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Germplasm evaluation and different control strategies against *Rhizoctonia solani* in potato

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Abstract

Black scurf and stem canker caused by the soil-borne basidiomycete *Rhizoctonia solani* (teleomorph *Thanatephorus cucumeris*) remain major constraints to potato production worldwide. This study combined morphological and ITS-based molecular characterisation of *R. solani* isolates from black-scurf-affected tubers in Faisalabad, Pakistan, with screening of 50 potato germplasm entries and comparative evaluation of four chemical fungicides, six plant growth-promoting rhizobacteria (PGPR), *Trichoderma harzianum* and six aqueous plant extracts. Five isolates (Rs1-Rs5) were confirmed as *R. solani* by ITS sequencing and deposited in GenBank (PZ952552-PZ952556). Neighbour-Joining phylogeny placed them within the *R. solani*/*T. cucumeris* clade. Germplasm responses ranged from resistant (FD3536, FD63-1, FD78-36; disease incidence 6-8%) to highly susceptible (Simply Red=93%). *In vitro*, Cabrio Top and Antracol completely inhibited mycelial growth at 50 and 100 ppm. *Pseudomonas aeruginosa* (OR741775) produced 100% growth inhibition and *P. putida* (PX496465) 89.6-93.6%; *T. harzianum* (PZ955200) gave 45.4-47.8% inhibition in dual culture. Among extracts, *Azadirachta indica* at 15% yielded the highest inhibition (41.9%). In a subsequent pot trial on the susceptible cultivar Kashmir, *P. aeruginosa*, *P. putida*, Cabrio Top, Antracol, Miravis Duo, *T. harzianum*, *A. indica* and *Calotropis procera* reduced disease incidence and severity to zero; *P. aeruginosa* and *P. putida* also produced the highest tuber numbers (10 and 9 per plant). Multivariate analyses confirmed the superiority of selected *Pseudomonas* strains. The results identify useful host resistance and effective chemical and biological agents that can contribute to integrated management of potato black scurf.

Keywords: Biological control · Black scurf · Germplasm resistance · Plant extracts · Potato · *Rhizoctonia solani*

1 Introduction

Among the essential food crops across the planet, potato (*Solanum tuberosum* L.) plays a key role in ensuring food security in temperate and subtropical production systems (Devaux *et al*. 2020; Devaux *et al*. 2021). The significance of potato stems from its highly adaptive nature, short growth cycle, and high nutritional value it possesses. In Pakistan, potato is cultivated on an area of 0.33 million hectares and produces over 7.9 million tonnes potatoes every year (Hasan 2024). Unfortunately, there are numerous soil- and tuber-borne diseases that reduce yield and quality, making it hard to achieve high productivity.

One of the soil-borne diseases that are particularly damaging is black scurf and stem canker caused by *Rhizoctonia solani* Kühn (teleomorph *Thanatephorus cucumeris* (A.B. Frank) Donk). This pathogen survives as sclerotia and mycelium in the soil and seed tuber, invades the underground parts of the plant, and produces dark sclerotia that appear on the tuber. Yield losses in potatoes under favorable conditions range from 30 to 50%, and even lower disease severity may lead to severe economic losses due to downgrading (Banville 1989; Rafiq et al. 2024). The disease is favored by cool and moist soils and is extremely hard to control since it is persistent and has a very wide host range.

R. solani exists as a complex of species that are classified into the anastomosis groups (AGs). AG-3 is the most prevalent group associated with potato populations (specifically AG-3PT), which is considered to be the outbreak agent for black-scurf and stem-canker globally (Lehtonen et al. 2008; Ferrucho et al. 2012; Woodhall et al. 2022). For epidemiological studies and rational disease management, proper identification is essential. Morphological identification employs a number of colony characteristics, sclerotia formation, and the characteristic of right-angle branching of the hyphae with constrictions present at the branching points (Sneh et al. 1991). Despite its usefulness, its reliability in AG assignment is limited. The sequencing of the internal transcribed spacer (ITS) of ribosomal DNA and its subsequent phylogenetic analysis is used as a reliable complementary technique (Iradukunda et al. 2022; Chaudhary et al. 2023). The basis for sustainable management is the utilization of resistant hosts. It has been revealed in a number of screening studies that different potato cultivars and breeding lines possess different levels of resistance with there not being any significant level of immunity in commercially available varieties (Naz et al. 2008; Zhang et al. 2014; Rafiq et al. 2024). Identification of resistant or moderately resistant germplasm therefore remains a priority for breeding programmes and for the design of integrated disease-management systems that reduce reliance on chemical inputs.

Despite the fact that chemical fungicides still play a vital role in the control of *R. solani*, the use of single modes of actions leads to the formation of resistant populations, additional expenses and negative environmental consequences (Acharya et al. 2018; Islam et al. 2024). Biological agents like plant growth-promoting rhizobacteria (PGPR) of *Pseudomonas* spp. and fungal antagonists of *Trichoderma harzianum* are used as complementary tools for antibiosis, competition, mycoparasitism and systemic resistance induction (Grosch et al. 2005; Brewer and Larkin 2005; Tariq et al. 2010). Besides, plant extracts rich in bioactive compounds have shown variable levels of antifungal effect against *R. solani*; however, their effectiveness depends greatly on plants' species, concentration and extraction technique (Moslem and El-Kholie 2009; Bokhari et al. 2014).

Besides the yield reduction, the economic consequences of the black scurf include its ability to create a continuous cycle of infection due to infested tubers and sclerotia which can survive in soil for several years. Repeated cultivation of potatoes increases the number of the inoculum in intensive potato-growing system. Thus, it is possible to state that the use of host resistance, biological and decreased chemical fungicides is needed in order to achieve sustainability.

Limited studies have been conducted which involve evaluating chemical, bacterial, fungal, and botanical antagonists on the same population of *R. solani* and validating the best candidates in pots. This study attempts to incorporate the following four stages into its framework: (i) morphological and molecular characterization of the *R. solani* isolates collected from Faisalabad area where potatoes are

cultivated; (ii) evaluation of 50 local potato varieties in response to the disease; (iii) evaluation of four fungicides, six PGPR strains, *T. harzianum* and six plant extracts *in-vitro*; and (iv) validation of the best treatment's antagonists in pots.

2. Methods and materials

2.1 Isolation and morphological characterisation

Samples of diseased potato tubers (those showing black scurf symptoms) were taken from the different sites including experimental plot of Ayub Agricultural Research Institute (AARI), Vegetable Markets, Local Sellers, and from the Farmers fields in 2025 (Supplementary Fig. 4). Pieces of tissue were prepared by surface sterilization with 1% sodium hypochlorite for 1 minute, followed by rinsing three times with sterile distilled water and placed on potato dextrose agar (PDA). The cultures obtained were incubated at temperature 25±2 °C. Pure cultures were established through hyphal-tip transfer. The colony characteristics, formation of sclerotia and microscopic features of the fungus were recorded according to standard description (Sneh et al. 1991; Tsror 2010).

2.2 Molecular identification and phylogeny

From five isolates (Rs1-Rs5), genomic DNA extraction was performed through a modified CTAB method. ITS region was amplified using the universal primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′). The sequences were then sequenced. The sequences obtained were compared with existing sequences available in GenBank through BLASTn. The sequences were assigned accession numbers PZ952552-PZ952556. Phylogenetic tree construction was performed using the Neighbour-Joining method (with 1,000 bootstrap replications) through MEGA software using the sequences of both *R. solani* and *T. cucumeris* as reference sequences.

2.3 Germplasm screening

Fifty potato germplasm entries which were collected from the Potato Research Institute, AARI, Faisalabad were examined under controlled field infestations applying a randomized complete block design with three replications. *R. solani* was multiplied on cleaned wheat grains to prepare an inoculum. At harvesting, disease incidence (DI) and severity (DS) were rated according to the 0-5 scale mentioned in Supplementary Tables S12 and Fig. 1. The screening of germplasm was conducted for two consecutive years.

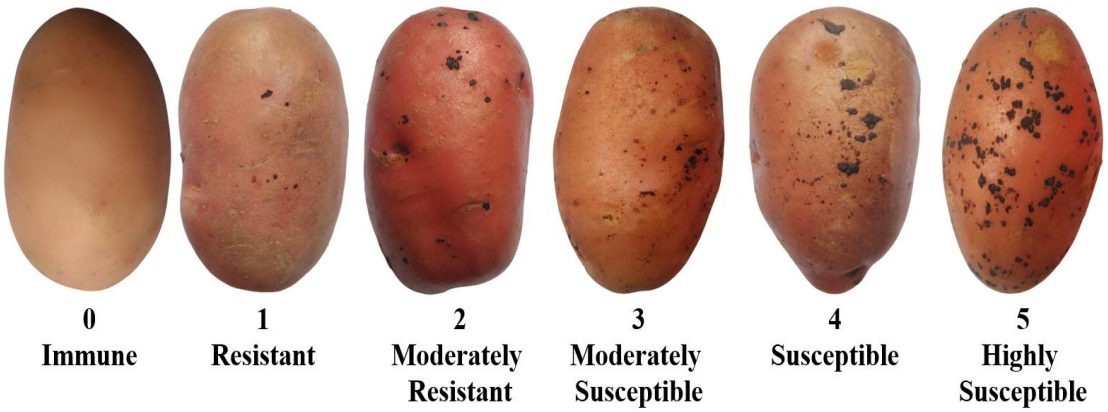


Fig. 1 Disease severity rating scale for the black scurf of potato.

2.4 *In-vitro* antifungal assays

Four fungicides (Cabrio Top, Antracol, Miravis Duo, and Defeater) at concentrations of 50 and 100 ppm were tested by the poisoned-food technique. Six PGPR strains: *Pseudomonas aeruginosa* (GenBank accession OR741775), *P. fluorescens* (QB001296), *P. putida* (PX496465), *Agrobacterium fabrum* (OR553483), *Enterobacter cloacae* (OR559983), and *Gluconacetobacter liquefaciens* (QB001796) were tested. The fungus *Trichoderma harzianum* (GenBank accession PZ955200) was studied by dual culture on PDA. Six aqueous leaf extracts (*Azadirachta indica*, *Nerium oleander*, *Calotropis procera*, *Olea europaea*, *Citrullus colocynthis*, and *Cannabis sativa*) were tested at concentrations of 5, 10, and 15% (w/v). The measurement of radial growth was conducted at 3 and 7 days along with the calculation of percent growth inhibition (PGI) as compared to the control using formula:

$$\% \text{ Growth Inhibition} = \frac{(C-T)}{C} \times 100$$

wherein C is radial growth of control and T is radial growth of treatment. Complete randomized design protocol was followed with three replications for all experiments. All *in vitro* experiments were repeated twice for the confirmation of effectiveness against *R. solani*.

2.5 Pot assay

The best treatments obtained from *in vitro* studies (Cabrio Top, Antracol, Miravis Duo, *P. aeruginosa*, *P. putida*, *P. fluorescens*, *T. harzianum*, *A. indica* and *C. procera*) were tested on susceptible variety Kashmir in a pot experiment under the field environment. A complete randomized design with five replications was used. The pathogen inoculum was incorporated by drenching into soil before 15 days of plantation. The treatments were applied also by drenching in soil before 7 days of plantation. The parameters assessed were disease incidence, disease severity (Supplementary Tables S12 and Fig. 1) and tuber number per plant during harvesting.

2.6 Statistical analysis

Statistical analysis of data was performed using analysis of variance (ANOVA) procedure. Mean separation was done using Tukey's HSD at $P \leq 0.05$. Multivariate analysis procedures consisting of principal component analysis, hierarchical clustering and correlation matrix were used to visualize the relationship among treatments. Analyses were conducted in R and Statistix 8.1.

3 Results

3.1 Morphological and molecular identification

Organisms originating from tubers affected by black sclerotia produced colonies having rapid growth characteristics on potato dextrose agar medium containing dark-colored and irregular shapes sclerotia (Fig. 2A a-c). By microscopic observation, septated hyphae were found having clear right-angle branching and constriction points at the points of branching (Fig. 2A d), which matched the expected characteristics of *R. solani*. These morphological traits helped in the preliminary identification but were not sufficient to characterize at the population level.

High-quality genomic DNA was extracted from the isolates Rs1-Rs5. The amplified ribosomal RNA gene ITS produced the expected sized fragments (Fig. 2B a-b). After BLASTn analysis all five sequences were identified as *R. solani*. The sequences were submitted in GenBank with the accession numbers given within Supplementary Table S1. Phylogenetic analysis using Neighbour-joining method, showed that the isolates of Pakistan belonged to *R. solani*/*T. cucumeris* clade (Fig. 2C). Among the

isolates, Rs3 clustered with the reference sequences from Russia, USA, Mauritius and Belgium while Rs1, Rs2, Rs4 and Rs5 had tie with the isolates in China (Bootstrap value 53-89%). The first available ITS sequences identified from *R. solani* from Faisalabad area of Pakistan were included in the current study.

The phylogenetic placement of the Pakistan isolates of the fungus agrees with previous studies, indicating that AG-3 lineages predominate in potato-associated populations of *R. solani* in temperate and subtropical areas. The existence of local reference sequences will be useful in future diagnostics and population studies aimed at monitoring the pathogen movement and shifts in the procedure of the anastomosis groups.

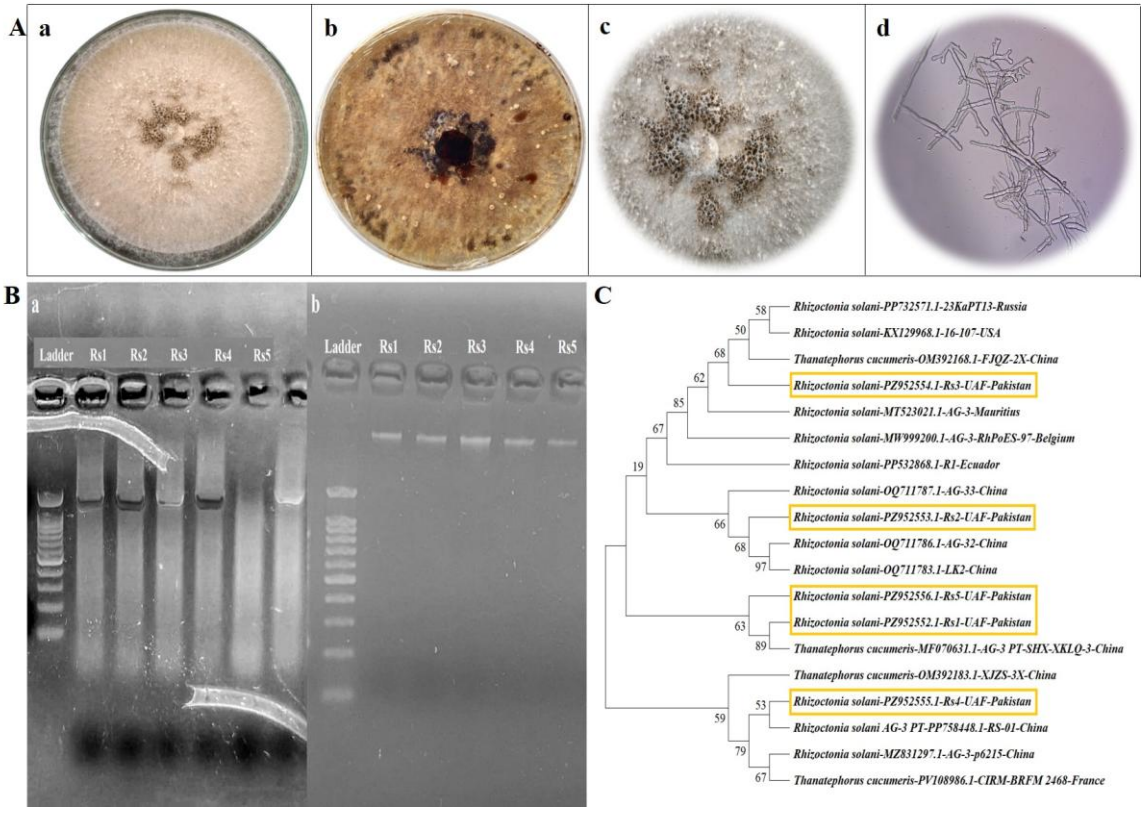


Fig. 2 Morphological and molecular identification of the *R. solani* isolates from potatoes infected by black scurf. A(a-b) Pure cultures on PDA showing colony morphology; (c) dark sclerotium; (d) microscopic view of the septate hyphae branching at right angles (400×). (B) The ITS PCR profiles. (C) Neighbour-Joining phylogenetic tree incorporating the isolates Rs1-Rs5 belonging to the *R. solani*/*Thanatephorus cucumeris* clade.

3.2 Variation in potato germplasm response

The 50 germplasm accessions showed a broad spectrum of reactions to *R. solani* infections created under laboratory conditions (Table 1; Fig. 3). Incidence varied from 6% (FD3536) to 93% (Simply Red) and severity from 0.8% to 72%. There were three resistant, eleven moderately resistant, sixteen moderately susceptible, nineteen susceptible and one highly susceptible entry (Simply Red) accessions in the test group (Table 1). There were no immune accessions. Hierarchical cluster analysis was able to distinguish resistant and susceptible accessions similarly to the numerical classification (Fig. 3).

The evident quantitative variability shows that partial resistance can be effectively selected from the local germplasm pool. Resistant accessions had not only low incidence but also low sclerotial production in infected tubers indicating their ability to restrict both infection frequency and colonisation by the pathogen. This type of accessions may have high value as parents in breeding programs and part of integrated management systems aimed at decreasing the inoculum level. Complete individual data are provided in Supplementary Table S2.

The lack of immune lines among the 50 germplasms analyzed is an indication of the quantitative inheritance of resistance to *R. solani* as indicated in previous screening programs. However, the detection of three resistant lines and eleven moderately resistant lines is an important genetic material that can be used locally in breeding. This will increase the degree of resistance of future varieties in the region.

Table 1 Overall germplasm response category according to disease rating scale against *Rhizoctonia solani*.

Rating	Disease severity (%)	Response	Germplasm/Varieties	Total
0	No disease symptoms	Immune	-	0
1	< 1% tuber surface affected	Resistant	FD3536, FD63-1 and FD78-36.	3
2	1 to 10% tuber surface affected	Moderately Resistant	FD74-21, FD76-18, FD61-3, Sadaf, SL15-10, SL14-15, SL5-2, V14, V15, CK-40, FD73-73.	11
3	11 to 20% tuber surface affected	Moderately Susceptible	FD-78-51, SL13-43, SL-52, ETAB, SHL-RED, Punjab, Cardinal, Diamont, FD 35-36, FD76-67, Cosmo, SL9-14, Sutlej, 7450, 9814, 391202-158.	16
4	21 to 50% tuber surface affected	Susceptible	394005-115, 394028-37, 394032-16, 394055-40, 396240-21, 396266-33, FSD White, FD 48-1, FD71-1, Lady Roseta, SHL-WHI, 9808, 393574-61, Sante, Multa, Ruby, FD77-4, FD74300, Kashmir	19
5	> 50% tuber surface affected	Highly Susceptible	Simply Red	1

Experiment was conducted for two consecutive years.

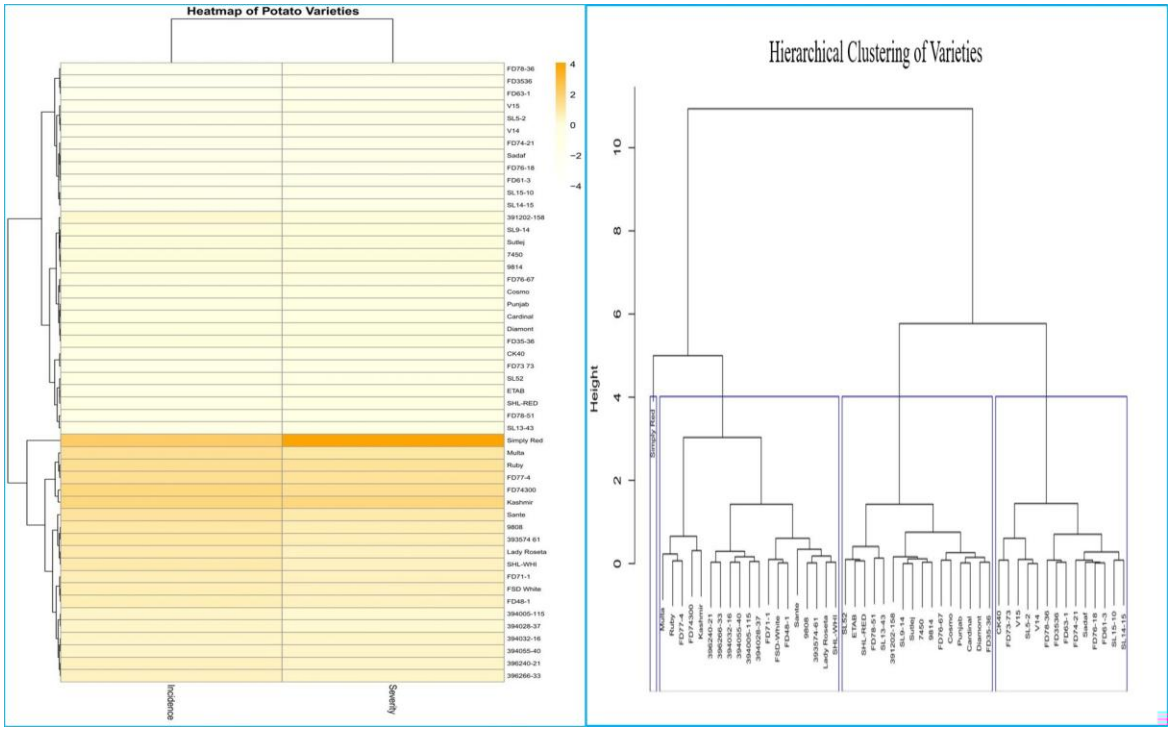


Fig. 3 Distribution of potato germplasm among disease response categories against *Rhizoctonia solani* and hierarchical clustering based on disease incidence and disease severity.

3.3 Comparative in-vitro antifungal activity

Chemical treatment, incubation time, and their interaction importantly influenced the radial growth of *R. solani* ($P \leq 0.001$). Cabrio Top and Antracol led to complete inhibition of the mycelial growth (100% inhibition) at both 50 and 100 ppm already after 3 and 7 days (Fig. 4A). This 100% inhibition proves that both formulations were able to obtain a fungicidal effect during the experiment and to maintain its effectivity within the whole incubation time. Miravis Duo showed complete inhibition of *R. solani* after 3 days of the experiment, while the residual activity had decreased significantly after 7 days of incubation (40.6-50.1%) stating that the residual effectiveness of this formulation is not so high. Defeater demonstrated the lowest residual effectiveness after the 7-day incubation period (the values are presented in Supplementary Tables S3-S4).

P. aeruginosa (OR741775) stood out among the six PGPR strains by producing 100% inhibition at both evaluation intervals. Meanwhile, *P. putida* (PX496465) reached 89.6-93.6% inhibition level, and *P. fluorescens* (QB001296) resulted in 90.1-92.3% inhibition (Fig. 4B). The rest of the strains, namely *Agrobacterium fabrum* (OR553483), *Enterobacter cloacae* (OR559983), and *Gluconacetobacter liquefaciens* (QB001796), were less successful (Supplementary Tables S5-S6). This observation is in line with the known ability of fluorescent pseudomonads to achieve sufficient antibiosis, siderophore-mediated competition, and produce cell wall degrading enzymes.

It was noticed that dual culture application of *Trichoderma harzianum* (PZ955200) restrained pathogen growth by 45.4% after three days since the application and appeared also to be effective in lowering pathogen growth by 47.8% after the seventh day of using it (Supplementary Table S8). The mycelial growth (mm) due to *T. harzianum* was presented in Supplementary Table S7.

Plant extract activity was significantly influenced by concentration. Only *A. indica* at 15% showed noticeable inhibition (by 36.7% at 3 days of application, and 41.9% at 7 days of application), while the results for *C. procera* at the same 15% of concentration were significantly lower (22.0% and 12.2%). The remaining extracts showed no measurable inhibition under the tested conditions (Fig. 4C; Supplementary Table S10). The mycelial growth (mm) by plant extracts were presented in Supplementary Table S9. The strong concentration dependence highlights the importance of optimising extraction methods and application rates if botanical products are to be developed further.

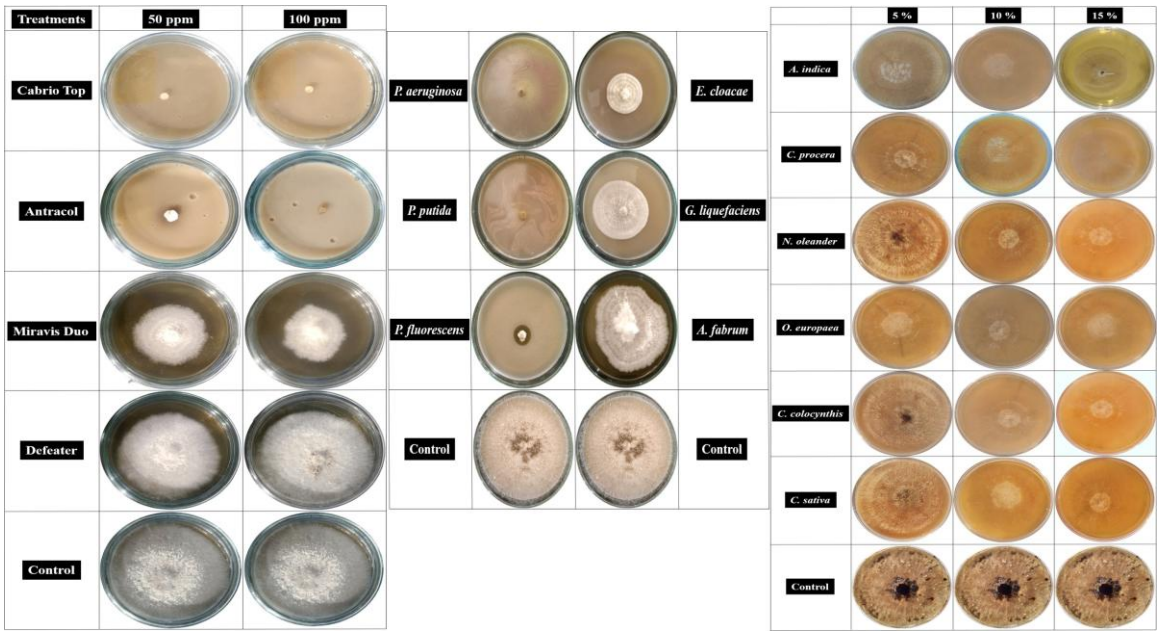


Fig. 4 Representative *in-vitro* assays of treatments against *Rhizoctonia solani*. (A) Chemical fungicides; (B) PGPR treatments; (C) aqueous plant extracts. Detailed mycelial growth and percent growth inhibition data are provided in Supplementary Tables.

3.4 Pot validation of selected treatments

Both treatment effects on tuber number and disease incidence were extremely significant with P being less than 0.001 (Supplementary Table S11). Mean tuber number varied from 3 (positive control) to 10 (*P. aeruginosa*) (Supplementary Table S11). Eight treatments showed disease incidence at 0% (*P. aeruginosa*, *P. putida*, Cabrio Top, Antracol, Miravis Duo, *T. harzianum*, *A. indica*, and *C. procera*). *P. fluorescens* resulted in 60% incidence and 20% severity whereas in the positive control disease incidence was 81% and severity was 40% (Table 2). It is worth mentioning that both the most effective *Pseudomonas* isolates gave not only the lowest visible disease incubation but also the highest tuber number which implies that they had a combined effect on suppression of the disease and promotion of growth unlike purely fungicidal treatments.

Assessment of principal components allowed us to identify effective treatments from the positive control and the treatment with *P. fluorescens* that had moderate effectiveness (Fig. 5). It was established that incidence and severity were positively correlated with one another allowing us to reveal that they were negatively correlated with tuber number. Multivariate observations confirm the results obtained in univariate analysis.

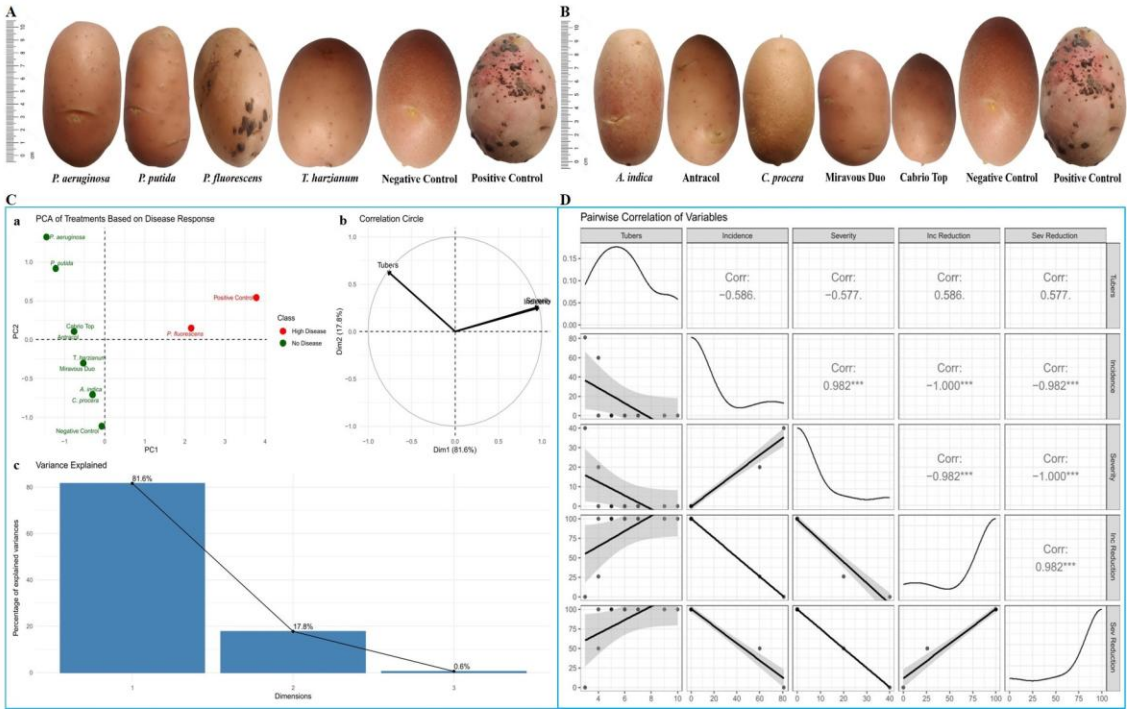


Fig. 5 Pot validation and multivariate interpretation of treatment responses against *Rhizoctonia solani*. (A-B) Tuber production and disease responses; (C) principal component analysis; (D) pairwise correlation matrix.

Table 2 Effect of selected treatments on disease incidence (%) and disease severity (%) under pot conditions.

Treatments	DI (%)	DS (%)	DR	Response
P. aeruginosa	0	0	0	Complete suppression
P. putida	0	0		
Cabrio Top	0	0		
Antracol	0	0		
T. harzianum	0	0		
Miravis Duo	0	0		
A. indica	0	0		
C. procera	0	0		
P. fluorescens	60	20	3	Moderately Susceptible
Positive Control	81	40	4	Susceptible
Negative Control	0	0	0	No disease

DI=Disease Incidence, DS=Disease Severity, Values in the DI and DS column are the mean of 5 replicates.

4 Discussion

The present research combined the identification of pathogens, assessments of their resistance to various hosts, and a comparison of treatments that could eliminate *Rhizoctonia solani* from potatoes. The relationships of the features of the isolates obtained were in accordance with the notorious criteria for

diagnosing *R. solani*, which are characterized by the fast growth of the colonies on PDA, forming of irregular and dark sclerotia and right-angle branching of the hyphae with some constrictions when they branch off (Sneh et al. 1991; Tsror 2010; Chaudhary et al. 2023). The sequencing of ITS region and the phylogenetic position within the clade of *R. solani* /*Thanatephorus cucumeris* allowed the identification of all five isolates and provided access to the first reference sequences from the area of Faisalabad, which are available to the entire public (PZ952552-PZ952556). Although ITS phylogeny alone does not allow definitive anastomosis-group assignment, the clustering pattern is consistent with the predominance of AG-3 lineages.

The germplasm evaluation showed high variability in susceptibility to the disease. The three resistant entries (FD3536, FD63-1, and FD78-36) possessed high resistance and very low incidence of disease. It is generally accepted that resistance against *R. solani* in potatoes occurs in a polygenic manner, rather than resulting from a single major resistance locus (Naz et al., 2008; Zhang et al., 2014; Rafiq et al., 2024). Some cellular processes that might be responsible for this quantitative resistance are cell wall lignification, increased levels of phenolics either in baseline state or in response to the pathogen, production of PR proteins, and enhanced activities of defenders such as chitinases, β-1,3-glucanases, and peroxidases (M'hamdi et al., 2012; Parveen et al., 2023). These processes create both physical barriers to hyphae entry and chemical surrounding, preventing fungi from colonizing the plant and forming sclerotia. The present research, however, did not quantify the above-mentioned biochemical markers, which makes them theoretical and supported by literature, but not proven.

In terms of chemical fungicides, full and lasting growth inhibitions were shown by Cabrio Top and Antracol at all concentrations and assessment times. Cabrio Top is based on a QoI fungicide pyraclostrobin that is bound to the Qo site of the cytochrome bc1 complex (Complex III) of the fungal mitochondrial electron-transport chain, preventing electron transfer and collapsing the protonation gradient alongside rapid depletion of cellular ATP, causing energy starvation and lack of hyphal elongation (Brown et al. 2021; Islam et al. 2024). The active ingredient acts in multiple sites of the pathogen, broadening the range of activity and minimizing the chances of resistance development. Antracol (propineb) is a multi-site dithiocarbamate that reacts with sulphydryl-containing enzymes participating in respiration and intermediate metabolism. The residual activity decline of the last Miravis Duo fungicide after a week indicates either progressive degradation of the active component or limited physiological adaptation of surviving hyphae (Panozzo et al. 2023; Maaoui et al. 2025).

Pseudomonas aeruginosa and *P. putida* demonstrate excellent performance both *in vitro* and in pot conditions because of the underlying mechanisms that function at the cellular level of the pathogen and the plant-microbe interface. A variety of fluorescent pseudomonads produce secondary metabolites, such as phenazines, pyrrolnitrin, pyoluteorin, hydrogen cyanide and 2,4-diacetylphloroglucinol. Secondary metabolites lead to interruption into the stability of membranes of fungi, inhibition of mitochondrial respiration and to the formation of reactive oxygen species which damage major cellular compounds (Tariq et al. 2010; Mrabet et al. 2013). Also, high-affinity siderophores trap iron in the rhizosphere, depriving *R. solani* of the required trace element for developing and living (Grosch et al. 2005; Fathi et al. 2021). Another group of extracellular hydrolytic enzymes, including chitinases, β-1,3-glucanases and proteases enables to digest chitin-glucan cellular wall matrix of the fungus and leads to hyphal lysis

(Panicker and Sayyed 2022). In addition to direct fighting against the pathogen, these bacteria stimulate systemic resistance in the host by means of jasmonic-acid and ethylene signaling pathways (Mehmood et al. 2021; Darbon et al. 2024). Thus, *P. aeruginosa* and *P. putida* possess the ability to combat diseases simultaneously while increasing crop yield (Lal et al. 2022; Lan et al. 2024).

Trichoderma harzianum achieved moderate suppression in dual culture (45-48%) but completely inhibited disease occurrence in pot tests. *Trichoderma* employs its main cellular mechanism of action, that is mycoparasitism, which includes host hyphae recognition, coiling, appressorium-like bodies production, and the release of enzymes that breach the cell wall of the invading fungus *R. solani* (Brewer et al. 2005; Kanak Sirari et al. 2015). The fungal antagonism is supported by the competition for nutrients and space in the rhizosphere and the production of antifungal secondary metabolite compounds. The comparative efficacy of *Trichoderma* in soil is explained by its ability to colonize root zone and preserve its antagonistic properties for a longer period of time (Larkin and Brewer 2020). The modest efficacy of *Azadirachta indica* and *Calotropis procera* extracts is related to their high content of bioactive secondary metabolites. The use of neem limonoids results in disruption of fungal cell membrane integrity and inhibition of mitochondrial processes and with chitin synthesis blocking (Moslem and El-Kholie 2009; Bokhari et al. 2014). *Calotropis* species also contain alkaloids, flavanoids, tannins, and phenolic compounds which affect membrane permeability and metabolism (Sanaullah et al. 2018; Kansal et al. 2025).

In conclusion, the outcome concerning the control of *R. solani* pathogen emphasizes that its elimination can be achieved through the combined effects of different cellular mechanisms, including the disruption of the pathogen metabolism via QoI and multi-site fungicides (Brown et al. 2021; Islam et al. 2024), damage to the membranes and cell walls by selected pseudomonads and *Trichoderma* species (Tariq et al. 2010; Brewer and Larkin 2005), iron competition and host-plant resistance activated by rhizobacteria (Grosch et al. 2005; Mehmood et al. 2021), and suppression of pathogenic morphogenesis via plant components (Moslem and El-Kholie 2009; Bokhari et al. 2014). The integrated scheme involving resistant or moderately resistant germ-plasm together with selected *Pseudomonas* strains, *Trichoderma harzianum*, and reasonable use of effective fungicides seems to be a sustainable method to control black scurf (Kiptoo et al. 2021; Delai et al. 2024). The next step in this investigation is to conduct field-scale trials that would confirm the durability and the economic feasibility of all proposed combinations.

Combining molecular identification with multi-treatment assessment is especially pertinent in cases where local pathogen populations have not been previously described-characterized. By putting ITS sequences and bacterial accessions into the public database, this study adds reference work that may be useful for other laboratories to perform diagnostics and analyze changes in *R. solani* populations associated with potatoes. From a practical view of the situation, the dual benefits of disease prevention and increase in the number of tubers reached with the use of *P. aeruginosa* and *P. putida* are especially appealing for resource-poor production systems. Unlike chemical fungicides that mainly inhibit the development of the pathogen, these rhizobacteria seem to make plants better even in the presence of the pathogen. Further research should quantify the contribution of the above-mentioned mechanisms in field conditions and evaluate how stable these advantages are through different seasons and soils.

The fact that plant extracts only showed some level of effectiveness in recent tests does not take away their cheapness, ease of access, and positive environmental effects. With improving extraction methods and composition formulation, the effectiveness of these extracts may become much higher. The utilization of plant extracts with the participation of other compatible biological agents may lead to the emergence of additional synergetic impact. Besides direct influence upon the growth of pathogens, the outstanding performance of some strains of *Pseudomonas* under pot conditions indicates that these bacteria may influence the composition of the rhizosphere microbiome or induce the host's defence reaction that helps to prevent any establishment of pathogens. Such multifactorial mechanism of action is a characteristic of triumphal biocontrol agents.

The study results are of importance to seed-certification programs. The provision of locally adapted resistant materials and very effective seed or soil treatment may decrease the risk of establishing and spreading of *R. solani* through contaminated seed tubers, thus reducing its inoculation potential across the region and landscape. The introduction of these techniques into national guidelines for potato farming would significantly contribute to better control of the disease in the region. In general, the multi-stage research methodology used in this project starts from studying pathogens to germplasm screening, compares and tests the results in pots to make it possible to create a systematic approach for detection and identification of the options of efficient control. It has been proved that the efficiency of the treatments tested is stable in various eco-systems such as laboratory and pot. Thus, they can be used for field tests. In general, it can be concluded that resistant germplasm combined with fungicides from this project will be an effective tool for establishing local integrated control measures against potato black scurf.

5 Conclusion

In Faisalabad, scientists obtained five *R. solani* isolates from potato tubers that are known to be suffering from black scurf disease. Based on both morphological and molecular features, they categorized them and deposited them on GenBank as PZ952552-PZ952556. In the process of screening 50 available germplasm materials, the scientists identified three resistant lines such as FD3536, FD63-1, and FD78-36 that show low disease incidence along with limited sclerotial development. Furthermore, using Cabrio Top, Antracol, and two bacterial strains, which are *P. aeruginosa* (OR741775) and *P. putida* (PX496465), the scientists achieved complete inhibition of mycelial growth in vitro. For eight tested treatments, which indicated zero disease incidence and severity, the scientists carried out pot experiments with the usage of these bacterial strains. These results are very valuable as they provide resistant germplasm materials and efficient chemical and biological means that can serve as the groundwork for measures against the black scurf disease. Further testing in different locations is recommended to confirm the results of the studies carried out.

List of Abbreviations

Abbreviation	Full form
AG	Anastomosis Group
ANOVA	Analysis of Variance
AARI	Ayub Agricultural Research Institute

DI	Disease Incidence
DS	Disease Severity
DR	Disease Rating
HSD	Honestly Significant Difference
ITS	Internal Transcribed Spacer
PDA	Potato Dextrose Agar
PGPR	Plant Growth-Promoting Rhizobacteria
PGI	Percent Growth Inhibition
QoI	Quinone outside Inhibitor
RCBD	Randomized Complete Block Design
SI	Supplementary Information

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Author contributions

Muhammad Usman conceived and designed the research, performed the majority of the experiments, collected and analysed the data, prepared the figures and tables, and wrote the original draft of the manuscript. Muhammad Ehetisham-Ul-Haq contributed to methodology, resources and formal analysis. Muhammad Kamran and Usama Bin Arif assisted with experimental work and data collection. Amjad Abbas contributed to methodology and validation. Usama Ahmad contributed to investigation and visualisation. Shabana Ehsan provided resources and contributed to writing-review & editing. Huma Abbas contributed to conceptualisation, supervision, project administration, writing-review & editing and funding acquisition. All authors approved the final version of the manuscript.

Statements and Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Availability of data and material

ITS sequences of *R. solani* isolates are available in GenBank under accession numbers PZ952552-PZ952556. The *T. harzianum* isolate is deposited under PZ955200. PGPR strains are deposited under the following accessions: *P. aeruginosa* OR741775, *P. fluorescens* QB001296, *P. putida* PX496465, *A. fabrum* OR553483, *E. cloacae* OR559983 and *G. liquefaciens* QB001796. Full germplasm data and detailed *in-vitro* tables are provided in the Supplementary Information.

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Figure Legends

Fig. 1 Disease severity rating scale for the black scurf of potato.

Fig. 2 Morphological and molecular identification of the *R. solani* isolates from potatoes infected by black scurf. A(a-b) Pure cultures on PDA showing colony morphology; (c) dark sclerotium; (d) microscopic view of the septate hyphae branching at right angles (400×). (B) The ITS PCR profiles. (C) Neighbour-Joining phylogenetic tree incorporating the isolates Rs1-Rs5 belonging to the *R. solani*/*Thanatephorus cucumeris* clade.

Fig. 3 Distribution of potato germplasm among disease response categories against *Rhizoctonia solani* and hierarchical clustering based on disease incidence and disease severity.

Fig. 4 Representative *in-vitro* assays of treatments against *Rhizoctonia solani*. (A) Chemical fungicides; (B) PGPR treatments; (C) aqueous plant extracts. Detailed mycelial growth and percent growth inhibition data are provided in Supplementary Tables.

Fig. 5 Pot validation and multivariate interpretation of treatment responses against *Rhizoctonia solani*. (A-B) Tuber production and disease responses; (C) principal component analysis; (D) pairwise correlation matrix.

Table Legends

Table 1 Overall germplasm response category according to disease rating scale against *Rhizoctonia solani*.

Table 2 Effect of selected treatments on disease incidence (%) and disease severity (%) under pot conditions.

Supplementary Files

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