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Efficacy of plant products and animal products for management of *Sarocladium oryzae* (Sawada) gams and hawks worth causing sheath rot disease in rice

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Abstract
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. Inhibition of radial growth varied significantly with different botanicals and animal products with their concentrations. Seven different plant products weeds such as Neem (*Azadirachta indica*), Garlic (*Allium sativam*), Ginger (*Zingiberofficinale*), Mehanthi (*Lawsonia inermis*), Kizhanelli (*Phyllanthus niroori*), Turmeric (*Curcuma longa*) and Notchi (*Vitex negundo*) were tested against *S. oryzae* was carried out by Poisoned food technique, Paper disc and Agar well method. Among the extracts, Mehanthi at the highest concentration (20%) was found to be minimum mycelial growth was recorded 11.10 mm and maximum reduction was recorded 42.17 and 39.45 mm. Seven different animal products such as Cow dung, Goat dung, Buffalo dung, Sheep dung, Hen litter, Cow urine and Buffalo urine were tested against *S. oryzae* was carried out by Poisoned food technique, Paper disc and Agar well method. Among the extracts, Cow dung and Hen litter at the highest concentration (20%) was found to be minimum mycelial growth was recorded 11.13 mm and maximum reduction was recorded 39.79 and 40.86 mm.

Keywords: Antifungal activity, animal products, plant products, *Sarocladium oryzae*, rice

Introduction
Rice is a global grain that is grown in about 89 nations and it is a stable food for more than half of the global population (Bodh and Rai, 2015) [5]. Rice crop suffers from a number of fungal, bacterial, viral and nematode disease. Sheath rot incited by present study. *Sarocladium oryzae* (Sawada) Gams and Hawksworth, is gaining importance due to widespread occurrence in almost all rice growing areas of the world. The fungus is detected frequently during routine seed health testing and causes empty grain production (Kulwanth and Mathur., 1992) [11] and glume discoloration (Sachan and Agarwal., 1995) [21] and also seed discoloration (Reddy *et al*., 2000) [20]. It also causes poor grain filling and reduction in seed germination (Vidhyasekaran *et al*., 1984) [28]. Seeds from infected panicles became discolored andsterile (Mew and Gonzales., 2002) [12]. Sheath rot disease, inflicts damage to the upper most flag leaf sheath by infecting the sheathing covering the young panicle at the booting stage. The young panicles are generally affected leading to an increase in the number of chaffy, discoloured and shriveled grains, and a reduction in weight and number of healthy grains (Najeeb *et al*., 2008) [14]. Lesions are oblong or irregular oval spots (0.5 to 1.5 cm length and 0.3 to 0.5 cm width) with an undulated dark brown margin and gray or light- brown centers or dark reddish – brown diffuse margin or the lesion may form an irregular target pattern (Tasugi and Ikeda., 1956) [26]. Use of fungicides to control the diseases causes several adverse effect *ie*, Development of resistance in the pathogen, Residual toxicity, Pollution in environment, High cost etc., The organic control of soil borne pathogens is a potential alternative to the use of chemicals. Grasela *et al*., 1990 [9] 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. The systematic search of plants for antifungal activity has shown that plants extracts have the ability to inhibit spore germination and mycelial growth in many species (Natarajan and Lalithakumari. 1987; Singh and Dwivedi., 1987) [15, 24]. Many plant extracts are reported to specifically inhibit the germination of fungal spores (Babu *et al*., 2001) [4]. Disease management through animal products is very important aspects to minimize the cost of cultivation. Cow dung is very effective's manures for reducing the bacterial and fungal pathogenic disease.

Akhtar and Malik (2000) [2] and Gamiliel *et al*., 2000 [6] reported that organic manure reduce disease incidence caused by a wide range of plant pathogens including bacteria, fungi, and nematode species. Therefore, application of cowdung in proper and sustainable way can enhance not only productivity of yield but also minimizing the chances of disease. Combination of animal excrements showed increased fungicidal activity against pathogens of several crops.

Materials and method

Isolation

The diseased samples were washed thoroughly with tap water. Small portion of infected parts containing healthy as well as diseased tissues were cut in to 0.5 cm pieces with the help of sterilized scalpel blade. These pieces were then surface sterilized with 1 percent sodium hypochlorite solution for 1 minute with 3subsequent changes in sterilized water to remove traces of the chemical. The pieces were then transferred aseptically to Petri dishes containing sterilized potato dextrose agar (PDA) and incubated at 28°C under BOD incubator. The Petri dishes were examined at regular time intervals for fungal growth radiating from the infected pieces.

Purification

In each Petriplate about 20 ml PDA medium was poured after supplementing with pinch of Streptomycin sulphate, to avoid bacterial contamination. One 9mm mycelial disc from a freshly isolated culture was transferred aseptically to the solidified PDA in each Petri plate. Adequate numbers of sub culture transformation were separately made for further purification. The finally purified culture of the pathogen was used for the studies of cultural characteristics of *S. oryzae*.

Evaluation of plant extracts against *S. oryzae* under *in-vitro*

Preparation of plant extracts

Fresh plant products were used for extraction. The collected plant part was washed with tap water followed by sterile distilled water. It was then processed with sterile distilled water @ one ml/g of tissue (1:1 w/v) with a pestle and mortar and filtered through a double layered cheese cloth. This formed the standard plant extract solution (100 per cent).

Poisoned food technique

Several plant products such as Neem, Garlic, Ginger, Mehanthi, Kizhanelli, Turmeric and Notchi were used for the study. The effect of plantproducts on the radial growth of the pathogen was studied by Poisoned food technique (Schmitz., 1930) [22]. Required quantity of plantproducts were mixed with autoclaved and cooled PDA just before pouring into Petri dishes, so as to obtain the required concentrations viz.,5%, 10%,15%, 20%. The medium was then dispensed uniformly into 90mm diameter Petri plates and inoculated with 9mm mycelia disc of the pathogen from 7 days old culture with their mycelial disc side down. Pathogen inoculated in unamended medium served as control. The growth of the fungus was monitored by measuring the radial growth in mm every each till the fungus covers the plates completely in control plates. The per cent inhibition (PI) of the fungus over control was calculated using the following formulae.

$$PI=(A-B)/A\times100$$

Where, A is colony diameter of the fungus in control plates (mm) and B is colony diameter of the fungus in treated plates (mm).

Agar well method (Thongson *et al*., 2004) [27]

Plant products extracts like 5%, 10%, 15%, 20% individually were added to the sterilized potato dextrose agar medium and thoroughly mixed just before plating. These mixtures individually were immediately poured into sterilized Petri plates and were allowed to solidify. A 9 mm of PDA disc was removed by using cork borer to form wells.1 ml of spore suspension was poured into well. All these were carried out under aspectic conditions. The plates were incubated at 28±2ºC for 10 days. Potato dextrose agar medium without animal products served as the control. Three replications were maintained. The radial growth of the colony was measured. The per cent inhibition of the growth was calculated.

Paper disc method (Saha *et al.*, 1995)

Spore suspension of the fungi was prepared from a ten day oldculture with sterile distilled water. Various concentrations like 5, 10, 15and 20 per cent of plant extracts were made. Twenty ml of PDA medium was seeded with three ml of spore suspension (1×10^6 spores/ml) of the fungus and was allowed to solidify. Sterile filter paper discs (10 mm)were dipped separately in known concentrations of treatment and placed equidistantly over the seeded medium. Three replications were maintained for each treatment. The plates were incubated at 28 ± 2°C for 10days. The inhibition zone of the fungal growth around the treated paper discs was measured and recorded.

Evaluation of animal products against *S. oryzae* under *in-vitro*

Preparation of animal products

Preparation of animal dung extracts (Sundar raj *et al.*, 1996) [25]

Selected animal dungs (Cow dung, Buffalo dung, Goat dung, Sheep dung Hen litter) were collected, shade dried for one week and made into powder. The powdered animal dungs were soaked in sterile distilled water separately @ 2 ml/g (2:1 w/v) and kept overnight. The materials were then filtered through cheese cloth. This formed the standard animal dung extracts solution (100 per cent). After extraction they were subjected to low speed centrifuge (5000 rpm for 5 mins) and the clear supernatant were used for the further studies.

Preparation of animal urine (Raja and Kurucheve., 1998) [18]

Freshly collected urine was used as such forming the standard extract (100 per cent). The extract was diluted by adding equal amount of distilled water to form required concentrations.

Poisoned food technique

Several animal products such as Cow dung, Cow urine, Buffalo dung, Buffalo urine, Goat dung, Sheep dung and Hen litter were used for the study. The effect of animal products on the radial growth of the pathogen was studied by Poisoned food technique (Schmitz., 1930) [22]. Required quantity of animal products were mixed with autoclaved and cooled PDA just before pouring into Petri dishes, so as to obtain the required concentrations viz.,5%, 10%,15%, 20%. The medium was then dispensed uniformly into 90mm diameter Petri plates and inoculated with 9mm mycelia disc of the pathogen from 7 days old culture with their mycelial disc side

down. Pathogen inoculated in unamended medium served as control. The growth of the fungus was monitored by measuring the radial growth in mm every each till the fungus covers the plates completely in control plates. The per cent inhibition (PI) of the fungus over control was calculated using the following formulae.

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Result and discussion

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

In present study seven plant extracts were tested against *S. oryzae* and presented in Table 1. All the tested plant extracts registered appreciable inhibition in colony growth. Among seven plant extracts, Mehanthi (*Lawsonia inermis*) at a highest concentration (20%) was found to be the maximum was recorded 11.10 mm inhibiting the mycelial growth of *S. oryzae* by poisoned food technique. Plant extracts were selected and evaluated for the antimicrobial activity by agar well and paper disc method. The extract of Mehanthi (*Lawsonia inermis*) at a highest concentration (20%) was found to be the maximally reduced in agar well and paper disc method was recorded 42.17 and 39.45 mm inhibition zone respectively. It was followed by a highest concentration (20%) of Neem which recorded 39.12 and 36.67 mm Table 2.Generally all plant extracts inhibited the mycelia growth of pathogen in the present study. This statement has been confirmed by several workers. Plant extracts as potential antifungal agents are being exploited against several diseases. Natural products from many plants were known to control plant pathogens (Mitra *et al*., 1987) [13] including *Sarocladium oryzae* (Jeeva *et al*., 1992; Selvaraj *et al*., 1994) [10, 23]. Antifungal activity of clove extract which caused complete growth inhibition of *Rhizoctonia solani* causing root rot of pea (Abdulaziz *et al*., 2010) [1] and sheath blight of rice (Anil sehajpal *et al*., 2009) [3] was also reported.

In-vitro* evaluation of animal extracts against *S. oryzae

In present study seven animal extracts were tested against S. *oryzae* and presented in Table 3. All the tested animal extracts registered appreciable inhibition in colony growth. Among animal extracts cow dung at a highest concentration (20%) was found to be the maximum was recorded 11.13 mm percent inhibiting the mycelial growth of *S. oryzae* by poisoned food technique. Animal extracts were selected and evaluated for the antimicrobial activity by agar well and paper disc method. The extract of cow dungat a highest concentration (20%) was found to be the maximally reduced in agar well and paper disc method was recorded 39.79 and 40.86 mm inhibition zone respectively. It was followed by a highest concentration (20%) of Hen litter which recorded 37.51 and 39.15 mm inhibition (Table 4). Generally all animal extracts inhibited the mycelia growth of pathogen in the present study. This statement has been confirmed by several workers. Cow urine at 20 per cent concentration completely inhibited the mycelia growth of *S. oryzae* (Rajendra Prasad 2001) [19]. Hen litter extracts at 20 per cent concentration completely inhibited the mycelia growth of *M. phaseolina* and *S. rolfsii* (Senthilkumar., 2000). Animal excreta containing stronger and higher levels of antimicrobial principles will be effective at low concentrartions (Gerardezhilan and Kurucheve., 1994; Raja and Kurucheve., 1997) [8, 17].

Table 1: Effect of various plant extracts on mycelial growth of *S. oryzae* by poison food technique under in-vitro condition.

S. No	Plant extracts	Mycelial growth of *S. oryzae* (mm)							
		5%	Percent inhibition over control	10%	Percent inhibition over control	15%	Percent inhibition over control	20%	Percent inhibition over control
1.	Neem- *Azadirachta indica*	39.46^{f}	56.15	34.78^{fg}	61.35	27.36^{ef}	69.60	12.56^{ef}	86.04
2.	Garlic- *Allium sativam*	41.79^{e}	53.56	35.67^{ef}	60.36	29.67^{de}	67.03	14.25^{e}	84.16
3.	Ginger- *Zingiber officinale*	47.30^{c}	47.44	41.46^{bc}	53.93	35.98^{bc}	60.02	19.28^{c}	78.57
4.	Mehanthi- *Lawsonia inermis*	37.70^{f}	58.11	32.58^{g}	63.80	25.81^{f}	71.32	11.10^{f}	87.66
5.	Notchi- *Vitex negundo*	44.15^{d}	50.94	37.20^{de}	58.66	31.57^{d}	64.92	16.47^{d}	81.70
6.	Turmeric- *Curcuma longa*	50.31^{b}	44.10	43.67^{b}	51.47	37.30^{b}	58.55	21.68^{b}	75.91
7.	Kizhanelli- *Phyllanthus niroori*	45.89^{cd}	49.01	39.28^{cd}	56.35	34.29^{c}	61.90	17.35^{cd}	80.72
8.	Control	90.00^{a}	-	90.00^{a}	-	90.00^{a}	-	90.00^{a}	-
SEd CD (p=0.05)		2.219		2.245		2.468		1.995	

*Values are means of three replications. In a column, means followed by a common letter are not significantly different at 5% level by DMRT's

Table 2: Antifungal activity of Plant extracts against *S. oryzae* under in-vitro condition.

S. No	Plant extracts	Inhibition Zone (mm)									
		Paper disc method					Agar well method				
		5%	10%	15%	20%	Mean	5%	10%	15%	20%	Mean
1.	Neem- *Azadirachtaindica*	34.57^{b}	36.20^{b}	38.82^{b}	39.12^{a}	37.17	33.02^{a}	34.13^{b}	34.98^{b}	36.67	34.70^{b}
2.	Garlic- *Alliumsativam*	32.71^{c}	34.27^{c}	35.78^{c}	37.33^{c}	35.02	31.35^{b}	32.16^{c}	33.23^{c}	35.12	32.96^{b}
3.	Ginger- *Zingiberofficinale*	27.21^{f}	29.34^{e}	31.89^{e}	32.56^{e}	30.25	27.14^{d}	28.05^{e}	28.78^{e}	29.12	28.27^{d}
4.	Mehanthi- *Lawsoniainermis*	35.80^{a}	38.21^{a}	40.35^{a}	42.17^{a}	39.13	34.01^{a}	36.98^{a}	38.78^{a}	39.45	37.30^{a}
5.	Notchi- *Vitexnegundo*	30.35^{d}	33.56^{c}	37.57^{b}	39.35^{b}	35.20	29.93^{bc}	30.66^{cd}	31.12^{d}	32.68	31.09^{c}
6.	Turmeric- *Curcumalonga*	25.67^{g}	26.98^{f}	28.66^{f}	30.41^{f}	27.93	24.98^{e}	25.29^{f}	27.45^{f}	28.68	26.60^{d}
7.	Kizhanelli - *Phyllanthusniroori*	28.35^{e}	31.70^{d}	33.77^{d}	35.36^{d}	32.29	29.12^{c}	29.79^{d}	30.12^{d}	31.55	30.14^{c}
8.	Control	0.00^{h}	0.00^{g}	0.00^{g}	0.00^{g}	0.00	0.00^{f}	0.00^{g}	0.00^{g}	0.00	0.00^{e}
CD (p=0.05)		0.86	1.07	1.52	1.55		1.53	1.58	1.30	1.64	

*Values are means of three replications. In a column, means followed by a common letter are not significantly different at 5% level by DMRT's

Table 3: Effect of various animal extracts on mycelial growth of *S. oryzae* by poison food technique under in-vitro condition.

S. No	Animal extracts	Mycelial growth of*S. oryzae* (mm)							
		5%	Percent inhibition over control	10%	Percent inhibition over control	15%	Percent inhibition over control	20%	Percent inhibition over control
1.	Hen litter	40.67^{e}	54.81	31.20^{f}	65.33	23.71^{f}	73.65	12.54^{g}	86.06
2.	Cow urine	42.23^{e}	53.07	34.89^{e}	61.23	25.65^{e}	71.50	14.16^{f}	84.26
3.	Cow dung	39.86^{e}	55.71	28.51^{g}	68.32	20.45^{g}	77.27	11.13^{h}	87.63
4.	Sheep dung	50.08^{c}	44.35	41.12^{c}	54.31	32.89^{b}	63.45	21.30^{c}	76.33
5.	Goat dung	53.23^{b}	40.85	44.25^{b}	50.83	33.18^{b}	63.13	24.41^{b}	72.87
6.	Buffalo dung	45.21^{d}	49.76	37.23^{d}	58.63	28.34^{d}	68.51	16.49^{e}	81.67
7.	Buffalo urine	48.66^{c}	45.93	38.12^{d}	57.64	31.23^{c}	65.30	19.96^{d}	77.82
8.	Control	90.00^{a}	-	90.00^{a}	-	90.00^{a}	-	90.00^{a}	-
CD (p=0.05)		2.37	-	2.23	-	1.66	-	1.09	-

*Values are means of three replications. In a column, means followed by a common letter are not significantly different at 5% level by DMRT's

Table 4: Antifungal activity of Animal extracts against *S. oryzae* under in-vitro condition.

S. No	Animal extracts	Inhibition Zone (mm)									
		Paper disc method					Agar well method				
		5%	10%	15%	20%	Mean	5%	10%	15%	20%	Mean
1.	Hen litter	32.54^{a}	33.77^{a}	35.10^{a}	37.51^{a}	34.73	33.24^{b}	34.76^{b}	36.81^{b}	39.15^{ab}	35.99
2.	Cow urine	30.19^{b}	31.24^{b}	33.39^{b}	36.01^{b}	32.70	31.42^{c}	33.10^{b}	35.76^{bc}	37.83^{bc}	34.52
3.	Cow dung	33.56^{a}	34.78^{a}	36.14^{a}	39.79^{a}	36.06	35.82^{a}	36.57^{a}	38.34^{a}	40.86^{a}	37.89
4.	Sheep dung	22.12^{e}	24.65^{e}	27.28^{e}	29.70^{e}	25.93	23.84^{f}	25.57^{e}	28.38^{e}	30.08^{e}	26.96
5.	Goat dung	20.35^{f}	23.13^{e}	24.45^{f}	27.72^{f}	23.91	20.14^{g}	22.55^{f}	24.95^{f}	27.03^{f}	23.66
6.	Buffalo dung	27.24^{c}	29.30^{c}	31.23^{c}	34.21^{c}	30.49	29.14^{d}	32.04^{c}	33.24^{c}	36.72^{c}	32.78
7.	Buffalo urine	24.37^{d}	26.32^{d}	28.72^{d}	31.13^{d}	27.63	26.36^{e}	28.93^{d}	31.29^{d}	33.66^{d}	30.06
8.	Control	0.00^{g}	0.00^{f}	0.00^{g}	0.00^{g}	0.00	0.00^{h}	0.00^{g}	0.00^{g}	0.00^{g}	0.00
CD (p=0.05)		1.16	1.55	1.20	1.47	-	1.59	1.47	1.50	1.76	-

*Values are means of three replications. In a column, means followed by a common letter are not significantly different at 5% level by DMRT's

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