RESEARCH ARTICLE

EFFICACY OF MEDICINAL PLANT LEAF EXTRACTS, OILS AND BIOAGENTS AGAINST *RHIZOCTONIA SOLANI* CAUSING AERIAL BLIGHT OF SOYBEAN

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Abstract: Soybean (*Glycine max* (L.) Merrill) is one of the most important oil seed crop of India .It was wonder of the twentinth century. Soybean ranks first among world oilseed with an annual production of about 105 mt. In Chhattisgarh, the crop is grown over an area of 0.82 m ha with production and productivity of 0.73 mt and 891 kg/ha, respectively which are much lower than national average. Soybean aerial blight caused by *Rhizoctonia solani* is a most important oilseed disease. The disease appears during July-August and is characterized by sudden and complete death of the plants. This disease is very destructive and causes heavy losses to the tune of 35-60 % in warm and humid parts of the countries. Antifungal activity of different medicinal plant leaf extracts, oils and *Trichoderma spp* were studied under *in vitro* condition. Out of fifteen medicinal plants studied, the leaf extracts of Butch significantly inhibited the mycelial growth of *Rhizoctonia solani* under *in vitro* conditions. Among the medicinal oils, Eucalyptus and Neem oils were found to significantly inhibit the mycelial growth of *Rhizoctonia solani* at 5% concentrations. Among the antagonists, maximum mycelial growth inhibition was caused by *Trichoderma harzianum* (74.81%) followed by *Trichoderma viride* (67.40%) while *Trichoderma spp.* (mushroom isolates) was least effective against *Rhizoctonia solani*.

Keywords: Soybean, *Rhizoctonia solani*, Antifungal compound, *Trichoderma spp.*

INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) is one of the most important oil seed crops of India .It was wonder of the twentieth century. Soybean ranks first among world oilseeds with an annual production of about 105 mt. Among the different growing countries of the world, USA, China, Brazil, Argentina and India are major producers which accounts for more than 90% of the world's acreage (Taware *et al.*, 2007). Soybean is mainly grown during Kharif season in sandy loam to clay loam soil in Chhattisgarh. In Chhattisgarh, th crop is grown over an area of 0.82 m ha with production and productivity of 0.73 mt and 891 kg/ha, respectively which are much lower than national average. (Anonymous, 2006). Soybean aerial blight is a most important oilseed disease. The disease appears during July-August and is characterized by sudden and complete death of the plants. This disease is very destructive and causes heavy losses to the tune of 35-60 % in warm and humid parts of the countries (Patel *et al.*, 1998). Although various fungicides have shown promising results in controlling the aerial blight of soybean but the phytotoxicity and fungicidal residue problems leading to the environmental pollution are the major constraints in disease management. Substantial emphasis is being given these days on using eco-friendly approaches for controlling plant diseases. Several medicinal plants especially neem, eucalyptus and butch were reported to be one of the best alternatives to synthetic fungicides. In same context, an attempt was made through this investigation, to evaluation of different antifungal compounds against *Rhizoctonia solani* causing aerial blight of soybean.

MATERIAL AND METHOD

Leaf extracts of medicinal plants

Antifungal activity of fifteen medicinal plant leaf extracts was studied under *invitro*. The medicinal plants viz., Lemon grass (*Cymbopogan flaxuosus*), Bhringraj (*Wadelia chinensis*), Kalmegh (*Andrographis paniculata*), Ashwagandha (*Withania somnifera*), Satawar (*Asparagus racemosus*), Butch (*Acorus calamus*), Mandukparni (*Centella asiatica*), Bramhi (*Bacopa moniari*), Patchouli (*Pogostemon patchouli*), Vantulsi (*Hyptis suaveolens*), Eucalyptus (*Eucalyptus globulus*), Besrum (*Ipomea spp*), Neem (*Azadirachta indica*), Karanj (*Pongamia pinnata*) and Datura (*Datura stramonium*) were used. PDA without extract was used as control. The procedure for preparation of leaf extract medium was same as for standard PDA medium. Twenty gm leaves of each medicinal plant were taken in 100ml water and boiled till they were softened. Softened medicinal plant leaves were cursed in pastel and mortar, and then extract was filtered. Two gm of dextrose and two gm agar- agar were mixed in filtered leaf extracts and volume was made up to 100 ml followed by autoclaving at 15 lbs pressure for 20 minutes. In each sterilized petriplates 20 ml media was poured and allowed to solidify. A 5 mm disc from 4 days old culture of test fungus was placed in the centre of medium. Three replications were maintained for each treatment along with a control. The inoculated petriplates were incubated in the BOD incubator at 27±2 ^{o}C and observation were recorded at 3 and 5

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days after incubation. The percent growth inhibition of pathogen was calculated as below:

$$\text{Percent growth inhibition} = \frac{\text{Growth of test pathogen} - \text{Growth of test pathogen in control plate in presence of leaf extract}}{\text{Growth of test pathogen in control plates}} \times 100$$

Oils of medicinal plants

Antifungal activities of different medicinal plant oils were studied under *in vitro* condition. The medicinal oils *viz.,* Alsi (*Linum usitaticimum*), Til (*Sesamum indicum*), Neem (*Azadirachta indica*), Eucalyptus (*Eucalyptus globulus*), Arandi (*Ricinus communis*), Mahua (*Maduca indica*), Karanj (*Pongamia pinnata*) and Mustard (*Brassica compestris*) were used. PDA without oil was used as control. To evaluate the bio efficacy of medicinal oils at 5 % concentration, 5 ml of each oil was mixed in 95 ml PDA in each conical flask of 250 ml capacity. There after autoclaving was done at 15 lbs for 20 minutes. Twenty ml media was poured in each of the sterilized petriplates of 90 mm diameter and allowed to solidify. On solidification, 5 mm disc of 3 days old culture of test fungus was placed in the centre of the plates. Three replications were kept in each treatment along with control. Inoculated petriplates were incubated in the BOD incubator at 27±2 ^{o}C and observations were recorded at 1, 2 and 3 days after inoculation and the percent growth inhibition of pathogen was calculated as described above for leaf extracts..

Bioagents

The pure cultures of *Trichoderma viride* and *Trichoderma harzianum* were obtained from Department of Plant Pathology, Indira Gandhi Agricultural University, Raipur, Chhattisgarh. The culture of *Trichoderma spp*. (Mushroom isolates) were obtained from paddy straw mushroom beds. The antagonistic activity of these isolates against *R. solani* was evaluated by dual culture technique. An amount of 20 ml sterilized melted PDA was poured in 90 mm diameter petriplates. After solidification of medium, 5 mm disc of the antagonist and the test pathogen were separately cut with the help of a sharp sterilized cork borer from the edge of 3 days old culture and placed in straight line at distance of 5 mm from the edge. In control plates antagonist was replaced with the test fungus. The inoculated petriplates in triplicate were incubated at 27±2 ^{o}C. Observation was recorded 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 bellow.

$$\text{Percent growth inhibition} = \frac{\text{Growth of test pathogen} - \text{Growth of test pathogen in control plate in presence of } \textit{Trichoderma spp.}}{\text{Growth of test pathogen in control plates}} \times 100$$

RESULT AND DISCUSSION

Leaf extracts of medicinal plants

Fifteen medicinal plant leaf extracts were evaluated to study the antifungal activity on the growth of *R. solani* at 3 and 5 days after inoculation (Table 1). The per cent inhibition in mycelial growth of *R. solani* ranged from 12.83 % to 87.71 %. The maximum inhibition in mycelial growth was recorded in the extract of Butch (87.71%) followed by Eucalyptus *(*75.93 %). Both treatments were statistically at par with each other at 3 DAI. The percent mycelial growth inhibition by different plant extracts at 5 DAI ranged between 0.00 to 87.04 %. The maximum mycelial growth inhibition was recorded in plant extract of Butch (87.04 %) followed by Eucalyptus (66.66 %). These two leaf extracts were found to be significantly superior to leaf extracts of other medicinal plants (Table 1). Tiwari *et al.* (2007) also tested the efficacy of medicinal plant extracts *in vitro* against *R. solani* and reported that out of 950 extracts, *Acorus calamus* (Butch) was highly effective against *R. solani* at different concentrations (1%, 5% and 10%). Ansari (1995) also reported fungistatic activity of Eucalyptus extract against *R. solani*. Similarly Reddy *et al.* (2002) reported that extract of *Eucalyptusglobulus, Allium sativam* and *Zingiberoffinale* caused 61 to 100 % inhibition of the mycelial growth of *R. solani* causing root rot of chickpea. Sharma *et al.* (2005) tested the efficacy of eight plant extracts against *R. solani in vitro* and reported that *Eucalyptus globulus* inhibited 85% mycelial growth at 10% concentration. These results clearly suggest that butch and eucalyptus can be used as best alternatives to synthetic fungicides and can be incorporated in Integrated Disease Management (IDM) module to manage the aerial blight of soybean.

Oils of medicinal plants

Eight medicinal plant oils were evaluated for the effect on the growth of *R. solani* at 5 % concentration (Table 2). Maximum mycelial growth inhibition was recorded in Eucalyptus oil (100 %) followed by Neem (86.78, 71.85 and 49.26 %) at 1, 2 and 3 days after inoculation respectively. These two medicinal plant oils were found significantly superior to rest of the tested medicinal plant oils (Table 2). Madhukar and Reddy (1989) also reported that Eucalyptus oil completely checked the fruit rot diseases of guava caused by *R. solani* and anthracnose caused by

Pestalotiopsis versicolor. Similarly Singh *et al.* (1989) evaluated 6 oils of medicinal plants for their antifungal activity against *Sclerotium rolfsii* and 10 soil inhabiting fungi. Out of these, the oil of *Azadirachta indica* was most effective followed by *Eucalyptus globulus*. These results clearly suggest that eucalyptus and neem can be used as best alternatives to synthetic fungicides and can be incorporated in Integrated Disease Management (IDM) module to manage the aerial blight of soybean.

Bioagents

The data presented in Table 3 revealed that all the isolates of *Trichoderma* inhibited mycelial growth of *R. solani* by 55.77 to 74.81 percent over control. Minimum mycelial growth of *R. solani* was recorded in *T. harzianum* (22.67mm) followed by *T. viride* (29.34mm). It is concluded from the above data that *T. harzianum* isolates was most effective species to inhibit the mycelial growth of *R. solani*. These observations are agreement with the finding of Sharma and Shankran (1996) and Talanca (1999) who also reported the antagonistic activity of *Trichoderma spp*.. These results clearly suggest that *T.harzianum* can be used as potential biocontrol agent against *R. solani* and can be incorporated in Integrated Disease Management (IDM) module to manage the aerial blight of soybean. Ray *et al.* (2007) also tested the efficacy of bio-agents under *in vitro* condition. Among the bio-agents, *T. harzianum* found most effective as it inhibited the mycelial growth of *R. solani* after 96 hr of incubation followed by *T. viride* and *P. flourescens* where 82.43 and 80.36 mm growth were observed, respectively. Sarojaini and Nagmani, (2007) and Cundom *et al.* (2003) tested the antagonistic potential of *Trichoderma* isolates against *Rhizoctonia solani* and found that all the isolates inhibited the mycelial growth of *R. solani* in dual cultures.

Table 1. Evaluation of leaf extracts of medicinal plants against *Rhizoctonia solani* under *in-vitro* condition

S.N.	Medicinal plants	3 DAI**		5 DAI**	
		Mycelial growth (mm)*	% inhibition	Mycelial growth (mm)*	% inhibition
1	Lemongrass	35.66	42.78	57.50	36.11
2	Bhringraj	44.50	28.60	90.00	0.00
3	Kalmegh	45.50	27.00	90.00	0.00
4	Ashwagandha	28.83	53.74	53.33	40.74
5	Satawar	54.33	12.83	90.00	0.00
6	Butch	7.66	87.71	11.66	87.04
7	Mandukparni	47.50	23.79	90.00	0.00
8	Brahmi	31.16	50.00	59.16	34.26
9	Patchouli	47.66	23.53	90.00	0.00
10	Vantulsi	38.16	38.77	81.66	9.26
11	Eucalyptus	15.00	75.93	30.00	66.66
12	Besrum	46.66	25.14	90.00	0.00
13	Neem	51.66	17.11	90.00	0.00
14	Karanj	46.66	25.14	90.00	0.00
15	Datura	36.66	41.18	64.16	28.71
16	Control	62.33		90.00	
	S Em±	3.14		1.23	
	CD (5%)	9.1		3.6	

* Means of three replications

** Days after inoculation

Table 2. Evaluation of medicinal oils against *Rhizoctonia solani in-vitro* condition

Medicinal oils	1 DAI**		2 DAI**		3 DAI**	
	Mycelial growth (mm)*	% inhibition	Mycelial growth (mm)*	% inhibition	Mycelial growth (mm)*	% inhibition
Alsi	22.00	45.45	50.00	44.44	78.66	12.60
Til	24.33	39.67	51.66	42.60	82.66	8.15
Neem	5.33	86.78	25.33	71.85	45.66	49.26
Eucalyptus	0.00	100.00	0.00	100.00	0.00	100.00
Arandi	25.00	38.01	53.00	41.11	90.00	0.00

Mahua	7.00	82.64	31.33	65.18	53.33	40.74
Karanj	19.00	52.88	40.33	55.18	63.66	29.26
Mustatd	24.00	40.49	56.33	37.41	76.66	14.82
Control	40.33		90.00		90.00	
S Em±	1.47		0.62		1.74	
CD (5%)	4.4		1.9		5.2	

* Means of three replications
** Days after inoculation

Table 3. Effect of *Trichoderma spp* on mycelial growth of *Rhizoctonia solani*

Trichoderma species	**Dual culture (mycelial growth mm)***		**% Inhibition**
	*Trichoderma**	*Rhizoctonia **	
Trichoderma viride	60.66	29.34	67.40
Trichoderma harzianum	67.33	22.67	74.81
Trichoderma spp (Mushroom isolates)	52.00	38.00	57.77
Control	90.00	90.00	
CD (5%)	2.3	2.3	
S Em±	0.70	0.70	

*Mean of three replication

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