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 being infected by numerous diseases. For managing these diseases, fungicide 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 the 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 is observed in *Trichoderma*@15%(66.30%)followed by cow urine @15%(52.59%). In Bakanae disease the maximum inhibition observed in *Trichoderma*@ 15%(58.89%)followed bycow urine@15%(49.26%).

Keywords:Trichoderma,Culture filtrates, Basmati Rice,Propiconazole 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 (60%) followed by Uttar Pradesh (17.1%) and Punjab (16.1%). 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 (*Drechsleraoryzae/ Helminthosporiumoryzae*),bakanae disease (*Fusarium fujikuroi*), blast (*Pyricularia oryzae*) and sheath blight(*Rhizoctonia solani*).

Recently diseases like sheath rot, stem rot and grain discoloration which was 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 % 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 that each year, 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 controlling plant diseases is the application of biological control and organic farming production system 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 [2] and fragrance (aroma) in basmati rice may be upgraded.

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 enhancement of plant growth regulators suppressionof 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 cowurine and culture filtrate of *Trichoderma*aretested 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.

Comment [WU1]: Expand full form

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% sodium hypochlorite solution for 15- 20 seconds and washed by 3 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. In each Petri dish, 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.

Comment [WU2]: Sterile water

2.3 Culture filtrate of *Trichoderma* isolates S-13

Trichoderma isolates 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 isolates was filtered through Whatman No.1 filter paper. Filtrates were evaluated against mycelial growth of pathogen through poison food technique.

2.4Evaluation 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% EC were evaluated at by food poison technique [10]. PDA medium was amended with different concentration of cow urine, metabolites of *Trichoderma* isolate S-13 and Propiconazole 25% EC sterilized by autoclaving and poured to labelled Petri plates. 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 [11].

Comment [WU3]: Trichoderma isolates are temperature sensitive or not. Bu autoclaving, metabolites of Tichoderma may denature. Kindly clarify for your self.

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

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. Result and Discussion

Under *in vitro* conditions, the efficacy ofc ow urine and culture filtrates of *Trichoderma* isolate S-13(5,10 and 15 per cent) concentrationswas tested against the*Bipolarisoryzae,* and *Fusariumfujikuroi.*The outcomes revealed notablesuppression of the mycelial growth of *Bipolarisoryzae,* and *Fusariumfujikuroi* in all treatment tested, as compared to thecontrol. In*Bipolarisoryzae*mycelium growth, among all treatments,the highest mycelial growth inhibition over control wasobserved in *Trichoderma* culture filtrates 15% (66.30%) followed by cow urine 15% (52.59%).The lowest mycelial growth inhibitionobserved in cow urine 5% (5.78%) followed bycompared to the control at 120 hr(Table 1; Fig 1). In their study, evaluated efficacy of culture filtrate of *Trichodermaharzianum* against brownspot of rice and identified that 70 to 90% of mycelial inhibition [12].

In other hand, *Fusariumfujikuroi*,maximum percent mycelium inhibition was observed in *Trichoderma* culture filtrates 15% (58.89%) followed by cow urine 15% (49.26). The lowest mycelial growth inhibition observed in cow urine 5% (21.86%)compared to the control at 120 hr(Table 2 & Fig.2).The similar study observed by [13, 14] evaluated efficacy of cow urine at different concentrations (5%,10% and 15%) against*Fusariumoxysporum.* Among these concentration cow urines at 15% concentration was most effective and the maximum mycelium suppression of (78.57%) was observed. Similarly, [15]identified that 20% of *Trichoderma* culture filtrates was inhibit (100%) mycelial growth of *Fusariummoniliformae*.

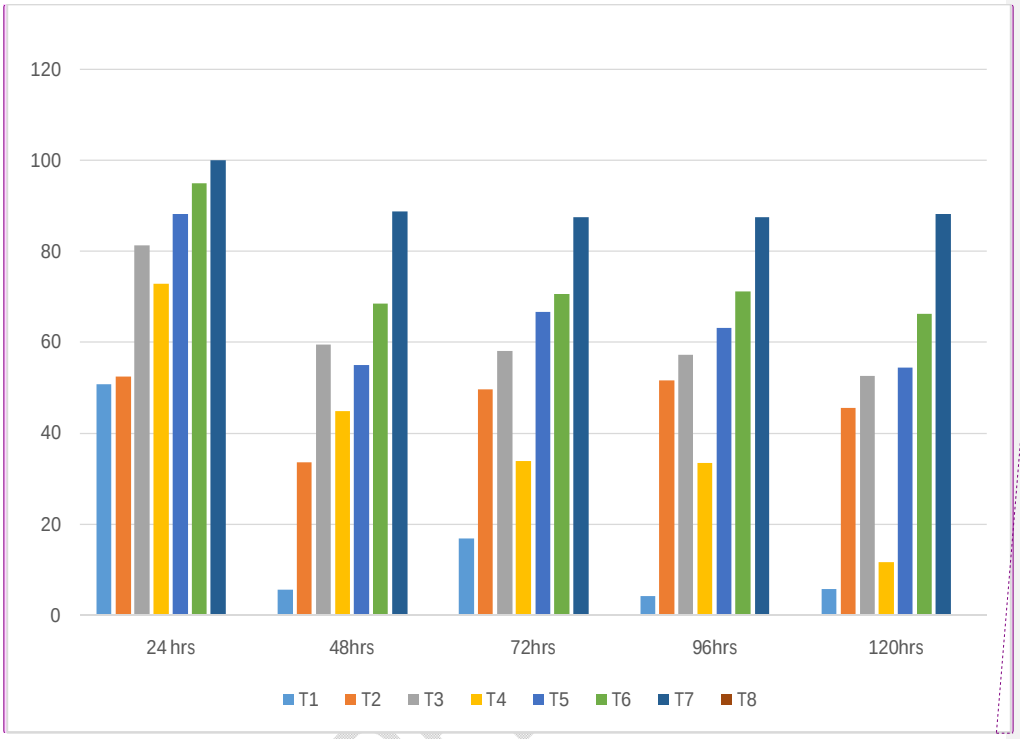
Table No. 1 Effect of cow urine and culture filtrates of *Trichoderma* isolate- S13on *Biopolarisoryzae*

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	5%	0.96	50.84	2.80	5.63	4.90	16.95	6.86	4.19	8.48	5.78
T2	Cow urine	10%	0.93	52.57	1.96	33.70	2.96	49.71	3.46	51.63	4.90	45.56
T3	Cow urine	15%	0.36	81.34	1.20	59.56	2.46	58.19	3.06	57.21	4.26	52.67
T4	*Trichoderma*S-13	5%	0.53	72.90	1.63	44.96	3.90	33.90	4.76	33.49	7.96	11.56
T5	*Trichoderma* S-13	10%	0.23	88.15	1.33	55.07	1.96	66.66	2.63	63.26	4.10	54.44
T6	*Trichoderma* S-13	15%	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	

Comment [WU4]: Provide units

Comment [WU5]: The statistical analysis is not enough since zero values are present in table. Percent root transformation may be applied. Please check

Fig. No. 1 Effect of cow urineand culture filtrates of *Trichoderma* isolateS-13 on *Biopolarisoryzae*



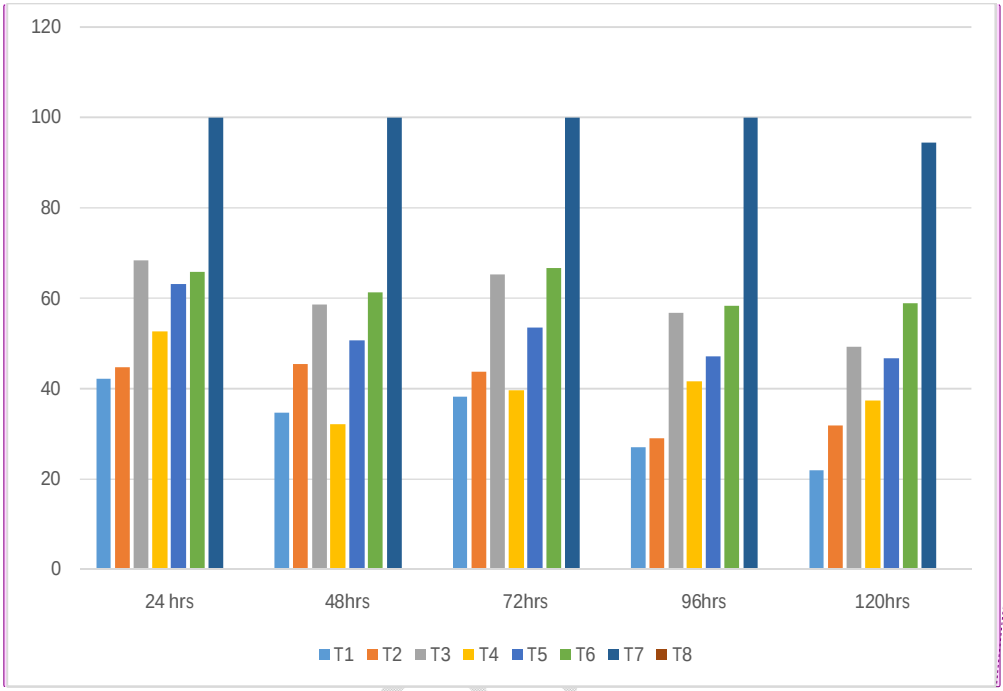
Comment [WU6]: Please provide axis titles and error bars etc

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

Treatment	Treatment Detail	Concentration	24h	Percent inhibition	48h	Percent inhibition	72h	Percent inhibition	96h	Percent inhibition	120h	Percent inhibition
T1	Cow urine	5%	0.73	42.15	1.63	34.68	2.96	38.19	4.96	26.96	7.03	21.86
T2	Cow urine	10%	0.70	44.75	1.36	45.32	2.70	43.75	4.83	28.93	6.13	31.86
T3	Cow urine	15%	0.40	68.43	1.03	58.68	1.66	65.27	2.93	56.87	4.56	49.26
T4	*Trichoderma*	5%	0.60	52.64	1.70	32.00	2.90	39.58	3.96	41.66	5.63	37.41
T5	*Trichoderma*	10%	0.46	63.14	1.23	50.68	2.23	53.48	3.60	47.06	4.80	46.67
T6	*Trichoderma*	15%	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	

Comment [WU7]: The statistical analysis is not enough since zero values are present in table. Percent root transformation may be applied. Please check

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



Comment [WU8]: Please provide axis titles and error bars etc

Reference

1. Adhikary, S. K., Das, P. K., Risam, M., Sarker, B. C. and Sultana, S.(2013) evaluation of botanicals and cow urine on in vitro mycelial growth and sporulatin of *bipolarisoryzae* causing brown spot of rice. *Bangladesh journal of agriculture and environment* 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) Chryseobacteriumbalustinum 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, AbdElkodous M, El-Batal AI. The effective antagonistic potential of plant growth-promoting rhizobacteria against Alternariasolani causing early blight disease in tomato plant. ScientiaHorticulturae. 2020; 266: 109289.
10. Nirmalkar VK, Said PP, Kaushik DK. Efficacy of fungicides and bio-agents against Pyriculariagresia 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, E. P., Cabrera, A. B., Virelles, B. M., Reyes, Y. S., Mora, M.L., Guerra, S. M. and Hernández, M. Efficiency of Trichodermaharzianum (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 (Bipolarisoryzae) by using Trichodermaviride 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 Fusariumfujikuroi causing rice bakanae disease from Odisha and in vitro management. ORYZA-An International Journal on Rice. 2018;55(1):214-23.