	15.88	17.62	17.40	18.50	20.18	19.02	18.10
Average of bio-control	17.18			19.33			
Average of variety	Abaa=18.69		Barcelona=18.43		Adna=17.65		
L.S.D 0.05	Bio-control=0.955		Pathogen=1.350		Variety=1.169		Interaction=3.307

Table 4. Effect of *Rhizoctonia* on the root growth length (cm) of wheat varieties

Treatments	Without bio-control agent			With bio-control agent			Average
	Abaa	Barcelona	Adna	Abaa	Barcelona	Adna	
Control	9.10	8.05	8.15	10.42	9.30	9.15	9.03
Rhizoctonia 1	5.33	4.30	4.30	6.92	6.95	6.42	5.70
Rhizoctonia	6.25	5.48	5.27	7.50	6.70	7.02	6.37

2							
Rhizoctonia 3	5.60	4.58	4.62	6.68	6.95	6.62	5.84
Average of bio-control	5.92			7.55			
Average of variety	Abaa=7.22		Barcelona=6.54		Adna=6.45		
L.S.D 0.05	Bio-control=0.402		Pathogen=0.569		Variety=0.493		Interaction=0.804

Table 5. Effect of *Rhizoctonia* on the fresh weight of the vegetative mass (g) of wheat varieties

Treatments	Without bio-control agent			With bio-control agent			Average
	Abaa	Barcelona	Adna	Abaa	Barcelona	Adna	
Control	0.36	0.24	0.24	0.49	0.33	0.34	0.33
Rhizoctonia 1	0.17	0.13	0.12	0.25	0.20	0.19	0.18
Rhizoctonia 2	0.22	0.19	0.14	0.32	0.25	0.18	0.22
Rhizoctonia 3	0.23	0.17	0.15	0.30	0.23	0.28	0.23
Average of bio-control	0.20			0.28			
Average of variety	Abaa=0.29		Barcelona=0.22		Adna=0.20		
L.S.D 0.05	Bio-control=0.0201		Pathogen=0.0284		Variety=0.0246		Interaction=0.0696

Table 6. Effect of *Rhizoctonia* on the fresh weight of the total root (g) of wheat varieties

Treatments	Without bio-control agent			With bio-control agent			Average
	Abaa	Barcelona	Adna	Abaa	Barcelona	Adna	
Control	0.18	0.13	0.12	0.26	0.21	0.19	0.18
Rhizoctonia 1	0.08	0.07	0.09	0.11	0.13	0.13	0.10
Rhizoctonia	0.12	0.11	0.08	0.21	0.18	0.16	0.14

2							
Rhizoctonia 3	0.11	0.09	0.10	0.15	0.15	0.12	0.12
Average of bio-control		0.11			0.16		
Average of variety	Abaa=0.15		Barcelona=0.13		Adna=0.12		
L.S.D 0.05	Bio-control=0.0123		Pathogen=0.0175		Variety=0.0151		Interaction=0.0428

Table 7. Effect of *Rhizoctonia* on the dry weight of vegetative parts (g) of wheat varieties

Treatments	Without bio-control agent			With bio-control agent			Average
	Abaa	Barcelona	Adna	Abaa	Barcelona	Adna	
Control	0.077	0.040	0.047	0.100	0.090	0.115	0.078
Rhizoctonia 1	0.025	0.025	0.020	0.082	0.050	0.030	0.038
Rhizoctonia 2	0.032	0.035	0.025	0.062	0.070	0.045	0.045
Rhizoctonia 3	0.032	0.022	0.027	0.065	0.060	0.060	0.044
Average of bio-control		0.034			0.069		
Average of variety	Abaa=0.059		Barcelona=0.049		Adna=0.046		
L.S.D 0.05	Bio-control=0.0050		Pathogen=0.0071		Variety=0.0061		Interaction=0.0178

Table 8. Effect of *Rhizoctonia* on the dry weight of the total root (g) of wheat varieties

Treatments	Without bio-control agent			With bio-control agent			Average
	Abaa	Barcelona	Adna	Abaa	Barcelona	Adna	
Control	0.045	0.037	0.32	0.082	0.065	0.067	0.055
Rhizoctonia 1	0.010	0.012	0.010	0.030	0.032	0.027	0.020

Rhizoctonia 2	0.020	0.017	0.012	0.035	0.027	0.025	0.022
Rhizoctonia 3	0.017	0.012	0.015	0.030	0.030	0.037	0.023
Average of bio-control		0.020			0.040		
Average of variety	Abaa=0.033		Barcelona=0.029		Adna=0.028		
L.S.D 0.05	Bio-control=0.0025		Pathogen=0.0036		Variety=0.0089		Interaction=0.0089

The percentage and severity of infection

The infection rate decreased from 37.08% to 19.06%, and the infection severity decreased from 41.02% to 17.48% with the addition of the biofungus (Tables 9 and 10), confirming its high efficacy. The significant decrease in infection rate (from 37.08% to 19.06%) and infection severity (from 41.02% to 17.48%) reflects high biological efficacy under soil conditions indicating that the biofungus was able to establish itself and compete in a complex, but not entirely sterile environment. This is an important indicator because the success of biological control agents is often influenced by their interactions with the soil microbiome. These results support the findings of Chopra et al. (8) regarding the ability of biotic factors to induce a microbial balance that reduces the density of pathogenic inoculum in the soil.

Significant differences in susceptibility were also observed between studied varieties when the Aba'a variety exhibiting relatively higher tolerance compared to the Adna and Barcelona varieties. This may be attributed to genetic differences in cell wall thickness, germination rate, or the level of defensive phenolic compounds. This supports the concept of the three-way interaction between plant, pathogen and biotic agent (13). This three-way interaction is a crucial element in the success of integrated management programs. Integrating fungal bio-control agents into programs of integrated management is a strategic option for achieving sustainable agriculture and reducing reliance on chemical pesticides (32). Overall, the study results confirm that the use of *Trichoderma* is an effective and sustainable tool for managing root rot and seedlings death in wheat, and multi-environment field trials are recommended to evaluate the consistency of performance under varying field conditions. Recent studies indicate that *Trichoderma* fungi not only act as antibiotics against pathogenic fungi but also play a regulatory role in the rhizosphere microbiome, promoting soil ecosystem stability and reducing disease inoculum density. Pacheco-Trejo et al. (24) demonstrated that the interaction between *Trichoderma* and the plant leads to the reprogramming of gene expression associated with systemic resistance. Poveda (26) also reported that *Trichoderma* stimulate the production of phenolic compounds and defensive enzymes in plants thereby increasing resistance to soil-borne diseases including *R. solani*. These findings support the results of Zin and Badaluddin (33) regarding the efficacy of *Trichoderma harzianum* in reducing damping-off disease by over 60% under field conditions. In a recent study, Gutiérrez-Chávez et al. (12) indicated that the use of *Trichoderma* leads to improved absorption of micronutrients, especially iron and zinc, which positively impacts vegetative growth and dry weight, consistent with the results of current study. As Gupta and Bar (11) explained, the antagonistic mechanism does not rely solely on the secretion of hydrolytic enzymes, but also includes the secretion of signaling molecules that stimulate plant resistance via the jasmonic acid pathway. Sharma (29) indicated that the integrating biological control within integrated pest management programs reduces the need for chemical pesticides by up to 40% without affecting productivity. More recent study by Mukherjee et al., (21) has shown that modern genetically enhanced *Trichoderma* strains exhibited a higher ability to colonize roots and increased biological control efficiency against *Rhizoctonia*.

Table 9. Effect of *Rhizoctonia* on the percentage of infection of wheat varieties

Treatments	Without bio-control agent			With bio-control agent			Average
	Abaa	Barcelona	Adna	Abaa	Barcelona	Adna	
Control	00	00	00	00	00	00	00
Rhizoctonia 1	48.75	50.00	60.00	25.00	21.25	23.75	38.12
Rhizoctonia 2	43.75	38.75	57.50	28.75	22.50	23.75	35.83
Rhizoctonia 3	51.25	35.00	60.00	32.50	20.00	31.25	38.33
Average of bio-control	37.08			19.06			
Average of variety	Abaa=28.75		Barcelona=23.44		Adna=32.03		
L.S.D 0.05	Bio-control=2.087		Pathogen=2.951		Variety=2.556		Interaction=7.229

Table 10. Effect of *Rhizoctonia* on the severity of the infection of wheat varieties

Treatments	Without bio-control agent			With bio-control agent			Average
	Abaa	Barcelona	Adna	Abaa	Barcelona	Adna	
Control	00	00	00	00	00	00	00
Rhizoctonia 1	42.00	52.50	51.75	18.75	23.25	20.00	34.71

Rhizoctonia 2	52.00	49.25	54.50	18.25	22.75	31.25	38.00
Rhizoctonia 3	65.00	57.00	68.25	24.75	25.25	25.50	44.29
Average of bio-control		41.02			17.48		
Average of variety	Abaa=27.59		Barcelona=28.75		Adna=31.41		
L.S.D 0.05	Bio-control=1.657		Pathogen=2.344		Variety=2.030		Interaction=5.741

Conclusion

Root diseases caused by *R.* solani is a critical threat to the productivity of wheat worldwide. Current study determined that the Rh3 isolate was the most virulent on all studied varieties, while, Aba'a variety exhibiting relatively higher tolerance compared to the Adna and Barcelona. *Trichoderma* appeared to show high antagonistic activity against the pathogenic fungus. Vegetative characteristics of wheat improved significantly with the use of the biological agent as well as the incidence and severity of infection decreased considerably. Biological control can be incorporated into integrated disease management programs as the *Trichoderma* fungi can be considered an effective way to reduce seed rot and wheat seedling death caused by *R. solani*. They improve germination rates, reduce infection and promote vegetative growth making them a sustainable option for combating fungal diseases in the fields.

Conflict of interest

The authors declare no conflict of interest.

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