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Evaluation of bio-agents and botanical *in-vitro* against root rot of chilli (*Capsicum annuum* L.) caused by *Rhizoctonia solani*

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Abstract
Botanicals and bio-agents have antagonistic activity against fungal pathogens. In present study Botanicals and bio-agents were evaluated by poison food technique and dual culture technique against *Rhizoctonia bataticola* to check their efficacy. Among the botanicals and bio-agents, maximum mycelium inhibition was recorded in *T. viride* (82.82%) followed by *T. harzianum* (77.98%), *P. fluorescens* (79.26%), neem oil (82.69%) and Neem leaf Extract (73.79%). Among all treatments *T. viride* showed highest inhibition percentage of mycelia growth of *Rhizoctonia bataticola*.

Keywords: Bio-agents, botanical, root rot, *Rhizoctonia solani*

Introduction
Chilli (*Capsicum annuum* L.) a member of family solanaceae is mainly cultivated for its green fruits as vegetable and for the dry chilli as the spice of commerce. It has also acquired a great importance because of the presence of 'oleoresin', which permits better distribution of color and flavor in foods (Chattopadhyay *et al*., 2011) [3].

Chilli is known to suffer from as many as 83 different diseases, of which more than 40 are caused by fungi. Among the fungal diseases, damping off caused by *Pythium aphanidermatum*, *Rhizoctonia solani* etc. are important (Rangaswami, 1958) [10]. Among the fungal diseases, root rot of chilli caused by *R. solani* has attained the economic importance. *R. solani* is one of the most destructive plant pathogen, causes various maladies starting from seed decay, damping-off, root and stem rot, canker, sheath blight and rot in monocots and dicots. It has remarkable capacity to remain in soil saprophytically in diversity with wide host range and lack of sharp differentiation among its specialized strains, *R solani* possess great difficultly to develop resistance in crop varieties. It can cause up to 33.2 percent disease incidence of the seedling in greenhouse condition and main field 40.2 percent (Rini and Sulochana, 2006) [11].

Though chemical measures are recommended based on the previous studies on *R. solani* the new fungicides along with earlier proven fungicides need to be evaluated to find out the superior fungi toxicants with which the disease can be managed. Presently, greater emphasis should be placed on biological control of soil borne pathogens, in order to reduce the environmental hazards, to avoid the development of resistant strains and to reduce the cost of cultivation. In view of the adverse effect of fungicides to the environment and increasing interest in sustainable agriculture, biological control has been reported as an attractive possibility for management of soil-borne plant pathogens. Hence, an integrated approach plays an important role in the effective management of root rot of chilli caused by *R. solani.*

Materials and Methods
Isolation and identification of pathogen
Root was collected from infected chilli plants bearing characteristic symptoms. Seedlings usually showed a soft, yellow patch which becomes black at the ground level. On pulling out the infected plant, the entire root system was found to be rotten. These root symptoms after mounting on slide was examined under microscope to confirm the presence of *Rhizoctonia* sp.

Pathogenicity of *Rhizoctonia solani*

The root rot infected plants of chilli showed yellowing and withering of upright foliage. The whole plant wilted and dried up later on. When pulled up, the root system was found to be partially or fully decayed. The infection was extended up to lower portion of the stem at ground level and a dark brown discoloration of the affected portion of the stem was observed. Closer view revealed the presence microscopic examination revealed the presence of fungus which was identified as *Rhizoctonia solani* Kuhn. (Agrios, 1936 and Singh, 2011) [2, 13]. Pathogenecity of the fungus was tested using Koch's Postulates.

Maintenance of the culture

The fungus was sub cultured on PDA slants and allowed to grow at 27 ± 1 °C for 15 days. Such slants were preserved in refrigerator at 5 °C and sub cultured once in two months. This pure culture was used for further study.

Mass multiplication of pathogen

The pathogen *Rhizoctonia solani* was mass-multiplied on the sorghum grains. Grains of sorghum were soaked in the boiling water for 30 minutes and autoclaved at 15 lbs/ inch^2 pressure for half an hour for two consecutive days. On cooling, each flask was inoculated with mycelial bit (3 mm) of *R. solani* under aseptic conditions and culture was incubated at 25 ± 2 °C until the whole medium was covered with mycelium.

Inoculation of pathogen

The experimental pot was put on appropriate place and soil was brought to a fine tilth on completion of field preparation and levelling, plots were divided in to three replication plot size (4 m^2). Inoculums prepared by mass culturing was incorporated 5g/kg in field and mixed well, 10 days before transplanting.

***In-vitro* evaluation of bio-agents and botanical**

Preparation of plant extracts

The fresh leaves were grounded in a pestle and mortar by using sterile distilled water. The extract was filtered through double layered muslin cloth and made to the required concentration by adding distilled water.

Poisoned food technique

Nine millimetre diameter disc of *Rhizoctonia solani* was kept at the centre of each Petri plate containing the extracts and fungicide of required concentration dissolved in PDA. Three replications were maintained. The plates were incubated at 27±1 °C for seven days and colony diameter was recorded. Percent inhibition of mycelial growth was calculated by using the formula given by Vincent (1947) [16].

***In-vitro* evaluation of bio-agents**

Antagonistic microorganisms like, *Trichoderma harzianum*, *Trichoderma viride* and *Pseudomonas fluorescens* were evaluated for their antagonistic properties against *Rhizoctonia solani* by dual culture technique. Twenty millilitre of PDA was poured into sterile Petri plates. Fungal antagonists were evaluated by inoculating the pathogen at one side of the Petri plate and the antagonist was inoculated at exactly opposite side of the same plate by leaving 3-4 cm gap. For this actively growing cultures were used. In case of bacterial antagonist's evaluation, two mycelial discs of pathogen were inoculated and bacterial antagonist was streaked in the centre of the plate. One control was maintained where in only test fungus was grown. The treatments were replicated three times. The plates were incubated for seven days at 27±1 °C. after incubation, the colony diameter of *R. solani* was recorded. Percent inhibition was calculated by using the formula given by Vincent (1947) [16].

$$\text{Percent inhibition of colony} = \frac{C - T}{C} \times 100$$

Where:
C = Colony diameter in control
T = Colony diameter in treatment

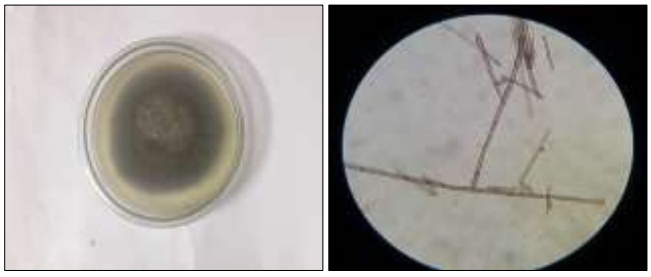

Plate 1: Pure culture microscopic view of *Rhizoctonia solani*

Results and Discussion

In vitro analysis of Mycelial growth (cm) of *R. solani* as affected by different treatments at different hours interval.

	Treatments	24 hrs.	48 hrs.	72 hrs.	96 hrs.	120 hrs.	144 hrs.	168 hrs.
T_0	Untreated check	2.82	2.86	4.42	5.42	6.12	7.12	7.86
T_1	Neem leaf extract	1.35	1.41	1.53	1.65	1.81	1.95	2.06
T_2	Neem oil	0.30	0.46	0.76	0.82	1.0	1.10	1.36
T_3	*Trichoderma harzianum*	1.18	1.23	1.28	1.46	1.53	1.66	1.73
T_4	*Trichoderma viride*	0.73	0.85	0.88	1.06	1.15	1.21	1.35
T_5	*Pseudomonas fluorescens*	1.15	1.21	1.28	1.36	1.45	1.51	1.63
T_6	Treated check (Carbendazim)	0	0.20	0.20	0.30	0.60	0.90	0.96
	F-test	S	S	S	S	S	S	S
	S. Ed. (±)	0.696	0.857	0.829	0.745	0.617	0.819	0.450
	C. D. (5%)	2.110	2.599	2.513	2.261	1.872	2.484	1.365

Mycelial growth (cm) at 168 hours after inoculation

At 168 hours after inoculation Minimum mycelial growth (cm) was recorded in T_4- *Trichoderma viride* (1.35 cm) followed by T_2- Neem oil (1.36 cm), T_5- *Pseudomonas fluorescens* (1.63 cm), T_3- *T. harzianum* (1.73 cm), T_1- Neem leaf extract (2.06 cm) as compared to treated check T_6- Carbendazim (0.96 cm) and untreated check T_0- (7.86 cm).

Table 1: Percent inhibition of *Rhizoctonia solani* as affected by different treatments

Treatments		Percent inhibition
T_0	Untreated check	0
T_1	Neem leaf extract	73.79
T_2	Neem oil	82.69
T_3	*Trichoderma harzianum*	77.98
T_4	*Trichoderma viride*	82.82
T_5	*Pseudomonas fluorescens*	79.26
T_6	Control (carbendazim)	87.78

Percent inhibition of *Rhizoctonia solani* as affected by different treatments

At maximum Percent inhibition was recorded in T_4- *Trichoderma viride* (82.82%) followed by T_2- Neem oil (82.69), T_5- *Pseudomonas fluorescens* (79.26%), T_3- *T. harzianum* (77.98%), T_1- Neem leaf extract (73.79%) as compared to treated check T_6- Carbendazim (87.78%) and untreated check T_0- (0).

Antagonistic activity of *Trichoderma viride, Pseudomonas fluorescens* and *T. harzianum* were investigated by dual culture method on PDA. Data reveals that, *T. viride, P. fluorescens* were potential antagonists of *Rhizoctonia solani* forming a clear zone of inhibition. On microscopic examination hyphae of antagonists were observed coiling and oppressed around hyphae of *R. solani*.

All the treatments were found statistically significant over control. The results of the present study are in accordance to the findings of the Madhavi and Bhattiprolu (2011) [7], Malhotra *et al.* (2011) [9], Tariq *et al.* (2009) [15], Abdel-Monaim *et al.* (2012) [1] and Subash *et al.* (2013) [14]. They reported that the inhibition of *R. solani* due to *Trichoderma* spp. may have been due to secretion of extracellular cell degrading enzymes such as chitinase B-1, 3-glucanase, cellulose and lectin, which may have helped mycoparasites in the colonization of their host. The inhibition of pathogen may also be attributed to the production of secondary metabolites by antagonists such as glioviridin, viridian and gliotoxin (Shabir and Rubina, 2010; Kalmesh and Gurjar, 2002) [12, 5]

Conclusion

From the findings it is concluded by *in vitro* study application of bio control agents and botanicals will be significantly promising and applicable as an alternative to synthetic chemicals and low efficiency and harmful methods for control of root rot of chilli caused by *Rhizoctonia solani*. Microorganisms that have fast growth in the rhizosphere are best for antagonism of pathogen.

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