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Akhilesh Kumar Kulmitra
Department of Plant Pathology, University of Agricultural Sciences, GKVK, Bangalore-560065

Neha Sahu
Department of Soil Science and Agricultural Chemistry, Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.)

Durgesh Kumar Sahu
Department of Plant Pathology, Indira Gandhi Krishi Vishwavidyalaya, Raipur (C.G.)

VB Sanath Kumar
Department of Plant Pathology, University of Agricultural Sciences, GKVK, Bangalore-560065

Correspondence
Akhilesh Kumar Kulmitra
Department of Plant Pathology, University of Agricultural Sciences, GKVK, Bangalore-560065

In vitro evaluation of animal products against *Pyricularia oryzae* (Cav.) causing rice blast disease

Akhilesh Kumar Kulmitra, Neha Sahu, Durgesh Kumar Sahu and VB Sanath Kumar

Abstract
The present *In-vitro* study was conducted at pathology laboratory, University of agricultural sciences, GKVK, Bangalore, Karnataka, India during February 2016 to evaluate animal products against rice blast disease caused by *Pyricularia oryzae* (Cav.). The five different animal products *viz.,* cow milk, cow urine, butter milk, cow ghee and cow dung were tested at three different concentrations of 10, 20 and 30% by using the poisoned food technique. Among the five different animal products, highest percent inhibition of mycelial growth of fungus was recorded in cow ghee (96.33, 96.50 and 98.90%) at all the concentration tested with mean of 97.24 percent followed by butter milk with the inhibition of 86.07, 87.93 and 88.73 percent respectively with mean of 87.57 percent. Minimum inhibition was observed in cow dung, with 45.37, 53.67 and 67.17 percent inhibition at 10, 20 and 30 percent concentration respectively with a mean of 55.40 percent. In general, the inhibition of radial growth of fungus increased with increase in concentration of each animal product.

Keywords: Animal products, mycelial growth, percent inhibition, poisoned food technique, *Pyricularia oryzae*

1. Introduction
Rice is central to the lives of billions of people around the world. Rice was originally cultivated in tropical Asia, the oldest record dating 5000 years BC, Possibly the oldest domesticated grain (~10,000) years but then extended also to temperate regions [14]. Rice is the most important staple food in Asia. More than 90% of the world's rice is grown and consumed in Asia, where 60% of the world's population lives. Rice accounts for between 35-60% of the caloric intake of three billion Asians [6].

Calories from rice are particularly important in Asia, especially among the poor, where it accounts for 50-80% of daily caloric intake [10]. Asia accounted for 60% of the global population, about 92% of the world's rice production, and 90% of global rice consumption.85% of the rice that is produced in the world is used for direct human consumption. Rice can also be found in cereals, snack foods, brewed beverages, flour, oil, syrup and religious ceremonies to name a few other uses [10].

Rice is grown under many different conditions and production systems, but submerged in water is the most common method used worldwide. Rice is the only cereal crop that can grow for long periods of time in standing water [4]. The flooded rice paddy is a field of aquatic biodiversity, providing a home for fish, plants, amphibians, reptiles, mollusks, and crustaceans, which many of can be used as a means to incorporate protein into the diets of poor and malnourished people in low and middle income countries that farm rice [9].

The world's estimated rice production is 496.0 million metric tons during 2016 [2]. India is the largest rice growing country accounting for about one third of the world acreage under the crop. In India's annual rice production is 103.6 million tons during 2016 [2]. Rice is grown throughout India in all the states. The major rice growing states of India are West Bengal, Uttar Pradesh, Bihar, Madhya Pradesh, Orissa, Andhra Pradesh, Karnataka and Chhattisgarh [8].

Rice suffers from many diseases caused by fungi, bacteria, viruses, phytoplasma, nematodes and other non-parasitic disorders [10]. Among the fungal diseases, blast is considered as a major threat to rice production because of its wide spread distribution and its destructiveness under favourable conditions [11]. The Commonwealth Mycological Institute has recorded its presence from 85 countries throughout the world [10]. Paddy blast is generally considered as the principal disease of rice and is caused by a fungus belonging to the Ascomycete *Pyricularia*

oryzae Cavara (teleomorph= *Magnaporthe grisea* (Hebert) Barr Comb nov.) [10, 15]. Losses due to the blast disease may range up to 90 percent depending upon the component of the plant infected. *M. grisea* infects above ground parts of the plant, but neck blast and the panicle blast are the most damaging phases of the disease and have been shown to significantly reduce yield, grain weight and milling quality [4]. The pathogen may infect all the above ground parts of a rice plant at different growth stages *viz.,* leaf, collar, node, internodes, base or neck and other parts of the panicle and sometimes the leaf sheath. A typical blast lesion on a rice leaf is gray at the centre, has a dark border and it is spindle-shaped [3].

2. Material and methods

The present *In-vitro* study was conducted at pathology laboratory, University of agricultural sciences, GKVK, Bangalore, Karnataka, India during February 2016 to evaluate animal products against rice blast disease caused by *Pyricularia oryzae* (Cav.).

2.1 Collection of animal products

Fresh sample of each test product (Table 1) were collected from dairy unit of University of agricultural sciences, Bangalore, then prepare 10, 20 and 30 percent concentrations of each animal product.

2.2 Experimental set up

To study the anti-fungal activity of animal products (Table 1), the poison food technique was followed, to prepare 10, 20 and 30 percent concentrations of animal product. Ten, twenty and thirty ml of stock solution was mixed with 90, 80 and 70 ml of sterilized molten PDA medium respectively. The medium was thoroughly shaken for uniform mixing of the extract. Twenty ml of molten media was poured into 90mm sterilized petriplates. Each plate was inoculated with 5mm mycelial disc taken from the periphery of seven day old *P. oryzae* culture and incubated at 28 ± 1^0 C till the growth of colony touched the periphery in the control plate. The disc was placed upside down in the centre of the petriplates, so that the mycelium was in direct contact with the medium poisoned with the requisite animal product at required concentration.

Three replication were maintained in each treatment. Suitable control plates were maintained where in culture discs were incubated into the centre of PDA plates without animal product. Mean colony diameter in each case was recorded by taking the diameter of the colony in two directions. Radial growth of the fungus was measured and percent inhibition of mycelial growth over control was calculated by using the formula given by [13].

$$I = \frac{(C-T)}{C} X\ 100$$

Where,

I= Percent inhibition

C= Growth of the fungus in control

T= Growth of the fungus in treatment

Table 1: List of animal products used for *in vitro* evaluation against *P. oryzae*

Sl. No.	Animal products
1	Cow urine
2	Cow dung
3	Cow butter milk
4	Cow ghee
5	Cow milk

2.3 Statistical analysis

Analysis and interpretation of the experimental data was done by using completely randomized design (CRD) and Factorial CRD for laboratory studies ANOVA [5, 7].

3. Results and Discussion

Efficacy of five animal products *viz.,* cow milk, cow urine, butter milk, cow ghee and cow dung were tested at three different concentrations (10, 20 and 30%) each by following poisoned food technique. The percent inhibition over control was worked out based on the test fungal growth in control plate. The results thus obtained are presented in Table 2 and depicted in Fig. 1 and Plate 1.

Among the all five animal products the highest percent inhibition of mycelial growth was recorded in cow ghee (96.33, 96.50 and 98.90%) at all the concentration (10, 20 and 30%) with mean of 97.24 percent followed by butter milk with the inhibition of 86.07, 87.93 and 88.73 percent at 10, 20 and 30 percent concentration respectively with mean of 87.57 percent. In cow milk 42.10, 53.83 and 82.73 percent inhibition at 10, 20 and 30 percent concentrations with mean 59.50 percent was recorded respectively. In cow urine 44.13, 44.57 and 84.30 percent inhibition with mean 57.44 percent was recorded at 10, 20 and 30 percent concentrations respectively. Minimum inhibition was observed in cow dung, with 45.37, 53.67 and 67.17 percent inhibition at 10, 20 and 30 percent concentration respectively with a mean of 55.40 percent.

Disease management through animal products is very important aspects to minimize the cost of cultivation. Among five animal products, cow ghee recorded maximum mycelial inhibition of 96.33, 96.50 and 98.90 percent at all the concentration 10, 20 and 30 percent tested with mean of 97.24 percent followed by butter milk with the inhibition of 86.07, 87.93 and 88.73 percent at 10, 20 and 30 percent concentration respectively with mean of 87.57 percent. Minimum inhibition was observed in cow dung, with 45.37, 53.67 and 97.17 percent inhibition at 10, 20 and 30 percent concentration respectively with a mean of 55.40 percent. The results obtained are confirmatory with the findings of [1, 12] who recorded the maximum inhibition of mycelial growth of *P. oryzae* in cow ghee.

Table 2: *In vitro* evaluation of animal products against rice blast fungus *P. oryzae*

Treatments	Animal products	Mean percent mycelial inhibition			
		Concentration of animal products (%)			
		10	20	30	Mean (%)
T1	Cow milk	42.10	53.83	82.73	59.50
T2	Cow urine	44.13	44.57	84.30	57.44
T3	Butter milk	86.07	87.93	88.73	87.57
T4	Cow ghee	96.33	96.50	98.90	97.24
T5	Cow dung	45.37	53.67	67.17	55.40
		Animal products	**Concentration**	**T X C**	
	SEm±	0.21	0.16	0.36	
	C.D at 1%	0.82	0.63	1.42	
	CV %	**1.8**			

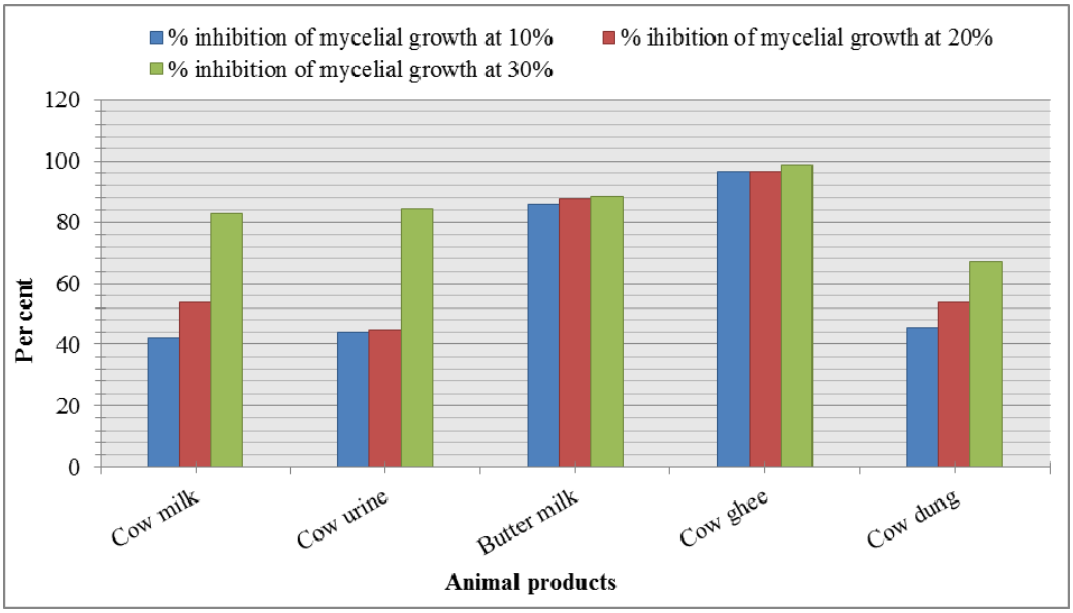

Fig 1: Effect of animal products on the mycelial growth of *P. oryzae*

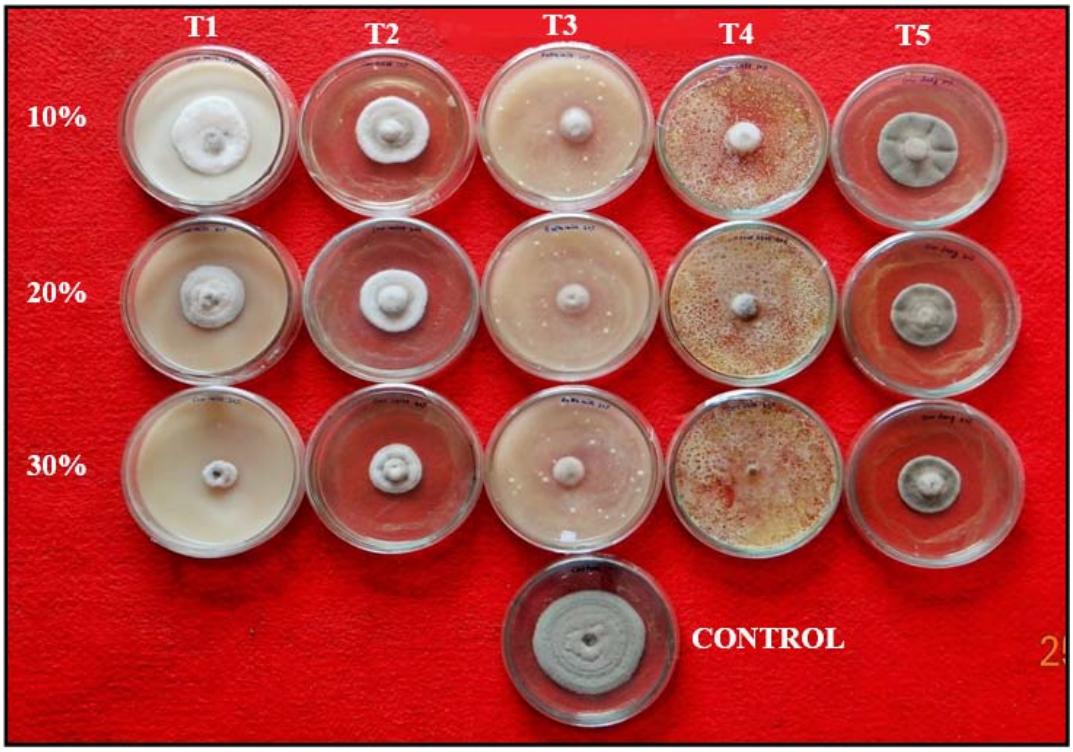

Plate 1: Effect of animal products on the mycelial growth of *P. oryzae*

Legend:
T1 cow milk
T2 cow urine
T3 butter milk
T4 cow ghee
T5 cow dung

4. Conclusion

Among the five different animal products, highest percent inhibition of mycelial growth of fungus was recorded in cow ghee (96.33, 96.50 and 98.90%) at all the concentration tested with mean of 97.24 percent followed by butter milk with the inhibition of 86.07, 87.93 and 88.73 percent respectively with mean of 87.57 percent. Minimum inhibition was observed in cow dung, with 45.37, 53.67 and 67.17 percent inhibition at 10, 20 and 30 percent concentration respectively with a mean of 55.40 percent. In general, the inhibition of radial growth of fungus increased with increase in concentration of each animal product. However in the present study cow ghee showed maximum mycelial inhibition which is attributed to presence of proteases which have inhibitory effect on *P.oryzae*.

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