Available Online at EScience Press
Plant Protection
ISSN: 2617-1287 (Online), 2617-1279 (Print)
http://esciencepress.net/journals/PP

MANAGEMENT OF CHILI ROOT ROT PATHOGEN, RHIZOCTONIA SOLANI, WITH POTENTIAL RHIZOBACTERIA

[a]Abdul Mateen, [a]Mirza Abid Mehmood, [a]Muhammad Ashfaq, [b]Abu Bakar Saddique, [a]Rana Binyamin, [c]Muhammad Mahmood Ahmed, [a]Hafiz Hamza Naeem
[a] Plant Pathology, Institute of Plant Protection, Muhammad Nawaz Shareef University of Agriculture, Multan, Pakistan.
[b] Institute of Plant Breeding and Biotechnology, Muhammad Nawaz Shareef University of Agriculture Multan, Pakistan.
[c] Institute of Biochemistry, Biotechnology and Bioinformatics, The Islamia University of Bahawalpur, Pakistan.

ARTICLE INFO

Article history
Received: 13th August, 2022
Revised: 17th September, 2022
Accepted: 20th October, 2022

Keywords
Capsicum annuum
Rhizoctonia solani
Incidence
Prevalence
Rhizobacteria

ABSTRACT

Chili (*Capsicum annuum* L.) belongs to the solanaceae family. It is a major vegetable crop grown on a wide scale in Asia. Nearly 20% of Pakistan's total vegetable producing land is utilized for cultivating chili. Various plant pathogens including viruses, fungi, bacteria and nematodes are responsible for causing diseases in chili crop. Among fungal diseases, root rot is an important fungal disease which is caused by *Rhizoctonia solani*. In the present study, a survey of different localities of Multan district were conducted and plants with prominent root rot symptoms were collected and subjected to characterization. Moreover, the disease incidence and prevalence was calculated in surveyed localities. Highest disease incidence i.e. 20.77% was evident in samples collected from Tatay Pur and the lowest disease incidence (7.59%) was observed in samples of Suraj Miani. Morphological identification of root rot samples was carried out based on growth, shape, color, and hyphae structure and confirmed the association of *R. solani*. Koch's postulates were confirmed after pathogenicity test which was accomplished by stem application method. About 10 rhizobacteria were isolated from rhizospheric soils of healthy plants and dual culture technique was used to manage the *R. solani* mycelial growth under *in vitro* conditions. Our results revealed that rhizobacteria 7 (RB7) exhibited the highest inhibition percentage 54.81% while the lowest inhibition percentage i.e. 40% was evident using rhizobacteria 4 (RB4) compared to control. This study helped us to provide the alternatives to the chemical fungicides that could be explored under field conditions against root rot disease as they are ecofriendly and has less impact to the human life.

Corresponding Author: Mirza Abid Mehmood
Email: abid.mehmood@mnsuam.edu.pk

INTRODUCTION

Chili (*Capsicum annuum* L.) is a major crop cultivated around the world. It is used to make medicines and is consumed fresh as well as in dried form. The genus *Capsicum*, which includes the chili pepper, belongs to the Solanaceae family. There are 31 reported species of chilies and the prominent species are *C. chinense*, *C. pubescens*, *C. annuum*, *C. frutescens*, and *C. baccatum* (Sanogo, 2003). It is considered as one of the most imperative vegetable crop and also often known as "wonder spice." If the soil is properly drained, chilies may be grown in a range of soil types. The best soil for growing chilies is a light loamy or sandy loam that is high in lime and organic matter (Solanki et al., 2014).

Africa, North Africa, Asia, Europe, and Latin America are the major chili-producing countries (Sanogo, 2003).

Numerous plant pathogens including viruses, fungus, bacteria, and nematodes disrupt the metabolic pathways of the chili and lead to a variety of illnesses (Ahmad et al., 2017; Asghar et al., 2020; Ashfaq et al., 2014; Aslam et al., 2017; Hyder et al., 2020; Riaz et al., 2018; Shahbaz et al., 2015; Tariq-Khan et al., 2020; Tariq-Khan et al., 2017). The most concerning diseases of chili that significantly reduce output include downy mildew, collar rot, *Phytophthora* blight, fruit and root rot, purple blotch, and anthracnose as well (Agrios, 2005). The absence of capsaicin in chili fruits which is the primary bioactive substance in chili and produces the pungent flavor is caused by fungi. The chili crop is harmed by a number of diseases and pests, which significantly reduce yield (Agrios, 2005). *R. solani* is a soil-borne pathogen that affects lower stems and roots of plants, causing stem and stolon canker, collar rot, and damping off (Parmeter, 1970). The fungus is a facultative parasite that lives on crop wastes as micro-sclerotia in agricultural soils (Ramusi, 2008). The name "*Rhizoctonia*," which translates as "root killer," comes from the Ancient Greek words (rhiza, "root") + (ktonos, "murder"). The genus developed into a diversified group of ascomycetes, basidiomycetes, and imperfect fungi, enabling the inclusion of several unrelated species (Stalpers and Andersen, 1996). In 1815, the *Rhizoctonia* genus was first described (Haque and Parvin, 2021). Infected potatoes were the first source of *Rhizoctonia solani* Kühn's discovery in 1858. Mycelium from *R. solani* is colorless while it is young, but as it develops and ages, it turns brown (Ogoshi, 1975; Tu and Kimbrough, 1975).

In soil, several bacterial and fungal genera can be identified. They engage in a variety of biotic activities to keep the soil ecosystem active for nutrient turnover and long-term crop production (Ahemad and Khan, 2009; Chandler et al., 2008). Rhizosphere bacteria that may colonize the root environment fall under the category of rhizobacteria. The term "rhizosphere" refers to the small region of soil immediately around the root system (Walker et al., 2003; Zablotowicz et al., 1991). These challenges have been significant barriers for regional agriculture. On the other hand, local food production is crucial in determining whether or not there is an improvement in global food security (Adesemoye and Egamberdieva, 2013).

Chemical fungicides are of limited benefit, not very effective and pose negative effects on human health and environment (Iqbal and Mukhtar, 2020). In contrast, biological control agents are eco-friendly with the ability to suppress or inhibit pathogen populations (Chow et al., 2018; Shahzaman et al., 2017; Shahzaman et al., 2016). Therefore, the biocontrol agents are alternatives to chemical fungicides for managing phyotpathogens. Rhizopsheric soil is a rich source of microbes having beneficial potential like biocontrol, plant growth promotion, nitrogen fixation and mycorrhizal association. Keeping in view, the importance of other pathogen and their association with chili plants, a survey for incidence and prevalence of root rot pathogens associated with chili plants that are being grown in Multan district was carried out and the plants representing root rot symptoms were characterized morphologically. Moreover, rhizospheric soil samples from the healthy plants were collected and subjected to the isolation of rhizobacteria. These isolated rhizobacteria were used for *in vitro* management of root rot pathogen.

MATERIALS AND METHODS

Survey of chili field

In the Multan district, surveys were conducted in the five main chili-growing regions i.e. Suraj Miani, Tatay Pur, Qadir Pur Rawan, Jalalpur Pirwala, and Shujabad. Based on root rot symptoms, the incidence and frequency of the disease at random locations within chili fields laid out in a Z pattern were calculated using the below given formula (Asad et al., 2009).

$$Incidence\ \% = \frac{Number\ of\ infected\ plants}{Total\ number\ of\ plants} \times 100$$

Similarly, the disease prevalence of each area was calculated as per the given formula

$$Prevalence\ \% = \frac{Number\ of\ infected\ fields}{Total\ number\ of\ fields\ observed} \times 100$$

Collection of samples

Chili plants infected with root rot pathogen while healthy plants with no disease symptoms were uprooted to collect the rhizospheric soil samples. Each sample was preserved in a polypropylene bag and shifted to the Diagnostic Laboratory at Muhammad Nawaz Shareef University of Agriculture, Multan where they were kept at 4°C (Mishra et al., 2018).

Isolation of root rot pathogen and rhizobacteria

For isolation of a fungal pathogen, the PDA media (potato starch 20 g, dextrose 20 g, agar 20 g and distilled water 1000 mL) was prepared. The root

samples were surface sterilized using 1% solution of sodium hypochlorite (NaOCl) followed by three washings in distilled water. Later, the root samples were placed on PDA plates. These plates were then incubated at 28°C and growth was observed with 24 h interval (Madhavi and Bhattiprolu, 2011).

Rhizosphere soil was taken and passed through sieve and the serial dilution of 10^{-3} was prepared. With the help of micropipette, 200 µL from 10^{-3} dilution was taken and spread onto the petri plate containing Nutrient Agar media (Yeast extract/ Beef extract 3 g, peptone 5 g, NaCl 5 g, Agar 15 g and distilled water 1000 mL) with the help of spreader. The petri plates were then wrapped and incubated at 28°C. The growth was observed after 24 h (Madhavi and Bhattiprolu, 2011) and the colony forming units (cfu) of each bacterium was calculated by the following formula (Sieuwerts et al., 2008).

$$Colony\ forming\ units\ (cfu/ml) = \frac{\text{Number of colonies} \times \text{Dilution factor}}{\text{Volume of culture plate}}$$

After serial dilution, streaking of morphologically distinct bacterial colonies was done to obtain single culture of bacteria.

Morphological characterization

The isolated fungal pathogen was characterized morphologically based on colony color, shape of hyphae and growth pattern (Hyder et al., 2018).

Pathogenicity test

To confirm Koch's postulates, fungal inoculum was prepared by collecting mycelium from 7-day old culture grown in PDA media and homogenized in 0.5 L of sterile distilled water and mixed for 5 min and the suspension was adjusted to 10^5 cfu/mL using hemocytometer (Mannai et al., 2018). The fungal isolate was tested on chili variety WP-1335 to confirm its association with root rot disease. Fifteen days old chili plants were tested in three replications where 6 plants per replication were treated by using this inoculum by following stem application method and plants without treatment served as control. The pathogen was re-isolated and compared with original culture. The data of total number of plants and infected plants was calculated (Sumalatha et al., 2018).

Management with potential rhizobacteria

Bacterial isolates were tested *in vitro* for their ability to suppress the development of *R. solani*, a globally prevalent soil-borne phytopathogenic fungus. A single colony of each bacterial isolate was picked and streaked equidistantly across the PDA plate edges in three replications. Between two parallel streaks of the tested bacterium, a 5 mm diameter mycelial agar plug from a 7-day-old culture of *R. solani* and plates without bacterial streak were kept as control. Plates were incubated at 25°C (Torres et al., 2016). The percentage of growth inhibition was calculated by comparing the diameter of mycelial growth on bacteria-inoculated plates to the diameter of mycelial growth on control plates by using following formula.

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

Where C is mycelial growth in control plate, T is mycelial growth in test organism's inoculated plate and I is inhibition of mycelial growth (Landa et al., 1997).

Statistical Analysis

The colony forming units and mycelial growth inhibition was statistically analyzed using ANOVA and the means were compared using multiple comparison test (Tukey's HSD test) (Steel et al., 1997).

RESULTS

The survey of different locations of Multan district revealed that the highest (20.77%) incidence of root rot was evident in Tatay Pur followed by Qadir Pur Rawan, Shujabad, Jalalpur Pirwala while the least (7.59%) was observed in Suraj Miani (Table 1).

Table 1: Root rot of chili incidence in different growing areas of Multan.

Location	Total Plants	Infected Plants	Disease Incidence %
Suraj Miani	158	12	7.59
Shujabad	162	15	9.25
Jalalpur Pirwala	153	14	9.15
Qadir Pur Rawan	155	21	13.54
Tatay Pur	154	32	20.77
Total	782	94	12.02

Figure 1: Isolated pathogen from roots infected with root rot (A) Purified culture of *R. solani* (B).

Colony morphology like shape and color of colony and growth pattern was similar to *R. solani* fungus. When *R. solani* mycelium was young, it was colorless but as it grew and matured subsequently turned into brown color. Young vegetative hyphae were branched at right angles at the cell's distal septum and constricted at their junction or a short distance from it (Figure 2).

R. solani showed slow growth rate and filled the 90 mm plate in ten days. The average growth of three replications on daily basis was 0.6 mm, 1.2 mm, 1.7 mm, 2.2 mm, 3.05 mm, 4.05 mm, 5.2 mm, 6.5 mm, 7.7 mm, and 8.5 mm (Figure 3).

Figure 2: Mycelial growth of *R. solani* observed under microscope.

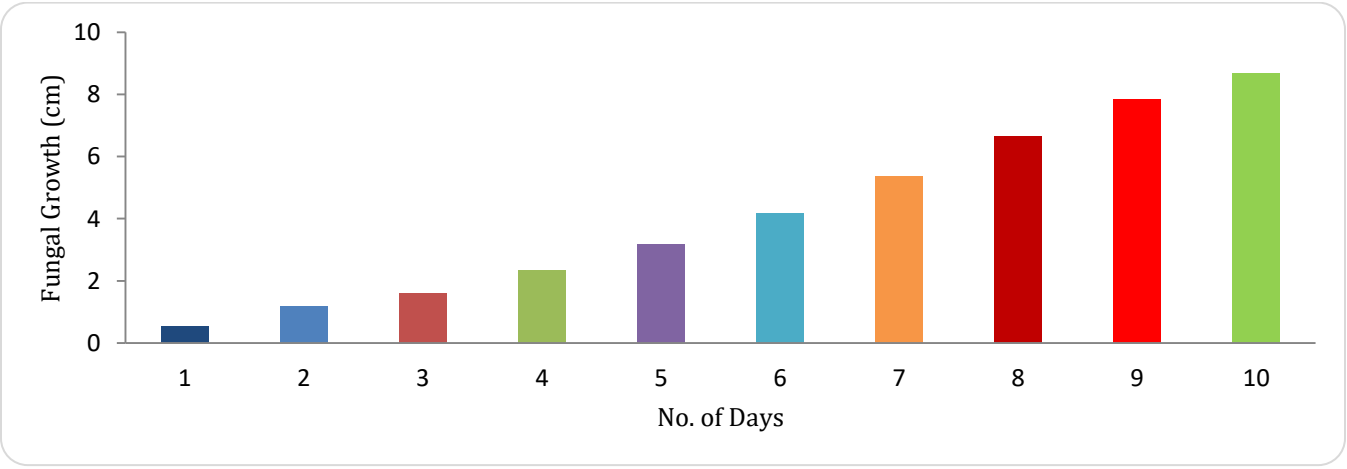

Figure 3: Mycelial growth of *R. solani* observed for ten days.

Isolation of rhizobacteria

About 200 µL aliquot from 10^{-3} serially diluted samples was spread on NA media and colony forming units/mL were calculated (Figure 4). There was a statistically non-significant difference in colony forming units of soil samples collected from different locations of Multan district. Precisely, highest colony forming units (4.43×10^6) were obtained from soil samples of Tatay Pur while the least (3.80×10^6) were present in Suraj Miani samples (Table 2). The morphologically distinct colonies were streaked on fresh NA plates to obtain single colonies. After 12 h, bacterial single colonies developed and showed whitish, creamy, yellowish colors and ten rhizobacteria with distinct morphology were isolated.

Figure 4: Bacterial growth of samples obtained from Suraj Miani (A) Qadir Pur Rawan (B) and Tatay Pur (C) of district Multan using serial dilution technique.

Table 2: Colony forming units of rhizobacteria isolated from different locations of district Multan.

Sr. No	Location	Colony forming units (cfu)
01	Suraj Miani	3.80×10^6
02	Qadir Pur Rawan	3.83×10^6
03	Jalalpur Pirwala	4.40×10^6
04	Shujabad	4.30×10^6
05	Tatay Pur	4.43×10^6

Pathogenicity test

Pathogenicity test was done by stem application method on fifteen days old chili plants and after 3-4 days the plants showed root rot symptoms like light to dark brown lesions formed followed by drooping and wilting of leaves and death of plant occur (Figure 5). The re-isolated fungus was confirmed with already isolated fungi in terms of colony color, growth pattern etc. which confirmed the association of *R. solani* with the root rot samples collected in this study.

Figure 5**:** Symptoms appeared after 4 days (A), root rot occurred after 10 days (B) and healthy plant (C).

Management of *R. solani* with potential rhizobacteria

The bacterial cultures were streaked equidistantly across the PDA plate edges containing 5 mm plug of *R. solani* and all rhizoacteria inhibited the mycelial growth of *R. solani* (Figure 6). The inhibition percentage varied in samples streaked with different rhizobacteria.

Different rhizobacteria inhibited the mycelial growth of *R. solani* and showed statistically significant difference while Tukey's HSD test revealed that RB1, RB2, RB3, RB6, RB7, RB8, and RB10 were statistically non-significant but they were significant with RB4, RB5, and RB9 (Figure 7). Rhizobacteria 7 (RB7) showed the highest (54.81%) mycelial inhibition of *R.solani* followed by RB2 and RB3 with 54.07% mycelial inhibition. RB6 exhibited 51.85% against mycelial inhibition of *R. solani*. RB8 and RB10 showed 51.11% and 50.37% inhibition against mycelial growth of *R. solani*, respectively. RB1 inhibition rate was 49.62%. RB5 exhibited 47.40% inhibition rate. RB9 inhibition rate was 43.70% while RB4 inhibition rate against the mycelial growth of *R. solani* was lowest (50.37%). While the RB7 exhibited highest inhibition percentage (54.81%) against *R. solani* while RB4 revealed lowest inhibition percentage (40%).

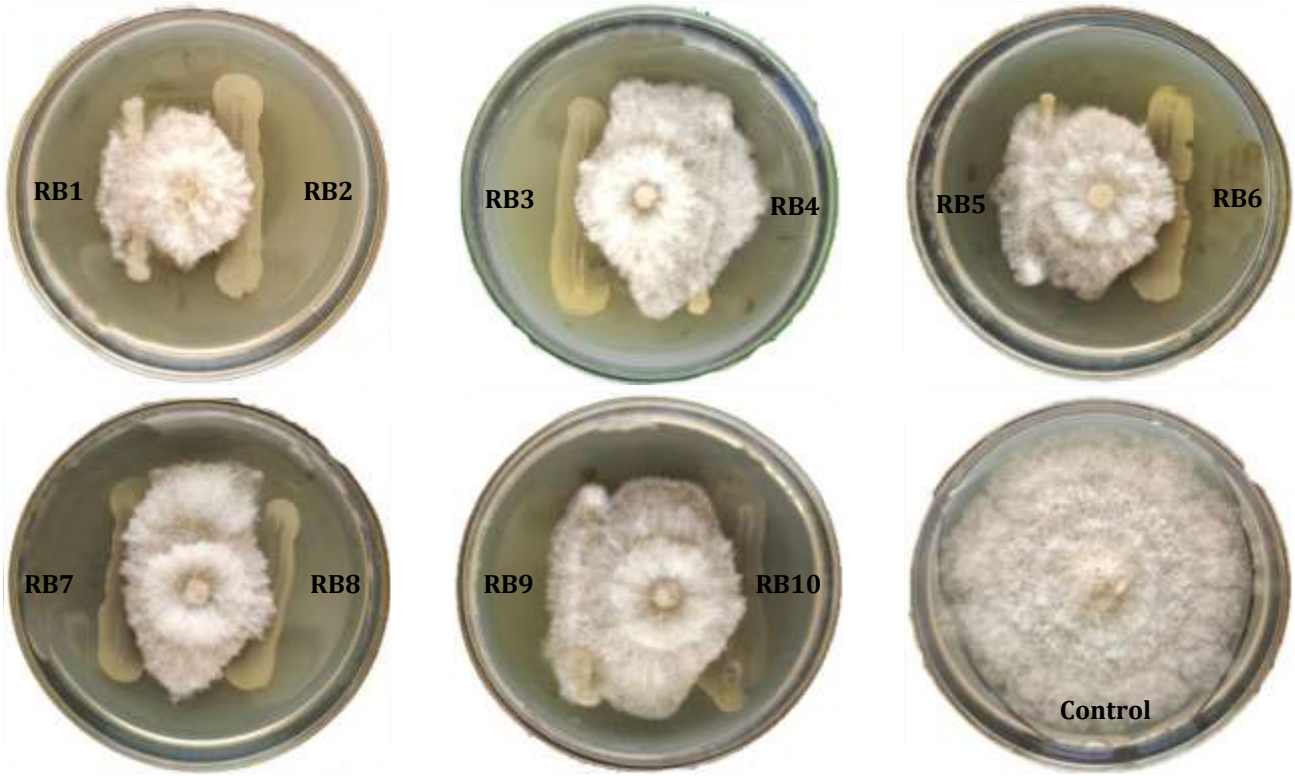

Figure 6: Different rhizobacteria showing inhibition against *R. solani* and control plate showing growth of *R. solani*.

DISCUSSION

One of the most important crops in Asian countries is chili that is being grown widely. It is consumed in raw, dried, or processed form and used in pharmaceuticals (Sanogo, 2003). People used chili that serves as a natural taste and colorant in the food sector (Vinaya et al., 2009). Numerous diseases and pests harm the chili crop, drastically reducing productivity (Agrios, 2005). A significant problem affecting the productivity of chilies is the soil-borne fungus *R. solani*, which causes stem canker in young transplants and root rot disease in seedlings (Valle et al., 2001). Additionally, mature chili plants are affected, which may cause wilting and plant death from stem canker and root rot along the soil line (Sanogo, 2003). The fungus, a facultative parasite, feeds on crop wastes in agricultural soils as micro sclerotia (Ramusi, 2008). In the present study, a survey of five separate chili growing areas of Multan district was conducted. The highest disease incidence was calculated in Tatay Pur

(20.77%) and lowest in Suraj Miani (7.59%). An earlier study carried out a survey of various places in the Pothwar area showed that *R. solani* infection was present at every site.

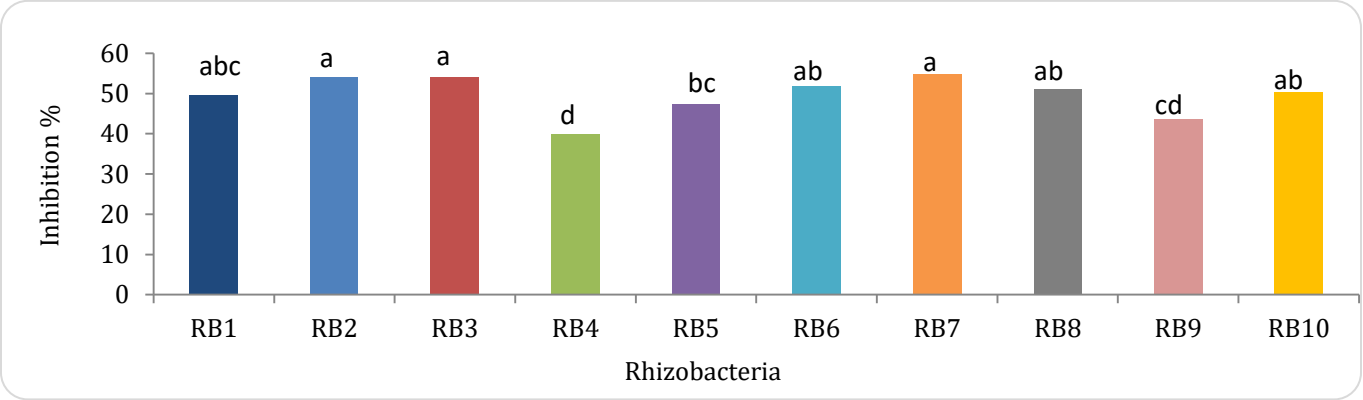

Figure 7: Average inhibition percentage of all the Rhizobacteria.

The highest mean disease incidence in the district of Rawalpindi was found in Kahuta (35.9%), while Hazro in district Attock had the lowest mean disease incidence (25.4%). Furthermore, a thorough survey in Uttar Pradesh was done where infection of *R. solani* in different villages was ranged from 5-15% in district Allahbad, Faizabad, Etawah and Mirzapur; 15% in Gaurabibipur of district Sultanpur, 15-20% in district Amethi; 10-25% in district Kanpur, 25% in district Pratapgarh, 25-45% in district Jaunpur (Mishra et al., 2018). The present study is in agreement with aforementioned studies as disease incidence occurring not only in Pakistan but also in India and Vietnam (Saba et al., 2022).

R. solani previously showed a white colony due to cultural and physical characteristics, followed by brownish mycelium with irregular forms and slightly melanized hyphae. In addition, microscopic analysis showed that the hyphae branch at a 90° angle that the septa develop near to the start of the hyphal branches that there are no clamp connections, and that the hyphae do not contain conidia (Al-Fadhal et al., 2019). Mycelium size ranged from tiny to big (0.2-2 mm), and their color ranged from light brown to dark brown. In isolate number 7, the largest mycelial diameter (0.25-2 mm) was found. Mycelium was often formed by isolates in the center of the colony. Mycelium might sometimes be seen all over the Petri plate. Five groups of *R. solani* isolates were categorized based on morphological features (Sharma et al., 2005). The present study is in agreement with the earlier findings, as the fungal growth showed whitish colony that was around the samples. While, no dark brown colony was evident in our study, that is contrary to the aforementioned studies. In the present study, pathogenicity test was done by stem application method on fifteen days old chili plants and after 3-4 days the plants were inoculated with *R. solani* that showed root rot symptoms like seedlings wilts and rotted tap root and lesions formed at early stage and turned reddish brown. Hence the association of *R. solani* with root rot samples was confirmed.

In the present study, ten rhizobacteria were isolated from rhizosphere soil and used for the management of *R. solani in vitro* and all of these rhizobacteria showed inhibition percentage against *R. solani* in which RB7 showed the highest inhibition percentage (54.81%) and RB4 showed the lowest inhibition percentage (40%). Similarly, in comparison to pathogen-inoculated and control, all examined bacterial treatments increased the inhibition percentage of *R. solani* inoculated seedlings that emerged, with the exception of isolates *B. glathei* 35, *P. huttiensis* 69, and *B. subtilis* 263. Using the isolates *P. putida* 227 and *B. pumilus* 420, this improvement was increased by 57.14%. In comparison to the control, post-emergence damping-off of pepper seedlings infected with *R. solani* was reduced by the eight investigated bacterial isolates. In fact, *P. aureofaciens* 314 and *P. putida* 227 treatments fully repressed the manifestation of the disease, with *B. pumilus* 420 treatments reducing this parameter by 40%. Disease-suppressing abilities of *P. aureofaciens* 31 and *B. subtilis* 263 were equivalent to those of the reference strain (*P. fluorescens*), where

damping-off was reduced by 30% in comparison to the untreated control. Isolates of *P. aureofaciens* 314 was more efficient than the reference strain. The degree of *Rhizoctonia* root rot was observed 75 days after planting and did not substantially change in response to tested antagonistic treatments. Though statistically insignificant, *P. aureofaciens* 31 lowered disease severity by 50%, followed by *B. pumilus* 420, *P. aureofaciens* 314, and *P. putida* 227, which reduced this parameter by 40% when compared to infected and untreated control (Mannai et al., 2018). These results are in agreement with the present study where we confirmed that the isolated rhizobacteria showed inhibitory effects on mycelial growth of *R. solani*. These results could be helpful in future to replace the chemical fungicides that are able to develop resistance and pose toxic effects on human health and environment while rhizobacteria based biocontrol agents would be eco-friendly with minimum or zero chances of resistance development.

AUTHORS' CONTRIBUTION

AM and MAM conceived and designed experiments, AM collected and processed samples, performed experiments, MAM, MA and ABS contributed reagents/materials/analysis tools, AM and MAM analyzed the data, AM, MAM, MMA, HHN prepared figures and/or tables, AM, MAM, MA, ABS, RB, HHN and MMA wrote the manuscript, All the authors read and approved the final draft.

CONFLICT OF INTEREST

The authors declare no conflict of interest

REFERENCES

Adesemoye, A.O., Egamberdieva, D., 2013. Beneficial effects of plant growth-promoting rhizobacteria on improved crop production: prospects for developing economies Bacteria in agrobiology, Crop Productivity. Springer, pp. 45-63.

Agrios, G.N., 2005. Plant Pathology. Elsevier.

Ahemad, M., Khan, M.S., 2009. Effect of insecticide-tolerant and plant growth-promoting Mesorhizobium on the performance of chickpea grown in insecticide stressed alluvial soils. Journal of Crop Science and Biotechnology 12, 217-226.

Ahmad, A., Ashfaq, M., Mukhtar, T., Malik, S.I., 2017. An insight into recombination in *CP* gene of tomato infecting Chilli Veinal Mottle Virus isolate from Pakistan. International Journal of Biosciences 11, 48-54.

Al-Fadhal, F.A., AL-Abedy, A.N., Alkhafije, D.A., 2019. Isolation and molecular identification of *Rhizoctonia solani* and *Fusarium solani* isolated from cucumber (*Cucumis sativus* L.) and their control feasibility by *Pseudomonas fluorescens* and *Bacillus subtilis*. Egyptian Journal of Biological Pest Control 29, 1-11.

Asad, J., Hussain, M.A., Aslam, M.N., Mukhtar, T., Ashfaq, M., 2009. Incidence and prevalence of bacterial wilt of chili in Punjab, Pakistan. Mycopath 13.

Asghar, A., Mukhtar, T., Raja, M.U., Gulzar, A., 2020. Interaction between *Meloidogyne javanica* and *Ralstonia solanacearum* in chili. Pakistan Journal of Zoology 52, 1525-1525.

Ashfaq, M., Iqbal, S., Mukhtar, T., Shah, H., 2014. Screening for resistance to cucumber mosaic cucumovirus in chilli pepper. Journal of Animal and Plant Sciences 24, 791-795.

Aslam, M.N., Mukhtar, T., Ashfaq, M., Hussain, M.A., 2017. Evaluation of chili germplasm for resistance to bacterial wilt caused by *Ralstonia solanacearum*. Australasian Plant Pathology 46, 289-292.

Chandler, D., Davidson, G., Grant, W., Greaves, J., Tatchell, G., 2008. Microbial biopesticides for integrated crop management: an assessment of environmental and regulatory sustainability. Trends in Food Science and Technology 19, 275-283.

Chow, Y.Y., Rahman, S., Ting, A.S.Y., 2018. Interaction dynamics between endophytic biocontrol agents and pathogen in the host plant studied via quantitative real-time polymerase chain reaction (qPCR) approach. Biological Control 125, 44–49.

Haque, M., Parvin, M., 2021. Sugar beet, it 'disease *Rhizoctonia* root rot, and potential biological agents. International Journal of Agriculture Biology 37, 96-101.

Hyder, S., Gondal, A.S., Rizvi, Z.F., Ahmad, R., Alam, M.M., Hannan, A., Ahmed, W., Fatima, N., Inam-ul-Haq, M., 2020. Characterization of native plant growth promoting rhizobacteria and their anti-oomycete potential against *Phytophthora capsici* affecting chilli pepper (*Capsicum annum* L.). Scientific Reports 10, 1-15.

Hyder, S., Inam-Ul-Haq, M., Ahmed, R., Gondal, A.S., Fatima, N., Hanan, A., Zhao, Y.F., 2018. First Report

of *Phytophthora capsici* Infection on Bell Peppers (*Capsicum annuum* L.) from Punjab, Pakistan. International Journal of Phytopathology 7, 51-51.

Iqbal, U., Mukhtar, T., 2020. Inhibitory effects of some fungicides against *Macrophomina phaseolina* causing charcoal rot. Pakistan Journal of Zoology 52, 709-715.

Landa, B.B., Hervás, A., Bettiol, W., Jiménez-Díaz, R.M., 1997. Antagonistic activity of Bacteria from the chickpea rhizosphere against *Fusarium Oxysporum* f. sp. Ciceris. Phytoparasitica 25, 305-318.

Madhavi, G.B., Bhattiprolu, S., 2011. Integrated disease management of dry root rot of chilli incited by *Sclerotium rolfsii* (Sacc.). Internation Journal of Plant Animal Environmental Sciences 1, 31-37.

Mannai, S., Jabnoun-Khiareddine, H., Nasraoui, B., Daami-Remadi, M., 2018. *Rhizoctonia* root rot of pepper (*Capsicum annuum*): Comparative pathogenicity of causal agent and biocontrol attempt using fungal and bacterial agents. Journal of Plant Pathology and Microbiology 9, 431-439.

Mishra, A., Ratan, V., Trivedi, S., Dabbas, M., Shankar, K., Singh, A., Srivastava, Y., 2018. Survey of anthracnose and wilt of chilli: A potential threat to chilli crop in central Uttar Pradesh. International Journal of Pharmacognosy and Phytochemistry 7, 1970-1976.

Ogoshi, A., 1975. Grouping of *Rhizoctonia solan*i Kuhn and their perfect states. Review of Plant Protection Research 8, 93-103.

Parmeter, J.R., 1970. *Rhizoctonia solani*, biology and pathology, Univ of California Press.

Ramusi, T.M., 2008. Biological and chemical control of fungal seedling diseases of cowpea. University of Pretoria.

Riaz, T., Ashfaq, M., Malik, A.H., Mukhtar, T., Hafiz, I.A., 2018. An insight into genetic variability and host response of Pakistani isolate of Chilli veinal mottle virus (ChiVMV) infecting chilli pepper. International Journal of Biosciences 12, 302-312.

Saba, S., Mukhtar, T., Haq, M.I., Malik, S.I., 2022. Occurrence of damping off of chili caused by *Pythium* spp. in the Pothwar region of Pakistan. International Journal of Phytopathology 11, 125-234.

Sanogo, S., 2003. Chile pepper and the threat of wilt diseases. Plant Health Progress 4, 23.

Shahbaz, M.U., Mukhtar, T., Haque, M.I., Begum, N., 2015. Biochemical and serological characterization of *Ralstonia solanacearum* associated with chilli seeds from Pakistan. International Journal of Agriculture and Biology 17, 31-40.

Shahzaman, S., Gardazi, R.A., Inam-ul-Haq, M., Mastoi, A.H., Hayat, S., Ahmed, R., 2017. Optimization of nutritional conditions and effect of time and temperature for the production of bacterial biocontrol agents. International Journal of Biosciences 11, 141-146.

Shahzaman, S., Inam-Ul-Haq, M., Bibi, S., Sufyan, M., Altaf, A., Mehmood, U., Ahmed, R., 2016. Bio-efficacy of *Pseudomonas fluorescens* isolated from chickpea fields as plant growth promoting rhizobacteria. International Journal of Biosciences 9, 138-146.

Sharma, M., Gupta, S.K., Sharma, T.R., 2005. Characterization of variability in *Rhizoctonia solani* by using morphological and molecular markers. Journal of Phytopathology 153, 459-456.

Sieuwerts, S., De Bok, F.A., Mols, E., De Vos, W.M., Van Hylckama Vlieg, J., 2008. A simple and fast method for determining colony forming units. Letters in Applied Microbiology 47, 275-278.

Solanki, M.K., Singh, R.K., Srivastava, S., Kumar, S., Kashyap, P.L., Srivastava, A.K., Arora, D.K., 2014. Isolation and characterization of siderophore producing antagonistic rhizobacteria against *Rhizoctonia solani*. Journal of Basic Microbiology 54, 585-597.

Stalpers, J.A., Andersen, T.F., 1996. A synopsis of the taxonomy of teleomorphs connected with *Rhizoctonia* sl. *Rhizoctonia* species: Taxonomy, molecular biology, ecology, pathology and disease control. Springer.

Steel, R.G.D., Torrie, J.H., Dicky, D.A., 1997. Principles and Procedures of Statistics, A Biometrical Approach. 3rd Edition, McGraw Hill, Inc. Book Co., New York. 352-358.

Sumalatha, N., Pushpavathi, B., Jagadeeshwar, R., Reddy, R., 2018. Studies on Blight of Tomato Incited by *Rhizoctonia solani*. International Journal of Current Microbiology and Applied Sciences 7, 1050-1059.

Tariq-Khan, M., Mukhtar, T., Munir, A., Hallmann, J., Heuer, H., 2020. Comprehensive report on the prevalence of root-knot nematodes in the Poonch division of Azad Jammu and Kashmir, Pakistan. Journal of Phytopathology 168, 322–336.

Tariq-Khan, M., Munir, A., Mukhtar, T., Hallmann, J., Heuer, H., 2017. Distribution of root-knot nematode species and their virulence on vegetables in northern temperate agro-ecosystems of the Pakistani-administered territories of Azad Jammu and Kashmir. Journal of Plant Diseases and Protection 124, 201-212.

Torres, M.J., Brandan, C.P., Petroselli, G., Erra-Balsells, R., Audisio, M.C., 2016. Antagonistic effects of Bacillus subtilis subsp. subtilis and *B. amyloliquefaciens* against *Macrophomina phaseolina*: SEM study of fungal changes and UV-MALDI-TOF MS analysis of their bioactive compounds. Microbiological Research 182, 31-39.

Tu, C., Kimbrough, J.W., 1975. Morphology, development, and cytochemistry of the hyphae and sclerotia of species in the *Rhizoctonia* complex. Canandian Journal of Plant Science 53, 2282-2296.

Valle, R.V., Aguilar, M.M.M., Ruiz, J.D.J.L., 2001. Sintomatología y Géneros de Patógenos Asociados con lasPudriciones de la Raíz del Chile (*Capsicum annuum* L.) en el Norte-Centro de México. Revista Mexicana de Fitopatología 19, 175-181.

Vinaya, H., Rao, M., Yashoda, H., Mohankumar, H., 2009. Status of seed borne incidence of anthracnose of chilli in northern Karnataka and evaluation of seed health testing methods for the detection of *Colletotrichum capsici*. Journal of Agriculture Sciences and Technology 22, 807-809.

Walker, T.S., Bais, H.P., Grotewold, E., Vivanco, J.M., 2003. Root exudation and rhizosphere biology. Plant Physiology 132, 44-51.

Zablotowicz, R.M., Tipping, E.M., Lifshitz, R., Kloepper, J.W., 1991. Plant growth promotion mediated by bacterial rhizosphere colonizers. The rhizosphere and plant growth. Springer.