Original Research Article

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

ABSTRACT

In the course of the study, native bioagents isolated from the rhizosphere of chilli crop was used to manage the soil-borne pathogens *Rhizoctonia solani*.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 rhizosphere of Chilli and screened *in vitro* by evaluating their antagonistic potential against *Rhizoctonia solani*, 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 case of *Trichod*erma harzianum (71.98%) followed by*Trichod*erma *viride* (62.54%) and among the bacterial isolates maximum inhibition was recorded in 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 the radial growth of *Rhizoctonia solani* causing Root rot disease in chilli.*Rhizoctonia solani* was established as a causal organism of chilli. Use of Bio-control agents is 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 *Rhizoctonia solani* showed that significantly reduced the mycelial growth of *Rhizoctonia solani in vitro.*

Key Words: *Bio-control, Chilli, Rhizoctonia solani, 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 *Rhizoctonia solani* which causes root rot disease leading to significant crop losses and reduction of yield. It was found to record 33.2 percent disease incidence of the seedling in greenhouse condition and 40.20 percent in 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 biocontrol agents such as, *Trichoderma*, *Bacillus, Psedomonas* isolates have gained popularity in recent years. In the pursuit of environmentally friendly and locally adapted solutions, this study focuses on the in vitro screening of native microflora residing in the rhizosphere, with the aim of identifying potential biocontrol agents against *Rhizoctonia solani*. Indigenous microflora have demonstrated the capacity to act as antagonists against soilborne pathogens, thereby offering a promising avenue for sustainable disease management [5,6].).The rhizosphere region influenced by root secretions is known to harbor a diverse community of microorganisms, including fungi, that play crucial roles in plant health. Guide the evaluation process, ensuring the reliable identification of biocontrol candidates [7,8]. The importance of genetic characterization in understanding the diversity and taxonomy of fungal isolates, a critical step in elucidating the potential biocontrol 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 *Rhizoctonia solani*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, 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, conducted during the experiment [12].

2.2 Isolation of rhizospheric microflora from chilli rhizosphere

Soil samples were collected from different ecosystems of the chilli 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 different colonies appearing on the plates were purified in the Potato Dextrose Agar (PDA).

2.3 Dual culture Technique

The antagonistic activity of fungal and bacterial microflora against *Rhizoctonia solani* was assessed by dual culture technique [14] on potato dextrose agar (PDA) medium. Seven days old test pathogen and fungal biocontrol agent were used in this experiment. Five mm size mycelial disc was cut from test 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 plate. Individual growth of the pathogen on 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 7th day of inoculation and mycelia inhibition calculated according to given 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*Rhizoctonia solani* [16]. A 5mmsampleofthetestpathogenfroma seven-dayoldculturewasplaced at thecentreofa90mmPetriplatecontaining PDA medium. Four different bacterial isolates from respective 24-hour old cultures werestreaked onfoursides ofthe Petriplateoppositetoeachotherand at1cmfromtheperiphery.Theseplateswereinoculated at26±1 ^{o}C.Aplate inoculated withonly*Rhizoctonia solani*wasmaintainedascontrol.Theinhibitionof mycelialgrowthofthetestpathogenwasobserved.Radial growth of the pathogen was recorded and percent inhibition was calculated by using 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 rhizospheric microflora from different chilli rhizosphere

The antagonistic ability of each microflora isolate against *Rhizoctonia solani* 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 made 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].

3.2Antagonism of *Trichoderma* isolates against Rhizoctonia solani

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 isolate 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 *Trichoderma harzianum* (71.98%), followed by *Trichoderma viride* (62.54%). However, *Trichoderma* spp. totally reduced *Rhizoctonia solani* after 7 days of inoculation. This finding aligns with numerous reports that have asserted that *T. harzianum, T. virens* and *T. hamatum* exhibits high efficacy in suppressing the mycelial growth of soil-borne, seed borne, phyllosphere and storage plant pathogens on PDA [18,19].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-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]. *Trichoderma harzianum* was shown to have the greatest mycelial growth inhibition of *Rhizoctonia solani* (64.81%) [21].Numerous workers have reported similar findings about the inhibition of *R. solani's* mycelial growth, which is harmful to chillies and certain other host plants, by distinct microbial antagonists such as *Trichoderma* spp. [22, 23].

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

Sl No.	Name of fungal isolates	Radial growth of *Rhizoctonia solani* (mm)			Inhibition over control on 168hrs(%)
		72hrs	120hrs	168 hrs	
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	*Fusarium solani*	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 Rhizoctonia solani

The assessment of antagonistic activity between *Rhizoctonia sol*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 recorded the growth reduction of 66.42%. The assay showed marked retardation of pathogen growth. This result correlates with 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 *Rhizoctonia solani*[24]. Maximum inhibition showed by *Pseudomonas fluorescens* (41.48 %), this was statistically at par with *Bacillus subtilis* (34.81%) against the *Rhizoctonia solani* causing root rot disease in chilli [25].

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

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

4. CONCLUSION

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

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