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## Management of *Rhizoctonia solani* Kühn causal agent of web blight of soybean under *in vitro* condition

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**Abstract**

In the present study, the management of *Rhizoctonia solani* Kühn causal agent of web blight of soybean under *in vitro* condition is discussed. One of the techniques employed for the *in vitro* management of the pathogen were by using eight plant leaf extracts i.e. Ashoka (*Saraca asoca*), Neem (*Azadirachta indica*), *Aloe vera* (*Aloe barbadensis*), Onion (*Allium cepa*), Tulsi (*Ocimum tenuiflorum*), China rose (*Hibiscus rosa-sinensis*), Parthenium (*Parthenium hysterophorus*), Calotropis (*Calotropis procera*). The highest percent inhibition of *R. solani* over control was observed in extract of Ashoka. Second method was of using five fungicides viz., Contaf (Hexaconazole 5% SC), Saaf (Carbendazim 12%+ Mancozeb 63% WP), Tilt (Propiconazole 25% EC), Taspas (Propiconazole 13%+Difencconazole 13.9% SC) and Nativo (Trifloxystrobin 50%+Tebuconazole 25% WG) against *R. solani* under *in vitro* condition by poison food technique. Saaf was found to be the most effective as no mycelial growth was observed. Third method was Dual culture technique using *Trichoderma viride* which exhibited significant inhibition of *R. solani* as compared to control. The percent inhibition was converted to 1-5 scale which indicated the reaction type. In the experiment, the reaction type was found to belong to 2 scale i.e. *Trichoderma* overgrew at least two thirds of the medium surface.

**Keywords:** *Rhizoctonia solani*, *in vitro* management, leaf extract, fungicides, *Trichoderma viride*

**Introduction**

Soybean is a world's first rank crop as a source of vegetable oil. It contains about 40 per cent protein, well balanced in essential amino acids such as lysine, leucine, lecithin, 20 per cent oil rich with poly unsaturated fatty acid specially Omega 6 and Omega 3 fatty acids, 6-7 per cent total mineral, 5-6 per cent crude fiber and 17-19 per cent carbohydrates (Chauhan *et al.*, 1988) [2]. Soybean is mainly grown during Kharif season in sandy loam to clay loam soil in Chhattisgarh. Soybean plant is known to suffer from many diseases such as *Rhizoctonia* web blight, anthracnose, rust, *Cercospora* leaf spot, target spot, bud blight, yellow mosaic and some non-parasitic diseases due to excesses and deficiencies of trace elements (Sinclair, 1982) [10]. Among the fungal diseases *Rhizoctonia* web blight is one of the most important foliar disease caused by *Rhizoctonia solani* Kühn, anastomosis group 1 (AG-1). The disease occurs in most tropical soybean production regions, including portions of Brazil, India, Japan etc. Among the fungal diseases *Rhizoctonia* web blight is one of the most important disease which attack the soybean plant towards maturity and directly affect on yield of soybean particularly in humid and warm part of the country and 35-60 percent yield losses have been estimated by Ray *et al.*; (2007) [8]. In India, it was first reported from Pantnagar in 1967 (Mukhopadhyay and Singh, 1984) [7]. Since then it has spread to other soybean growing states (Anonymous, 2007) [1]. Symptoms of web blight include leaf spot, leaf blight and defoliation. Infected leaves appear as water soaked at initial condition of the disease. In severe condition of the disease leaves become blighted completely and dark brown sclerotia are formed in infected leaves and petioles.

**Materials and Methods**

All *in-vitro* studies on *Rhizoctonia solani* were conducted in the laboratory of Department of Plant Pathology, IGKV, Raipur.

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## Management of *Rhizoctonia solani* under in vitro condition

### 1.) By Leaf extracts

Antifungal activity of eight plant leaf extracts were studied under *in vitro* condition taking plant leaf dextrose agar medium. The following plant viz., Ashoka (*Saraca asoca*), Neem (*Azadirachta indica*), Aloe vera (*Aloe barbadensis*), Onion (*Allium cepa*), Tulsi (*Ocimum tenuiflorum*), China rose (*Hibiscus rosa-sinensis*), Parthenium (*Parthenium hysterophorus*), Calotropis (*Calotropis procera*) were used. PDA without extract was used as control.

Percent inhibition of mycelial growth was calculated by the following formula- Inhibition % =  $C-T/C \times 100$

Where

C = Diameter of fungus colony (mm) in control plate T = Diameter of fungus colony (mm) in treated plate

### 2.) Evaluation of different fungicides against *Rhizoctonia solani* under in-vitro condition

Five fungicides viz., Contaf (Hexaconazole 5%SC), Saaf (Carbendazim 12%+ Mancozeb 63%WP), Tilt (Propiconazole 25%EC), Taspas (Propiconazole 13%+Difencconazole 13.9% SC) and Nativo (Trifloxystrobin 50%+Tebuconazole 25% WG) were tested against *R. solani* *in vitro* condition by poison food technique. 100 ml potato dextrose agar medium was sterilized in conical flask of 150 ml capacity. The fungicides were separately incorporated aseptically in molten PDA to make 100 ppm concentration. The amended medium was then poured in sterilized petriplates. Five mm discs of the test pathogen were cut with the help of sterilized cork borer from the margin of 4 days old culture and than placed centrally in each of the petriplates. The disc was placed in inverted position to allow the contact of the fungus with the medium. The inoculated petri plates without fungicides served as control. The inoculated petriplates were incubated in the BOD incubator at  $27 \pm 2$  °C and the diameter of the pathogen was measured after 5 days of incubation with the help of scale in mm. Per cent growth inhibition under the influence of different fungicides was calculated. Percent inhibition of mycelial growth was calculated by the following formula-

$$\text{Inhibition \%} = C - T / C \times 100$$

Whereas

C = Diameter of fungus colony (mm) in control plate T = Diameter of fungus colony (mm) in treated plate

### 3.) Evaluation of bioagent against *Rhizoctonia solani* under in-vitro condition

The pure culture of *Trichoderma viride* was obtained from Biocontrol lab, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur. It's antagonistic activity against *R. solani* was evaluated by dual culture technique. Six replications were maintained. The inoculated petriplates were incubated at  $27 \pm 2$  °C Observations were recorded on

growth of the interacting fungi from which % inhibition was calculated. Percent inhibition was also then converted to 1-5 scale which indicated the reaction type. 1: *Trichoderma* completely overgrew the pathogen and covered the entire medium surface; 2: *Trichoderma* overgrew at least two thirds of the medium surface; 3: *Trichoderma* and pathogen each colonized approximately one half of the medium surface (more than one third and less than two thirds) and neither organism dominate to each other; 4: The pathogen colonized at least two thirds of the medium surface and appeared to withstand encroachment by *Trichoderma*; 5: The pathogen completely overgrew the *Trichoderma* and occupied the entire medium surface

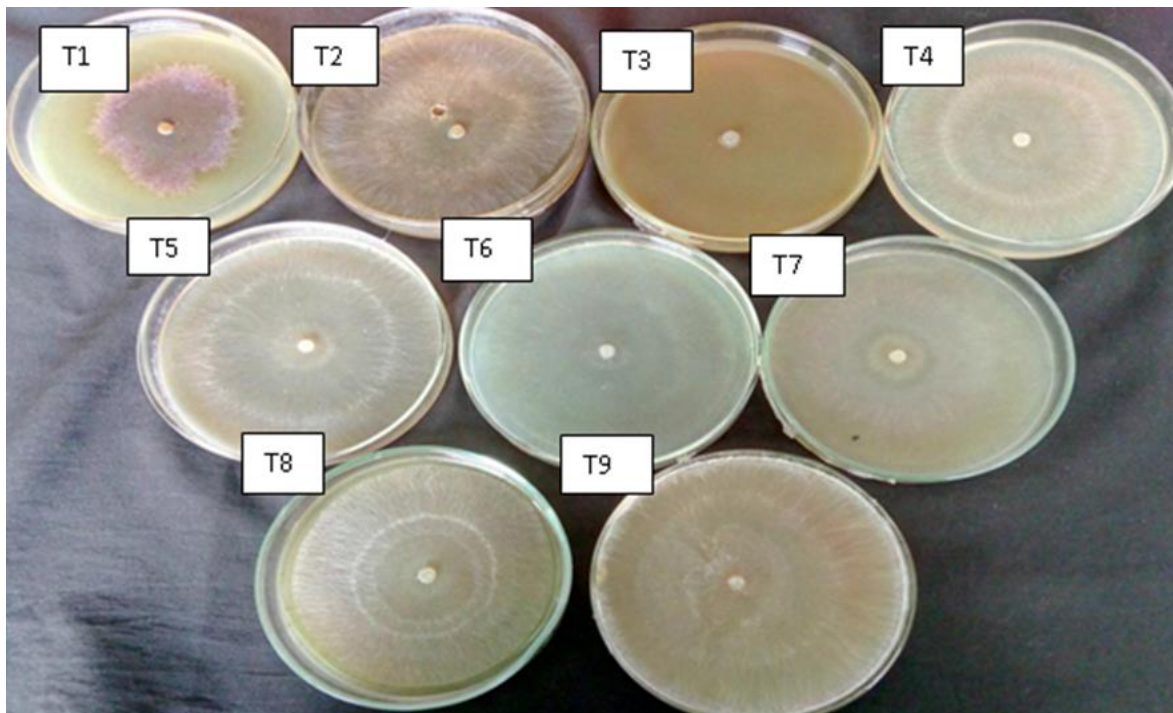
Observation was made on the radial growth of the antagonist and test pathogen when the fungus in control plate reached to rim of the plate. The per cent growth inhibition of the test pathogen in presence of antagonist was calculated over control as below.

$$\text{Per cent growth Inhibition} = \frac{\text{Growth of test pathogen in control plate} - \text{Growth of test pathogen in presence of } Trichoderma \text{ sp.}}{\text{Growth of test pathogen in control plates}} \times 100$$

## Results and Discussion

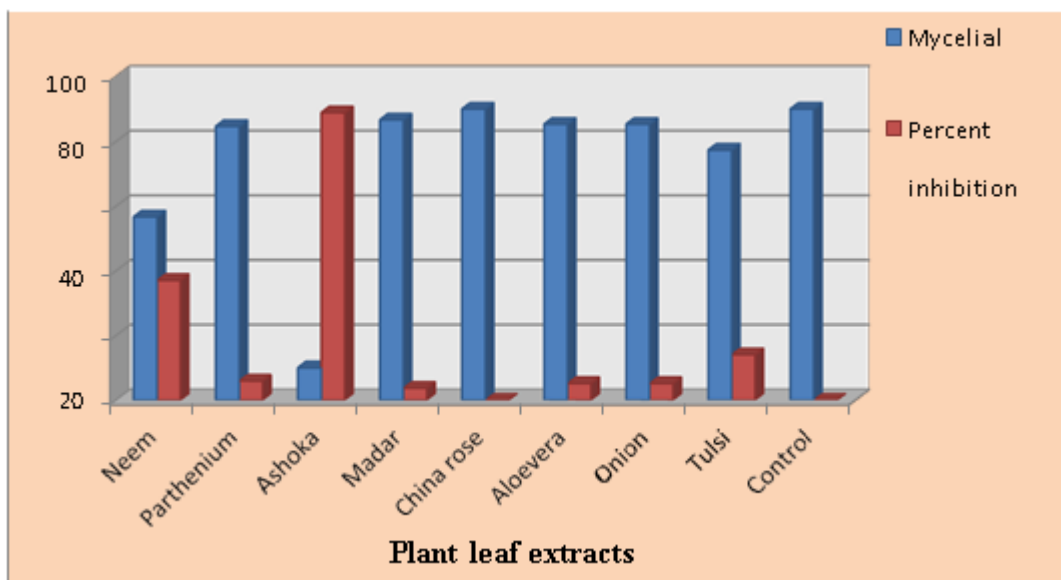
### Inhibitory effect of different plant leaf extract on radial growth of *Rhizoctonia solani* under in vitro condition

Eight plant leaf extract were evaluated to know their antifungal activity on the growth of *Rhizoctonia solani* at 5 days after inoculation. It is observed that the mycelial growth differs significantly with respect to different plant leaf extracts. The percent inhibition of *R. solani* was in accordance with that of mycelial growth. Mycelial growth of *Rhizoctonia solani* was ranged from 10 mm to 90 mm. The least mycelial growth of *Rhizoctonia solani* was recorded in the extract of Ashoka (10 mm) which was followed by neem (56.66 mm) and tulsi (77.33 mm). The mycelial growth obtained in case of extracts of Parthenium (84.66 mm), aloe vera (85.33 mm), onion (85.33 mm), calotropis (86.66 mm) did not show any significant difference and were statistically at par with each other. The growth of *R. solani*, in extracts of Parthenium, aloe vera, calotropis and onion was also significantly more compared to the extracts of Ashoka, neem and tulsi but growth was at par with each other, whereas significantly higher growth was found in case of leaf extract of China rose (90 mm) and control (90 mm). The highest percent inhibition of *R. solani* over control was observed in extract of Ashoka. Similar results have been confirmed by Kaur *et al.* (2009) [4] who evaluated the antifungal effect of 44 plant extracts and 8 plant oils against the pathogen *Rhizoctonia solani* by disc diffusion method. Out of 44 plants tested, 36 plant extracts showed varied degree of antimicrobial effect at different concentrations against the pathogen whereas 8 plant extracts, viz. *Abrus precatorious*, *Acacia auriculiformis*, *Bougainvillea glabra*, *Convolvulus arvensis*, *Hibiscus rosa-sinensis*, *Morus alba*, *Thevatia peruviana*, and *Withania somnifera* did not exert any effect



T1 = Neem, T2= Parthenium, T3= Ashoka, T4= Madar, T5= Chinarose, T6=Aloevera, T7=Onion, T8= Tulsi, T9= Control

**Fig 1:** *In vitro* evaluation of different plant leaf extracts on the radial growth of *R.solani*



**Fig 2:** Effect of different plant leaf extracts on the mycelial growth of *R. solani* and on percent growth inhibition of *R. solani* under *in vitro* condition

**Table 1:** Effects of different plant leaf extracts on radial growth of *Rhizoctonia solani* under *in vitro* condition

Treatment	Plant leaf extract	Mycelial growth (mm)	Percent inhibition over control	No. of sclerotia production per plate
T1	Neem	56.66	37.04	***
T2	Parthenium	84.66	5.93	****
T3	Ashoka	10.00	88.88	-
T4	Madar	86.66	3.71	**
T5	China rose	90.00	-	**
T6	Aloevera	85.33	5.18	**
T7	Onion	85.33	5.18	****
T8	Tulsi	77.33	14.07	****
T9	Control	90.00	-	*****
	SE (m) ±		0.770	
	CD (5%)		2.313	

Average of three replications

\*=20 sclerotia

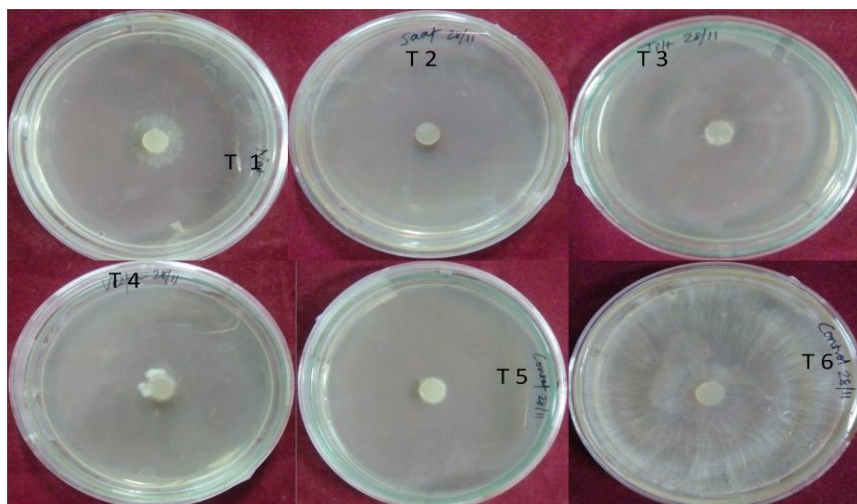
- = no sclerotia

### Evaluation of different fungicides against *Rhizoctonia solani* by poison food technique under *in-vitro* condition.

Five fungicides at 100 ppm concentration were tested under *in-vitro* condition against *Rhizoctonia solani* and observations on inhibition of radial growth and sclerotial production were recorded. The growth of *R. solani* observed ranged from 0 to 90 mm. No mycelial growth was recorded in case of Saaf (0 mm). The mycelial growth was observed to be at par with each other in case of Tilt (6.33 mm) and Contaf (6.33 mm). Significant difference in mycelial growth was seen in case of Taspa (8.33mm) and Nativo (12mm). The highest mycelial growth was recorded in case of control (90 mm). Sclerotia production was not observed in case of Saaf, Tilt and Contaf. Very less sclerotia were observed in case of Nativo and

Taspa. The highest percent inhibition (100%) over control was observed in case of Saaf.

The results are in confirmation with earlier workers, Dutta and Kalha (2011) evaluated five fungicides *viz.*, carbendazim 50 WP, hexaconazole 5 EC, propiconazole 25 EC, Saaf 75 WP and vitavax 75 WP @ 10, 25, 50, 100, 200 and 500 ppm each. Among the fungicides Saaf (carbendazim 12% + mancozeb 63%) was the most effective fungicide which registered cent per cent inhibition of the mycelial growth of *R. solani*. Srinivas *et al.* (2013) did *in-vitro* evaluation of fourteen different groups of fungicides against *Rhizoctonia solani*. Metalaxyl (0.1%), Mancozeb (0.1%), Tricyclazole (0.1%), Thiophenate methyl (0.1%), Carbendazim + Mancozeb (0.1%) were proved to be most effective in inhibiting the growth of the fungus.



T1 = Tebuconazole 50% + Trifloxystrobin 25% WG (Nativo), T2 = Carbendazim 12% + mancozeb 63% WP (Saaf), T3 = Propiconazole 25% EC (Tilt), T4 = Propiconazole 13% + Difenconazole 13.9% SC (Taspa), T5 = Hexaconazole 5% EC (Contaf), T6 = Control

**Fig 3:** *In vitro* evaluation of fungicides (100ppm) against *R. solani*

**Table 2:** Evaluation of fungicides against *Rhizoctonia solani* under *in vitro* condition

Treatment	Fungicide	Mycelial growth (mm) <sup>a</sup>	Percent inhibition over control	No. of sclerotia produced per plate
T1	Tebuconazole 50%+ Trifloxystrobin 25% WG (Nativo)	12.00	86.66	*
T2	Carbendazim 12%+ mancozeb 63% WP (Saaf)	0.00	100	-
T3	Propiconazole 25% EC (Tilt)	6.33	92.96	-
T4	Propiconazole 13%+Difenconazole 13.9% SC (Taspa)	8.33	90.74	*
T5	Hexaconazole 5% EC (Contaf)	6.33	92.96	-
T6	Control	90.00	-	*****
	SE (m) ±	0.471		
	CD (5%)	1.469		

a = Average of three replications

\* = 20 sclerotia

- = no sclerotia

### Antagonistic effect of *Trichoderma sp.* against *Rhizoctonia solani* under *in-vitro* condition.

*In vitro* antagonistic potential of *Trichoderma sp.* (*Trichoderma viride*) was studied against fungal plant pathogen *Rhizoctonia solani* following dual culture method and was assessed after 7 days of growth.

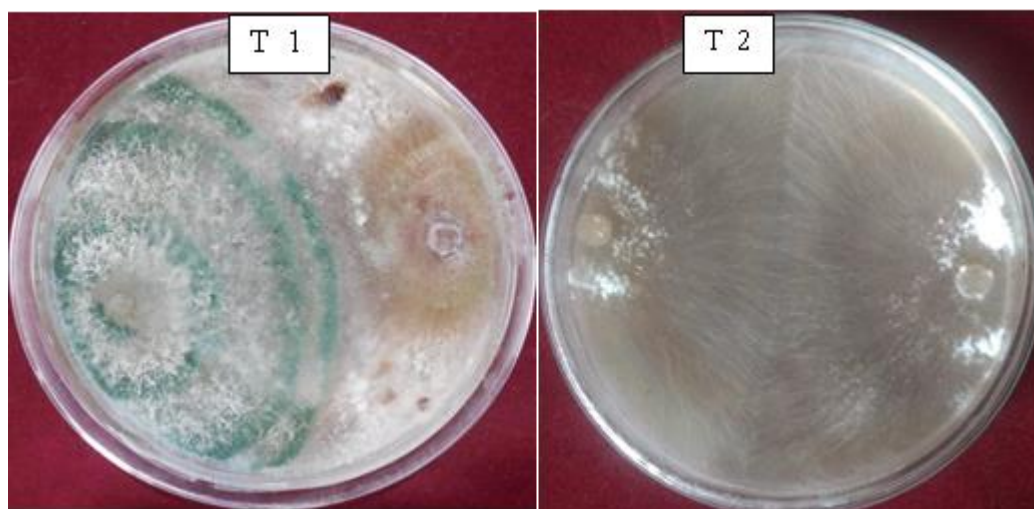
Dual culture technique using *Trichoderma viride* exhibited significant inhibition of *R. solani* as compared to control. A clear visible inhibitory zone was observed in the region of confluence between *T. viride* and *R. solani*. The percent inhibition was converted to 1-5 scale which indicated the reaction type. In the experiment, the reaction type was found

to belong to 2 scale i.e. *Trichoderma* overgrew at least two thirds of the medium surface.

Earlier reports of Kumar and Tripathi (2011) [5] stated that *Trichoderma* significantly checked the growth of *Rhizoctonia solani* are in support to the observation during the present investigation. Besides this several lines of evidence indicate the antagonistic / mycoparasitic behavior of different species of *Trichoderma*. Kapil and Kapoor (2005) [3] reported that the culture filtrate of *T. viride* inhibited the mycelial growth of *Sclerotinia sclerotiorum* due to production of antibiotic like substance. Lee and Wu (1984) [6] observed that *T. viride* produced metabolites that inhibited the mycelial growth of *Sclerotinia sclerotiorum*. Shalini and Kotasthane (2007) [9]

screened seventeen *Trichoderma* strains against *Rhizoctonia solani* *in vitro*. All strains including *T.harzianum*, *T.viride* and

*Trichoderma aureoviride* were more or less inhibited the growth of *R. solani*.



T1= Dual culture technique with *T. viride* on left and *R.solani* on right T2=Control

**Fig 4:** Evaluation of antagonistic effect of *Trichoderma viride* on *Rhizoctonia solani* under *in vitro* condition

**Table 3:** Antagonistic effect of *T. viride* on *R. solani* under *in vitro* conditions

Sl. No.	<i>Trichoderma</i> sp.	Radial growth (mm)*	Inhibition %
		<i>Rhizoctonia solani</i>	
1	<i>Trichoderma viride</i>	22.5	75 (2)
2	Control	90	-

\*Average of six replications; Figures in parenthesis indicate reaction type as a measure of mycoparasitism or antagonism

### Conclusion

Study on management of *Rhizoctonia solani* under *in vitro* condition was carried out at the laboratory of Department of Plant Pathology, IGKV, Raipur. In case of plant leaf dextrose agar medium of 8 different plant leaf extracts the Ashoka extract was found to be the most effective as the least mycelial growth of *Rhizoctonia solani* was recorded in the extract of Ashoka (10 mm). Among the five fungicides at 100 ppm concentration that were tested under *in-vitro* condition against *Rhizoctonia solani*, no mycelial growth was recorded in case of Saaf (0 mm). The growth of *R. solani* observed ranged from 0 to 90 mm. Sclerotia production was not observed in case of Saaf, Tilt and Contaf. Very less sclerotia were observed in case of Nativo and Taspa. Dual culture technique using *Trichoderma viride* also exhibited significant inhibition of *R. solani* as compared to control.

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