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Bioefficacy of Fungicides, Botanicals and Biocontrol Agents Against Sarocladium Oryzae, Incitant of Rice Sheath Rot.

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Abstract: *Among the eight fungicides evaluated in vitro using poisoned food technique at five different concentrations, hexaconazole, tebuconazole+trifloxistrobin, carbendazim and mancozeb recorded 100% inhibition in the growth of S. oryzae at all the five concentrations tested. Isoprothiolone, kasugamycin, tricyclazole and validamycin varied in their inhibitory effect on S. oryzae at different concentrations. Among the six plant extracts tested with five concentrations each, bulb extract of Allium sativum was most effective in inhibiting the growth of S. oryzae (63.4%), while leaf extract of Ocimumbasilicum recorded least inhibition (3.9%). Inhibition in the radial growth of S. oryzae due to extracts of Eucalyptus, Curcuma, Aloe vera and Allium cepa was in between Allium sativum and Ocimumbasilicum. None of the seven isolates of Trichoderma and eight isolates of Pseudomonasfluorescensshowed significant antagonistic effect on the growth of S. oryzae.*
Key Words*: Biocontrolagents, Botanical extracts, Fungicides, Rice sheath rot,Sarocladiumoryzae*

I. Introduction

Sheath rot of rice is a serious disease accounting for heavy toll of rice production. The disease was first reported in Taiwan in 1922 and later it has been reported in all countries in South Asia. In India, sheath rot was first reported in 1973 and the losses due to the disease were found to be ranging from 50 to 65 per cent (Ravishankar and Revanna, 2008). Severe outbreaks of sheath rot causing considerable yield losses were reported in the Indian state of Punjab during 1978-79. In Andhra Pradesh, sheath rot was found to be severe in Godavari, Nellore and Chittoor districts, causing 80 to 85 per cent yield loss (Bhaskaret al., 2002)necessitating research on sheath rot management. Lakshmanan (1984) reported thattridemorph at 100 ml + carbendazim at 100 g/ha or tridemorph alone at 200 ml reduced the sheath rot intensity and increased the yield over the control significantly. However, Lewin and Vidhyasekaran (1987) reported that all the 12 fungicides tested against sheath rot pathogen S.oryzaeinhibited mycelial growth in vitro.Chaliganjewaret al. (2010) found that P. fluorescens, T. viride, T. harzianumand B. subtiliswhen sprayed at booting stage significantly reduced sheath rot disease incidence in rice.Ahmed et al., (2013) found garlic extract effective in inhibiting the radial growth of S. oryzaein vitro.

II. Material And Methods:

Pathogen Sarocladiumoryzae was isolated from rice fields of Agricultural Research Station, Nellore, Andhra Pradesh and cultures of Trichoderma and Pseudomonasfluorescens from the Department of Plant Pathology, S V Agricultural College, Tirupati, Andhra Pradesh. Poison food technique was used for evaluating bioefficacy of fungicides and botanicals, and dual culture technique was used for assessing antagonistic potential of biocontrol agents.

Eight fungicides commonly used in rice ecosystem,viz., carbendazim,hexaconazole, isoprothiolone,kasugamycin,mancozeb,tricyclazole, validamycin and tebuconazole+trifloxistrolin were evaluated for their efficacy against S.oryzae by poison food technique.

For testing bioefficacy of botanicals, plant material from Allium cepa(bulbs), Allium sativum(cloves), Eucaluptusodorata(leaves), Aloe vera(fleshy stem), Oscimumbasilicum(leaves) and Curcuma longa (processed rhizomes) were collected and washed thoroughly in tap water followed by washing in distilled water. The plant tissue was ground with sterile water at the rate of 1g plant material in 1ml of water using a pestle and mortar, and the macerate was filtered through a muslin cloth to get the crude extract. The extract of each plant species was tested at five concentrations,viz., 5, 10, 15, 20 and 25 per cent for its effect against the growth of S. oryzaeby poisoned food technique.

In dual culture plates (Dennis and Webster, 1971), four 5 mm culture discs of S. oryzae were inoculated at four sides on the periphery about 1cm from the edge of the Petri dishes and in the centre 5mm disc of Trichoderma was inoculated. For interactions between S. oryzae and fluorescent Pseudomonads, three day old individual culture of fluorescent Pseudomonad is streaked with the help of inoculation loop around the disc of S. oryzae in the form of three straight lines forming a triangle.Monocultured plates served as check. Observation on the radial growth of S. oryzae was recorded.

Per cent inhibition of S. oryzaewas calculated by using the following formula given by Vincent (1927):

$$I = 100(C-T)/C$$

Where, I= Per cent Inhibition
C= Radius of the colony of S. oryzaein control plates
T= Radius of the colony of S. oryzaein treatments

III. Results And Discussion

Effect Of Fungicides Against Radial Growth Of Sarocladium Oryzaein Vitro

All the fungicides significantly inhibited the growth of S. oryzae compared to control (2.4 cm). Carbendazim, hexaconazole, tebuconazole+trifloxistrobin, and mancozeb recorded 100% in inhibiting the growth of S. Oryzae (Table 1).

Tricyclazole, isoprothiolone, kasugamycin and validamycin at 600ppm, 1500ppm, 2000ppm and 2500ppm concentrations respectively, recorded more than 50% inhibition in the radial growth of S.oryzae. However, Tricyclazole at 200, 300, and 400ppm concentrations, isoprothiolone at 300, 600, 900 and 1200ppm concentrations, kasugamycin at 500, 1000, and 1500ppm conmcentrations and validamycin at 400, 800, 1200 and 1600ppm concentrations recorded less than 50% inhibition in the radial growth of S. oryzae NLR isolate.

The fungicide kasugamycin at 500ppm and 1000ppm concentrations recorded least inhibition (1.6%) in the of growth of S. oryzae followed by validamycin at 400 ppm concentration (2.9%).

The results of the present investigation revealed that the fungicides hexaconazole, tebuconazole + trifloxistrobin, carbendazim and mancozeb were the most effective fungicides inhibiting the growth of S.oryzae. Tebuconazole + trifloxistrobin and carbendazim were recommended to sheath rot at field conditions (ANGRAU, 2013-14). The present investigation is in accordance with the reports published by Laxmanan (1984), Lewin and Vidyasekaran (1987), Prasad et al. (2011), Sharma et al. (2013) and Venkateswarlu and Chauhan (2004) as carbendazim and mancozeb were effective in inhibiting the growth of S. oryzaein vitro

IV. Effect Of Botanicals On The Radial Growth Of S. Oryzaein Vitro.

All the botanical extracts significantly inhibited the growth of S. oryzaecompared to the control (2.7cm) (Table 2).

Over all the concentrations, clove extract of Allium sativum was found to be the most effective in inhibiting the growth of S. oryzae (63.4%) followed by leaf extracts of Eucalyptus (38.9%), rhizome extracts of Curcuma longa (22.4%), stem extracts of Aloe vera (17.0%) and bulb extract of Allium cepa (4.9%). The leaf extract of Ocimumbasilicum recorded the least mean inhibition (3.9%) of S. oryzaegrowth.

Over all the botanical extracts, 25% concentration of plant extract brought about the highest inhibition of growth (51.6%) followed by the extracts at 20% concentration (32.0%). The extracts at 5% concentration recorded the lowest inhibition of growth (8.5%). Thus, increase in the concentration of the extract increased inhibitory effect on the growth of S. oryzae

Interactions between botanical extracts and their concentrations were found to be significant. The differences among the extracts in inhibiting the growth of S. oryzae were affected by concentrations at which they are used. Extract of Allium sativum at 20% and 25% concentration inhibited the growth of S. oryzae by 100% and it was superior to the other plant extracts tested at all the concentrations. Ocimumbasilicum at 5% concentration was least effective against the growth of S. oryzae.

Irrespective of the type of plant extracts used,the inhibition was less than 50% at 5% and 10% concentrations.

The results of the present investigation revealed that the extracts of Allium sativum was the most effective in inhibiting the growth of S. oryzae followed by those of Eucalyptus odorata, Curcuma longa, Aloe vera and Ocimumbasilicum.

Present investigation was in accordance with Ahmed et al. (2012) who reported sensitivity of S. oryzae towards Allium sativum. Chaliganjewaret al. (2010a) reported sensitivity of S. oryzae towards Ocimumbasilicum and Allium cepa.However,in the present investigation, Allium sativum was found superior to Allium cepa and Osimumbasilicum in inhibiting the growth of S. oryzaein vitro.

V. Effect Of Trichoderma Spp On The Radial Growth Of S. Oryzaein Vitro

Use of biocontrol agents against plant pathogens does not pose any hazard to environment, but also prevents the possibility of resistance development in targeted plant pathogens. Hence, in the present investigation, antagonistic effect of Trichodermaspp and Pseudomonas fluorescence on the growth of S. oryzae was studied in vitro using dual culture method and the results are presented here with (Table 3).

All the seven test isolates of Trichodermaspp except T. harzianum GRT 6 significantly inhibited the growth of S. oryzae compared to controlwith inhibitory effect which ranging from 5.5% to 66.6%. Thus variation was observed among different isolates of Trichoderma in antagonising S. oryzae.

Among all the isolates, T. viride GRT 2 was most inhibitory to the growth of S. oryzae with 66.6% inhibition followed by T. viride GRT 3 with 38.9% inhibition and T. viride GRT 1 with 27.7% inhibition. The least inhibition was recorded in T. harzianum GRT 6 (0.0%) followed by T. koningii GRT 4 and T. koningii GRT 7 with 5.5% inhibition. Several reports were published on the in vitro antagonistic efficacy of Trichodermaagainst S. oryzae (Paneerselvam and Saravanamuthu (1996), Srinivas and Ramakrishnan (2003), Gopalakrishnan and Valluvaparidasan (2006), Bag et al. (2010), Chaliganjewaret al. (2010b), Selvaraj and Panneerselvam (2011)).

None of the eight Pseudomonas fluorescence isolates tested showed any inhibitory effect on S. oryzae growth in the present investigation. However, Sakthivel and Gnanamanickam(1986a), Sakthivel and Gnanamanickam (1987), Gopalakrishnan and Valluvaparidasan (2006), Bag et al. (2010), Chaliganjewaret al. (2010b), Manidipaet al. (2013) reported that Pseudomonas fluorescence as an effective antagonist against S. oryzae in vitro.

In the present investigation, fungicides hexaconazole, tebuconazole + trifloxistrobin, carbendazim and mancozeb, Allium sativum among the botanicals were found effective in inhibiting the growth of S. oryzae. As none of the test isolates of Trichoderma and fluorescent Pseudomonads were effective, rigorous screening of more number of antagonistic isolates is required.

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Table1. Effect of fungicides on the radial growth of Sarocladiumoryzaein vitro

S. No.	Fungicide	Concentration (ppm)	Colony radius of S. oryzae(cm)	Percent inhibition*
1	Tricyclazole	200	1.8	25.0 (28.8)
		300	1.5	37.5 (37.8)
		400	1.4	41.6 (40.2)
		500	1.2	50.0 (45.0)
		600	1.1	52.9 (46.6)
2	Isoprothiolone	300	2.1	12.5 (19.9)
		600	1.3	45.8 (42.6)
		900	1.3	45.8 (42.6)
		1200	1.3	45.8 (42.6)
		1500	1.1	55.8 (48.2)
3	Kasugamycin	500	2.4	1.66 (3.9)
		1000	2.4	1.66 (3.9)
		1500	1.5	37.5 (37.3)

		2000	1.2	50.0 (45.0)
		2500	1.1	55.8 (48.2)
4	Validamycin	400	2.3	2.9 (7.8)
		800	1.8	22.8 (28.5)
		1200	1.5	37.5 (37.6)
		1600	1.3	45.8 (42.6)
		2000	1.2	51.7 (45.8)
5	Hexaconazole	400	0.0	100 (90.0)
		800	0.0	100 (90.0)
		1200	0.0	100 (90.0)
		1600	0.0	100 (90.0)
		2000	0.0	100 (90.0)
6	Tebuconazole + Trifloxistrolin	80	0.0	100 (90.0)
		160	0.0	100 (90.0)
		240	0.0	100 (90.0)
		320	0.0	100 (90.0)
		400	0.0	100 (90.0)
7	Carbendazim	200	0.0	100 (90.0)
		400	0.0	100 (90.0)
		600	0.0	100 (90.0)
		800	0.0	100 (90.0)
		1000	0.0	100 (90.0)
8	Mancozeb	500	0.0	100 (90.0)
		1000	0.0	100 (90.0)
		1500	0.0	100 (90.0)
		2000	0.0	100 (90.0)
		2500	0.0	100 (90.0)
9	Control		2.4	000 (00.0)
	S.Em ±			2.2
	CD (P = 0.01)			6.1
	CV (%)			6.0

Figures in parenthesis are arc sine transformed values
Each treatment replicated thrice

Table 2. Effect of botanical extracts on radial growth of Sarocladiumoryzaein vitro

S.No	**Botanical Extract**	**Inhibition of growth of S. Oryzae (%)**					
		Concentration of botanical extract					
		5%	**10%**	**15%**	**20%**	**25%**	**Mean**
1	Allium sativum	16.0 (23.6)	38.2 (37.9)	62.9 (52.4)	100 (90.0)	100 (90.0)	63.4 (58.8)
2	Allium cepa	4.9 (10.2)	3.7 (11.1)	3.7 (11.1)	4.9 (12.6)	7.4 (11.9)	4.9 (11.9)
3	Ocimumbasilicum	0.0 (0.0)	2.5 (7.3)	2.5 (7.3)	2.5 (7.3)	12.3 (20.5)	3.9 (8.6)
4	Eucalyptus odorata	18.2 (18.2)	19.8 (26.3)	20.9 (27.2)	57.7 (49.6)	77.8 (61.9)	38.9 (36.7)
5	Aloe vera	3.7 (11.1)	9.9 (18.1)	11.1 (19.4)	11.1 (19.4)	49.3 (44.6)	17.0 (22.5)
6	Curcuma longa	8.6 (17.0)	12.3 (20.5)	12.3 (20.5)	16.0 (22.6)	62.9 (52.4)	22.4 (26.6)
	Mean	8.5 (13.3)	14.4 (20.2)	18.9 (23.0)	32.0 (33.6)	51.6 (47.3)	
		S.Em ±	**CD (P= 0.01)**	**CV(%)**			
	Botanical Extract	1.02	2.90	14.43			
	Concentration	0.93	2.65				
	Interaction	2.29	6.50				

Figures in parenthesis are arc sine transformed values
Each treatment replicated thrice

Table3. Effect of Trichoderma spp. on the radial growth of Sarocladiumoryzaein vitro.

Trichoderma isolate	**Colony radius of S. oryzae(cm)***	**Percent inhibition***
T. viride GRT1	0.4	27.7 (31.5)
T. viride GRT2	0.2	66.6 (54.7)
T. viride GRT3	0.4	38.9 (38.4)
T. koningii GRT4	0.6	5.5 (8.01)
T. harzianum GRT 5	0.5	11.1 (16.0)
T. harzianum GRT 6	0.6	0.0 (0.0)
T. koningii GRT7	0.6	5.5 (8.0)
Control (S.oryzae)	0.6	00.0 (0.0)
	SEm ±	0.03
	CD (P = 0.01)	0.08
	CV (%)	10.45

*Mean of three replications
Figures in parentheses are arc sine transformed values
Observations were recorded 5 days after inoculation