

Original Research Article

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Studies on *Trichoderma viride* Formulations against Pod Blight and Leaf Spot Disease of Soybean

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ABSTRACT

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In the present study the effect of *Trichoderma viride* Pers. formulations against *Myrothecium* leaf spot of soybean caused by *Myrothecium roridum* and Pod blight of soybean caused by *Colletotrichum truncatum* in the field of soybean are observed. *Trichoderma viride* was effective in reducing the radial growth of *M. roridum* and *C. truncatum* under *in-vitro* condition with dual culture technique. The lowest disease severity of pod blight (7.43% and 16.80%) and *Myrothecium* leaf spot (2.57% and 8.46%) of soybean was observed in Bavistin seed treatment at 37 days after sowing and 52 days after sowing but the seed treatment with leachates + 3 spray of leachates (15%) shown lowest disease severity at 67 days after sowing.

Introduction

Soybean [*Glycine max* (L.) Merrill] is one of the most important oil seed crops of India. Soybean crop is known to suffer from many diseases. Two of such diseases are *Myrothecium* leaf spot and pod blight of soybean. *Myrothecium* leaf spot is one of the important diseases which is caused by *Myrothecium roridum* Tode ex. Fries. *M. roridum* is a facultative parasite with a large number of plant hosts, including vegetables, fruits and ornamental plants. Initial symptoms of *M. roridum* appear as small round or oval, brown spots developed with dark brown

margin on leaves in the infected plant. It causes yield loss in soybean crops to a considerable amount.

Pod blight of soybean incited by *Colletotrichum truncatum* (Schw.) Andrus and Moore, is a serious disease in almost all soybean growing areas of the world including India and thereby causing qualitative and quantitative losses to the tune of 30 to 70 % (Lenne, 1992; Backman *et al.*, 1982; Chandrasekaran and Rajappan, 2002). The fungus *C. truncatum* is recognized as one of the most destructive and widespread seed-borne pathogen of soybean, especially in

warm and humid areas (Ploper and Backman, 1992). The soybean crop is susceptible to *C. truncatum* at all stages of development particularly from bloom to pod fill.

Trichoderma spp. employs various mechanisms to affect disease control and improve all plant health (Singh *et al.*, 2004). *Trichoderma* spp. proved to be effective in reducing the mycelial growth of *M. roridum* causing disease in teak saplings (Murthy *et al.*, 2004).

Begum *et al.*, (2008) evaluated the fungal biocontrol agents *in-vitro* for their antagonistic potential against *C. truncatum* isolated from soybean seeds, and found that the *Trichoderma* spp. strongly inhibited the growth of *C. truncatum* in dual culture tests.

Keeping in view the importance of these two diseases and loss to the crop, the present experiment was conducted in the Department of Plant Pathology, IGKV, Raipur. *T. viride* were also tested for their antagonistic performance against *M. roridum* and *C. truncatum*.

Biological control is an alternative approach to the chemical fungicides and it may be a safe, effective and ecofriendly method for plant disease management. Several species of *Trichoderma* have been reported as biocontrol agent for management of fungal diseases of plants. *Trichoderma* species restrict the growth of pathogens with the production of antifungal compounds.

Formation of specialized structures, and degradation of the fungal cell wall, followed by the assimilation of its cellular content. process known as mycoparasitism. *Trichoderma* species was the most common fungal biological control agents that have been commonly used in agriculture for fungal pest control.

Materials and Methods

Treatment details

Three formulations of *T. viride* i.e. powder base (CG Trichom), capsule (CG Tricap) and one liquid formulation leachates were used as seed treatment against soybean diseases. Leachate of *T. viride* was also evaluated as foliar spray under natural field condition during *kharif* season.

Sample collection and isolation of fungal pathogens

The infected soybean leaf or pod sample showing typical symptoms were collected for the research work from the experimental field of Department of Plant Pathology situated in the Research Farm, campus of Indira Gandhi Krishi Vishwavidyalaya, Raipur (Chhattisgarh). Tissue segment method was used for isolation of pathogenic fungus describe by Rangaswami and Mhadevan, 2011.

Evaluation of antagonists against pathogens

The antagonistic effect of *T. viride* against *Colletotrichum truncatum* and *Myrothecium roridum* pathogens *in vitro* was evaluated using the dual culture technique. The procedure were performed as per suggested by Mishra *et al.*, 2011. Seven days old culture of 5 mm diameter mycelium disk of *Trichoderma viride* and test pathogens were placed on the potato dextrose agar (PDA) plate opposite to each other equal distance from periphery. The potato dextrose agar plate inoculated with 5 mm disk of fungal test pathogen served as control. The plates were incubated at $28 \pm 2^{\circ}\text{C}$.

The colony diameter of *Trichoderma viride* and targeted fungal pathogens were measured at two locations, right angle to each other and the average diameter was calculated.

Percent inhibition of mycelial growth of targeted fungal pathogens over control was calculated by following formula.

$$\text{Percent growth inhibition} = \frac{R1 - R2}{R2} \times 100$$

Where,

R1 = Radial growth of pathogen in control (mm).

R2 = Radial growth of pathogen in dual culture experiment (mm).

Evaluation of different formulations of *T. viride* on disease severity of soybean diseases

An experiment conducted to find out the effect of different formulations of *Trichoderma viride* on disease severity of soybean diseases during *kharif* season at research farm I.G.K.V., Raipur with 8 treatments and 3 replications.

The observations were recorded for disease severity of pod blight of soybean caused by *C.truncatum* and Myrothecium leaf spot disease caused by *M. roridum*.

Percent disease Index was recorded by percent infected plant and calculated by the formula as given below

PDI was calculated using the formula of Wheeler (1969) as given here

$$\text{Percent Disease Index (PDI)} = \frac{\text{Sum of individual rating}}{\text{Number of leaves examined}} \times \frac{100}{\text{maximum disease rating}}$$

Results and Discussion

During study, the pod blight and leaf spot infected soybean plant samples were collected during the *kharif* season from soybean experimental field of Department of Plant Pathology situated in the research farm, Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.). These infected samples were used for isolation of the causal pathogens. Isolation of pathogens was carried out aseptically from the infected tissue by employing tissue isolation technique. The antagonistic activity of *T. viride* against *Colletotrichum truncatum* under *in-vitro* condition by dual culture technique and data presented in Table 2 and Plate 1. It is evident from Table 1 that *T. viride* inhibited the mycelial growth of *C. truncatum*. The maximum growth inhibition percent was 75.17% recorded after 4 days (96 hrs) of inoculum. Thus the result showed that *T.viride* can efficiently inhibit the mycelium growth of the fungal pathogen.

The antagonistic activity of *Trichoderma viride* against *Myrothecium roridum* under *in-vitro* condition by dual culture technique and data presented in Table 3 and Plate 2. It is evident from Table 2 that *T. viride* inhibited the mycelial growth of *M. roridum*. The maximum growth inhibition of *Myrothecium roridum* by *T. viride* recorded by 81.11% after 6 days (144 hrs). Thus the result showed that *T. viride* can efficiently inhibited the mycelium growth of fungal pathogen.

Under field condition by using different *T. viride* formulations observed that the severity of pod blight and myrothecium leaf spot diseases of soybean were reduced. The disease severity of myrothecium leaf spot disease of soybean was recorded after 37, 52 and 67 days after sowing and results are given in Table 4. At 37 DAS, the lowest disease severity was found in case with T4 (2.57%). The lower disease severity was also found in T1 (5.28%),

T3 (5.97%), T5 (6.01%), T2 (6.37%), T6 (6.40%) and T7 (6.49%) which are statistically at par with each other. And the highest disease severity was recorded in control (8.52%). At 52 DAS, significantly lowest disease severity was noticed in T4 (8.46%). And the treatments T7 (10.65%) and T6 (10.98%) were significantly at par with each other (Fig. 1 and 2).

Table.1 Treatments details

S. NO.	Treatments	Doses / kg seed
1.	CG Trichom seed treatment	10 g
2.	CG Tricap (Capsule) seed treatment	2 Capsule
3.	Seed treatment with leachates (20 min before sowing)	15%
4.	Bavistin seed treatment	2 g
5	Seed treatment with leachates + 1 spray of leachates (30 DAS)	15%
6.	Seed treatment with leachates + 2 spray of leachates (30 and 45 DAS)	15%
7.	Seed treatment with leachates + 3 spray of leachates (30, 45 and 60 DAS)	15%
8.	Control	Untreated seed

DAS- days after sowing

Table.2 Antagonist effect of *Trichoderma viride* on *Colletotrichum truncatum* in dual culture technique

Sr. No.	Incubation Period (hrs)	Radial growth of pathogen		Percent growth inhibition
		control (mm)	dual culture (mm)	
1	24	13.33	13.00	2.48
2	48	48.00	20.67	56.94
3	72	79.33	21.00	65.53
4	96	90.00	22.35	75.17

*Average of three replication

Table.3 Antagonist effect of *Trichoderma viride* on *Myrothecium roridum* in dual culture technique

Sr. No.	Incubation Period (hrs)	Radial growth of pathogen		Percent growth inhibition
		control (mm)	dual culture (mm)	
1	24	7.33	7.00	4.50
2	48	19.33	10.33	46.56
3	72	38.67	13.33	65.53
4	96	56.33	15.67	72.18
5	120	74.00	16.67	77.50
6	144	90.00	17.00	81.11

*Average of three replication

Table.4 Effect of *T. viride* formulations on disease severity of myrothecium leaf spot of soybean

Treatments	Treatments details	Disease severity (%)		
		37 DAS*	52 DAS	67 DAS
T1	CG Trichom seed treatment	5.28 (13.24)**	12.88 (21.01)	20.89 (27.18)
T2	CG Tricap (Capsule) seed treatment	6.37 (14.59)	13.40 (21.45)	22.68 (28.34)
T3	Seed treatment with leachates 15%	5.97 (13.99)	12.78 (20.89)	22.26 (28.12)
T4	Bavistin seed treatment	2.57 (9.19)	8.46 (16.89)	17.30 (24.54)
T5	Seed treatment with leachates + 1 spray of leachates (15%)	6.01 (14.18)	13.25 (21.33)	25.43 (30.26)
T6	Seed treatment with leachates + 2 spray of leachates (15%)	6.40 (14.64)	10.98 (19.32)	19.33 (26.07)
T7	Seed treatment with leachates + 3 spray of leachates (15%)	6.49 (14.74)	10.65 (19.01)	15.57 (23.18)
T8	Control	8.52 (16.96)	19.92 (26.50)	39.69 (39.03)
	SE(m)±	0.61	0.53	1.50
	CD at (5%)	1.86	1.62	4.59

DAS – days after sowing

* Means of three replications

** Arc sine transformation in parenthesis

Table.5 Effect of *T. viride* formulations on disease severity of pod blight of soybean

Treatments	Treatments details	Disease severity (%)		
		37 DAS*	52 DAS	67 DAS
T1	CG Trichom seed treatment	10.45 (18.84)**	17.84 (24.97)	30.21 (33.32)
T2	CG Tricap (Capsule) seed treatment	11.24 (19.57)	19.40 (26.11)	31.64 (34.21)
T3	Seed treatment with leachates 15%	11.85 (20.12)	21.73 (27.77)	32.68 (34.83)
T4	Bavistin seed treatment	7.43 (15.80)	16.80 (24.18)	29.04 (32.58)
T5	Seed treatment with leachates + 1 spray of leachates (15%)	12.13 (20.35)	22.46 (28.28)	33.44 (35.31)
T6	Seed treatment with leachates + 2 spray of leachates (15%)	11.65 (19.94)	20.35 (26.80)	30.31 (33.39)
T7	Seed treatment with leachates + 3 spray of leachates (15%)	11.92 (20.18)	20.81 (27.12)	27.45 (31.57)
T8	Control	15.72 (23.33)	30.65 (33.60)	41.65 (40.16)
SE(m) ±		0.53	0.72	1.53
CD at (5%)		1.61	2.22	4.68

DAS – days after sowing

* Means of three replications

** Arc sine transformation in parenthesis

Plate.1 Bioefficacy of *Trichoderma viride* against *Colletotrichum truncatum* under *in vitro* condition (Left: *T. viride* against *C. truncatum* , Right: *C.truncatum*)

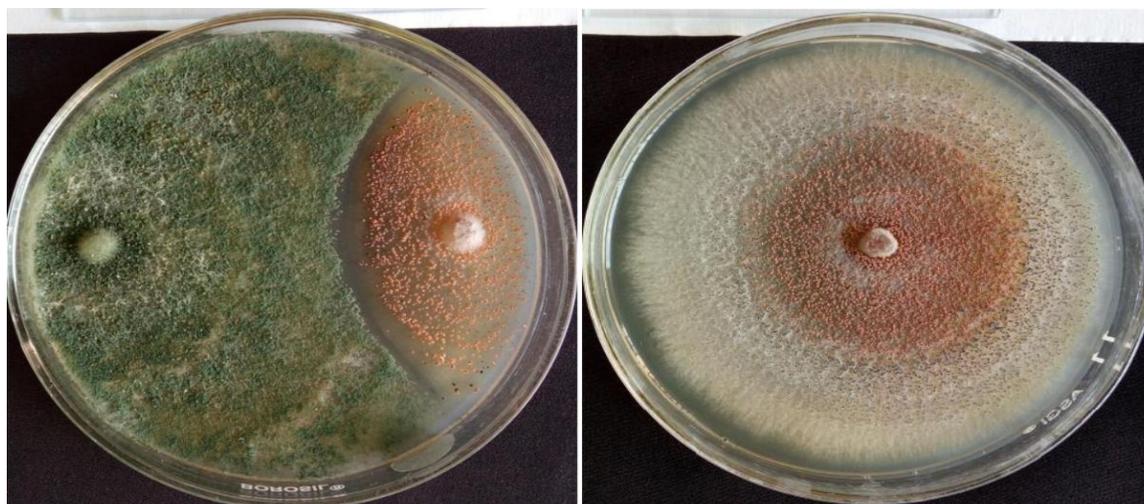


Plate.2 Bioefficacy of *Trichoderma viride* against *Myrothecium roridum* under *in vitro* condition (Left: *T.viride* against *M.roridum*, Right: *M.roridum*)



Fig.1 Effect of *T. viride* formulations on disease severity of pod blight of soybean

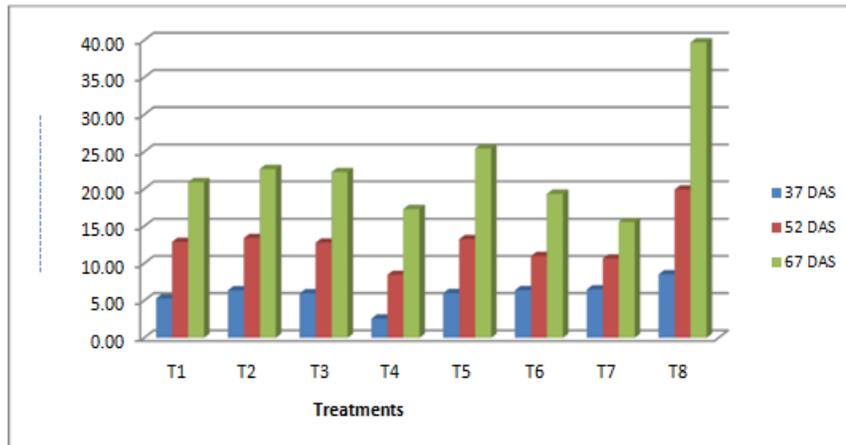
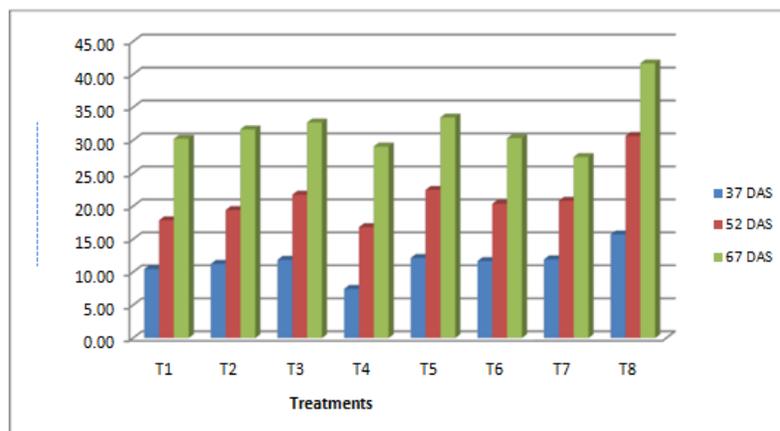


Fig.2 Effect of *T. viride* formulations on disease severity of myrothecium leaf spot of soybean



The highest disease severity was recorded in control (19.92%). At 67 DAS, significantly less severity of myrothecium leaf spot diseases was noticed in case of T7 (15.57%), followed by T4 (17.30%), T6 (19.33%) and T1 (20.89%) in all treatments as compared to control (39.69%). It was also revealed from the result that almost all the treatments inhibited disease severity after each treatment whenever *Trichoderma* formulations were used. The result are in agreement with the finding of Murthy *et al.*, (2004) observed that *Trichoderma* sp. gave the lowest mean disease severity percentage of *Myrothecium roridum* on teak.

Disease severity of anthracnose was reduced after 37, 52 and 67 days after sowing and results are given in Table 5. At 37 DAS, the lowest disease severity was found in T4 (7.43%). The treatments T1 (10.45%), T2 (11.24%), T6 (11.65%), T3 (11.85%) and T7 (11.92%) significantly at par with each other. And the highest disease severity was recorded in control (30.65%).

At 52 DAS, the lowest disease severity was noticed in T4 (16.80%) followed by T1 (17.84%). Significantly lower disease severity was also reported in T2 (19.40%), T6 (20.35%) and T7 (20.81%) and at par with each other. The highest disease severity was recorded in control (30.65). At 67 DAS, significantly less severity of pod blight disease was noticed in case of T7 (27.45%) followed by T4 (29.04%), T1 (30.21%), T6 (30.31%) and T2 (31.64%) in all treatments as compared to control (41.65%). It was also revealed from the result that almost all the treatments inhibited disease severity after each treatment whenever *Trichoderma* formulations were used. The result is in agreement with the finding of Bankole and Adebajo (1996) observed that *T. viride* as a spore suspension foliar spray significantly reduced *C. truncatum* incidence in the field. Similar finding was also

recorded by Amin *et al.*, 2014, that disease incidence of anthracnose of bean on plots treated seeds with *T. viride* was 66.7% over untreated control (93.3%); Severity of same pathogen on plots treated seeds with same bio-agent was 57.9% over untreated control (86.3%).

T. viride was evaluated under *in-vitro* condition to test antagonistic performance in dual culture with *Colletotrichum truncatum* (75.17%) and *Myrothecium roridum* (81.11%) radial growth inhibition at 4th and 6th DAS respectively and the lowest disease severity of Myrothecium leaf spot and pod blight of soybean was observed in Bavistin seed treatment at 37 DAS and 52 DAS but the seed treatment with leachates + 3 spray of leachates (15%) showed lowest disease severity at 67 DAS.

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