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BIOLOGICAL CONTROL OF ROOT ROT FUNGI IN COWPEA

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SUMMARY

The fungal species isolated from infected seedlings showed the appearance of root rot fungi, including *Rhizoctonia solani*, *Fusarium solani*, and *Macrophomina phaseolina*. The evaluation of two biological control agents, *Trichoderma viride* and *Trichoderma harzianum,* showed their ability to inhibit pathogenic fungi, and as per Bell's scale, the inhibition percentage ranged from 1% to 2%. Both biological controls, *T. harzianum* and *T. viride,* significantly reduced the incidence of infection caused by the fungi. *T. harzianum* reduced the incidence of *R. solani* (20%), which was at par with *T. viride* (33.33%) reduction of *R. solani*, and a reduction in *M. phaseolina* and *F. solani* with *T. viride* amounted to 30.00% and 33.33%, respectively, compared with the control treatment inoculated with the fungus only (80.00%). Results also revealed that these two types lessened infection severity, where the highest decrease in infection severity with *F. solani* was 0.30 and 0.20 for *T. viride* and *T. harzianum*, respectively. In addition, both biological controls also positively affected the plant growth characteristics. *T. harzianum* with *M. phaseolina* increased shoot height (22.20 cm) compared with the pathogenic fungus treatment (16.46 cm), respectively. Moreover, the *T. harzianum* with *M. phaseolina treatment* significantly raised root length and dry weight compared with the control treatment.

Keywords: Cowpea (*Vigna sinensis* L.), *F. solani, M. phaseolina, R. solani*, *T. viride*, *T. harzianum*

Key findings: In cowpea (*Vigna sinensis* L.), the root rot infection caused by fungi, including *Rhizoctonia solani*, *Fusarium solani*, and *Macrophomina phaseolina,* gained significant reduction and control by bioagents fungi (*Trichoderma viride* and *Trichoderma harzainum*).

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INTRODUCTION

Cowpea (*Vigna sinensis* L.) is an essential legume crop in Iraq, growing in the spring and autumn seasons; however, it is native to Africa (Manganiello *et al*., 2018). Its planting is also advantageous to increase soil fertility and improve its properties (Mohammed and Ridha, 2020). Like other legumes, cowpea is rich in nutrients, including proteins, carbohydrates, and minerals (Tan *et al.,* 2012). Cowpea is one of the main products of family farming in semi-arid regions, such as Iraq, and is a vital protein source for low-income populations of rural areas (Alsaedi *et al*., 2017).

The cowpea crop is mainly prone to infection due to various soil fungi that infect the seeds before and after germination, causing seed rotting and decomposition, death of seedlings before and after emergence, and root rot, which increases its danger with its wide family range (Singh *et al.,* 2012a, b; Leyva *et al*., 2019). Recorded resistance to unsuitable environmental conditions and survival in soil and plant residues from one season to another and various fungal pathogens were in detail, including *Fusarium* spp., *Pythium* spp., *Phytophthora* spp., *Rhizoctonia solani,* and *Macrophomina phaseolina* (Anne *et al*., 2002; Babu *et al.,* 2007; Bunbury-Blanchette and Walker, 2019; Khan *et al.,* 2014; Sanches-Montesions *et al.,* 2021).

Biological control is one of the imperative research directions that can considerably be a practical solution to combat various plant diseases, especially knowing the risks of using chemical pesticides (Chamem *et al.,* 2021). Many organisms, including bacteria and fungi, are practically alternative biological control. The bio-agent *Trichoderma* is one of the most common fungi vital in this aspect. Reports on *Trichoderma* have stated it is a natural agent for numerous pathogenic fungi, including *R. solani*, *F. solani,* and *M. phaseolina* (Carrasco *et al.,* 2016). Several studies have indicated the efficiency of *Trichoderma* spp. for their diverse mechanisms in influencing the various pathogens (Singh *et al* ., 2014; Uinale *et al.,* 2013; Tarirai *et al.,* 2019; Rodriguez *et al.,* 2021).

In the fungal biological control, two chief action mechanisms exist. The first is direct, which includes antagonism, straight parasitism, competition for nutrient sources, and inhibition of pathogen enzymes (Carrasco *et al.,* 2016). The second is producing various lysis enzymes, increasing nutrient availability, absorbing and transferring minerals nourishing the plant, and complex organic material decomposition (Chamem *et al.,* 2021). Additional actions include enhancing the plant's tolerance to various adverse conditions and environmental stresses, promoting plant growth, and inducing plant resistance (Bunbury-Blanchette and Walker, 2019).

Therefore, the promising study aimed to a) isolate and identify the pathogenic fungi associated with cowpea root rot and seedling death, b) determine the efficiency of the fungal biological control, *Trichoderma viride* and *T. harzianum,* for their in vitro antagonism against root rot fungi, and c) evaluate the in vivo effectiveness of biocontrol fungal agents in reducing the cowpea root rot infection.

MATERIALS AND METHODS

Isolation and identification of the fungal pathogens

Isolation of pathogens from cowpea seedlings transpired, which has signs of seedling death. The seedlings' placement under running water for two hours continued with cutting the affected area. Cut pieces measuring 0.5 cm from the area adjacent to the infection zone bore superficial sterilization in 1% sodium hypochlorite for 2–3 min. Then, the pieces gained culturing in 9-cm Petri dishes containing potato dextrose agar (PDA) medium added with 100 mg/L antibiotic chloramphenicol. Plates containing five pieces underwent incubation at 25 °C ± 2 °C for five days. The purified fungal isolates proceeded an identification according to the approved taxonomic guide (Barnett and Hunter, 2006). Fungal isolates' culturing in slants continued storage at 4 °C before subsequent tests.

Table 1. Five-point Bell scale for the degree of parasitism.

Degree	Description
1	Fungal biological control covered all plate
2	Fungal biological control covered 2/3 of the plate
3	Fungal biological control cover 1/2 of the plate
4	The pathogen covers 2/3 of the plate
5	The pathogen covers 2/3 of the plate

Fungal inoculum *R. solani, F. solani,* and *M. phaseolina*

The fungal inoculum preparation used local millet seeds (*Panicum miliacum*), washed well and moistened for six hours, dried by filter paper, and placed at 100 g in a 250 ml glass beaker, moistened with sterile distilled water, then sterilized with autoclave for half an hour. Each flask inoculation had a 4 mm disc taken from the edge of the eight-day-old fungal colony, cultured on a PDA medium, and incubated at 25 °C ± 2 °C for 15 days, with shaking every two days by adding sterile distilled water (Fraval, 2005).

Biological control agents

Using isolates of two biological controls, *T. harzianum* (Th) and *T. viride* (Tv) comprised the study. These isolates came from the Plant Pathology Laboratory, Department of Plant Protection, College of Agriculture and Forestry, University of Mosul, Iraq.

Antagonistic ability test

Trichoderma spp. isolates evaluation occurred for their antagonism against pathogenic fungi by the Dual Culture Technique (DCT) on a PDA medium with three replications for each treatment. For the control treatment, both halves of the dish sustained inoculation with two discs of pathogenic fungi and incubated at 25 °C ± 2 °C, with the degree of antagonism determined (Table 1) (Bell *et al.*, 1982).

Greenhouse experiment

The study conducted at the Department of Plant Protection, College of Agriculture and Forestry, University of Mosul, Iraq, had a completely randomized design (CRD) used for the experiments, and the treatments included untreated and treated cowpea seeds. In treated seeds, two types of biological control agents (*T. viride* and *T. harzianum*) ensued in sterile soil or soil inoculated with pathogenic fungi separately. Filling plastic pots with one kilogram of disinfected soil inoculated with pathogenic fungi happened before planting the cowpea seeds with 3 g of pathogenic fungi inoculum/1 kg soil.

Cowpea seeds incurred 1 h soaking in a suspension of *Trichoderma* spp. spores at 10^4 spores/ml, with 5% molasses added as an adhesive and food base. The seeds in the control treatment, soaked in distilled water, also had 5% molasses added. Three days after treating the soil with the pathogenic fungi, five cowpea seeds followed planting in each pot, with three replications for each treatment. After four weeks of planting, calculations on the percentage of infection with seedling death after emergence and the severity of infection progressed (Rodriguez *et al.,* 2021).

The infected seedlings incurred reisolation to confirm the pathogenicity of the fungi used, with the average shoots’ height and roots’ length of plants, the average dry weight, and the plants’ ratio of S/R (vegetative to root length) estimated. All data analysis used the SAS statistical program, testing the averages by Duncan's polynomial method at the 5% probability level (ICSO, 2019).

RESULTS AND DISCUSSION

Isolation and identification of pathogen fungi

The isolation in cowpea seedlings showed the appearance of the fungi *Rhizoctonia solani*,

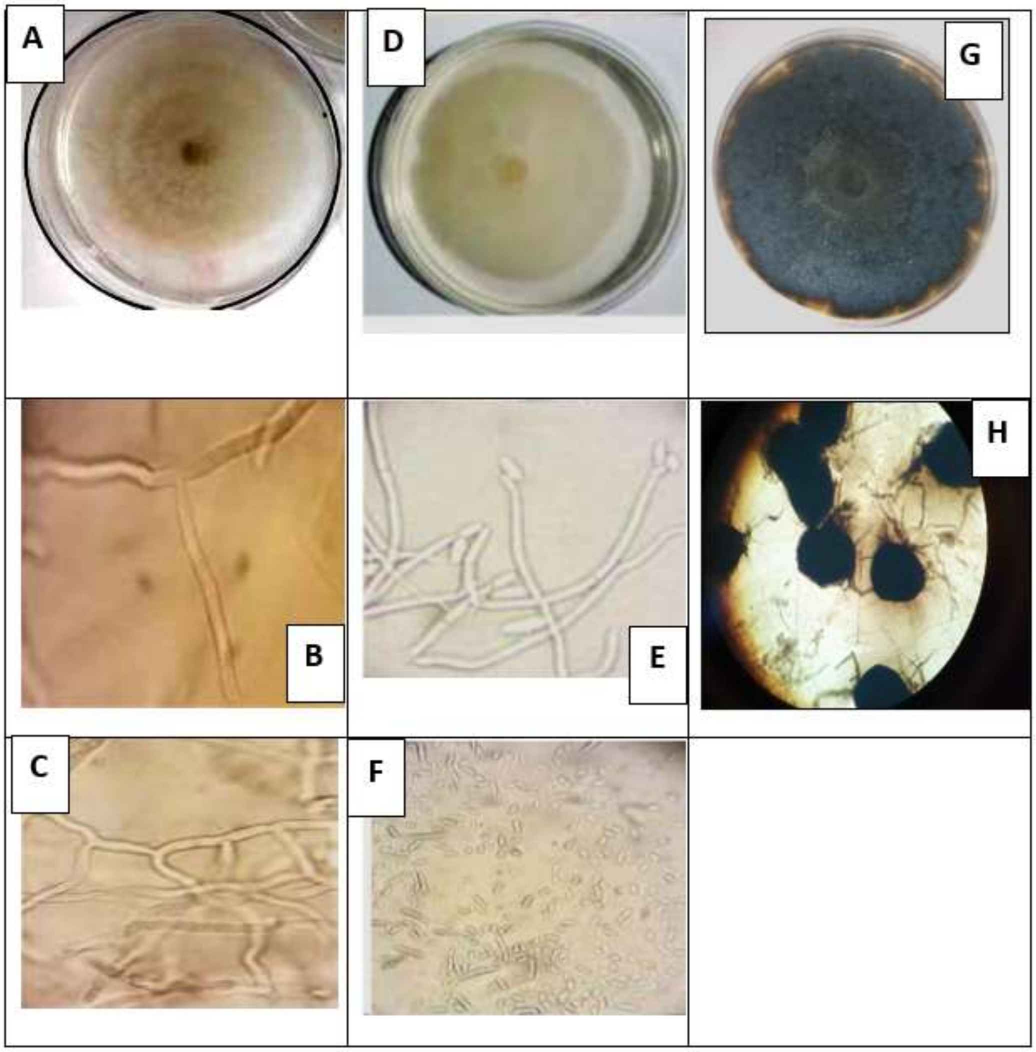

Figure 1. Microscopic examination of the pathogenic fungi under study showing A*: Rhizoctonia solani* with cottony white color colony; B, C: raised septate mycelium at a right angle; D*: Fusarium solani*, with creamy or violet-white colony; E, F: abundance of microconidia, macroconidia, and some chlamydospores; G: *Macrophomina phaseolina*, the colony with rapid growth, and H: large fruit bodies.

Fusarium solani, and *Macrophomina phaseolina*, with the isolated fungi also identified according to their phenotypic characteristics. The colony of *Rhizoctonia solani* has characteristics of a cotton white color; however, the color changed later to brown. The microscopic examination showed a raised and branch mycelium at a right angle, with dents at the branch emergence area, septate, and the cells characterized by barrel shape. The diameter of the colony was 8 cm after five days of incubation at 25 °C ± 2 ° C (Figure 1 A, B, and C).

Fusarium solani was distinguishable by its creamy and violet-white color, with an abundance of microconidia in its cylindrical to oval shape, with a high percentage presence of macroconidia and chlamydospores. The colony diameter was 8 cm after seven days of incubation at 25 °C ± 2 °C (Figure 1 D, E, and F). As for *Macrophomina phaseolina*, the colony was distinctive by its rapid growth, transparent

Table 2. Antagonism of the two biological control *T. viride* and *T. harzianum* against pathogenic fungi according to the Bell's scale (14).

Antagonism	*F. solani*	*R. solani*	*M. phaseolina*
T. viride	1	1	2
T. harzianum	2	1	1

Table 3. Effect of treatment with *Trichoderma* spp. on pathogenicity indicators (infection rate and severity) and growth indicators of cowpea plants inoculated with pathogenic fungi.

Treatments	Infection rate %	Infection severity	Shoot height	Root length	Plant dry weight	S/R ratio
F. solani	80a	0.29d	15.33ef	8.5f	8.73cd	1.73a
M. phaseolina	80a	0.79a	15.33ef	8.5f	8.73cd	1.73a
R. solani	80a	0.84a	15.33ef	8.5f	8.73cd	1.73a
F. solani + T. viride	33.33bc	0.30d	18.6cd	13.96bcd	11.13b	1.23cde
F. solani + T. harzianum	40b	0.20d	16.86cdef	11.80de	10.5b	1.43bc
M. phaseolina + T. viride	33.33bc	0.52b	15.66def	12.80cde	10.16b	1.20cde
M. phaseolina + T. harzianum	30bc	0.60bc	22.20a	19.03a	14.8a	1.13de
R. solani + T. viride	40b	0.72ab	18.73cd	15.00bc	9.10bc	1.23cde
R. solani + T. harzianum	20c	0.68abc	14.93f	13.30cde	8.46d	1.06e
T. viride	00d	0.00e	20.63ab	12.36cde	10.33b	1.60ab
T. harzianum	00d	0.00e	16.86cdef	12.6de	8.70cd	1.36bcd
Control	00d	0.00e	16.50cdef	10.76ef	10.60b	1.46abc

color at first, and later turning black centrally and then including the entire colony, with a growth of a high fluffy appearance. On microscopic examination, fruit bodies ranged from 72 to 163 microns with diameters, and the colony diameter was 8 cm after five days of incubation at 25 °C ±2°C (Figure 1 G and H). These results agreed with studies that referred to these fungi causing seedling death and root rot (Barnett and Hunter, 2006; Sanches-Montesions *et al.,* 2021; Rodriguez *et al.,* 2021).

Antagonistic ability test

The results referred to the two biological controls, *T. viride* and *T. harzianum,* showing the highest antagonistic ability against pathogenic fungi *R. solani*, *F. solani,* and *M. phaseolina* under laboratory conditions (Table 2). The degree of antagonism ranged at 1–2. Results indicated that *R. solani* bore most effects from both biological controls with a degree of antagonism of one according to the Bell scale in 1982. These results were consistent with previous findings in testing the antagonistic ability of the two biological controls, *T. viride* and *T. harzianum,* against fungal pathogens (Azevedo *et al.,* 2020; Hassan, 2023).

The antagonistic ability of *Trichoderma* against pathogenic fungi was due to several mechanisms, including direct parasitism on the mycelium, its growth rate compared with that of the pathogenic fungi colony, and the enzyme production, such as B-13-glucanase, protease, lipase, and chitinase that also degrade the pathogen cell walls. In addition, the breaking down ***of*** mycelium walls occurs due to the accumulation and adhesion of the biological control agent spores (AL-Lashi, 2003; Nerey *et al.,* 2010; Singh *et al.,* 2012a, b; Manganiello *et al.,* 2018).

The results further enunciated that the treatments of pathogenic fungi (positive control) led to the highest infection rate values and severity, which also significantly decreased when treated with the two biological controls, *T. harzianum* and *T. viride*. However, *T. harzianum,* interacting with *R. solani,* had the maximum reduction in infection rate from 80% to 20% and infection severity from 0.84 to 0.68. As for *T. viride*, it reduced the rate of infection in *M. phaseolina* from 80% to 33%

and the infection severity from 0.79 to 0.52 (Table 3). Past studies indicated that *Trichoderma* produces Gliotoxin, Glioviridin, and Peptaibols with inhibitory effects on many soil-borne pathogenic fungi (Dewan, 1989; Thilagavathi *et al.,* 2007). The relevant results were also analogous with past findings revealing that many plants treated with *Trichoderma* spp. showed a significant increase in growth parameters, such as shoot height and root length, number of lateral roots, and leaf area (Djonovic, 2005; Carrasco *et al.,* 2016; Onwubiko, 2020).

Concerning the growth indicators of cowpea plants, the variables gained significant influences from pathogenic fungi and biological controls. The results revealed that pathogenic fungi (without biological control factors) caused the highest reduction in shoot height and root length, dry weight, and shoot/root ratio. However, plants treated with biological controls, *T. viride* and *T. harzianum,* significantly enhanced plant growth parameters under study despite having the pathogenic fungi. Generally, the maximum values for shoot height and root length, dry weight, and shoot/root ratio were evident in the interaction treatment of pathogenic *M. phaseolina* with the biological control *T. harzianum.* This fungus was more effective in reducing the effect of *M. phaseolina phasiolina*, while *T*. *viride* had the highest influence in suppressing *R. solani* and *F. solani*. Speedeui *et al.* (2011) reported a relationship between the production of pyrone by *T*. *harzianum* and the ability to antagonize against *F. solani* outside the cell. The ability of *Trichoderma* spp. to produce Trichodermoi, Trichodermin, and Gliotoxine considerably inhibited various pathogenic fungi, including those causing death and rotting of seedlings, such as *M. phaseolina* (Harman, 2000; Khaledi and Taheri, 2016).

The studies also confirmed the efficiency of *Trichoderma* isolates showing a high parasitic, antagonistic, and competitive ability against the fungi that cause seedling death, root rot, and vascular wilt (Belete *et al*., 2015; Chamem *et al.,* 2021; ICSO, 2019), in addition to the positive effect of *Trichoderma* spp. on growth parameters of treated plants (Aziz *et al.,* 1997; Lopez-Bucio *et al.,* 2015).

CONCLUSIONS

The results authenticated the efficiency of fungal biological controls, i.e., *T. viride* and *T. harzianum,* in controlling the pathogenic fungi *F. solani*, *M. phaseolina,* and *R. solani* that cause seedling death and cowpea root rot. *Trichoderma* spp. showed the highest parasitic ability to pathogenic fungi. These mechanisms include invasion and sprouting on colonies of pathogenic fungi and the production of enzymes, degrading the pathogenic fungi mycelium, and producing volatile antifungal material and non-volatile growth inhibitory compounds. The results suggested the possible use of fungus isolates for the biological control of seedling death and cowpea root rot diseases.

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