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INTEGRATED DISEASE MANAGEMENT OF *RHIZOCTONIA SOLANI* ROOT ROT IN COMMON BEAN

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Abstract

Rhizoctonia solani was frequently isolated from diseased plants, and A 688 bp rDNA fragment was successfully amplified, which identified the isolate as *R. solani* CB1 (OR053863) with 96.44% genetic similarity to *R. solani* AG-4 HGIII RR2 (LC504765.1). *In vitro*, isolate CB1 caused 100% disease severity in bean seedlings. In the food poison method, fungicides exhibited substantial suppression in the growth of *R. solani.* Fungicide Nativo showed 52.2% growth inhibition when used @ 1 ppm. Ready Super at 10 ppm gave 72.5% growth suppression of the pathogen. In dual culture plate assays, *Trichoderma longibrachiatum* and *T. harzianum* suppressed *R. solani* growth by 66.7 and 60.4%, respectively. In pot experiment, *R. solani* showed 46.7% disease severity in positive control plants. The disease severity decreased to 6.7% with Nativo treatment, showing the best control. Fungicides and biocontrol agents significantly decreased disease severity and improved growth of bean plants compared to control. Biological agents, including *T. harzianum* and *T. longibrachiatum*, outperformed fungicides in improving plant length and dry weight.

Key words: *Phaseolus vulgaris*; Root rot; *Rhizoctonia solani*; Integrated disease management

Introduction

Phaseolus vulgaris L., commonly known as common beans, French beans, snap beans, green beans, and kidney beans, is a vital food legume crop globally. Beans are also a main and less expensive source of protein and contain a high amount of phaseolin. It provides essential vitamins, phenolic compounds, and carbohydrates and also has medicinal importance which may include oxidative stress and heart diseases (Kakar *et al*., 2019). In Sindh, the production of the beans has been reduced from 1255 tons in 2014-15 to 936 tons in 2018-19 (Talpur *et al*., 2020).

Rhizoctonia solani Kühn causes seed rot, seedling damping-off, stem rot, and crown and root rot in alfalfa. This root infecting fungus survives for long periods through its sclerotia and multiply well in warm, moist conditions, complicating management. Global yield losses in alfalfa range from 20% to 60%, with root rot alone accounting for 20-40% of annual losses, and incidence reaching 30-80% in some Chinese regions (Akber & Fang, 2024). One of the significant challenges to crop productivity is root rot disease caused by *R. solani* as root infecting fungus that affects multiple crops including beans, apples, canola, blueberries, tomatoes, peas and tobacco (Bodah, 2017). At least 64 hosts of *R. solani* including several leguminous plants have been reported from Pakistan (Mirza & Qureshi, 1978; Shahzad & Ghaffar, 1990). The pathogen is known for its competitive saprophytic capabilities, widespread occurrence and ability to infect plants during early development stages, leading to considerable yield losses (Tewoldemedhin *et al.*, 2006). The management of soil-borne pathogens such as *R. solani* has not been thoroughly studied in Pakistan (Parveen *et al.*, 2020).

Aphanomyces, *Fusarium*, *Pythium* and *Rhizoctonia* species are among the pathogens that cause root rot disease in bean plants, with *Rhizoctonia solani* being the most damaging. Leaf yellowing, stem drying, wilting, stunting, poor seedling development, and early defoliation are all symptoms of root rot (Singh & Schwartz, 2010; Kumar *et al.*, 2018). According to Dhami & Maharjan (2023), chemical fungicides have demonstrated efficacy against *R. solani* and other soil-borne fungi. Nonetheless, the need for sustainable agriculture and growing environmental concerns highlight the use of non-chemical plant disease management techniques. Plant pathogens have been successfully managed by biocontrol agents such as *Trichoderma*, *Gliocladium*, *Verticillium*, *Aspergillus*, *Paecilomyces*, *Penicillium*, *Pythium* and *Talaromyces*, which have benefits like host specificity, persistence in a variety of environmental conditions, and decreased ecological impact (Thambugala *et al.*, 2020). In order to control the root rot disease, increase crop yield, and lessen dependency on agricultural imports, Integrated Plant Disease Management (IPDM) combines chemical and biological methods. Despite its potential, *Phaseolus vulgaris* root rot disease has received limited attention in Pakistan. The purpose of the current study was to evaluate the effectiveness of six different chemical fungicides that are sold locally as well as the use of six antagonistic fungi to treat the disease caused by *R. solani* in *P. vulgaris*.

Materials and Methods

Experimentation site: The study was conducted under controlled laboratory conditions at the Department of Agriculture and Agribusiness Management, University of Karachi.

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Sampling & Isolation of pathogenic fungi: Plants showing symptoms such as wilt, chlorosis, dark brown root rot, or stunted growth were taken from diseased bean fields in the Sindh province (Thatta and Hyderabad regions). The diseased samples were brought to the PDRL Lab, UoK, and the agar plate method was used to isolate the pathogenic fungi from symptomatic plant parts of the common bean. The methodology outlined by Lamini *et al*., (2020) was followed during the isolation process. For additional examination, the isolated fungi were stored in slants of potato sucrose agar (PSA; potato 200 g, sucrose 20 g, agar 20 g, streptomycin sulphate 0.2 g, benzyl penicillin 100,000 units per liter).

Morphological and molecular characterization of the pathogen: The isolated plant pathogen was identified as *Rhizoctonia solani* using both molecular and cultural morphology methods. The microscopic features of the pathogen were studied after being cultivated on the PSA for two weeks at 26 ± 2°C (Parmeter, 1970).

In order to count number of nuclei in the *R. solani* cells, 6-8 drops of a wetting solution containing one mL of 85% lactic acid and 1000 mL of distilled water were applied to specific regions of the culture plate in order to prepare the *Rhizoctonia* culture for microscopic inspection. To ensure complete wetting, a sterile bent spatula was used to gently spread and rub these drops? over the fungal hyphae. One drop of 0.5% aniline blue in lactophenol was then added to the treated areas of the cultures to stain them. Both stained regions and tiny portions of unstained mycelium were carefully covered with coverslips. Following a staining period of 5-30 min., the samples were examined under a microscope at 400x magnification using the technique outlined by Herr (1979).

Molecular identification: Sequencing the ribosomal DNA (rDNA) region, which included the 5.8S gene, the internal transcribed spacer regions (ITS1 and ITS2), and nearby sections of the small unit that is a part of a large unit, allowed for the molecular identification of *R. solani*. Genomic DNA was extracted from pure fungal mycelia using the cetyltrimethylammonium bromide (CTAB) extraction protocol (Al-Fadhal *et al*., 2019). The quality and integrity of the isolated DNA were measured through electrophoresis on a 1% (w/v) agarose gel prepared in 1x TAE buffer (comprising TRIS base, acetic acid, and 0.5M EDTA). DNA bands were seen under UV light using a NIPPON GENETICS FAS-Digi PRO gel documentation system after SYBRTM Safe staining.

DNA amplification: The ribosomal DNA (rDNA) region, including the internal transcribed spacers (ITS1, and ITS2) together with the 5.8S gene, and bordered by the small subunit (SSU) and large subunit (LSU), was amplified using the universal primers ITS4 (5'-TCCTCCGC TTATTGATATGC-3') and ITS5 (5'-GGAAGTAAAA GTCGTAACAAGG-3'). Polymerase chain reaction (PCR) was performed in a total volume of 20 µl. The reaction mixture contained 2 µl of 10x PCR buffer, 1.2 µl of 1.5 mM $MgCl_2$, 0.2 µl of each primer (0.2 mM) and dNTPs, 1.5 unit of Taq DNA Polymerase, and approximately equal to 50 ng of template genomic DNA (2 µl). The volume was completed with nuclease-free water.

The following thermal profile was used for PCR amplification using a SimpliAmpTM Thermal Cycler (Applied Biosystems): initial denaturation at 94ºC for 2 min., followed by 35 cycles of denaturation at 94ºC for 30 seconds, annealing for 35 seconds (with the temperature determined by primer Tm), and extension at 72ºC for 30 seconds. The last extension phase of the treatment lasted for 7 min. at 72ºC (Al-Fadhal *et al.,* 2019). After electrophoresing the resultant amplicon, which was roughly 688 bp in size, on a 1.2% (w/v) agarose gel in 1x TAE buffer (containing 0.5M-EDTA, TRIS base, and acetic acid), it was stained using the SYBR™ Safe DNA gel strain. Using a FAS-Digi PRO gel documentation system (NIPPON Genetics), DNA bands were visible under UV light. Following purification with the MOLEQULE-ON® MQ PCR purification Kit (New Zealand), the PCR products were forwarded to Eurofins Genomics (Louisville, Kentucky, USA) for sequencing.

Sequencing analysis and multiple sequence alignment: The Finch TV (version 1.4) was used to analyze the electropherograms, and the resulting sequences were subjected to BLAST analysis through the NCBI database to determine similarities with known sequences. The obtained sequence was compared with closely related sequences found in the BLAST-NCBI database in order to determine the anastomosis group of the *R. solani* CB1 isolate. Using the neighbor-joining method and MEGA software, a phylogenetic tree was created. The evolutionary relationships were assessed using bootstrap analysis.

Pathogenicity test: The pathogenicity of *R. solani* was evaluated *In vitro* using the agar plate method (Basbagci & Dolar, 2020). Root and hypocotyl symptoms were assessed using a scale from 0 (no symptoms) to 5 (complete root and hypocotyl rot) to determine the disease severity (DS%). The scales took into consideration root length restriction, lesion formation, and different levels of discolorations. Based on DS% values, *R. solani* virulence was classified as highly virulent (51-100%), moderately virulent (21-50%), or less virulent (0-20%). This method provided a systematic approach for quantifying disease severity during pathogenicity testing (Basbagci *et al*., 2019).

***In vitro* effect of different antifungal chemicals against the colony growth of *R. solani*:** Using the poisoned food technique (Sharma & Dhruj 2018), six fungicides such as Fosetyl-Aluminium (Aliette), Propineb (Antacol), Tebuconazole, Trifloxystrobin (Nativo), Pyraclostrobin and Tebuconazole (Ready Super), Difenoconazole (Score) and Sulphur (Zaver), were tested for their efficacy against *R. solani.* The fungicides were applied at five concentrations: 1, 10, 100, 1,000 and 10,000 ppm. Vincent (1947) described a protocol for assessing the efficiency of each chemical fungicide in preventing the mycelial growth of *R. solani*. This approach calculates the growth inhibition% by comparing the average fungal growth observed in the untreated control (C) with that recorded in the fungicidal treatment samples (T) using the following formula:

$$\text{Growth inhibition \%} = \frac{C - T}{C} \times 100$$

***In vitro* interaction of *R. solani* with microorganisms:** Six biocontrol fungi viz., *Clonostachys rosea* (syn: *Gliocladium roseum*), *Paecilomyces variotii*, *Purpureocillium lilacinum* (syn: *Paecilomyces lilacinus*), *Trichoderma harzianum*, *T. longibrachiatum* and *T. virens* (syn: *Gliocladium virens*), were obtained from Plant Diseases Research Lab of the Department and assessed for their antagonistic impact against *R. solani* under *In vitro* conditions. The antagonistic activity was tested by the dual culture technique, and the percentage of growth inhibition was determined using the procedure described by Vincent (1947). To further investigate the interaction, light microscopy was used to observe the nature of mycoparasitic contact as reported by Yaqub & Shahzad (2005). Moreover, the time taken for each antagonist to make contact and then overgrow the pathogen was recorded.

Effects of selected antifungal chemicals and biocontrol agents on the growth of bean plants inoculated by *R. solani:* Based on the outcomes of *In vitro* testing, 3 fungicides *viz*., Nativo, Ready super and Score, and biocontrol agents *viz*., *T. harzianum*, *T. longibrachiatum* and *T. virens* were chosen for a supporting pot experiment. Pathogen and antagonist inocula were prepared by multiplying on sterilized sorghum seeds inoculated with 5-day old mycelial discs, incubated at 25 ± 2ºC for 6 days with 24-hour intermittent shaking. Steam sterilized soil was distributed into sterilized 25 cm clay pots @ one kg soil per pot. Prior to potting, inoculums of pathogen and biocontrol agents were prudently mixed into the potting soil at a concentration of 5% (w/w), using the protocol outlined by Lakhran & Ahir (2020). After the germination of a susceptible plant variety (Komal Green), the fungicide treatments i.e. Nativo 10 ppm, Ready super 10 ppm and Score 100 ppm were applied @ 50 ml per pot. A total of 11 treatments were performed with three replications of each treatment. After a 30-day growth period, various plant growth parameters were recorded, including length and fresh and dry weights of both shoots and roots. The disease severity was assessed using a 0-5 scoring measure with 0 designated = no infection and 5 indicating plant mortality (Basbagci & Dolar, 2020). The scale incorporated the number of affected plants ("n"), the disease score ("r"), the maximum score ("R") and total plants ("N").

$$\text{Disease severity \%} = \frac{\sum (n \times r)}{(R \times N)} \times 100$$

Statistical Analysis

Analysis of variance (ANOVA) was used to observe data both *In vitro* and pot experiments. Using Statistix version 8.1, treatment means were compared by the Tukey's Honesty Significant Difference (HSD) test at a 5% possibility level ($p<0.05$).

Results

Isolation, identification and pathogenicity test: The pathogen *R. solani* was isolated from diseased plant parts of common bean and found to be highly frequent. *R. solani* colonies exhibited a whitish to light brown appearance with abundant mycelial growth. Dark brown sclerotia were scattered throughout the culture, the hyphae were septate with large cells containing more than two nuclei.

Hyphal branching typically occurred at right angles, often accompanied by a slight constriction at the branch point. First septum in each branch was produced at some distance from the point of its origin. Additionally, some hyphae also produced moniloid cells. No conidia or spores were detected (Fig. 1). PCR amplification of fungal genomic DNA yielded a 688 bp fragment from the rDNA regions (Fig. 2). The sequence was identified using a BLAST search on the NCBI database, focusing on the ITS region of the 18S RNA gene. The resulting sequence was submitted to the NCBI GeneBank and allocated accession number OR053863. Comparative analysis showed that the *R. solani* isolate CB1 (OR053863) shared 98.54% genetic similarity with strain SQU14057 (MH025376.1), 96.44% similarity with member of the AG-4 and HGIII interspecific group, including the RR2 gene sequence (LC504765.1). Phylogenetic analysis placed isolate CB1 within clade A, clustering it closely with strain SQU14057 and the AG-4 HGIII RR2 genes group (Fig. 3). During *In vitro* agar plate assays, the *R. solani* isolate CBI demonstrated extreme virulence, causing 100% disease severity on common bean germinates (Fig. 4).

Impact of antifungal chemicals on mycelial growth of the pathogen: The results revealed that all the concentrations of the tested chemical fungicides significantly decreased radial colony growth (Figs. 5-6). Remarkably, at a concentration of 1 ppm, only Nativo and Ready Super suppressed the fungal growth of *R. solani*. The most effective fungicide was Nativo, which showed 100% growth suppression at 100 ppm, 81% at 10 ppm and 52.22% at 1 ppm. Score exhibited moderate effectiveness, inhibiting growth by 52.2% at 100 ppm and 30% at 10 ppm. In contrast to more effective treatments, Aliette, Antracol, and Zaver demonstrated relatively low antifungal activity, with none exceeding 50% growth inhibition even at higher concentrations. At 10,000 ppm, Aliette and Antracol inhibited fungal growth by 46.30% and 50% respectively, while Zaver showed only 58% inhibition at higher concentration of 10,000 ppm (Figs. 5 & 6).

***In vitro* impact of fungal antagonists on mycelial growth of soil-borne pathogen:** In a dual culture plate assay, *T. longibrachiatum* and *T. harzianum* showed the maximum inhibition in *R. solani* growth i.e. 66.67% and 60.44%, correspondingly (Figs. 7 & 8). Both *Trichoderma* species required two days to come in contact with the pathogen mycelium and exhibited a Type-**B** interaction, coiling around the pathogen after 10 days (Table 1, Figs. 8 & 9), *T. virens* showed a Type-**D** interaction, inhibiting *R. solani* growth by 47.11%. *P. lilacinum* exhibited the lowest inhibition i.e. 21.78%, forming a thin inhibition zone and a Type-**C** interaction with no overgrowth. In contrast, *C. rosea* and *P. variotii* caused minimal inhibition and showed Type-**A** interactions, with the pathogen overgrowing.

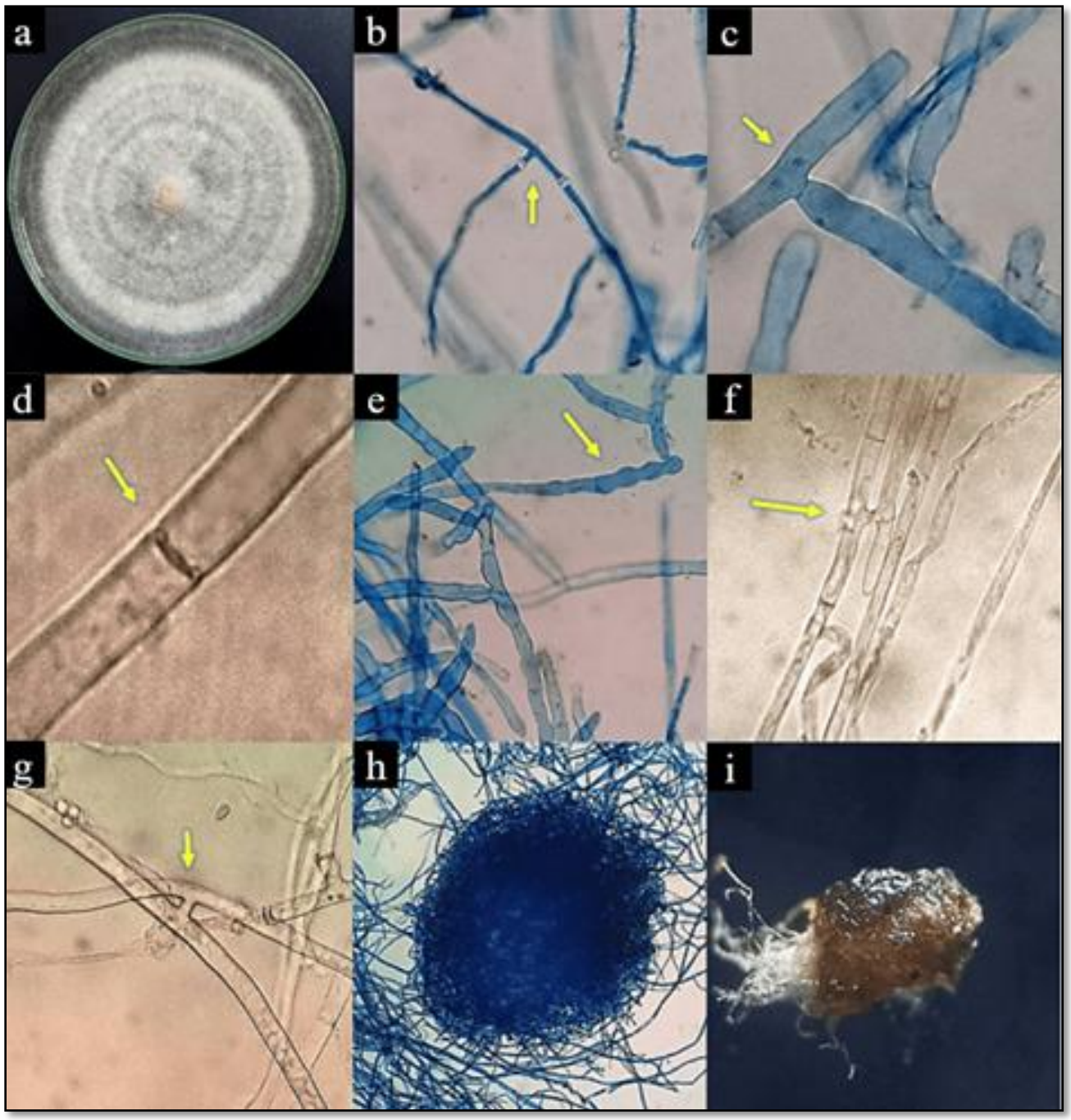

Fig. 1. Morphological characteristics of *R. solani*: **a**= colony growth on PSA medium, **b**= right angle branch, constriction at the base, and septum at some distance from base of the branch, **c**= multinucleate hyphae, **d-e**= moniloid cells, **f-g**= hyphal fusion, **h-I**= irregular sclerotia.

Efficacy of selected antifungal chemicals and fungal antagonists on growth of common bean plant inoculated with *R. solani*: The study revealed that both antagonistic organisms and fungicides significantly mitigated root rot disease in bean plants caused by *R. solani*. Infected plants in the positive control (PC) group exhibited characteristic root rot symptoms including dark brown hairless roots as observed under the microscope. In contrast, roots from the negative control (NC) group appeared healthy, white and covered with root hairs (Fig. 10). Mycelial growth of R. *solani* was clearly visible on infected roots, and the pathogen was successfully re-isolated from these roots on PSA medium (Fig. 11). However, no traces of the pathogen were found in the roots of NC plants. Nativo was the most successful fungicide since it reduced the disease severity to 6.7%, followed by Ready Super (13.3%) and Score (20%) as compared to 46.7% in control. Additionally, the antagonists were successful. Nevertheless, their effectiveness was marginally reduced, and the disease severity ranged from 20% to 33.3% (Fig. 12). Using biological agents, especially *T. harzianum* and *T. longibrachiatum*, greatly improved plant growth and decreased the severity of the disease. Furthermore, there were no signs of root rot in treatments that were not pathogen-inoculated. This study evaluated several aspects of plant growth in addition to disease control (Fig. 13). In comparison to the negative control (NC), the results showed that both fungicide treatments and biological control agents affected the growth of common bean plants. On the other hand, *R. solani* infection drastically reduced the length of the roots and shoots as well as the total biomass of the plants in the positive control

(PC) group. Among the fungicides tested, Score demonstrated a positive effect on plant growth. However, Nativo and Ready super led to a reduction in root and shoot length, likely due to phytotoxic effects at the applied concentrations, although they increased dry biomass of both root and shoot. On the other hand, all biological control agents significantly enhanced plant growth relative to fungicide treatments. *T. harzianum* and *T. longibrachiatum* were particularly effective, showing the greatest increases in both length and dry weight, followed by *T. virens*. *T. harzianum* was proved as the greatest effective treatment overall, exhibiting the maximum values for dry shoot weight, root length and dry biomass of shoots and roots confirming as the subsequently to be the most beneficial treatment promoting plant vigor and suppressing disease.

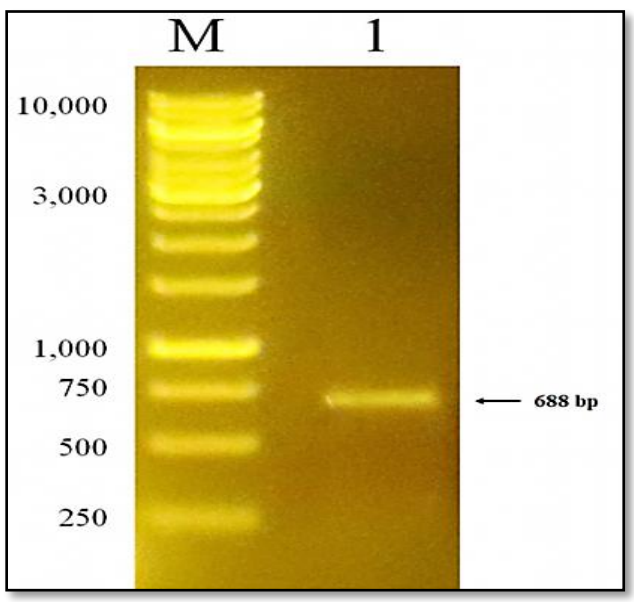

Fig. 2. Lane 1 shows the purified PCR amplification product using a *Rhizoctonia solani* DNA template. The Lane M contained 1 Kbp DNA ladder as a molecular size marker.

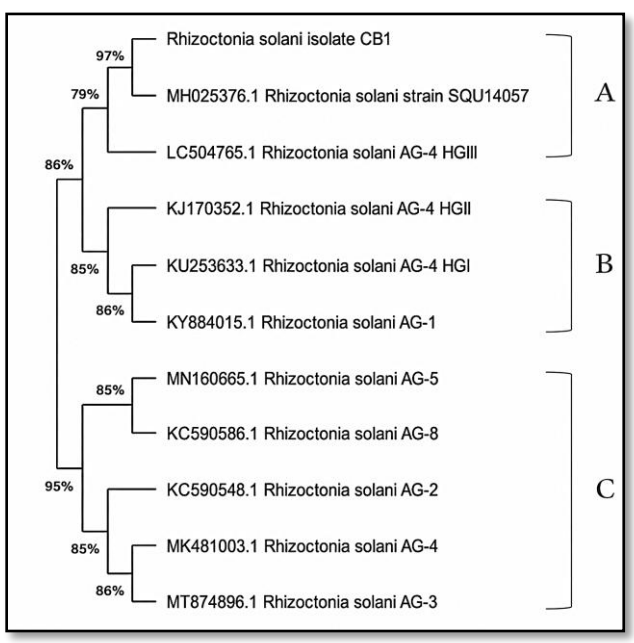

Fig. 3. Neighbor-joining tree illustrating the phylogenetic placement of *R. solani* isolate CB1 relative to other isolates, based on comparative analysis of internal transcribed spacer (ITS) sequences.

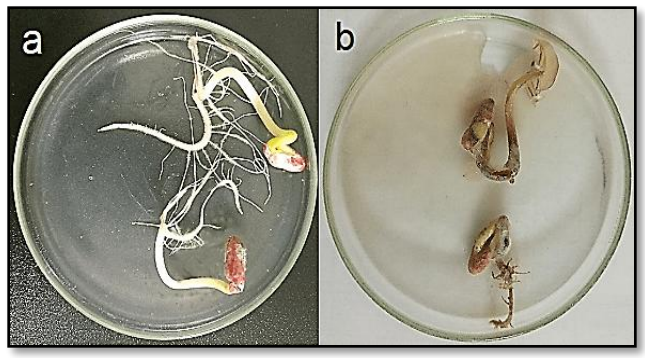

Fig. 4. Pathogenicity of *R. solani* on common bean observed ten days after inoculation: **a**= Control, **b**= infected beans seed with *R. solani*.

Discussion

In the current research, *R. solani* identified as the causal agent of root rot disease in *Phaseolus vulgaris*. Previous researches by Eken & Demirci (2004) as well as Basbagci *et al*., (2019) have also reported the widespread occurrence of *R. solani* in legumes, predominantly in beans and related plant species such as chickpea. Likewise, Marcenaro (2016) and Parsa (2016) successfully isolated *R. solani* from diseased common bean plants.

The fungal isolates were initially identified on morphological appearances. Among them, *R. solani* was further confirmed through (PCR), noticing close genetic connection with the anastomosis group 4 of *R. solani*. After that pathogenicity test proven that this isolate demonstrated 100% disease severity on growing common bean seeds, indicating it as an extremely dangerous strain. Parveen (2020), highlighted the ecological adaptability of *R. solani*, observing its capability to persist in plant tissue and debris, which serve as inoculum sources under satisfactory conditions, frequently foremost to substantial yield injuries. Eken & Demirci (2004) stated the pathogen *R. solani* extremely virulent pathogen to many common bean cultivars. Internationally, *R. solani* (AG-4) has been known as one of the greatest destructive and critical anastomosis groups affecting leguminous crops (Matloob & Juber, 2013). *Rhizoctonia solani* is known for its wide host range, accomplished of infecting over 250 plants species across diverse botanical families as well as diverse gardening crops (Senapati *et al.*, 2022).

The *In vitro* poison food technique exposed that the tested fungicides significantly restricted the mycelia growth of pathogen. Amongst the treatments, Nativo presented the maximum efficiency, followed by Ready Super and Score. Hunjan *et al.*, (2012) also stated that grouping of trifloxystrobin + tebuconazole was extremely effective against pathogen of *R. solani* under laboratory circumstances. Nativo, being a newer formulation, demonstrated strong fungal action and significant reduction of fungal colony growth at minimum concentrations (Sriraj *et al.*, 2014). Comparable results were noticed by Soomro *et al.*, (2015) who recorded that Difenoconazole resulted in significant fungal growth inhibition across all the checked concentrations.

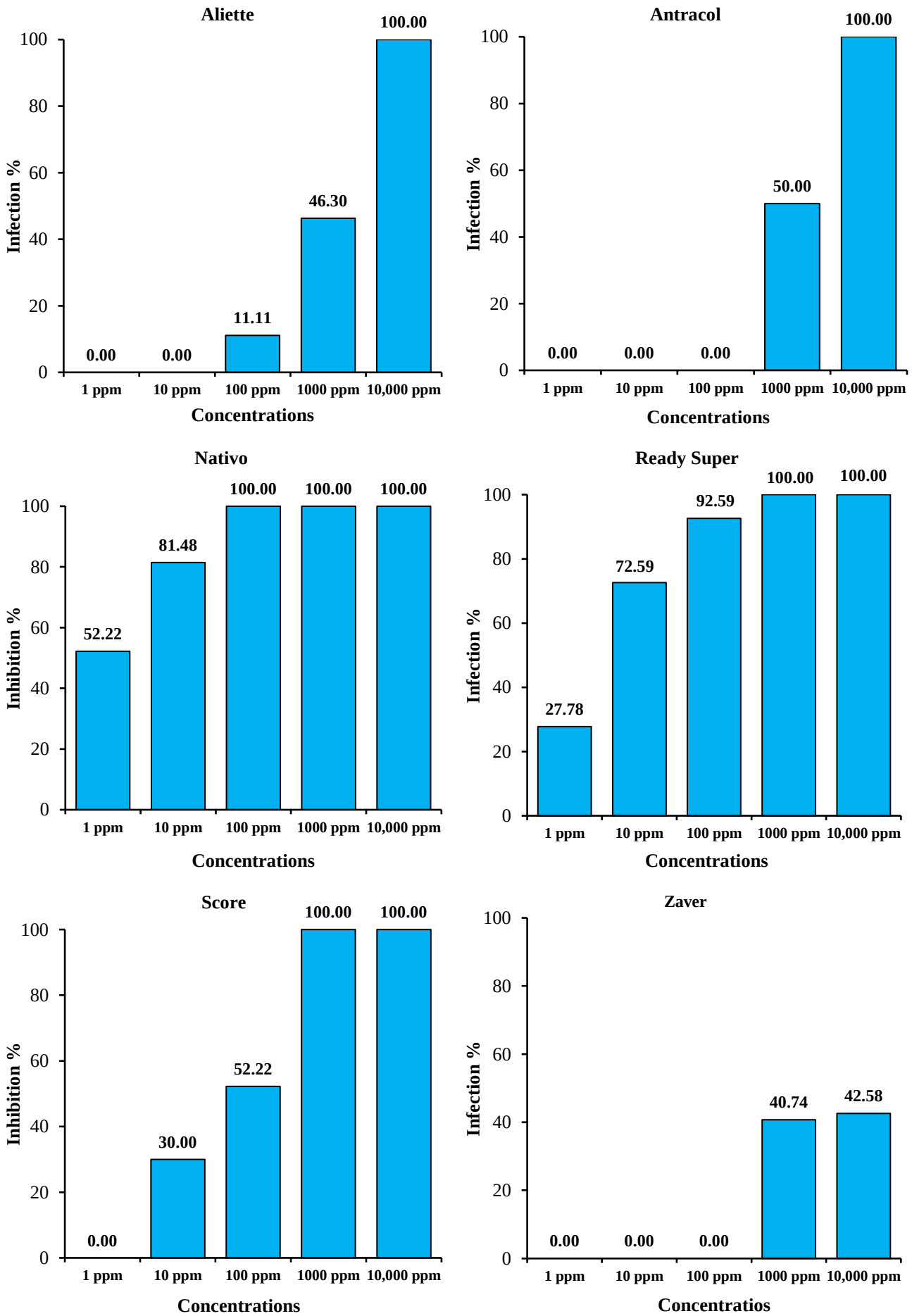

Fig. 5. *In vitro* impact of various antifungal chemicals on *R. solani* growth using poison food technique.

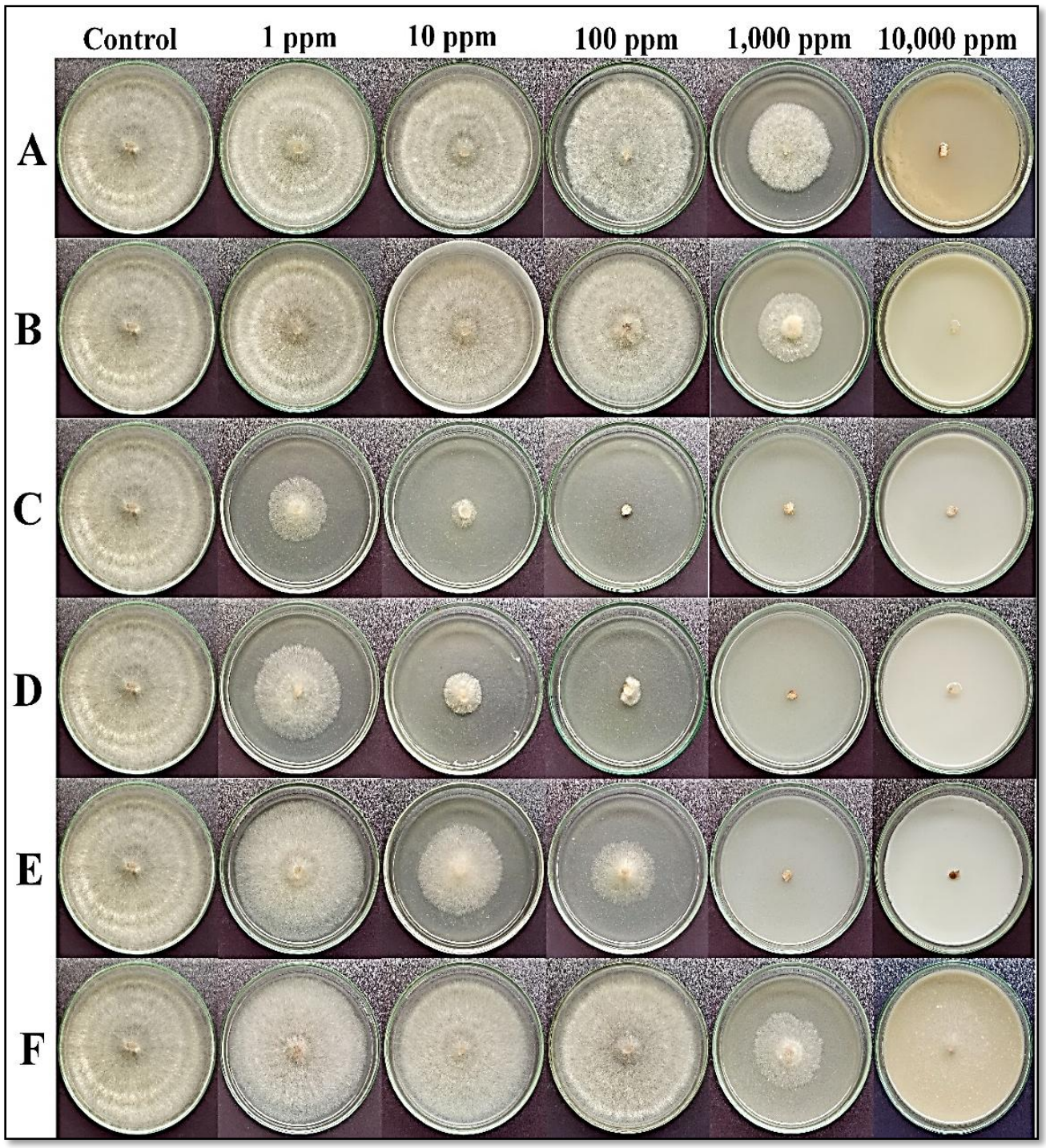

Fig. 6. *In vitro* impact of various antifungal chemicals on colony growth of *R. solani* after 4th days.
A = Aliette, **B** = Antracol, **C** = Nativo, **D** = Ready Super, **E** = Score, **F** = Zaver

Table 1. Interaction type, contact time and zone of inhibition by antagonistic microbes against *R. solani* in dual culture.

List of Antagonists	Time taken to contact (days)	Time taken to overlap (days)	Type of interaction
Trichoderma harzianum	2	10	B
Trichoderma longibrachiatum	2	10	B
Trichoderma virens	3	10	D
Clonostachys rosea	4	10	A
Paecilomyces variotii	3	10	A
Purpureocillium lilacinum	4	-	C

A: Colonies of test organism and pathogen met each other; The Pathogen overgrew the colony of test organism
B: Growth of pathogen inhibited; Test fungus produced coiling around mycelium of the pathogen
C: Colonies of test organism and pathogen met each other; No further growth of either the pathogen or the test fungus was observed
D: Colonies of pathogen and test fungi intermingled

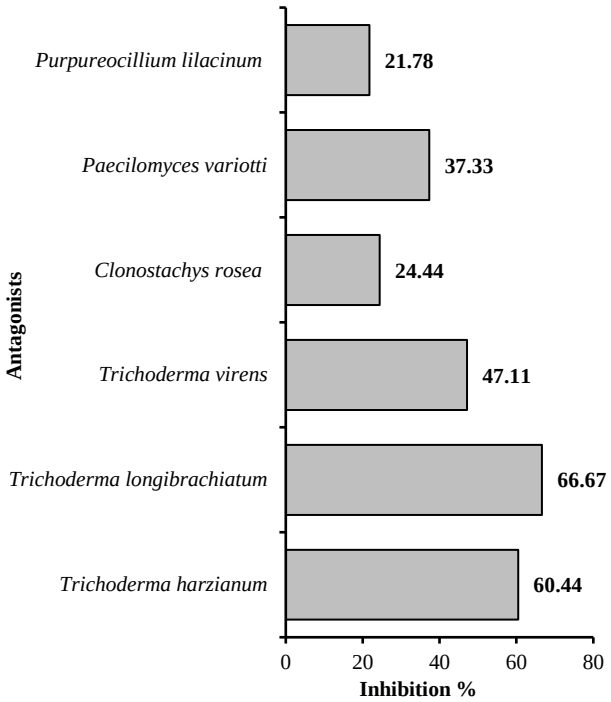

Fig. 7. *In vitro* inhibition percentage of *R. solani* against antagonists in dual culture technique.

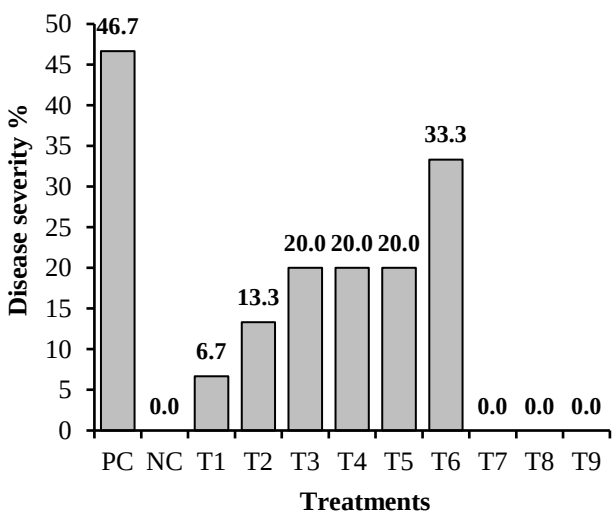

Fig. 12. Effect of antifungal chemicals and antagonist on the disease severity of root rot in common bean plant (pot experiment).
PC = Positive Control (inoculated with *R. solani*), **NC** = Negative Control, **T1** = *R. solani* + Nativo, **T2** = *R. solani* + Ready super, **T3** = *R. solani* + Score, **T4** = *R. solani* + *T. harzianum*, **T5** = *R. solani* + *T. longibrachiatum*, **T6** = *R. solani* + *T. virens*, **T7** = *T. harzianum*, **T8** = *T. longibrachiatum*, **T9** = *T. virens*

In the *In vitro* dual culture assay, all tested bio-agents showed variable degrees of inhibition against *R. solani*. Among them, *T. harzianum* and *T. longibrachiatum* demonstrated the highest inhibition percentages, followed by *T. virens* and *Paecilomyces variotii.* These findings are consistent with earlier reports highlighting the antagonistic potential of *T. harzianum, T. longibrachiatum,* and *T. virens* against *R. solani* (Anees *et al.*, 2010; Shalaby, 2022). Comparable antagonistic interactions were also observed in earlier studies (Walter & Lumsden, 2019 and Al-Mansoury & Salih, 2021). Similarly, *P. variotii* showed inhibitory effects on *R. solani*, as documented by Moreno-Gavra (2021). Among all antagonists, *Clonostachys rosea* exhibited lowest inhibition percentage in the present study. However, this contrasts with the observation of Salamone *et al*., (2018), who reported mycoparasitic behavior of *C. rosea*, and hyphal coiling around *R. solani* mycelia. *Purpureocillium lilacinum* showed limited direct inhibition but appeared to suppress the pathogens growth through the release of secondary metabolites, partially aligning with the findings of Constantin (2022). Shahzad & Ghaffar (1988) have also reported inhibitory action of *P. lilacinum* against isolates of *R. solani*.

The current study showed that biological control agents and chemical fungicides were equally effective in controlling common bean root rot. The severity of the disease was significantly reduced by all the tested antifungal substances, with Nativo being the most successful against *R. solani*, followed by Ready Super and Score. These findings are consistent with earlier researches, including Boukaew (2013), who reported 47-60% disease suppression in rice using chemical fungicides, and Sriraj *et al*., (2014), who emphasized the effectiveness of Nativo against pathogens that cause root rot. Nativo, a novel fungicidal formulation, proved particularly effective in controlling root rot (Sriraj *et al*., 2014). On the other hand, research by Sonakar (2014) showed that Propineb was only marginally successful in controlling *Rhizoctonia*. Similarly, Cotterill (1989) stated that Fosetyl-aluminum had not vital impact on *R. solani* of wheat with 3.6 disease severity.

The biological agents *Trichoderma harzianum* and *T. longibrachiatum* improved plant growth and decreased disease severity when compared to untreated controls, while *T. virens* was comparatively less effective. Among the biocontrol treatments, *T. harzianum* was most effective, promoting the greatest increases in dry shoot weight, root length and biomass accumulation. It was second only to the Score fungicide in terms of overall growth performance. These findings are supported by studies conducted by Asad *et al*., (2014) and Matloob & Juber (2013) who reported that *Trichoderma* species, significantly improved the growth and biomass of bean plants compared with untreated controls. Specifically, *T. harzianum* increased related biomass by up to 155% and reduced disease severity by 23.2%, outperforming even healthy control plants in biomass production. This finding is consistent with the findings of Khalaf *et al*., (2023), who showed how *T. harzianum* improved crop productivity and plant nutrition. Similar findings by Almeida (2007), based on microscopic analysis, showed that *Trichoderma* species displayed mycoparasitism behaviour, such as hyphal coiling around the thick hyphae of *R. solani*. Collectively, these results affirm the potential of *Trichoderma* species, predominantly *T. harzianum*, as effective and environmentally safer alternative to conventional fungicides for managing *R. solani* induced root rot in common bean crops.

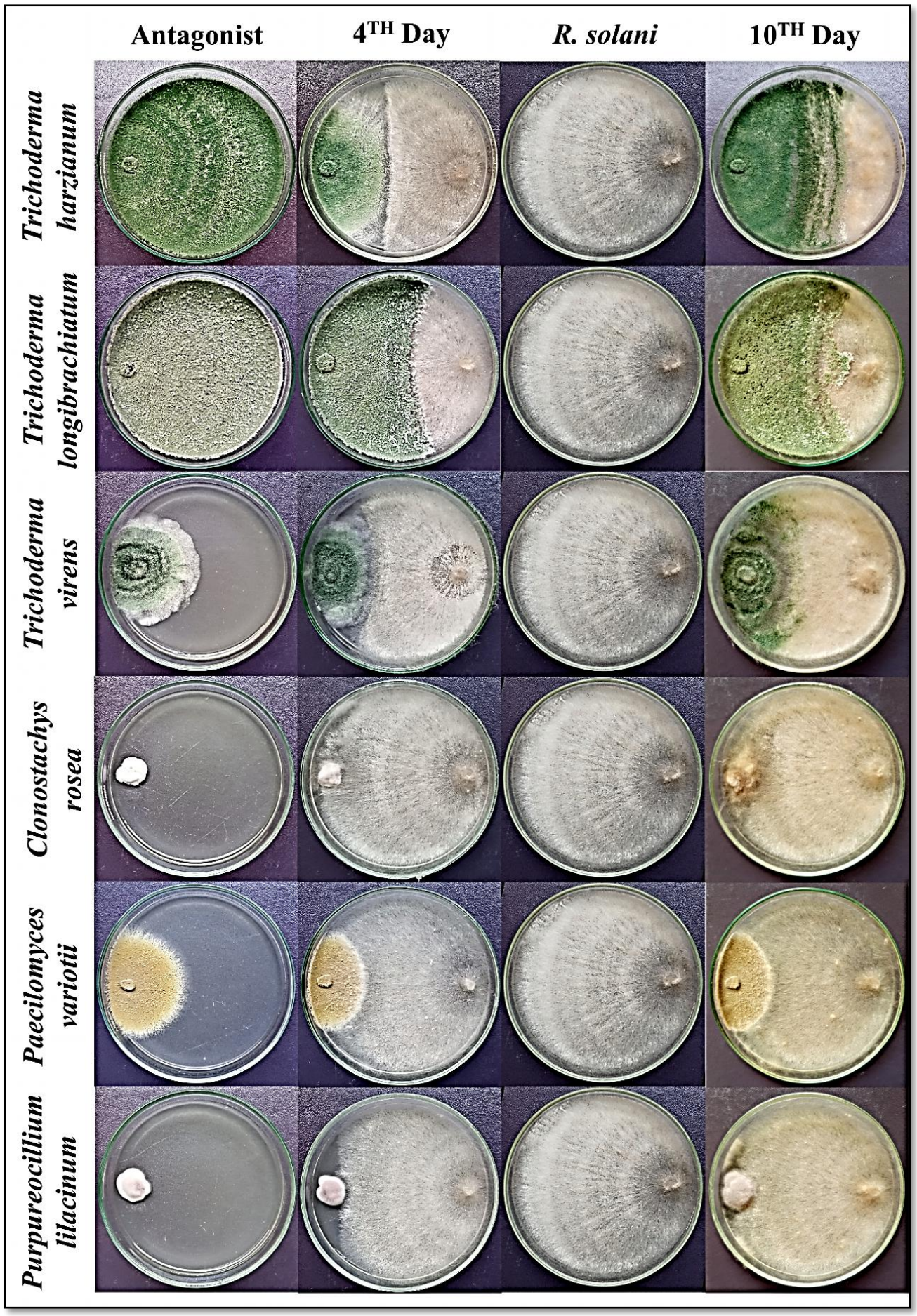

Fig. 8. Interaction between biocontrol agents and *R. solani* observed after four and ten days.

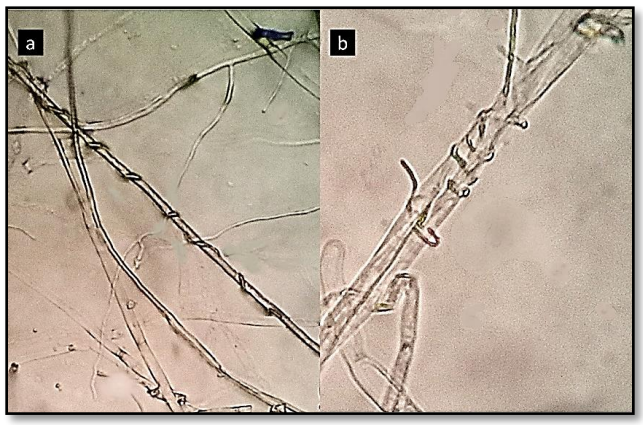

Fig. 9. Antagonistic interaction characterized by hyphal coiling around the pathogen by: **a**= *T. harzianum*, **b**= *T. longibrachiatum.*

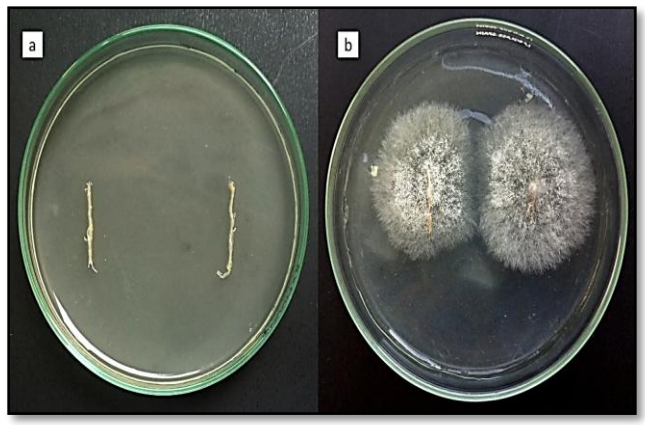

Fig. 11. Re-isolation of *R. solani* from root parts in pot experiment: **a**= isolation from negative Control (Healthy plants), **b**= isolation from positive Control (Infected plants).

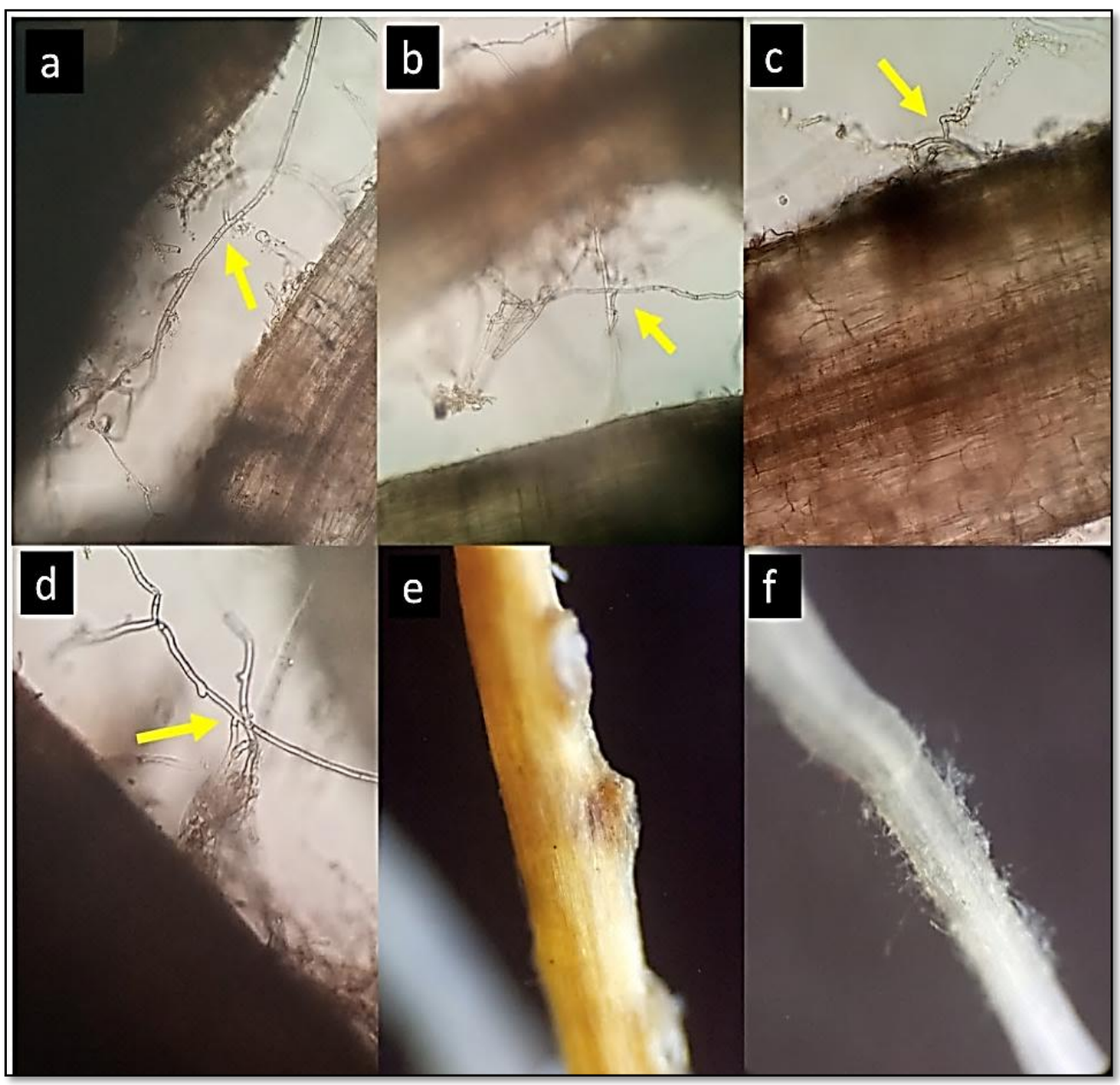

Fig. 10. Appearance of *R. solani* on roots of tested bean plant in pot experiment under microscope after 3 days incubation in water: **a-d** = hyphae of *R. solani*, **e** = infected root under microscope, **f** = healthy root under microscope.

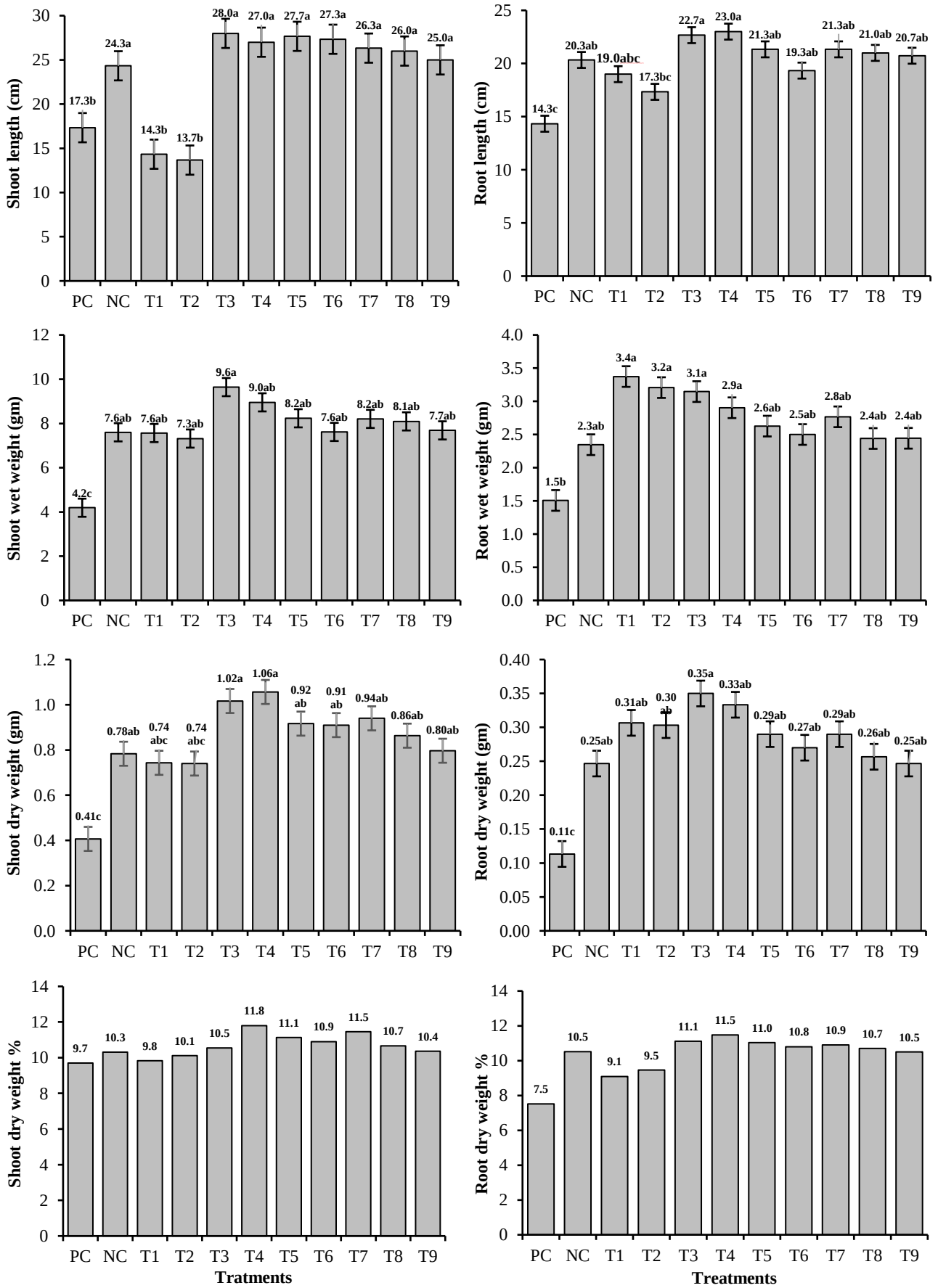

Fig. 13. Effect of antifungal chemicals and antagonist on the growth of common bean plants inoculated by *R. solani* (pot experiment). **PC** = Positive Control (inoculated with *R. solani*), **NC** = Negative Control, **T1** = *R. solani* + Nativo, **T2** = *R. solani* + Ready super, **T3** = *R. solani* + Score, **T4** = *R. solani* + *T. harzianum*, **T5** = *R. solani* + *T. longibrachiatum*, **T6** = *R. solani* + *T. virens*, **T7** = *T. harzianum*, **T8** = *T. longibrachiatum*, **T9** = *T. virens*. *Values with different letters are statistically different by Tukey's HSD multiple range Test ($p<0.05$).

Recommendations

R. solani CB1 is highly pathogenic strain, causing complete mortality in bean germinates. For effective management, both chemical and biological control methods should be considered. Among fungicides, Nativo at 1 ppm is recommended due to its ability of significantly inhibiting the pathogens growth (52.2%) and reducing the disease severity (6.7%) in pot trials. However, *Trichoderma* species have shown promising results in suppressing root rot in French bean and may offer further plant growth promoting benefits. It is recommended to explore and utilize such biological agents not only for disease control but also for their potential to improve plant health through additional mechanisms.

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Saboohi Raza: Molecular characterization of *Rhizoctonia solani.* **Samia Sattar:** Analyzed and interpretation of data.

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