

# Effect of cow urine and culture filtrates of *Trichoderma* on fungal pathogens of basmati rice

## Abstract

Rice (*Oryza sativa* L.) is the principle food crop for majority population of the world. Basmati rice is a unique crop of Himalaya comprising good quality characters and aroma. Basmati rice is being infected by numerous diseases. To manage these diseases, fungicides being used for years. This leads to the residual effects of fungicides on grains and soils so there is need for alternate approaches to the manage disease. Hence use of cow urine and *Trichoderma* is considered as the sustainable approach for management of important diseases of basmati rice. In this study we used different concentration of cow urine, *Trichoderma*, and Propiconazole (control check) against brown spot and bakanae disease of rice. Among these the maximum mycelial inhibition observed in *Trichoderma* @ 15per cent (66.33per cent) followed by cow urine @ 15per cent (52.59per cent). In Bakanae disease the maximum inhibition observed in *Trichoderma* @ 15per cent (58.89per cent) followed by cow urine @ 15per cent (49.26per cent).

**Keywords:** Basmati Rice, Bakane, Biopolarisoryzae, Trichoderma, Culture filtrates and cow urine

## 1. Introduction

Basmati rice is known as the 'Queen of Rice'. The name Basmati has been derived from its counterpart in Hindi, which translates into fragrance. "Basmati" is long grain aromatic rice grown for many centuries in a specific geographical area, in the Himalayan foothills of the Indian sub-continent, blessed with characteristics of extra-long slender grains that elongate at least twice their original size with a characteristic soft and fluffy texture upon cooking. This quality is derived from the amylose content in the rice. The aroma in Basmati arises from different compounds - hydrocarbons, alcohols, aldehydes and esters. A particular molecule is 2-acetyl-1-pyrroline.

India is the largest cultivar, consumer and exporter of basmati rice. India is the leading exporter of basmati rice to the global market. The country has exported 4.55 lakh million tonnes of basmati rice to the world for the worth of Rs. 38524.11 Crores during the year 2022-2023 (APEDA). The area of basmati rice production in India are in the state of Jammu and Kashmir, Himanchal Pradesh, Punjab, Haryana, Delhi, Uttarakhand and Western U.P. Largest area under basmati rice is in the state of Haryana (60per cent) followed by Uttar Pradesh (17.1per cent) and Punjab (16.1per cent). Rice production is affected by many biotic and abiotic stresses. Basmati varieties are particularly highly susceptible to pest and diseases. The major fungal diseases of Basmati rice that often cause great economic losses in Western Uttar Pradesh are brown leaf spot (*Drechslera oryzae*/ *Helminthosporium oryzae*), bakanae disease (*Fusarium fujikuroi*), blast (*Pyricularia oryzae*) and sheath blight (*Rhizoctonia solani*).

"Recently diseases like sheath rot, stem rot and grain discolorations which were minor and occurring sporadically are emerging and causing considerable yield loss. This is primarily due to climate change, crop intensification and continues growing of same crops. Growers are using synthetic toxicants (Fungicides) for management of these diseases which has resulted to 14 per cent of occupational injuries occurs as a result of exposure to pesticides and other agrochemical constituents" (ILO, 1996). "World Health Organization and United Nations Environment Programme surveyed, up to three million workers in agriculture experience severe poisoning due to pesticides, of which about 18,000 died" [1].

"Hence a possible way of managing plant diseases is the application of biological control and organic farming production system which aims at promoting and enhancing agro-ecosystem health, biodiversity, biological cycles and soil biological activities. In organic farming we constantly work to build a healthy soil that translates into healthy plants. Through organic farming, incidences of occurrence of disease and insects may be reduced; soil and grain quality improved and fragrance (aroma) in basmati rice may be upgraded" [2].

"The use of bio-enhancers in agriculture such as cow urine used in traditional farming that have been used to enrich soils, control pests and induce better plant vigor. These organic waste

products from cows are rich sources of microbial consortia, micronutrients, plant growth promoting substances and immunity enhancers" [3]. "Cow urine has several bioactive properties that enable it to be a fairly potent antioxidant, antibacterial, antidiabetic, antitumor, antiprotozoal, and molluscicidal" [4].

"*Trichoderma* is a ubiquitously-distributed genus of fungi that can be symbiotically associated with plant root" [5]. "The use of *Trichoderma* will enhance of plant growth; suppression of phytopathogens; nutrient mobilization in the rhizosphere; and enhancement of plants defense mechanisms" [6,7]. The fungal genus *Trichoderma* includes important species for production of antibiotics and enzymes and biocontrol activity against fungi. In this experiment cow urine and culture filtrate of *Trichoderma* are tested against major disease causing fungus of Basmati rice.

## **2. Material and Method:**

### **2.1 Collection of disease sample**

The disease sample of rice was collected from experimental area of Nematology Research Field, SVPUA&T, Meerut. The sample was brought to the laboratory for isolation of pathogen.

### **2.2 Isolation and purification of pathogens from diseased samples**

Infected plant parts of Basmati rice having the characteristics symptoms were collected for the isolation of pathogens. The infected plant parts were washed with sterilized water and cut into small sections containing both the disease and healthy looking tissue by sterilized scalpel. The sections were surface sterilized by dipping into 1 per cent sodium hypochlorite solution for 15- 20 seconds and washed by three changes of sterilized distilled water. Small sections of infected plant were then demysturized by placing on folds of sterilized blotting paper and transferred aseptically to Petri dishes containing the potato dextrose agar medium. Each Petri dish containing 5 pieces of each infected tissues were inoculated. The Petri dishes were incubated for  $28 \pm 2$  °C for 5-7 days for growth and sporulation of each pathogens associated with the diseased tissue [8,9]. After incubation, the growth were observed under the microscope for production of spores of different pathogens, the pathogen culture was purified and stored for further studies.

### **2.3 Culture filtrate of *Trichoderma* isolates S-13**

*Trichoderma* isolate S-13 were cultured on Potato Dextrose Agar medium. PDA disc of 5 mm size was added to flasks containing 200 ml of Potato Dextrose Broth (PDB). The inoculated flasks were incubated at  $26 \pm 2$  °C temperature in BOD. After 15 days of incubation, culture of all isolate was filtered through Whatman No.1 filter paper. Filtrates were evaluated against mycelial growth of pathogen through poison food technique.

### **2.4 Evaluation of Cow urine and *Trichoderma* isolate S-13 against Brown Spot and Bakanae disease of rice *in vitro***

The efficacy of cow urine, metabolites of *Trichoderma* isolate S-13 and Propiconazole 25 per cent EC were evaluated by food poison technique [10]. Sterilized PDA medium was amended with different concentrations of cow urine, metabolites of *Trichoderma* isolate S-13 and Propiconazole 25% EC and poured to labelled Petri plates. Actively growing fungal disc of 5 mm diameter were cut from periphery of 5 days old culture of each pathogen were inoculated aseptically on PDA plates poisoned with different concentration of cow urine, metabolites of *Trichoderma* isolate S-13 and Propiconazole. Potato dextrose agar medium without adding served as control. The plates were incubated for 7 days at  $28 \pm 2$  °C. Each treatment was replicated thrice. The diameters of the radial growth of colonies in each of the treatments were measured in four directions lengthwise and breadth wise and mean was calculated. The observations were made and compared with the check and per cent inhibition of mycelial growth was determined using the formula given by [11].

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

Where,

I = Per cent inhibition of mycelium

C = Colony diameter (cm) in control

T = Colony diameter (cm) in treatment

**Observation:** Percent Inhibition of Radial Growth

### 3. Result and Discussion

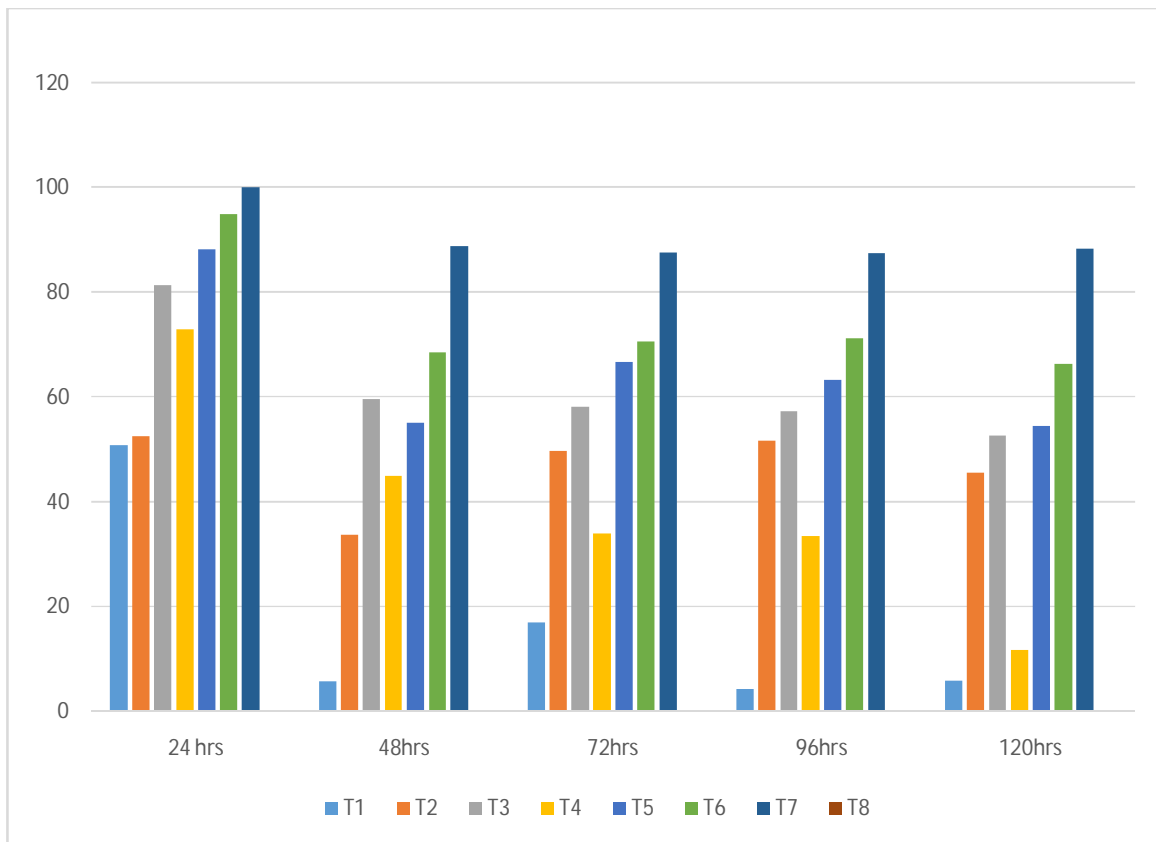
Under *in vitro* conditions, the efficacy of cow urine and culture filtrates of *Trichoderma* isolate S-13 (5, 10 and 15 per cent) concentrations was tested against the *Bipolaris oryzae*, and *Fusarium fujikuroi*. The outcomes revealed that, notable suppression of the mycelial growth of *Bipolaris oryzae*, and *Fusarium fujikuroi* in all treatment tested, as compared to the control. In *Bipolaris oryzae* mycelial growth, among all treatments, the highest mycelial growth inhibition over control was observed in *Trichoderma* culture filtrates 15 per cent (66.33 per cent) followed by cow urine 15 per cent (52.67 per cent). The lowest mycelial growth inhibition observed in cow urine 5 per cent (5.78 per cent) followed by compared to the control at 120 hr (Table 1; Fig 1). In their study, evaluated efficacy of culture filtrate of *Trichoderma harzianum* against brown spot of rice and identified that 70 to 90 per cent of mycelial inhibition [12].

In other hand, *Fusarium fujikuroi*, maximum percent mycelium inhibition was observed in *Trichoderma* culture filtrates 15 per cent (58.89 per cent) followed by cow urine 15 per cent (49.26). The lowest mycelial growth inhibition observed in cow urine 5 per cent (21.86 per cent) compared to the control at 120 hr (Table 2 & Fig.2). The similar study was observed by [13, 14] and they evaluated efficacy of cow urine at different concentrations (5 per cent, 10 per cent and 15 per cent) against *Fusarium oxysporum*. Among these concentration cow urines at 15 per cent concentration was most effective and the maximum mycelium suppression of (78.57 per cent) was observed. Similarly, [15] identified that 20 per cent of *Trichoderma* culture filtrates was inhibit (100 per cent) mycelial growth of *Fusarium moniliformae*. The Propiconazole @ 25 EC is used as positive control.

**Table No. 1 Effect of cow urine and culture filtrates of *Trichoderma* isolate- S13on *Biopolarisoryzae***

Treatments	Treatment Details	Concentration	24hr.	Percent inhibition	48 hr.	Percent inhibition	72 hr.	Percent inhibition	96 hr.	Percent inhibition	120 hr.	Percent inhibition
T1	Cow urine	5per cent	0.96	50.84	2.80	5.63	4.90	16.95	6.86	4.19	8.48	5.78
T2	Cow urine	10per cent	0.93	52.57	1.96	33.70	2.96	49.71	3.46	51.63	4.90	45.56
T3	Cow urine	15per cent	0.36	81.34	1.20	59.56	2.46	58.19	3.06	57.21	4.26	52.67
T4	<i>Trichoderma</i> S-13	5per cent	0.53	72.90	1.63	44.96	3.90	33.90	4.76	33.49	7.96	11.56
T5	<i>Trichoderma</i> S-13	10per cent	0.23	88.15	1.33	55.07	1.96	66.66	2.63	63.26	4.10	54.44
T6	<i>Trichoderma</i> S-13	15per cent	0.10	94.92	0.93	68.55	1.73	70.63	2.06	71.16	3.03	66.33
T7	Propiconazole 25 EC	50PPM	0.00	100.00	0.33	88.78	0.73	87.58	0.90	87.44	1.06	88.22
T8	Control		1.96	0.00	2.96	0.00	5.90	0.00	7.16	0.00	9.00	0.00
	C.D(P=0.05)		0.15		0.40		0.35		0.40		0.47	

**Fig. No. 1 Effect of cow urine and culture filtrates of *Trichoderma* isolate S-13 on *Biopolarisoryzae***

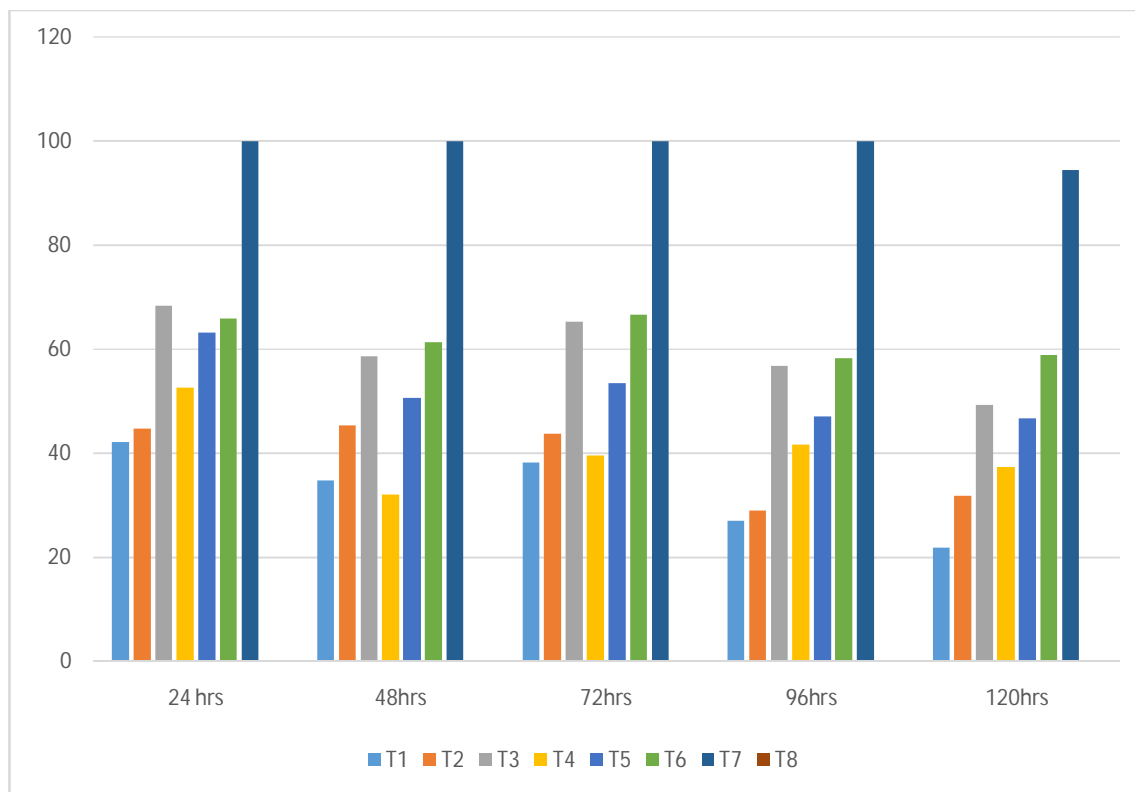


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**Table No. 2 Effect of cow urine, culture filtrates of *Trichoderma* isolate S-13 and on *Fusariumfujikuroi***

Treatment	Treatment details	Concentration	24hr.	Percent inhibition	48 hr.	Percent inhibition	72hr.	Percent inhibition	96hr	Percent inhibition	120hr.	Percent inhibition
T1	Cow urine	5per cent	0.73	42.15	1.63	34.68	2.96	38.19	4.96	26.96	7.03	21.86
T2	Cow urine	10per cent	0.70	44.75	1.36	45.32	2.70	43.75	4.83	28.93	6.13	31.86
T3	Cow urine	15per cent	0.40	68.43	1.03	58.68	1.66	65.27	2.93	56.87	4.56	49.26
T4	<i>Trichoderma</i> S-13	5per cent	0.60	52.64	1.70	32.00	2.90	39.58	3.96	41.66	5.63	37.41
T5	<i>Trichoderma</i> S-13	10per cent	0.46	63.14	1.23	50.68	2.23	53.48	3.60	47.06	4.80	46.67
T6	<i>Trichoderma</i> S-13	15per cent	0.43	65.82	0.96	61.32	1.60	66.67	2.83	58.34	3.70	58.89
T7	Propiconazole @ 25EC	50PPM	0.00	100.00	0.00	100.00	0.00	100.00	0.00	100.00	0.50	94.44
T8	Control		1.26	0.00	2.50	0.00	4.80	0.00	6.80	0.00	9.00	0.00
	C.D(P=0.05)		0.16		0.15		0.22		0.14		0.43	

**Fig. No. 2: Effect of cow urine and culture filtrates of *Trichoderma* isolate S-13 on *Fusariumfujikuroi***



### Conclusion

The use of bio-enhancers in agriculture such as cow urine used in traditional farming that have been used to enrich soils, control pests and induce better plant vigor. These organic waste products from cows are rich sources of microbial consortia, micronutrients, plant growth promoting substances and immunity enhancers.

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