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Research Article

ANTIFUNGAL ACTIVITY OF BOTANICALS AGAINST *SAROCLADIUM ORYZAE* CAUSING RICE SHEATH ROT DISEASE

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ABSTRACT
Rice sheath rot caused by *Sarocladium oryzae* to be a major constraints in rice production .Since the existing chemical control measures being costly and may favour development of resistance in pathogens. The potential alternative methods have been explored in the present studies. Forty plant extracts were tested against *Sarocladium oryzae*. Among these, *Spharanthus indicus*, *Lawsonia inermis, Brassica campestris, Jatropha curcas*, *Ricinus communis* and *Cymbopogan citrates* did not showed any effect. Among all the plant extracts *Eugenia caryophyllata* and *Eucalyptus globules* exhibited strong fungitoxicity at 50% concentration. Followed by, *Acorus calamus* and *Cinnamon zylanicum*. *Pavonia zylanica* exhibited least inhibition of pathogens. Out of the plant extracts tested, selectively 10 plant extracts which showed promising results were further evaluated at different concentrations (2%, 4%, 6%, 8% and 10%) against S. *oryzae.* In the present study, among the various plant extracts *Eugenia caryophyllata* and *Eucalyptus globules* at 10% concentration were observed to be the most effective in inhibitory the mycelial growth, bio-mass production, spore germination and germ tube length of the pathogen under *in vitro* condition.

Key words: - *In vitro,* antifungal activity, plant extract, *Sarocladium oryzae*, rice.

INTRODUCTION
Sheath rot of rice incited by *Sarocladium oryzae* (Sawada) W. Gams & D. Hawksw is seed borne and present in all rice growing countries worldwide. The disease is highly destructive in Tamil Nadu and other rice growing states of India (Lakshmanan, 1993). The fungus is detected frequently during routine seed health testing.The disease causes empty grain production (Kulwant and Mathur 1992) and glume discolouration (Sachan and Agarwal 1995). It also causes poor grain filling and reduction in seed germination (Vidyasekaran *et al*., 1984). Seeds from infected panicles became discoloured and sterile (Mew and Gonzales 2002). Use of fungicide to control diseases causes several adverse effects i.e. development of resistance in the pathogen, residual toxicity, pollution to the environment etc. Grasela *et al*. (1990) also reported that, despite advances in antifungal therapies, many problems remained to be solved for most antifungal drugs available. Therefore, it has become necessary to adopt eco-friendly approaches for enhancing crop yield and better crop health. Plants provide abundant resources of antimicrobial compounds and have been used for centuries to inhibit microbial growth (Jun-Dong *et al*., 2006). Flavanoids, triterpenoids, steroids and other phenolic compounds in plants have been reported to have antimicrobial activity (Rojas *et al*., 1992; Hostetmann *et al*., 1995).
The systematic search of higher plants for antifungal activity has shown that plant extracts have the ability to inhibit spore germination and mycelial growth in many fungal species (Natarajan and Lalithakumari, 1987; Singh and Dwivedi, 1987). Many plant extracts are reported to specifically inhibit the germination of fungal spores (Babu *et al*. 2001). The fungal pathogens of rice viz., *Sarocladium oryzae* and *Pyricularia oryzae* were also found to be controlled effectively by neem oil and neem seed kernel extract (Mariappan *et al*. 1995). Neem and Pungam oil based EC formulation developed by TNAU have been effective against sheath rot disease of rice under field condition (Narasimman *et al*. 1998). Hence, in the present study, plant extracts were tested *in vitro* against *S. oryzae* by preliminary bioassay screening. This study would

contribute to the acceptance of traditional medicine and to the solution of the growing problems of drug resistance by fungi.

MATERIALS AND METHODS

Collection of plant materials:

The fresh disease free leaves of 26 plant species were collected from different locations of Nagapattinam district; Tamilnadu, India and powdered form of 14 plant species were collected from SKM Pvt. Ltd. Coimbatore, India. The details are given in table.1.

Preparation of plant extracts

A cold water extract of the plant product was prepared by adding plant material and distilled water at 1:1 ratio (w/v), mixed well, filtered through muslin cloth and passed through bacterial filters under vacuum (Okigbo and Ogbnonnaya, 2006). The aseptically filtered extract formed the standard plant extract solution and was stored in refrigerator for further use. This standard extract was further diluted to the required concentration using sterile distilled water.

All the extracts were used at 50% concentration for screening antifungal activity. The plant species showed effectiveness in the preliminary screening were further diluted to different concentrations (2%, 4%, 6%, 8% and 10%) and tested against *S. oryzae* under *in vitro*.

Isolation of *S. oryzae*

Pathogen was isolated from infected leaf sheath collected from different locations of Nagapattinam district by direct plating technique (Ashura *et al.,* 1999). The infected tissue were surface sterilized for 10 minutes with sodium hypochlorite (2%), rinsed 5 times with sterile water to remove the disinfectant, dried on sterile paper and plated on potato dextrose agar medium and incubated at room temperature for 7 days. After incubation *Sarocladium oryzae* was identified and purified by single hyphal tip method and maintained on PDA slants for further study.

In vitro evaluation of plant extracts against *S. oryzae*

Radial growth

Efficacy of selected plant extract on the growth of *S. oryzae* was evaluated by using poisoned food technique. The standard plant extracts solution was mixed with PDA medium at the calculated quantity so as to get the required concentration (50%) of the plant extract and sterilized. Twenty ml of this mixture was poured into sterile Petri dishes and allowed to set. A 9 mm actively growing PDA culture disc of *S. oryzae* was placed at the centre of the medium and incubated at room temperature (28 ± 2^0C) for seven days. PDA medium without any plant extract served as control. For comparison Carbendazim (0.1%) was used and three replications were maintained for each treatment. The radial growth of the mycelium was measured seven days later or when the fungus fully covered in any one of the treatment plates. The results were expressed as per cent growth inhibition over control.

Bio-mass production

Fifty ml of PDA broth taken in 250 ml Erlenmeyer flasks were sterilized and amended with different concentrations (2%, 4%, 6%, 8% and 10%) of plant extracts and inoculated with mycelial disc (9 mm) of *S. oryzae* collected from the periphery of seven days old culture. The flasks were incubated at room temperature (28 ± 2^0C) for ten days and filtered through Whatman No. 42 filter paper. The fungal growth retained on the filter paper was dried in an oven at 105^0 C to a constant weight and the dry weight of mycelial biomass was recorded in mg.

Spore germination

Efficacy on spore germination was assessed by using spore germination assay. One drop from each plant extracts at different concentration (2%, 4%, 6%, 8% and 10%) was placed in the cavity of the depression slide and a drop of the conidial suspension (4×10^6 spores/ml) of *S. oryzae* prepared in sterile distilled water was added to the cavity and thoroughly mixed. The cavity slide was incubated in Petri dish- glass bridge chamber. Three replications were maintained for each treatment. For comparison Carbendazim (0.1%) was

used. The spore suspension in sterile distilled water served as control. The spores were observed for germination in three different microscopic fields and recorded after 48 h. Per cent inhibition of spore germination over control was calculated as per the formula described by Vincent (1947). Length of germ tube was measured by ocular micrometer and expressed in µ.

RESULTS AND DISCUSSION

In general the plant extracts showed varied degree of growth inhibition against *S. oryzae* whereas 6 plant extracts, viz. *Spharanthus indicus*, *Lawsonia inermis, Brassica campestris,* kattamanaku, *Ricinus*

Table 1. Anti fungal activity of plant extract at 50% conc. against *Sarocladium oryzae*

Sl.no	Plant extracts (50% conc.)	Vernacular name	Parts used	Radial growth (mm)	% of inhibition
1.	*Aadathoda vesica*	Adathodai	Leaves	27.5^{j}	69.4
2.	*Acalipha indica*	Kuppaimeni	Leaves	26.8^{j}	70.2
3.	*Acorus calamus*	Vasambu	Leaves	**7.0^{b}**	92.2
4.	*Allium cepae*	Vengayam	Bulb	26.2^{j}	70.8
5.	*Allium sativam*	Poondu	Bulb	26.4^{j}	70.6
6.	*Aloe vera*	Katralai	Leaves	27.5^{j}	69.4
7.	*Andrographis paniculata*	Nila vembu	Leaves	28.2^{j}	68.6
8.	*Aristalochia breateata*	Aadutheenda paalai	Leaves	28.0^{j}	68.8
9.	*Azadiracta indica*	Vembu	Leaves	26.9^{j}	70.1
10.	*Brassica campestris*	Mustard	Seed	-	-
11.	*Calotrophis jaijantia*	Erukan	Leaves	**15.0^{e}**	83.3
12.	*Capsicum annum*	Chilli	Leave	27.8^{j}	69.1
13.	*Catharanthus roseus*	Catharanthus	Leaves	28.3^{j}	68.5
14.	*Cinnamom zylanicum*	Elavangapattai	Bark	**9.3^{c}**	89.6
15.	*Cissus quadrangularis*	Perandai	Leaves	27.4^{j}	69.5
16.	*Coleus aromaticus*	Oama valli	Leaves	**26.1^{i}**	71.0
17.	*Curcuma longo*	Manjal	Rhizome	**12.7^{d}**	85.8
18.	*Cymbopogan citratus*	Lemon grass	Leaves	-	-
19.	*Datura stramonicum*	Umathai	Seeds	**23.2^{h}**	74.2
20.	*Eucalyptus globules*	Eucalyptus	Leaves	**4.5^{a}**	95.0
21.	*Eugenia caryophyllata*	Kirambu	Bud	**2.5^{a}**	97.2
22.	*Jatropha curcas*	kaattamanaku	Leaves	-	-
23.	*Lantana camera*	Road side weed	Leaves	26.5^{j}	70.5
24.	*Lawsonia inermis*	Henna	Leaves	-	-
25.	*Leucas aspera*	Thumbai	Leaves	27.4^{j}	69.5
26.	*Moringa oleifera*	Murungai	Leaves	28.3^{j}	68.5
27.	*Nerium oleander*	Apocynaceae	Leaves	26.4^{j}	70.6
28.	*Nigella sativa*	Karum seeragam	Seeds	27.0^{j}	70.0
29.	*Ocimum sanctum*	Tulsi	Leaves	**17.6^{f}**	80.4
30.	*Ocinum basilium*	Basil	Bulb	28.0^{j}	68.8

31.	*Pavonia zeylanica*	Palaver	Root	28.5[j]	68.3
32.	*Phyllanthus indica*	Nelli	Leaves	27.1[j]	69.8
33.	*Phyllanthus niroori*	Kizhanelli	Leaves	27.9[j]	69.0
34.	*Prosopis juliflora*	Karuvai	Leaves	26.7[j]	70.3
35.	*Psidium guajava*	Koyya	Leaves	26.6[j]	70.4
36.	*Ricinus communis*	Aamanakku	Leaves	-	-
37.	*Spharanthus indicus*	Kottai karanthai	Seeds	-	-
38.	*Vernnonia anthelmintica*	Kattuseeragam	Seeds	**20.2[g]**	77.5
39.	*Vitex negundo*	Notchi	Leaves	27.1[j]	69.8
40.	*Zingiber officinale*	Ginger	Rhizome	27.2[j]	69.7
41.	Carbendazim (0.1%)			6.0	93.3
42.	Control			90.0	-

communis and *Cymbopogan citratus* did not showed any effect. Among all the plant extract, *Eugenia caryophyllata* (97.2% growth reduction) and *Eucalyptus globules* (95.0% growth reduction) exhibited strong fungitoxicity. Followed by the extract of *Acorus calamus* and *Cinnamon zylanicum* with the mycelial growth of 7.0mm (92.2% growth reduction) and 9.3mm (89.6% growth reduction) respectively. *Pavonia zylanica* showed least inhibition of pathogen with the mycelial growth of 28.5 mm (68.3% growth reduction). Carbendazim (0.1%) used for comparison recorded 93.3% growth reduction with the minimum mycelial growth of 6.0mm (Table 1).

Out of the plant extracts tested, selectively 10 plant extracts which showed promising results were further evaluated at different concentrations (2%, 4%, 6%, 8% & 10%) against *S. oryzae*. The inhibition of growth of fungus increased with an increase in concentration of the aqueous extract of the test plants. Among the selected 10 plant extract tested against *S. oryzae* at different concentration, *Eugenia caryophyllata* and *Eucalyptus globules* (10 %) were on par showing biomass production of the pathogen. The mycelial dry weight was 211.8 mg (69.3% of growth reduction) and 212.7 mg (69.1 % of growth reduction) in these plant extracts as against 690.0 mg in control. These were followed by ten per cent extract of *Acorus calamus* and *Cinnamom zylanicum* with the mycelial dry weight of 215.0mg (68.8% growth reduction) and 217.8mg (68.4% growth reduction), respectively. Carbendazim (0.1%) used for comparison recorded 94.0% growth reduction with the minimum mycelial dry weight of 42.0mg (Table 2).

With regard to the spore germination, *Eugenia caryophyllata* and *Eucalyptus globules* at 10% concentration were on par showing spore germination and elongation of germ tube of the pathogen. The spore germination was 16.6% (81.3 per cent inhibition) and 18.7% (78.9 per cent inhibition), respectively and the germ tube length was 9.6 µ (89.2 per cent inhibition) and 10.2 µ (88.9 per cent inhibition) in these plant extracts as against 89.0% spore germination in control. These were followed by ten per cent extract of *Acorus calamus* and *Cinnamom zylanium* with the spore germination of 21.4% (75.9 per cent inhibition) and 24.1% (72.9 per cent inhibition), respectively and the germ tube length of 11.3 µ (87.3 per cent inhibition) and 12.2 µ (86.2 per cent inhibition) respectively. Per cent of spore germination and germ tube elongation was increasing when the concentration of the plant extract is increased. Carbendazim (0.1%) used for comparison recorded the minimum per cent spore germination (6.94%) and germ tube length (7.0 µ) (Table 3).

Table 2. Effect of selected plant extracts at different conc. on bio mass production of *Sarocladium oryzae*

Sl. No	Plant species	Mycelial dry weight (mg)					% of inhibition				
		2%	4%	6%	8%	10%	2%	4%	6%	8%	10%
1.	*Acorus calamus*	224.0[b]	221.5[b]	219.0[b]	216.8[b]	215.0[b]	67.5	67.8	68.2	68.5	68.8
2.	*Calotrophis jaijantia*	230.2[e]	229.5[e]	226.4[e]	224.4[e]	221.7[e]	66.6	66.7	67.1	67.4	67.8
3.	*Cinnamom zeylanicum*	226.4[c]	224.8[c]	222.1[c]	219.6[c]	217.8[c]	67.1	67.4	67.8	68.1	68.4
4.	*Coleus aromaticus*	239.3[h]	238.0[i]	234.1[i]	232.8[h]	230.1[i]	65.3	65.5	66.0	66.2	66.6
5.	*Curcuma longa*	228.3[d]	226.7[d]	224.5[d]	222.0[d]	219.8[d]	66.9	67.1	67.4	67.8	68.1
6.	*Datura stramonium*	237.3[g]	236.0[h]	232.2[h]	230.4[g]	227.7[h]	65.6	65.7	66.3	66.6	67.0
7.	*Eucalyptus globules*	220.2[a]	218.4[a]	216.0[a]	214.2[a]	212.7[a]	68.0	68.3	68.6	68.9	69.1
8.	*Eugenia caryphyllata*	218.[a]	216.7[a]	214.2[a]	212.0[a]	211.8[a]	68.3	68.5	68.9	69.2	69.3
9.	*Ocimum sanctum*	234.8[f]	232.3[f]	228.4[f]	226.7[f]	224.3[f]	65.9	66.3	66.8	67.1	67.4
10.	*Vernnonia anthelmintica*	235.3[f]	234.2[g]	230.3[g]	228.1[f]	226.5[g]	65.8	66.0	66.6	66.9	67.1
	Carbendazim (0.1%)	42.0					94.0				
	Control	690.0					-				

Table 3. Effect of selected plant extracts at different conc. on spore germination of *Sarocladium oryzae*

Sl. No	Plant species	Spore germination (%)					% of inhibition				
		2%	4%	6%	8%	10%	2%	4%	6%	8%	10%
1.	*Acorus calamus*	28.3[b]	27.6[b]	25.1[b]	23.5[b]	21.4[b]	68.2	68.9	71.7	73.5	75.9
2.	*Calotrophis jaijantia*	36.7[e]	35.3[e]	33.4[d]	32.0[e]	30.4[e]	58.7	60.3	62.4	64.0	65.8
3.	*Cinnamom zeylanicum*	31.2[c]	29.6[c]	27.2[b]	25.8[c]	24.1[c]	64.9	66.7	69.4	71.0	72.9
4.	*Coleus aromaticus*	45.3[i]	44.8[i]	43.8[h]	39.5[i]	37.6[i]	49.1	49.6	50.7	55.6	57.7
5.	*Curcuma longa*	33.6[d]	32.5[d]	30.1[c]	28.2[d]	26.8[d]	62.2	63.4	66.1	68.3	69.8
6.	*Datura stramonium*	43.9[h]	42.6[h]	41.3[g]	37.0[h]	35.2[h]	50.6	52.1	53.5	58.4	60.4
7.	*Eucalyptus globules*	26.3[a]	24.8[a]	22.6[a]	20.0[a]	18.7[a]	70.4	72.1	74.6	77.5	78.9
8.	*Eugenia caryphyllata*	25.1[a]	23.0[a]	20.4[a]	18.2[a]	16.6[a]	71.7	74.1	77.0	79.5	81.3
9.	*Ocimum sanctum*	38.0[f]	37.2[f]	36.4e	34.5[f]	33.0[f]	57.3	58.2	59.1	61.2	62.9
10.	*Vernnonia anthelmintica*	41.0[g]	39.2[g]	38.8[f]	36.5[g]	32.8[g]	53.9	55.9	56.4	58.9	63.1
	Carbendazim (0.1%)	6.94					92.2				
	Control	89.0					-				

Table 4. Effect of selected plant extracts at different conc. on germ tube elongation of *Sarocladium oryzae*

Sl. No	Plant species	Germ tube length (µ)					% of inhibition				
		2%	4%	6%	8%	10%	2%	4%	6%	8%	10%
1.	*Acorus calamus*	16.6[b]	15.2[b]	13.5[b]	12.0[b]	11.3[b]	81.3	82.9	84.8	86.5	87.3
2.	*Calotrophis jaijantia*	18.8[e]	17.9[e]	16.3[e]	15.2[e]	14.6[e]	78.8	79.8	81.6	82.9	83.5
3.	*Cinnamom zeylanicum*	17.2[c]	15.9[c]	14.3[c]	13.0[c]	12.2[c]	80.6	82.1	83.9	85.3	86.2
4.	*Coleus aromaticus*	23.6[i]	22.5[i]	21.1[i]	20.1[i]	19.5[i]	73.4	74.7	76.2	77.4	78.0
5.	*Curcuma longa*	18.1[d]	16.9[d]	15.3[d]	14.3[d]	13.5[d]	79.6	81.0	82.8	83.9	84.8
6.	*Datura stramonium*	22.9[h]	21.4[h]	19.8[h]	19.3[h]	18.3[h]	74.2	75.9	77.7	78.3	79.4
7.	*Eucalyptus globules*	16.2[a]	14.6[a]	12.7[a]	11.1[a]	10.2[a]	81.7	83.5	85.7	87.5	88.9
8.	*Eugenia caryphyllata*	16.0[a]	14.3[a]	12.5[a]	10.8[a]	9.6[a]	82.0	83.9	85.9	87.8	89.2
9.	*Ocimum sanctum*	19.8[f]	19.0[f]	17.3[f]	16.9[f]	16.2[f]	77.7	78.6	80.5	81.0	81.7
10.	*Vernnonia anthelmintica*	21.5[g]	20.8[g]	18.9[g]	18.1[g]	17.3[g]	75.8	76.6	78.7	79.6	80.5
	Carbendazim (0.1%)	7.0					92.1				
	Control	89.0					-				

Plant extracts as potential antifungal agents are being exploited against several plant diseases. Natural products from many plants were known to control plant pathogens (Grainage *et al*., 1987; Mitra *et al*., 1987) including *Sarocladium oryzae* (Jeeva *et al*., 1992; Selvaraj *et al*., 1994). Shivpuri *et al*. (1997) reported that the ethanol leaf extract of *Azadiracta indica*, *Datura stramonium*, Ocimum and *Polyalthiya longifolia* were toxic against *A. brassicola*, *C. capsici*, *F. oxysporum*, *R. solani* and *Sclerotinia sclerotiorum in vitro.*

In the present study among the various plant extracts *Eugenia caryophyllata* and *Eucalyptus globules* at 10% concentration were observed to be the most effective in inhibiting the mycelial growth, biomass production and spore germination of the pathogen under *in vitro* condition. Eugenol is the main component of clove oil which is one of the strongest inhibitors of enzyme processes and related compounds (Pepeljnjak *et al*. 2003). Antifungal and antibacterial activity of *Eucalyptus globules* may be due to the presence of eucalyptin, eucalypton and elligatannin compounds (Gutierrez *et al*. 1999). Antimicrobial activity of the clove oil can be attributed to the presence of an aromatic nucleus and a phenolic OH group that are known to be reactive and can form hydrogen bonds with –SH groups in the active sites of target enzymes, resulting in deactivation of enzymes in fungi (Velluti *et al*. 2003; Alma *et al*. 2007). Antifungal activity of clove extract which caused complete growth inhibition of *Rhizoctonia solani* causing root rot of pea (Abdulaziz *et al*. 2010) and sheath blight of rice (Anil sehajpal *et al*. 2009) was also reported.

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