



## RESEARCH PAPER

# Efficacy of fungicides, phytoextracts and cow urine against seed mycoflora of rice

R.G. Parmar\* and Dipan R. Patel

Department of Plant Pathology, B.A. College of Agriculture, Anand Agricultural University, Anand (Gujarat) India

**Abstract :** Rice (*Oryza sativa* L.) is an important cereal crop belongs to the family *Poaceae* and native to south-east Asia. Rice crop needs a hot and humid climate. It is best suited to regions which have high humidity, prolonged sunshine and assured supply of water. It is an indispensable cereal essentially used in daily Indian meal in the form of dal-rice, roti, many south Indian foods and alcoholic beverages. Rice suffers heavy yield losses from diseases caused by fungi, bacteria and viruses many of which are carried through seed. Major seed borne fungi infecting rice includes *Fusarium oxysporum*, *Fusarium moniliforme*, *Aspergillus niger*, *Aspergillus flavus*, *Alternaria padwickii* and *Curvularia lunata*. Among all the fungicidal treatments, carbendazim + mancozeb gave minimum per cent seed mycoflora (5.00%). Among all the phytoextracts treatments, minimum per cent seeds showed mycoflora was by neem (9.66%) and lowest mycelial growth and highest growth inhibition per cent found in 15% concentration cow urine.

**Key Words :** Mycoflora, *Aspergillus flavus*, Carbendazim + mancozeb, Phytoextracts

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## INTRODUCTION

Rice (*Oryza sativa* L.) belongs to family *Poaceae* and genus *oryza*. It is a plant of Asian origin. More than 90 per cent of the developing world's rice area is found in South and East Asia. Rice is one of the most important cereal crops of the country, extensively grown in most of states except a few like Himachal Pradesh, Rajasthan and Jammu and Kashmir. Rice is the major staple food for more than 70 percent of the Indian population. In Gujarat most of the area under rice crop is confined to Middle and South Gujarat. Seed borne pathogens can perpetuate from season to season through infected seed

(Zope and Thrimurty, 2004). Nearly 220 species of fungi besides 14 viruses, 12 species of bacteria and 2 phytoplasma are reported to be involved in rice diseases in tropical and sub-tropical countries (Raju, 2000). In India, nearly 10 per cent loss of rice is due to seed borne diseases (Pandey et al., 1988). Seed borne fungi play a vital role in reducing yield and deteriorating seed health status. Rice being a water loving crop, chances of fungal infection to developing seeds are relatively more. Rice seeds are known to harbour large number of mycoflora both within and on surface. Various species of *Alternaria*, *Bipolaris*, *Botrytis*, *Cephalosporium*, *Cercospora*, *Chaetomium*, *Cladosporium*, *Curvularia*, *Drechslera*,

\* Author for correspondence :

*Ephelis*, *Epicoccum*, *Fusarium*, *Gibberella*, *Helicocercus*, *Helminthosporium*, *Hemnoniella*, *Mycogone*, *Nigrospora*, *Phacetrichoconis*, *Phoma*, *Pseudocercospora*, *Pyrenochacte*, *Pyricularia*, *Rhizoctonia*, *Rhizopus*, *Sclerotium*, *Spermospora*, *Sphaeropsis*, *Stachybotrys*, *Stemphylium*, *Thielvia*, *Trichoconis* and *Verticillium* have been known to be seed-borne in rice seeds (Gangopadhyay, 1983).

## MATERIAL AND METHODS

Six fungicides viz. mancozeb 75 WP, copper oxychloride 50 WP, azoxystrobin 23 SC, carbendazim 50 WP, carbendazim 25 % + mancozeb 50 % WS and carboxin 37.5 % + Thiram 37.5 % WS were used for their toxicity to different seed mycoflora. The required concentration of fungicidal solution for seed treatment of each fungicide under the study was prepared on the basis of active ingredient available in the formulation. The seeds were treated with fungicidal solution as well as with the plant extract solution for 20 minutes. After treating the seeds, treated/coated seeds were placed in already poured PDA medium (20 ml/plate). For control/check seeds without treatments were placed in the PDA plates. After 5-6 days observations were recorded by calculating the mycoflora load of those particular rice cultivars with treated seeds as well as control.

For preparation of phytoextracts fresh and healthy leaves of different plants were collected and washed thoroughly with running tap water. These leaves were cut into small pieces and macerated in sterilized distilled water (1:1 w/v basis) by blender. Resulting crude extract of each plant was filtered through single layer of sterilized muslin cloth. Filtered extracts were considered as standard (100 %) solutions. The standard extracts were further diluted to the 10 % concentrations by adding required quantity of water. The procedures of phytoextracts were same as fungicidal treatment.

The fresh cow urine was collected from livestock research Station, AAU, Anand. Collected urine was considered as standard suspension (100 %). Cow urine was used for treating the seed by dipping.

### ***In vitro* study of the antifungal activity of cow urine against seed mycoflora of rice cultivars:**

Cow urine taken freshly, was filtered with the help Whatman's filter paper No. 1 under aseptic conditions in the laminar air flow chamber. The filtrate obtained was mixed with sterilized water to have the final

concentrations viz. 5%, 7.5%, 10% 12.5% and 15% and control, respectively. These concentrations were ready for use to check the efficacy of cow urine against seed mycoflora of rice cultivars. In control plate, no cow urine was mixed.

### ***In vitro* bioassay of cow urine:**

Effect of cow urine at different concentrations on the radial growth of the test fungus was evaluated by poisoned food technique on potato dextrose agar (PDA) medium. Different concentrations of cow urine were taken viz. 5%, 7.5%, 10%, 12.5% and 15%. The desired amount of the cow urine suspension was added in the medium and mixed thoroughly before plating. PDA medium was toxicated with cow urine and poured in each Petri plates. Subsequently a 5 mm mycelial disc of 4 days old culture of seed mycoflorawere cut with sterile cork borer and placed in centre of each Petri plates. The plates were incubated at 25± 2°C. The diameter of the fungal colony was measured after 4 days of incubation. After 4 days of incubation per cent inhibition was calculated by using the following formula given by Vincent (1947):

$$\text{Per cent inhibition} = \frac{C - T}{T} \times 100$$

where,

C = colony diameter in check

T = colony diameter on amended medium

## RESULTS AND DISCUSSION

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

### **Fungicides:**

Six fungicides were evaluated *in vitro* (mancozeb, copper oxychloride, azoxystrobin, carbendazim, carbendazim + mancozeb and carboxin + thiram) at their respective concentrations against seed mycoflora and results revealed significant differences in per cent seeds showing mycoflora (Table 1). However, carbendazim + mancozeb @ 0.3 per cent (T<sub>3</sub>) revealed minimum number of fungal species (3) and minimum per cent seeds showed mycoflora was 0.75 followed by carboxin + thiram @ 0.1 per cent (T<sub>6</sub>) and azoxystrobin @ 0.1 per cent (T<sub>3</sub>). Better performance of fungicide carbendazim can be attributed due to their systemic nature.

**Phytoextracts:**

*In vitro* evaluation five plant extracts were used (neem, tamarind, dhatura, naffatiya and ginger) at their respective against seed mycoflora revealed significant differences in per cent seeds showing mycoflora growth (Table 2). However, neem @ 0.3 per cent (T<sub>1</sub>) revealed minimum number of fungal species (3) and minimum per cent seeds showed mycoflora was 0.75 followed by

ginger @ 0.1 per cent (T<sub>5</sub>). Ketaet *et al.* (2019) studied on effect of neem (*Azadirachtaindica* A. Juss) leaf extract on the growth of *A. niger*, *A. flavus*, *A. fumigatus* and *A. nidulance*. The efficacy of the extracts was studied and determined by applying in different concentrations. Result shown that statistically, the ethanolic extract was more effective than aqueous extract in all cases. However, the effectiveness of the extracts was

**Table 1: Evaluation of fungicides against rice mycoflora by PDA plate method under *in vitro* condition**

Treatment no.	Treatment	Conc. (%) / Dose	Per cent seeds sowing mycoflora					Total fungal species	Total (%)
			<i>F. oxysporum</i>	<i>A. niger</i>	<i>A. flavus</i>	<i>C. lunata</i>			
T <sub>1</sub>	Mancozeb 75 WP	0.2	8.00	9.00	7.00	4.00	4	28.00	
T <sub>2</sub>	Copper oxychloride 50 WP	0.2	6.00	7.33	4.33	3.00	4	20.66	
T <sub>3</sub>	Azoxystrobin 23 SC	0.2	3.33	5.00	4.00	0.00	3	12.33	
T <sub>4</sub>	Carbendazim 50 WP	0.2	5.00	6.33	3.33	1.67	4	16.33	
T <sub>5</sub>	Carbendazim 25 % + mancozeb 50 % WS	0.2	1.67	2.33	1.00	0.00	3	5.00	
T <sub>6</sub>	Carboxin 37.5 % + thiram 37.5 % WS	0.2	4.00	3.67	2.67	0.00	3	10.34	
T <sub>7</sub>	Control		11.33	13.33	8.67	7.00	4	40.33	
		S. E. ±	0.22	0.36	0.25	0.13			
		C. D. (P=0.05)	0.66	1.08	0.76	0.38			
		C. V. %	6.73	9.19	9.86	9.75			

**Table 2: Effect of plant extracts against rice mycoflora by PDA plate method under *in vitro* condition**

Treatment no.	Treatments	Plant parts used	Conc. (%) / Dose	Seed mycoflora (%)				Total fungal species	Total (%)
				<i>F. oxysporum</i>	<i>A. niger</i>	<i>A. flavus</i>	<i>C. lunata</i>		
T <sub>1</sub>	Neem	Leaves	10	3.33	4.33	2.00	0.00	3	9.66
T <sub>2</sub>	Tamarind	Leaves	10	8.67	10.33	7.33	5.00	4	31.33
T <sub>3</sub>	Dhatura	Leaves	10	5.67	7.33	5.00	1.33	4	19.33
T <sub>4</sub>	Naffatiya	Leaves	10	6.33	8.67	4.33	3.00	4	22.33
T <sub>5</sub>	Ginger	Rhizome	10	4.33	5.67	3.33	0.00	3	25.67
T <sub>6</sub>	Control			11.67	16.67	8.67	7.00	4	44.01
			S. E. ±	0.33	0.33	0.27	0.14		
			C.D. (P=0.05)	1.03	1.03	0.84	0.42		
			C. V. %	8.66	6.54	9.22	8.66		

**Table 3: Effect of cow urine against rice mycoflora under *in vitro* condition**

Sr. No.	Cow urine (%)	Mycelial growth (mm)*				Growth inhibition (%)			
		Mycoflora				Mycoflora			
		<i>F. oxysporum</i>	<i>A. niger</i>	<i>A. flavus</i>	<i>C. lunata</i>	<i>F. oxysporum</i>	<i>A. niger</i>	<i>A. flavus</i>	<i>C. lunata</i>
1.	5.0	54.73	90.00	85.48	45.74	39.19	0.00	5.02	49.18
2.	7.5	48.38	90.00	75.74	37.05	46.24	0.00	15.84	58.83
3.	10.0	36.76	77.07	73.31	28.43	59.16	14.37	18.54	68.41
4.	12.5	21.24	52.26	68.47	15.72	76.40	41.93	23.92	82.53
5.	15.0	11.59	35.84	61.76	05.04	87.12	60.18	31.38	94.40
6.	Control (without cow urine)	90.00	90.00	90.00	90.00	-	-	-	-
		S.E.±	0.20	0.20	0.22	0.13			
		C.D. (P=0.05)	0.62	0.61	0.62	0.41			
		C.V.%	7.96	4.72	5.12	6.18			

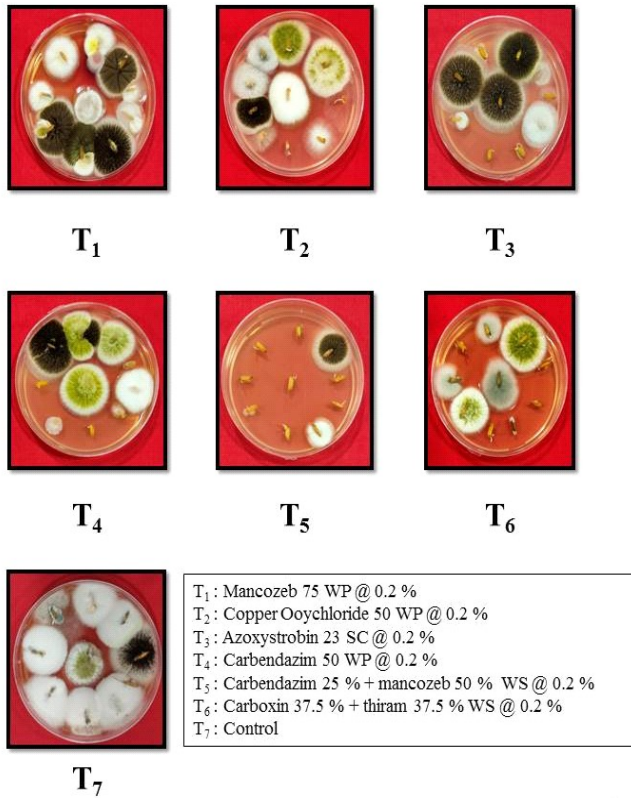


Fig. 1 : Management of seed mycoflora by fungicides

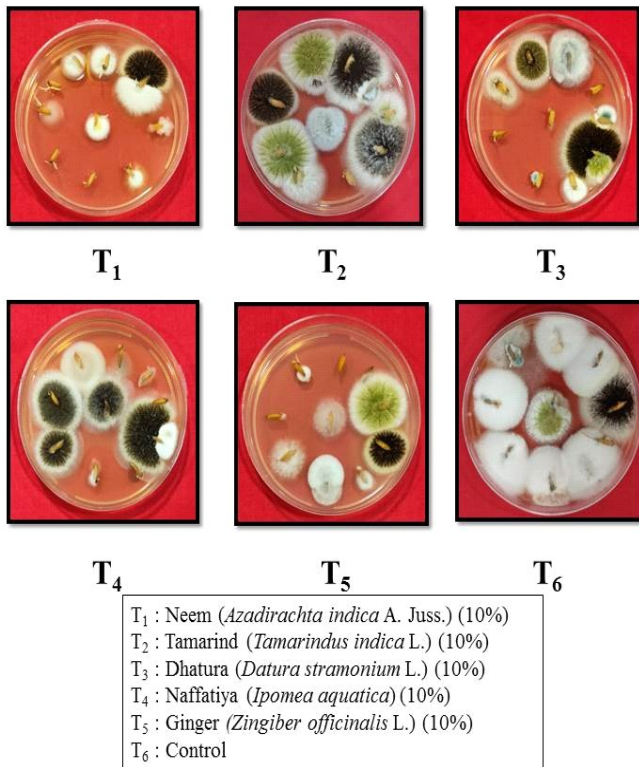


Fig. 2 : Management of seed mycoflora by phtoextracts

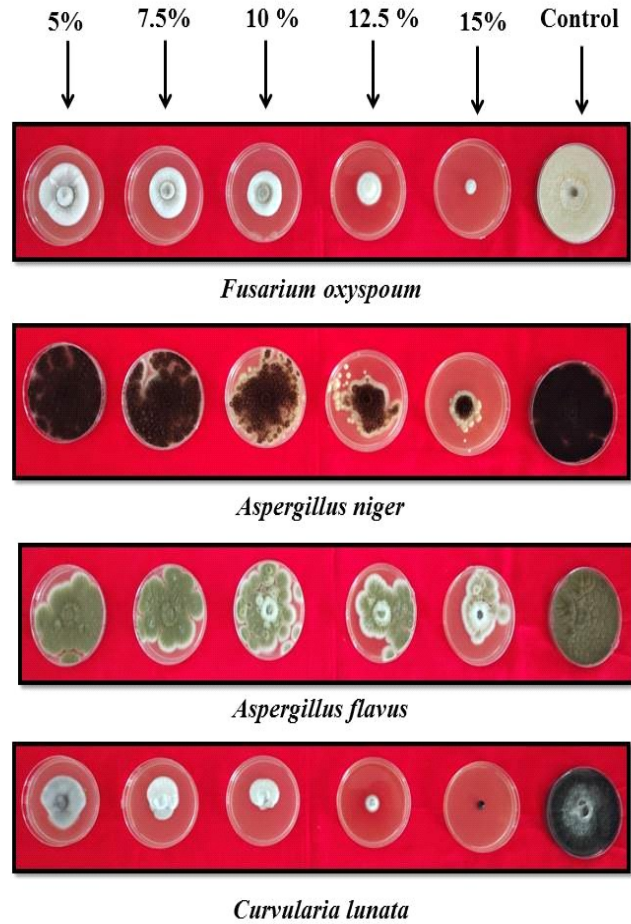


Fig. 3 : Management of seed mycoflora by cow urine

dependent on the concentration used.

**Cow urine:**

*In vitro* evaluation of cow urine at different concentrations (5, 7.5, 10, 12.5, and 15%) was evaluated against mycoflora. The data depicted in table 3 revealed that cow urine with the 15% concentration (T<sub>5</sub>) gave minimum mycelial growth (11.59, 35.84, 61.76 and 5.04 mm) and maximum growth inhibition (87.12, 60.18, 31.38 and 94.40%) with *F. oxysporum*, *A. niger*, *A. flavus* and *C. lunata*, respectively. Jandaiket *al.* (2015) studied antifungal activity of three different concentrations (5, 10, and 15%) of cow urine against three fungal pathogens (*F. oxysporum*, *R. solani*, and *S. rolfsii*). The extent of growth of test fungi in plates poisoned with cow urine was lesser when compared with the control plates. Finally they concluded that the cow urine has antifungal activities and the inhibitory activity can be used in the control of fungi.

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