RESEARCH COMMUNICATION

EVALUTION OF DIFFERENT ANTIFUNGAL COMPOUNDS AGAINST *RHIZOCTONIA SOLANI* CAUSING AERIAL BLIGHT OF SOYBEAN

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Abstrct: Soybean (*Glycine max* (L.) Merrill) is one of the most important oil seed crop of India . Soybean aerial blight caused by *Rhizoctonia solani* is a most important oilseed disease. The disease appears July-August and is characterized by sudden and complete death of the plants. Antifungal activity of different medicinal plant leaf extracts, oils and *Trichoderma spp* were studies under *in vitro* condition. The Out of fifteen medicinal plants leaf extracts, studies, the extract 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 observed by *Trichoderma harzianum* (74.81%) followed by *Trichoderma viride* (67.40%) while *Trichoderma spp.* (mushroom isolates) was least effective against *Rhizoctonia solani*.

Keywords: Aerial blight of soybean, *Rhizoctonia solani*, Antifungal compound, *Trichoderma spp.*

INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) is one of the most important oil seed crop of India .It was wonder of the twentinth century. Soybean rank first among world oilseed with an annual production of about 105 mt. Among the different growing countries of the world, USA, China, Brazil, Argentina and India are main which accounts 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, area, production and productivity of soybean are 0.82 m ha, 0.73 mt and 891 kg/ha, respectively which are much lower than national average (Anonymous, 2006b). Soybean aerial blight is a most important oilseed diseases. The disease appears July-August and is characterized by sudden and complete death of the plants. This disease is considered to be one of the most destructive and causes heavy losses in the yield particularly in warn and humid parts of the countries (Anwar *et al*., 1995). Yield losses can exceed 35-60 per cent and the disease is considered as economically important (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. Substancial emphasis is being given these days on using eco-friendly approaches for controlling plant diseases. Plant products are the best alternatives available today. Several; medicinal plant species have not been screened against plant pathogens. In same context, an attempt was made through this investigation, to evalution of different antifungal compounds against *Rhizoctonia solani* causing aerial blight of soybean.

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MATERIAL AND MATHOD

Leaf extracts of medicinal plants

Antifungal activity of fifteen medicinal plant leaf extracts were studies under *in vitro* condition taking plant leaf dextrose agar medium. The following medicinal plant 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 preparation of leaf extract medium was same as PDA medium. 20gm leaves of each medicinal plant were taken in 100ml water and boiled till it becomes 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 and then sterilization was done by autoclaving at 15 lbs pressure for 20 minutes. To avoid bacterial contamination a little amount of streptomycin sulphate was added at the time of pouring of media. 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 in each treatment along with a control. The inoculated petriplates were than incubated in the BOD incubator at 27±2 ^{o}C and observation were recorded at 3 and 5

days after incubation and calculated % growth inhibition of pathogen.

Medicinal oils

Antifungal activities of different medicinal plant oils were studied under *in vitro* condition taking potato dextrose agar medium. The following medicinal oil *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 with 5 % concentration 5 ml oils were mixed in 95 ml PDA in each conical flask of 250 ml capacity. There after autoclaving was done at 15 lbs for 20 minute. To avoid the bacterial contamination, a little amount of streptomycin sulphate was added at the time of pouring of media. 15-20 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 calculated % growth inhibition of pathogen.

Bioagents

The pure cultures of *Trichoderma viride* and *Trichoderma harzianum* were obtained from department of plant pathology. 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. Three replications were maintained. The inoculated petriplates were incubated at 27±2 ^{o}C. 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 bellow.

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

RESULT AND DISCUSSION

Leaf extracts of medicinal plants

Hot water leaf extracts of different medicinal plant species were evaluated to observe the inhibitory activity against *Rhizoctonia solani* under *in vitro* condition. Fifteen medicinal plant leaf extract were evaluated to study the antifungal activity on the growth of *Rhizoctonia solani* at 3 and 5 days after inoculation. The data presented in Table 1. It is clear from the data that the mycelial growth of *Rhizoctonia solani* differs significantly with respect to different medicinal plant leaf extracts used. The per cent inhibition in mycelial growth of *Rhizoctonia 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 %). They were statistically at par with each other at 3 DAI. Minimum inhibition in mycelial growth was recorded in Satawar (12.83 %) as comparison to control. The per cent mycelial growth inhibition at 5 DAI by different plant extracts 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 %). Bhringraj, Kalmegh, Satawar, Mandukparni, Patchouli, Besrum, Neem and Karanj were failed to inhibit the mycelial growth of *R. solani* . The results indicate that all plant extracts inhibited the growth of the fungus from 12.83% in Satawar to 87.71% in Batch after 3 days of inoculation respectively. The other plant extracts showing promising results against *R. solani* were Eucalyptus and Ashwagandha. Tiwari *et al.* (2007) also tested the efficacy of medicinal plant extracts *in vitro* against *Rhizoctonia solani* and reported that out of 950 extracts, *Acorus calamus* (Butch) was highly effective against *R. solani* at all concentration (1%, 5% and 10%). Similarly Reddy *et al.* (2002) reported that extract of, *Eucalyptus globulus, Allium sativam* and *Zingiber offinale* caused 61 to 100 percent inhibition of the mycelial growth of *Rhizoctonia solani* causing root rot of chickpea. Sharma *et al.* (2005) tested the efficacy of eight plant extracts against *Rhizoctonia solani in vitro* and reported that *Eucalyptus globulus* inhibited 85% mycelial growth at 10% concentration.

Medicinal oils

All the medicinal oils were superior in reducing the mycelial growth of *Rhizoctonia solani* over control at 5% (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. Minimum mycelial growth inhibition was recorded in Arandi (38.01%) at 1 DAI, Mahua (37.41%) at 2 DAI and Til (8.15%) at 3 DAI (Plate 8). Madhukar and Reddy (1989) reported that Eucalyptus oil completely checked the fruit rot diseases of guava caused by *Rhizoctonia solani* and anthracnose caused by *Pestalotiopsis*

versicolor. Coconut oil, castor oil and groundnut oil also effective in reducing the fruit rot of guava. Singh and Dwivedi observed the fungitoxic activity of the oils of *Eucalyptus globulus* against the sclerotial production of *S. rolfci.* 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.*

Bioagents

The data are presented in Table 3 revealed that all the isolates of *Trichoderma* in dual culture inhibited mycelial growth of *Rhizoctonia solani* and inhibition ranged from 55.77 to 74.81 per cent over control. A clear visible band was formed in the zone of contact between the two fungal growths. Minimum mycelial growth of *Rhizoctonia solani* was recorded in *Trichoderma harzianum* (22.67mm) followed by *Trichoderma viride* (29.34mm). Maximum mycelial growth of *Rhizoctonia solani* was recorded in *Trichoderma spp* (Mushroom isolates) (38mm). It is concluded from the above data that *Trichoderma harzianum* isolates was found most effective species to inhibit the mycelial growth of *Rhizoctonia solani.* 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) 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. Similarly Cundom *et al.* (2003) evaluated the antagonistic activity of nine isolates of *Trichoderma spp.* in dual culture and found that all the isolates significantly inhibited the mycelial growth of *R. solani* in dual culture.

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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