Original Research Article

Exploring the In Vitro Impact of Native Mycoflora on the Radial Growth of Rhizoctoniasolani, the Causative Agent of Root Rot Disease in Chilli

ABSTRACT

In the course of the study, native bioagents isolated from the rhizosphere of the chi~~l~~li crop ~~was~~ wereused to manage the soil-borne pathogens *Rhizoctoniasolani.*The study was conducted in the laboratory of the Department of Plant Pathology and Nematology, RPCAU, Pusa, Bihar in the year 2020-23.The soil microflora (fungal and bacterial) was isolated from the rhizosphere of Chi~~l~~li and screened *in vitro* by evaluating their antagonistic potential against *Rhizoctoniasolani*, and resultantly two fungal and two bacterial isolates were found most effective in inhibiting the mycelial growth of the pathogen over control. The maximum percent inhibition was recorded in the case of *Trichod*ermaharzianum (71.98%) followed by*Trichod*erma*viride* (62.54%) and among the bacterial isolates maximum inhibition was recorded in the case of RB1 that inhibit (69.38%), followed by RB6 (66.42%). Overall, these findings suggest that the combination of *Trichoderma* and *Bacteria* could be an effective and sustainable method for ~~reduceing~~ reducing the radial growth of *Rhizoctoniasolani* causing Root rot disease in chi~~l~~li.*Rhizoctoniasolani* was established as a causal organism of chi~~l~~li. ~~Use~~ The use of Bio-control agents is an eco-friendly approach and a good option to manage ~~the~~ soil-borne phyto-pathogens. These biological control agents either use the mechanism of antibiosis or mycoparasitism against the fungal pathogen. Evaluation of *Trichoderma*spp. and Bacterial isolates against *Rhizoctoniasolani* showed that significantly reduced the mycelial growth of *Rhizoctoniasolani in vitro.*

Key Words: *Bio-control, Chilli, Rhizoctoniasolani, Rhizobacteria, and Trichoderma.*

1. INTRODUCTION

Chili peppers, also known as *Capsicum annuum* are an important crop in agriculture with global production reaching over 36,286,643.77 tons[1]. This crop is widely cultivated and consumed in many parts of the world due to its culinary and medicinal properties. Chili peppers are rich in vitamin C, vitamin A, potassium, fiber, minerals, antioxidants, and other essential nutrients, making them a vital component of a balanced diet[2]. Additionally, they are used in the production of spices, sauces, and other food products contributing significantly to the food industry's economic value. However, chili pepper production is threatened by various diseases caused by fungal pathogens including *Rhizoctoniasolani*which causes root rot disease leading to significant crop losses and a reduction of yield. It was found to record 33.2 percent disease incidence of the seedlings in greenhouse conditions and 40.20 percent in the main field [3]. This pathogen is commonly found in soil and identified as both a seed and soil-borne pathogen [4]. Traditional control methods for this disease involve the use of chemical pesticides which pose a threat to the environment and human health. Therefore, there is an increasing demand for safer and sustainable approaches to manage this disease. As an alternative, the use of bio-control agents such as~~,~~*Trichoderma*, *Bacillus,* and*Pseudomonas* ~~*Psedomonas*~~ isolates ~~have~~ hasgained popularity in recent years. In the pursuit of environmentally friendly and locally adapted solutions, this study focuses on the in

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vitro screening of native microflora residing in the rhizosphere, ~~with the aim of identifying~~ to identify potential bio-control agents against *Rhizoctoniasolani*. Indigenous microflora have demonstrated the capacity to act as antagonists against soilborne pathogens, thereby offering a promising avenue for sustainable disease management [5~~,6~~, 6].).The rhizosphere region influenced by root secretions is known to harbor a diverse community of microorganisms, including ~~fungi, that~~fungi, which play crucial roles in plant health. Guide the evaluation process, ensuring the reliable identification of biocontrol candidates [7~~,8~~, 8]. The importance of genetic characterization in understanding the diversity and taxonomy of fungal isolates, is a critical step in elucidating the potential bio-control mechanisms [9].Through this in vitro screening approach, our study seeks to contribute valuable insights into the identification of indigenous microflora with the potential to mitigate *Rhizoctoniasolani*,induced root rot in chili peppers. The outcomes of this research hold promise for the development of sustainable and region-specific strategies for managing root diseases in chili cultivation.

2. MATERIALS AND METHODS

2.1 Isolation of pathogen

The roots were thoroughly washed with tap water to eliminate soil particles. Subsequently, roots were cut into small segments, each measuring approximately 0.5 cm, surface sterilization was done using a 0.1% mercuric chloride solution for 1 minute, and then these segments were washed three times with sterilized distilled water before being aseptically transferred to Potato Dextrose Agar (PDA) medium. The plates were then incubated at a temperature of 25 ± 2°C for 3 days to facilitate their mycelial growth. Purification of the cultures was achieved by using the hyphal tip method [10]. The subsequent analysis involved a comparison of various cultural and morphological characteristics of the isolated pathogen [11].The isolation of the pathogen from infected chili roots and seedlings~~,~~ was conducted during the experiment [12].

2.2 Isolation of rhizosphericmicroflora from chillirhizosphere

Soil samples were collected from different ecosystems of the chi~~l~~li rhizosphere in various locations at RPCAU, Pusa, and serial dilution technique was followed to isolate both fungal and bacterial microflora [13]. Ten grams of soil were taken from each sample and mixed with 90 ml of sterile distilled water to create a 100 ml suspension. One ml of the suspension was transferred to a new tube containing 9 ml of sterile distilled water. This process was repeated until a 10^{-8} dilution was achieved followed by one ml of sample suspension from each dilution was spread on nutrient agar (NA) plate media. The NA plates were then incubated for 24 hours at 28 ± 2°C. This allows the bacteria to grow and form colonies. After inoculation, individual colonies that developed on the plates were transferred to new NA plates. For isolation of *Trichoderma*,10^{-6} dilutions were prepared in sterilized distilled water and 1 ml diluted sample was poured on the surface of Trichoderma Selective Medium (TSM). Plates were inoculated at 28 ± 2°C for 96 h. ~~Morphologically~~morphologically different colonies appearing on the plates were purified in the Potato Dextrose Agar (PDA).

2.3 Dual~~culture~~ Culture Technique

The antagonistic activity of fungal and bacterial microflora against *Rhizoctoniasolani*was assessed by dual culture technique [14] on potato dextrose agar (PDA) medium. Seven-day~~s~~ -old test pathogen and fungal biocontrol agent were used in this experiment. A ~~Five~~ 5mm size mycelial disc was cut from the test

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pathogen and transferred on fresh PDA plates at one cm apart from the edge of the Petri plates whereas *Trichoderma*isolates were placed opposite to the test fungus one cm from the opposite edge of the plate. ~~Individual~~ The individualgrowth of the pathogen on the PDA medium was utilized as a control. The experiment comprised three replicates for each treatment, and the entire setup followed a completely randomized design. The plates were incubated at 25 ± 1°C for 7 days. Radial growth was recorded on the 7th day of inoculation and mycelia inhibition was calculated according to thegiven formula [15].

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

Where,

C = Radial growth of pathogen (mm) in check

T = Radial growth of pathogen (mm) in treatment

Similarly, the bacterial isolates were also evaluated for their antagonistic potential*invitro*against*Rhizoctoniasolani*[16] . A 5mmsampleofthetestpathogenfroma seven-day-oldculturewas~~placed at~~placed ~~the~~at the ~~centre~~ center~~of a~~of a90mmPetriplatecontaining PDA medium. Four different bacterial isolates from respective 24-hourold cultures werestreakedonfoursidesofthe Petriplateoppositetoeachotherand at1cmfromtheperiphery.Theseplateswereinoculated at26±1 °C.Aplate inoculatedwithonly*Rhizoctoniasolani*wasmaintainedascontrol.Theinhibitionof mycelialgrowthofthetestpathogenwasobserved.Radial growth of the pathogen was recorded and percent inhibition was calculated by using the following formula [14]:

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

Where,

C = Radial growth of pathogen (mm) in check

T = Radial growth of pathogen (mm) in treatment

3. RESULTS AND DISCUSSION

3.1 Isolation of rhizosphericmicroflora from different chilli rhizosphere

The antagonistic ability of each microflora isolate against *Rhizoctoniasolani* was assessed *in vitro*.Different isolates were obtained from several chilli rhizosphere ecosystems at RPCAU, Pusa, during ~~the course of~~ the investigation viz.*Aspergillus niger, A. flavus, A. fumigates, T. harzianum, T. viride, T. asperellum,Penicillium*sp.,*Alternaria*sp., *Fusarium*sp.*Trichoderma* isolates were cultivated on specific media that ~~was~~ weremade to favour the development of *Trichoderma*while preventing the growth of other fungi. On the other hand, nutrient agar was selected for facilitating bacterial growth [17].

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3.2Antagonism of *Trichoderma* isolates against Rhizoctoniasolani

Using a dual culture method, the antagonistic activity of *Trichoderma* and bacterial isolates was assessed against the test fungus. The test fungus and antagonist's radial growth were observed, and percentage inhibition was computed using this data. According to the findings shown in Table 1, all the isolates of *Trichoderma* were considerably better than the control at preventing the growth of the test fungus. After six days of inoculation, the highest percent inhibition was seen in the case of *Trichodermaharzianum*(71.98%), followed by *Trichodermaviride* (62.54%). However, *Trichodermaspp.* ~~totally~~ reduced *Rhizoctoniasolani* after 7 days of inoculation. This finding aligns with numerous reports that have asserted that *T. harzianum, T. virens*, and *T. hamatum* exhibit~~s~~ high efficacy in suppressing the mycelial growth of soil-borne, seed-borne, phyllosphere and storage plant pathogens on PDA [18~~,19~~, 19].Numerous enzymes that break down cell walls have been discovered to be secreted by *Trichoderma* strains during their mycoparasitic interactions with their hosts. Chitinases and β-1~~,3~~, 3-glucanases have been revealed to have a direct role in this regard, enabling them to pierce through their host fungus and draw resources for their ~~own~~ growth. In addition, it strongly inhibited sclerotia production and suppressed sclerotia germination of pathogen [20]. *Trichodermaharzianum* was shown to have the greatest mycelial growth inhibition of *Rhizoctoniasolani* (64.81%) [21].Numerous workers have reported similar findings about the inhibition of *R. solani's*mycelial growth, which is harmful to chi~~l~~lies and certain other host plants, by distinct microbial antagonists such as *Trichoderma*spp. [22, 23].

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Table 1: *In vitro* evaluation of antagonistic potential of rhizospheric fungal isolates against *Rhizoctoniasolani*

Sl No.	Name of fungal isolates	Radial growth of *Rhizoctoniasolani* (mm)			Inhibition over control on 168hrs(%)
		72hrs	120hrs	168hrs	
1	*Aspergillus niger*	25.33	32.33	31.68	31.96
2	*A. flavus*	23.67	30.78	32.87	34.56
3	*A. fumigatus*	22.53	24.78	26.88	44.56

4	*T. harzianum*	14.35	16.45	15.86	71.98
5	*T. viride*	12.34	18.34	22.45	62.54
6	*T. asperellum*	16.20	20.33	24.00	56.67
7	*Penicillium*sp.	24.67	32.45	38.33	16.67
8	*Alternaria*sp.	28.33	32.33	35.43	19.45
9	*Fusariumsolani*	30.67	31.67	26.67	40.73
10	Control	34.67	39.67	45.00	0
C.D. at 5%		1.51	2.01	2.25	--
C.V. (%)		2.35	2.58	3.45	--
S.Em (±)		0.39	0.41	0.37	--

3.2 Antagonism of Bacterial isolates against Rhizoctoniasolani

The assessment of antagonistic activity between *Rhizoctoniasol*ani and bacterial isolates was conducted through dual culture methods. According to the findings as shown in Table 2, Bacterial isolate RB1 was found to record maximum growth reduction of *R. solani*by 69.38% over control which was followed by bacterial isolate RB6~~that~~ which recorded ~~the~~ a growth reduction of 66.42%. The assay showed marked retardation of pathogen growth. This result correlates with the findings of other workers which revealed that *Bacillus subtilis* produces iturin and surfactin enzymes in the late phase of growth that inhibit mycelial growth of *Rhizoctoniasolani*[24]. Maximum inhibition was showed by *Pseudomonas fluorescens* (41.48 %), this was statistically at par with *Bacillus subtilis* (34.81%) against the *Rhizoctoniasolani* causing root rot disease in chi~~l~~li [25].

Table 2: *In vitro* evaluation of antagonistic potential of rhizospheric bacterial isolates against *Rhizoctoniasolani*

Sl No.	**Name of bacterial isolates**	**Radial growth of *Rhizoctoniasolani* (mm)**			**Inhibition over control after 168hrs (%)**
		72 hrs	**120hrs**	**168hrs**	
1	RB-1	11.67	14.33	13.67	69.38

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2	RB-2	14.67	39	44	1.5
3	RB-3	13.67	18.33	25.33	43.28
4	RB-4	18	22	25	44.02
5	RB-5	12.67	15	17	61.93
6	RB-6	12.67	13.53	14.38	66.42
7	RB-7	17.33	21.67	23.67	47
8	RB-8	15.33	38.33	44.33	0.76
9	RB-11	14.33	17.23	24.67	44.76
10	Control	25	38.33	44.67	0
C.D. at 5%		1.06	1.07	1.17	--
C.V. (%)		3.89	2.85	2.64	--
S.Em (±)		0.35	0.38	0.39	--

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4. CONCLUSION

The ability of native microflora against the phytopathogen*Rhizoctoniasolani* was investigated *in vitro* by dual culture technique. *T. harzianum* was the strong rhizospheric isolate in decreasing the radial development of *Rhizoctoniasolani*, which causes root rot disease in chillies, with a percentage of 71.98%. In contrast, RB1 (69.38%), an isolate of bacteria, was identified as the greatest inhibition of the test pathogen's mycelial growth. Howeverthis needs to be assessed further by contrasting the current isolates with different bioagents and fungicides.

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