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Effect of Cow Urine and Culture Filtrates of *Trichoderma* isolate S-13 on Fungal Pathogens of Basmati Rice

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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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 needs for alternate approaches to the manage disease. Hence use of cow urine and *Trichoderma isolate S-13* is considered as the sustainable approach for management of important diseases of basmati rice. In

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this study we used different concentration of cow urine, *Trichoderma isolate S-13*, and Propiconazole (control check) against brown spot and bakanae disease of rice. Among these the maximum mycelial inhibition observed in *Trichoderma isolate S-13* @15per cent (66.33per cent) followed by cow urine @15per cent (52.59 per cent). In Bakanae disease the maximum inhibition observed in *Trichoderma isolate S-13* @ 15per cent (58.89 per cent) followed by cow urine@15per cent (49.26 per cent).

Keywords: Basmati rice; Bakane; Biopolaris oryzae; 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/ Helmintho sporiumoryzae*), bakanae disease (*Fusarium fujikuroi*), blast (*Pyricularia oryzae*) an d 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 Tricoderma will enhance of plant growth; suppression of phytopathogens; nutrient mobilization in the rhizosphere; and enhancement of plants defense mechanisms" [6,7]. The fungal ge nus *Trichoderma* includes important species for production of antibiotics and enzymes and biocontrol activity against fungi. In this experiment cowurine and culture filtrate of *Trichoderma isolate S-13* are tested against major disease causing fungus of Basmati rice.

2. MATERIALS AND METHODS

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 1per cent sodium hypochlorite solution for 15- 20 seconds and washed by three changes of sterilized distilled water. Small sections of infected plant were then demoisturized 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±2 ^{0}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±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 25per 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 ± 2^0 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 Horsfall and Heuberger [11].

$$I=(C-T)/C\times100$$

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. RESULTS 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 wasobserved in *Trichoderma* culture filtrates 15per cent (66.33 per cent) followed by cow urine 15per cent (52.67 per cent).The lowest mycelial growth inhibition observed in cow urine 5 per cent (5.78per 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 broswn spot of rice and identified that 70 to 90per cent of mycelial inhibition [12].

Table 1. Effect of cow urine and culture filtrates of *Trichoderma* isolate- S13on *Biopolaris oryzae*

Treatments	Treatment Details	Concentration	24hr.	Per cent inhibition	48 hr.	Per cent inhibition	72 hr.	Per cent inhibition	96 hr.	Per cent inhibition	120 hr.	Per cent 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	*Trichoderma* 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	*Trichoderma* 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	*Trichoderma* 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	

Table 2. Effect of cow urine and culture filtrates of *Trichoderma* isolate S-13 on *Fusarium fujikuroi*

Treatment	Treatment details	Concentration	24 hr.	Per cent inhibition	48 hr.	Per cent inhibition	72 hr.	Per cent inhibition	96 hr.	Per cent inhibition	120 hr.	Per cent 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	*Trichoderma* 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	*Trichoderma* 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	*Trichoderma* 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	

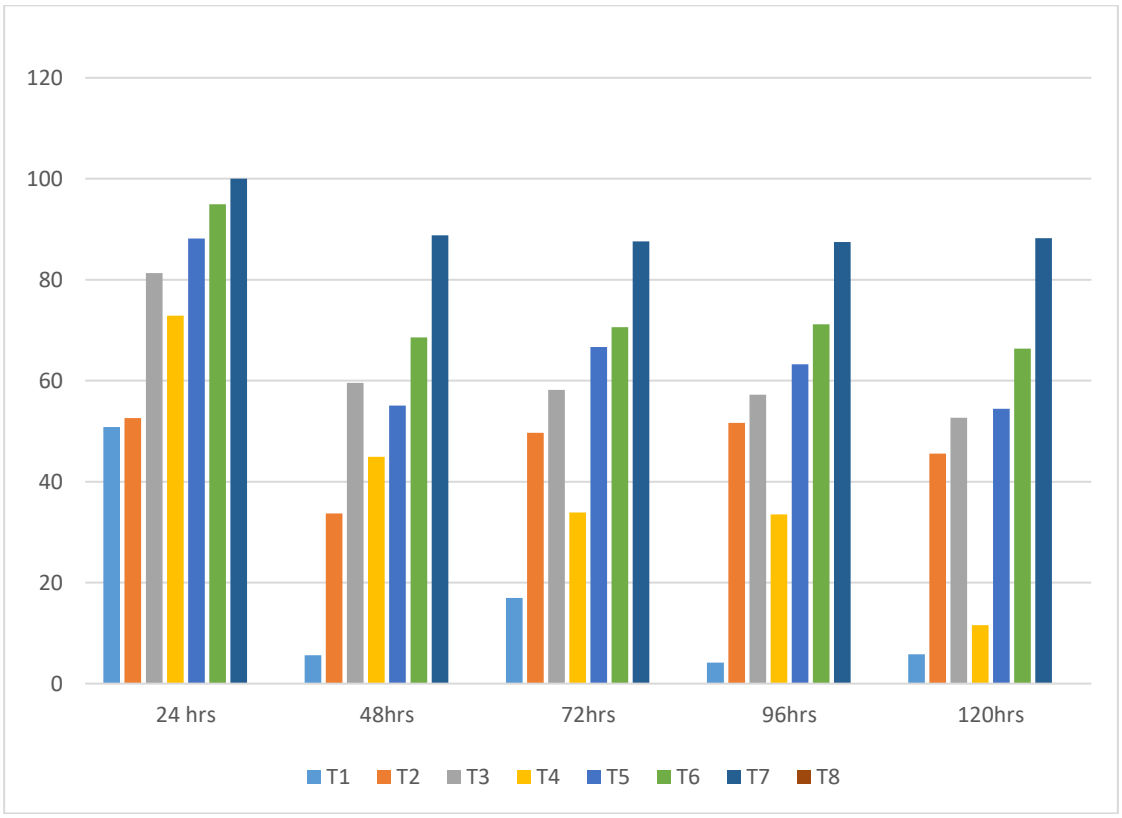

Fig. 1. Effect of cow urine and culture filtrates of *Trichoderma* isolateS-13 on *Biopolaris oryzae*

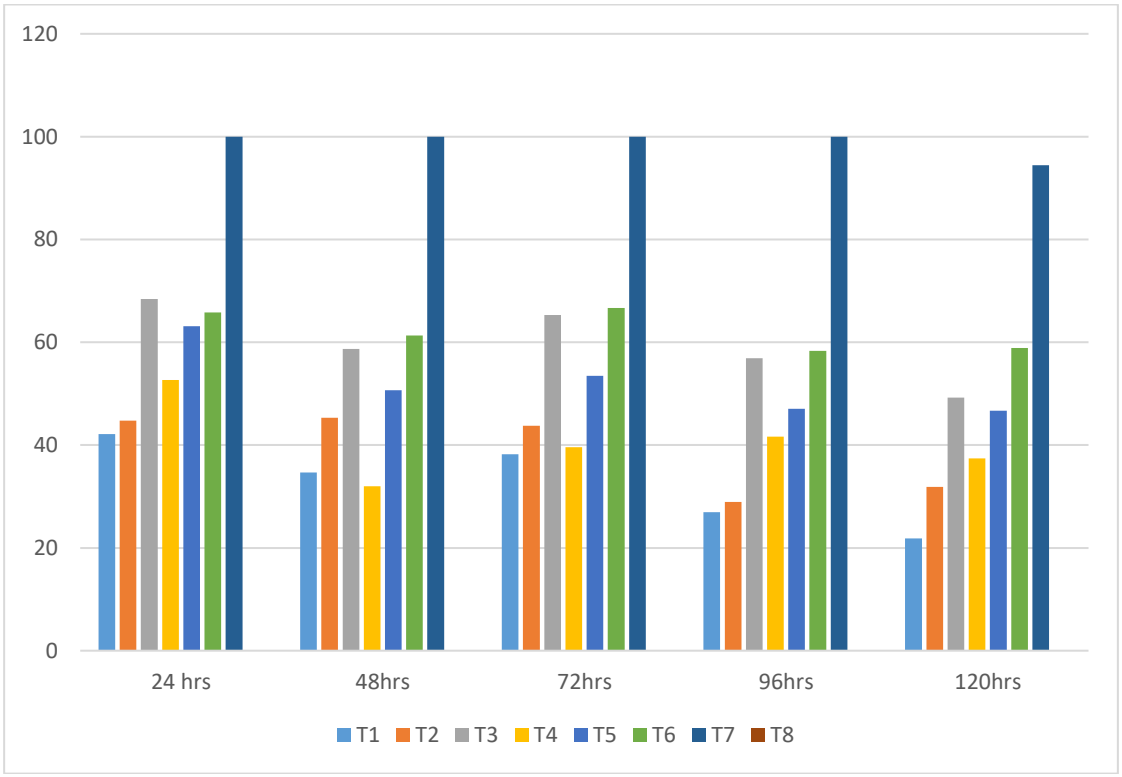

Fig. 2. Effect of cow urine and culture filtrates of *Trichoderma* isolate S-13 on *Fusarium fujikuroi*

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

4. 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.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Adhikary SK, Das PK, Risam M, Sarker BC, Sultana S. Evaluation of botanicals and cow urine on *in vitro* mycelial growth and sporulatin of *Bipolaris oryzae* causing brown spot of rice. Bangladesh Journal of Agriculture and Environment. 2013;9(2):29-34.
2. Stockdale EA, Lampkin NH, Hovi M, Keatinge R, Lennartsson EK, Macdonald DW, Padel S, Tattersall FH, Wolfe MS, Watson CA. Agronomic and environmental implications of organic farming systems. Adv Agron. 2001;70:261-327.
3. Pathak RK, Ram RA. Bio-enhancers: A potential tool to improve soil fertility, plant health in organic production of horticultural crops. Progressive Horticulture. 2013;45(2):237-54.
4. Rakesh KN, Dileep N, Nawaz NA, Junaid S, Kekuda PT. Antifungal activity of cow urine against fungal pathogens causing rhizome rot of ginger. Environment and Ecology. 2013 Jul;31(3):1241-4.
5. Vargas WA, Mandawe JC, Kenerley CM. Plant-derived sucrose is a key element in the symbiotic association between *Trichoderma virens* and maize plants. Plant physiology. 2009 Oct 1;151(2):792-808.
6. Bae H, Sicher RC, Kim MS, Kim SH, Strem MD, Melnick RL, Bailey BA. The beneficial endophyte *Trichoderma hamatum* isolate DIS 219b promotes growth and delays the onset of the drought response in Theobroma cacao. Journal of experimental botany. 2009 Jul 1;60(11):3279-95.
7. Dardanelli MS, Manyani H, González-Barroso S, Rodríguez-Carvajal MA, Gil-Serrano AM, Espuny MR, López-Baena FJ, Bellogín RA, Megías M, Ollero FJ. Effect of the presence of the plant growth promoting rhizobacterium (PGPR) *Chryseobacterium balustinum* Aur9 and salt stress in the pattern of flavonoids exuded by soybean roots. Plant and Soil. 2010 Mar;328:483-93.
8. Hanif A, Dawar S. Fungicidal effects of homeopathic drugs in the control of root rot fungi and growth of leguminous and non-leguminous crops. Int. J. Biol. Biotech. 2015;12(1):97-105.
9. Attia MS, El-Sayyad GS, Abd Elkodous M, El-Batal AI. The effective antagonistic potential of plant growth-promoting rhizobacteria against *Alternaria solani* causing early blight disease in tomato plant. Scientia Horticulturae. 2020;266:109289.
10. Nirmalkar VK, Said PP, Kaushik DK. Efficacy of fungicides and bio-agents against *Pyricularia gresia* in paddy and

yield gap analysis thought frontline demonstration. Int. J. Curr. Microbiol. App. Sci. 2017;6(4):2338-46.
11. Horsfall JG, Heuberger JW. Measuring magnitude of a defoliation disease of tomatoes. Phytopathology.1942;32(2):226-232.
12. Torres EP, Cabrera AB, Virelles BM, Reyes YS, Mora ML, Guerra SM, Hernández M. Efficiency of *Trichoderma harzianum* (strain A-34) and its culture filtrates on control of three rice fungal aerial diseases. Bioagro. 2018 Apr;30(1): 17-26.
13. Gomathinayagam S, Rekha M, Murugan SS, Jagessar JC. The biological control of paddy disease brown spot (*Bipolaris oryzae*) by using *Trichoderma viride in vitro* condition. Journal of Biopesticides. 2010; 3(1):93.
14. Holder DJ, Keyhani NO. Adhesion of the entomopathogenic fungus *Beauveria* (Cordyceps) *bassiana* to substrata. Applied and Environmental Microbiology. 2005 Sep;71(9):5260-6.
15. Raghu S, Yadav MK, Prabhukarthikeyan SR, Baite MS, Lenka S, Jena M. Occurance, pathogenicity, characterization of *Fusarium fujikuroi* causing rice bakanae disease from Odisha and *in vitro* management. ORYZA-An International Journal on Rice. 2018;55(1):214-23.

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