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Effect of Sterilization on Antifungal Properties of Various Components of Panchgavya-tested Against *Rhizoctonia solani* Causing Sheath Blight of Paddy

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A total of seven treatments of animal by-products which are used in the preparation of Panchgavya *viz.,* Cow Urine, Cow Dung, Dung + Urine (1:1), Dung + Urine (2:1), Cow curd, Cow ghee and Cow milk were screened against the test pathogen at 5, 10, 15 and 20 per cent concentrations under Sterilized and Unsterilized conditions. Under sterilized set of experiment significantly maximum inhibition of the mycelial growth was obtained in cow-urine (76.35%) followed by cow-ghee (41.57%), cow-dung + cow-urine (1:1) (38.43%), cow-curd (35.29%), cow-dung (27.88%) and cow-dung + cow-urine (2:1) (16.67%). No sclerotia formation was observed in higher concentrations except in cow-curd. Whereas under unsterilized condition, cow-urine, cow-curd, cow-dung+ cow-urine (1:1) showed 100% inhibition followed by cow-dung + cow-urine (2:1) (85.69%), cow-dung (84.71%) and Cow ghee (19.02%). Cent percent inhibition of sclerotia production was observed in all treatments at all concentrations except under cow-milk treatment. Cow-milk was found at par with untreated check in inhibiting mycelial growth of *Rhizoctonia solani* in sterilised as well as unsterilized experiment. For all treatments unsterilized set of experiment were found inhibiting much higher per cent mycelial growth of *R. solani* as compared to their sterilised counterparts.

Keywords: Animal by-product, Biorationals, Rice, Sheath blight, *Rhizoctonia solani*, Panchgavya.

India is one among the oldest agriculture practising country of the world. There are a lot of literature not only documenting this fact but provide hefty references to plant protection *viz.*, Vedas, Buddhist literature, Kautilya's Artha-sastra, Krishi-parashara Sangam literature, of Tamil, Agnipurana, Brihat samhita of Varahamihira, Surapala's Vrikshayurveda, Viswavallabha of Chakrapani Misra etc[1]. Use of animal by-product in protecting plants from various ailments was common in ancient form of soil culturing. Kunapajala (a unique liquid fertilizer-cum-plant protection material) was prepared from fermented animal wastes. Likewise use of cow's urine as a base material in various fermented anti- insect formulations has been well described[2-4]. Inert substances such as ash and brick-dust, cow dung and other animal wastes, and flours of grain were used for dressing of wounds, application of pastes over affected tree surfaces, drenching of soil with different materials, treating roots before transplanting, fumigation of trees and stored seeds, and spraying and dusting[1] but use of these plant protection measures has shown a diminishing trend after discovery of various synthetics. By over using these synthetic chemicals we have been

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disturbing balance of nature, which resulted in pesticide residue in food above specified levels, nitrate enrichment, soil and water pollution, diseases and disorders in living beings including human as well as animals[5-11]. Thus, question of the hour for every agriculturist today is how to feed growing population with simultaneous maintenance of quality and quantity of our natural resources. Panchgavya is one such product of five cattle by-product which have been used in Indian conventional agriculture, health and medicine[12-15]. Considering all these issues, an experiment was laid out to validate role of these conventional products in inhibiting growth of pathogenic fungi *R. solani* causing sheath blight of paddy.

MATERIALS AND METHOD

Different animal by-products were collected from different locations of Pantnagar for evaluation against *R. solani*. Details of animal by-products used in the present study are given in table 1.

Components of Panchgavya *viz.,* Cow Urine, Cow Dung (fresh), Cow curd, Cow ghee and Cow milk in addition of Dung + Urine (1:1), Dung + Urine (2:1), were screened against *R. solani* at 5, 10, 15 and 20 per cent concentrations.

The efficacy of selected animal by-products for their antifungal properties were tested by poisoned food technique[16].

The experiment was performed in two sets one with sterilized biorational and the other with unsterilized biorational , medium was kept sterilised for both the sets. Observation on colony diameter after 72 hours of incubation at 28±1^{0}C and Production of sclerotia after 120 hours of incubation was noticed.

Molten double strength potato dextrose agar medium was amended with individual product to get a final concentration of 5, 10 , 15, and 20 % (v/v) or (w/v). 95, 90, 85, 80 ml of PDA was poured in different sterilized 100 ml conical flask and volume was make up by the required bio-rational. After thorough mixing each biorational- product with PDA separately, was poured into each 80 mm Petri-dish. A control was also maintained without amending PDA with biorational. After solidification, the plates were inoculated by placing 5mm discs of 5 days old cultures of *R. solani.* Each treatment was replicated three times.

Inoculated Petri plates were incubated at 28± 1ºC and observations as colony diameter (mm) was recorded at every 24 hour interval and growth diameter of the pathogen (fungal growth) was measured and compared to control growth. Percent inhibition was calculated using the following formula:

$$I = \frac{C - T}{C} \times 100$$

Where,

I = Inhibition per cent
C = Colony diameter in check (mm)
T = Colony diameter in treatments (mm)

The data were analyzed statistically at the computer centre of G.B. Pant University of Agriculture and Technology, Pantnagar, using Completely Randomized Design (CRD). The treatments were compared by the means of critical differences (CD) at 5 per cent level of significance.

RESULTS AND DISCUSSION

The data recorded on colony diameter after 72 hours of incubation at 28±1^{0}C were non-significant only in treatments *viz.,* cow dung + cow urine and cow curd at 5 per cent concentration (under sterilised set of experiment) and cow milk (at all the tested concentrations and sterilised as well as unsterilized set of experiment). Rest all treatments (sterilised as well as unsterilized) at different concentrations were found statistically significant in inhibiting the mycelial growth of *R. solani*. (Table 2a; Fig.1)

A statistically highly significant difference as observed between sterilised and unsterilized sets of experiments except for that in case of cow milk where there is no inhibition at all

Table 1. Animal by-products (biorationals)

S. No.	Animal by- products
1	Cow milk
2	Cow curd
3	Cow ghee
5	Cow urine
6	Cow dung (fresh)
7	Cow dung (pat)
8	Cow urine + Cow dung

Table 2(a). Effect of different concentrations of biorationals on the growth of *Rhizoctonia solani* after 72 hour of incubation at 28± 1°C

S. No.	Treatments (Animal By-products)	Concentrations (%)															
		5				10				15				20			
		S		U		S		U		S		U		S		U	
		G	I	G	I	G	I	G	I	G	I	G	I	G	I	G	I
1	Cow Urine	28.50	66.47	18.30	78.43	23.00	72.94	00.00	100.00	21.00	75.29	00.00	100.00	20.10	76.35	00.00	100.00
2	Cow Dung (fresh)	71.00	16.47	35.00	58.82	67.30	20.82	33.60	60.39	65.80	22.58	24.80	70.78	61.30	27.88	13.00	84.71
3	Dung+Urine (1:1)	85.00	00.00	21.30	74.90	69.30	18.43	13.00	84.71	59.60	29.80	00.00	100.00	52.30	38.43	00.00	100.00
4	Dung+Urine (2:1)	85.00	00.00	41.00	51.76	78.00	08.24	29.70	65.69	74.80	11.96	25.50	70.39	70.80	16.67	12.17	85.69
5	Cow curd	85.00	00.00	07.30	91.37	66.50	21.76	00.00	100.00	58.80	30.78	00.00	100.00	55.00	35.29	00.00	100.00
6	Cow ghee	72.80	14.31	85.00	00.00	54.50	35.88	77.60	8.70	52.10	38.63	71.50	15.88	49.60	41.57	68.83	19.02
7	Cow milk	85.00	00.00	85.00	00.00	85.00	00.00	85.00	00.00	85.00	00.00	85.00	00.00	85.00	00.00	85.00	00.00
8	Check	85.00	00.00	85.00	00.00	85.00	00.00	85.00	00.00	85.00	00.00	85.00	00.00	85.00	00.00	85.00	00.00
CD at 5 % A×B×C :		For G: A : 1.10 B : 2.19 C : 1.55 A×B×C : 8.08								6.21	For I:A : 1.43 B : 2.86 C : 2.02						

S = Sterilized treatment, U = Unsterilized treatment, G=Average Colony diameter (mm), I= Average Inhibition per cent

Table 2b. Effect of different concentrations of biorationals on the production of sclerotia by *Rhizoctonia solani*after 120 hour of incubation at 28± 1°C

S. No	Treatments(Animal By-products)	Concentrations (%)							
		5		10		15		20	
		S	U	S	U	S	U	S	U
1	Cow Urine	-	-	-	-	-	-	-	-
2	Cow Dung (fresh)	-	-	-	-	-	-	-	-
3	Dung + Urine (1:1)	+	-	-	-	-	-	-	-
4	Dung + Urine (1:2)	+	-	-	-	-	-	-	-
5	Cow curd	++	-	+	-	+	-	+	-
6	Cow ghee	-	-	-	-	-	-	-	-
7	Cow milk	+++	++	++	++	+	+	-	-
8	Check	+++	+++	+++	+++	+++	+++	+++	+++

+++ Excellent production, ++ Good production, + Poor production, - No production

in all the concentrations under sterilised as well as unsterilized set of experiment.

It is worth to note all other treatments of unsterilized set of experiment gave significantly higher per cent inhibition than in sterilised set of experiment with an exception of cow ghee. Sclerotia production was completely checked by almost all un-sterilized set of treatments except cow-milk. However , under sterilized treatments, Dung + Urine (1:1), Dung + Urine (2:1) at 5 per cent concentrations produce very few sclerotia, good to poor sclerotial production was shown by sterilized curd and comparatively high sclerotial production was noticed in cow-milk in lower concentrations.

The data of efficacy of these biorationals on colony diameter and production of sclerotia of *R. solani* at different treatment and concentration in sterilised and unsterilized sets of treatment are presented below in table 2a and 2b.

Under sterilized set of experiment cow-urine was most effective in inhibiting colony diameter of *R.solani* followed by cow-ghee, combined product of cow-dung+ cow-urine (1:1), cow-curd, cow-dung, cow-dung+ cow-urine (2:1) and cow-milk.

Under un-sterilized set of experiment cow-urine performed best in inhibiting colony diameter of *R. solani* followed by cow-curd, cow-dung+ cow-urine (1:1) , cow-curd, cow-dung, cow-dung+ cow-urine (2:1), cow-ghee, cow-milk.

The results of the present investigation are accordance with study wherein, 3 year old cow-urine and cow-mattha at 10 per cent concentration inhibited the mycelial growth of *R.solani* **(17)**. Cent per cent Fungistatic activity of cow-urine have been reported by in fungi like *Penicillum notatum, Trichoderma viridae* and *Candida albicans* at 20 per cent concentration[18]. No mycelial growth of the pathogen in the medium amended with unsterilized cow dung and cowpat ash whereas, all the autoclaved cow dung preparations except cowpat ash did not have any inhibitory effect on the mycelial growth of *R.solani* at concentrations below 5 percent[19]. Cent per cent inhibition of mycelial growth when *Sclerotinia sclerotiorum* was incubated for 2, 3 and 4 days in cow-urine amended medium. When medium was amended

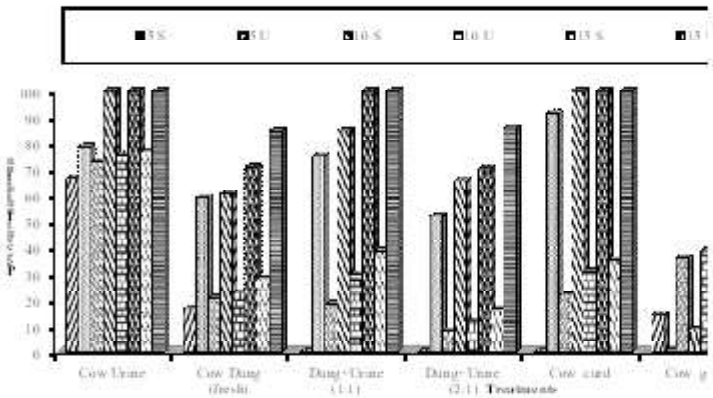

Fig. 1. Percent inhibition of mycelial growth of *Rhizoctonia solani* under different biorationals treatments at different concentrations

with cow-dung 64, 47.1 and 29.8 per cent inhibition was reported after 2, 3 and 4 days of incubation respectively[20].

Results of the present study on inhibitory effects of biorationals clearly indicate that sterilization have an important role to play in determining their efficiency to show anti-fungal activity. It was found that every treatment was exhibiting a significantly better inhibition of mycelial growth when used without sterilization. Inhibitory effect on sclerotia production accentuates deficiency of fungus to perennate.

The difference in sterilized and unsterilized treatments may be due to the presence of fungi toxic bacteria like *Pseudomonas* in unsterilized dung[21, 22]. Anti- microbial activity of cow-urine may be attributed to its phenolic contents as an increase in polyphenol oxidase and Peroxidase activity was reported by when cow urine was used[23, 24].

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