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In vitro Effect of Bio- Control Agents and Selected Botanical against Root Rot (*Rhizoctonia solani* Kuhn) of Chilli (*Capsicum annuum* L.)

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A B S T R A C T

To manage the disease an investigation at the Department of Plant Pathology, SHIATS-DU, Allahabad was carried out to evaluate the effect of bio-agents *viz. Trichoderma viride* @ 2%, *Pseudomonas fluorescens* @ 2%, *T. harzianum* @ 2%, botanical *viz.* neem leaf extract @ 5%, neem oil @ 5% and fungicide *viz.* carbendazim @ 0.2% against *R. solani* under *in-vitro* condition by dual culture and poisoned food technique. All the bio-agents and botanical were evaluated singly. All the treatments significantly inhibited the mycelial growth of *R. solani* as compared to untreated check. Maximum inhibition per cent of mycelia growth of *Rhizoctonia solani* was recorded in *Trichoderma viride* (82.82%) followed by neem oil (82.69%), *P. fluorescens* (79.26%), *T. harzianum* (77.98%) and neem leaf extract (73.79%) as compared to treated check (Carbendazim) (87.78%) and untreated check (0%).

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 is believed to be originated from South America. 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). Thus, chilli has diverse uses as spice, condiment, culinary supplement, medicine, vegetable and ornamental plant. The pharmaceutical application of capsaicinoid is attributed to its antioxidant, anticancer, antiarthritic, and analgesic properties (Akbar *et al.*, 2010).

India is largest exporter and major destinations are Malaysia, Sri Lanka, Bangladesh and Indonesia. Exports have touched record high of 2.81 lakh tonnes in 2012-13 and during April-Jun 2013 exports were 65500 tonnes. India earns about 2000 corers every year on export of chilli (Anonymous, 2014).

Chilli is known to suffer from as many as 83 different diseases, of which more than 40 are caused by fungi (Rangaswami, 1958). Among the fungal diseases, root rot of chilli caused by *R. solani* has attained the economic importance. The disease is difficult to manage as the pathogen has long saprophytic survival ability in soil. It can cause up to 33.2 percent disease incidence of the seedling in greenhouse condition and in main field 40.2 percent (Rini and Sulochana, 2006).

Materials and Methods

The in vitro experiment was laid out in completely randomized design (CRD) with six treatments bioagents *viz*. Trichoderma viride @ 2%, T. harzianum @ 2%, Pseudomonas fluorescens @ 2% and botanical *viz*. neem leaf extract, neem oil and fungicide *viz*. carbendazim @ 0.2% and three replications including untreated check.

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. Per cent inhibition of mycelial growth was calculated by using the formula given by Vincent (1947).

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 *Rhizoctonia solani* was recorded. Per cent inhibition was calculated by using the formula given by Vincent (1947).

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

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

Results and Discussion

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). All the treatments were found statistically significant over untreated check.

Table.1 Details of the treatments (*in-vitro*) with name and concentration

S. No.	Treatments	Replications			Conc. (%)	References	Technique used
T_0	Untreated check	R1	R2	R3	----	----	-----
T_1	Neem leaf extract	R1	R2	R3	5%	Rajput *et al.* (2011)	Poisoned food technique
T_2	Neem oil	R1	R2	R3	5%	Rajput *et al.* (2011)	Poisoned food technique
T_3	*Trichoderma harzianum*	R1	R2	R3	2%	Malhotra *et al*. (2011)	Dual culture technique
T_4	*Trichoderma viride*	R1	R2	R3	2%	Malhotra *et al*. (2011)	Dual culture technique
T_5	*Pseudomonas fluorescens*	R1	R2	R3	2%	Malhotra *et al*. (2011)	Dual culture technique
T_7	Treated check (Carbendazim)	R1	R2	R3	0.2%	Rehman *et al*. (2013)	Poisoned food technique

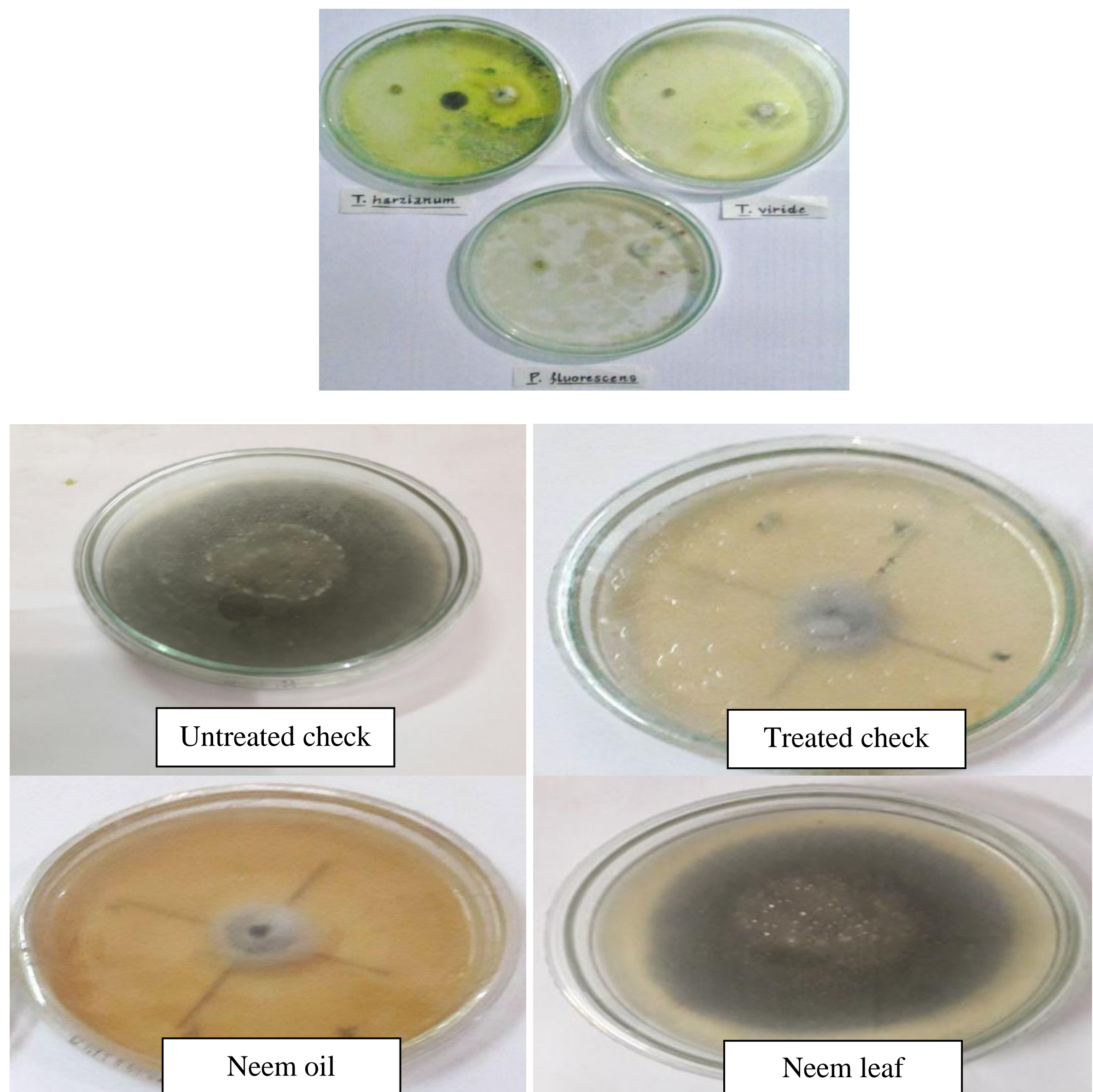
Table.2 Mycelial growth (cm) of *Rhizoctonia solani* as affected by different treatments at different hours interval

	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**

Table.3 Per cent inhibition of *Rhizoctonia solani* as affected by different treatments

Treatments		Per cent 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	Treated check (carbendazim)	87.78

Fig.1 Mycelial growth (cm) of *Rhizoctonia solani* 7 days after incubation as affected by different treatments



Per cent inhibition of *Rhizoctonia solani* as affected by different treatments

At maximum per cent 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 untreated check. The results of the present study are in accordance to the findings of the Madhavi and Bhattiprolu (2011), Malhotra *et al.,* (2011), Tariq *et al.,*

(2009), Abdel-Monaim *et al.,* (2012) and Subash *et al.,* (2013). 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; Patel *et al.,* 2014; Sab *et al.*, 2014).

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