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Characterization of cow urine for antimicrobial properties and plant growth promoter in rice (*Oryza sativa* L.)

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Abstract
A field experiment was conducted at Agricultural Research field, IGKV, Raipur, Chhattisgarh, during *kharif* 2023 to know the effect of various levels of RDF and cow urine application on yield of irrigated paddy (*Oryza sativa* L.) (Var: CG Devbhog) in clay loam soil. There were ten treatments laid out in Randomized Complete Block Design with three replications. Grain yield and straw yield were significantly higher (45.4 q/ha and 57.8 q/ha respectively), with application of 75% RDF + cow urine 30% twice (60 & 75 DAT) Which was on par with the application of 75% RDF + cow urine 20% twice (60 & 75 DAT) and 100% RDF (80:50:30 kg NPK/ha). *The number of IAA producing bacteria that was successfully purified was 04 isolates.* All isolates except FU2 were quantitatively measured in IAA producing *using Salkowski reagent*; concentration of IAA were ranged from 0.274 - 0.389 ppm. Characterization of cow urine was carried out to know the antifungal activity against fungal pathogens (*Fusarium oxysporum).* The extent of growth of test fungi in plates poisoned with cow urine was lesser when compared with the control plates. When the three concentrations of cow urine were compared, maximum growth suppression was observed in *Fusarium oxysporum* (53.33%) at 30% concentration of cow urine followed by 20% and 10%. Cow urine has antifungal activities and the inhibitory activity can be used in the control of fungi. The beneficial effect of cow urine on rice was also evaluated enhancing the grain yield.

Keywords: Cow urine, foliar spray, rice, IAA and antifungal activity

Introduction
In India, Rice (*Oryza sativa* L.), a staple food, is crucial for food security and livelihoods for millions. In Chhattisgarh, it occupies an average of 3.7 million hectares, with productivity ranging between 1.2 to 1.6 t/ha depending on rainfall.

The present hike in the price of chemical fertilizers has compelled Indian farmers to use natural fertilizer sources which are cost effective. Intensive cultivation with continuous use of higher doses of inorganic fertilizers significantly influences soil health, crop growth. Therefore, it is imperative to exploit the potential of organic manures, agricultural wastes, biofertilizers and their synergistic impact with chemical fertilizers for increasing productivity and sustainability.

Increase in microbial count of soil has been reported due to organic amendments *viz.,* FYM (Manqiang *et al.*, 2009) [16], compost, vermicompost (Kanan *et al.*, 2005; Swetha *et al*., 2009) [12, 24], cow dung and cow urine (Orwin *et al.,* 2010) [17] and Jeevamrut (Swetha *et al.,* 2009) [24]. In India, over 70% of farm households practice crop along with dairy farming. The abundant quantity of cattle excreta consisting of dung and urine is available. Though part of cattle dung is used as manure but cow urine usually drains out as waste material. However, cattle urine being a valuable manure (Khanal *et al.*, 2001) [14] is a rich source of nutrients like nitrogen, phosphate, potassium, calcium, magnesium, chlorite, and sulphate (Belie *et al.*, 2000) [3], which are available to farmers at free of cost. Being organic in nature it is ecofriendly and can be used in crops with no detrimental effect on environment and human health. It can be sprayed at critical growth stages to overcome slow release nutrients from organic sources, working as a plant growth promoter. Cow urine contains 95% water, 2.5% urea, and 2.5% salts, hormones, enzymes, and minerals (Bhadauria, 2002) [4] besides significant amounts of both nitrogen and potassium.

It has been reported that beneficial effect of farming system with inclusion of cow urine on several crops such as on mustard (Pradhan *et al*., 2016) [21], Maize & Sweet corn (Pande *et al.*, 2015) [19], and on vegetables Burubhai and Eribo, 2012; Keduka *et al.*, 2014 [6, 13]. It seems that cow urine application both as soil application and foliar spray is beneficial when use as a biofertilizer and biopesticide in crop production and it needs to be studies under existing climatic condition of Chhattisgarh. In this aspect no systematic studies carried out to find out response of rice to foliar spray of cow urine.

Therefore, a study has been planned to assess cow urine properties as plant growth enhancement by isolating IAA producing bacteria from cow urine, its antifungal activity and to evaluate the effect of various levels of fertilizer and cow urine on yield of rice.

Materials and Methods

A field experiment was conducted during kharif season of 2022-23 at the Agricultural Research Farm, IGKV, Raipur. The soil of the experimental site was sandy loam clay with neutral to slightly acidic/ alkaline in pH (5.5), medium in organic carbon (0.50%). The experiment was laid out in randomized complete block design with ten treatment combinations including one control replicated four times. The crop taken was rice, variety CGDevbhog. A spacing of 1mt between two replications and 75cm in between plots was maintained to serve as irrigation channel and working place. Plant to plant spacing of 20 x 10cm was maintained.

Well decomposed farm yard manure at the rate of 2t ha^{-1} was applied at the time of land preparation. Recommended dose of N: P: K at the rate of 80:50:30 kg NPK/ha incorporated in field in the form of Urea, SSP and Murat of Potash, respectively. One third the dose of N and full dose of P_2O_5 and K_2O were applied at time of transplanting and remaining one third dose of N was applied at 30 days after planting, again one third dose of N was applied at 45 days of transplanting. Foliar spray of cow urine was applied accordingly to treatment combination at three levels (10% 20% and 30%) was done at 30, 45 and 60 DAT. Cow urine was diluted with water as per the treatment concentration for foliar spray in the rice crop in field at three levels. The experimental field was kept free from weed by periodic weeding. Irrigations were given as and when required during crop growth period. All the recommended package of practices were adopted to raise healthy crop throughout the period of experiment. After 120 days of growth of rice, harvest grain yield and straw yields were recorded as per the treatment and replications

Cow urine collection: was collected from the dairy farm of IGKV, Raipur in a sterile container. Filtration of cow urine was done through Whatman No. 1 filterpaper to remove from dusts and other waste precipitated material and was stored in airtight container in the refrigerator for analysis purpose at 4 ºC.

The nutrient composition of cattle urine was analyzed. The pH and electrical conductivity of cow urine was determined (Jackson, 1973) [9]. Total nitrogen Total-P and Total-K in cow urine is commonly determined as per Page *et al* (1982) [18].

Antifungal activity test in cow urine

The antifungal activity of cow urine concentrations (5%, 10%, 15%) was studied in potato dextrose agar medium. Different cow urine concentrations were adjusted using distilled water. The medium was autoclaved and poured into sterilized Petri plates. 10mL of different concentrations of cow urine was amended in 10mL of potato dextrose agar medium and mixed thoroughly by stirring. Control was maintained in which distilled water was used instead of cowurine. A fungal species, *Fusarium oxysporum*, was isolated from infected tomato plants, showing symptoms of damping off and wilting.

The fungi were grown on the PDA medium to obtain the active culture. Fungal discs of 5mm diameter were taken from active cultures and transferred to PDA plates poisoned with cow urine. Incubated for 7 days at 28 ± 2 ºC temperature, colony diameters were measured (Dhingra and Kapoor, 1985) [7], and the percent of inhibition was calculated using a formula from Vincent (1947) [25].

Percent inhibition of mycelial growth (%) $I = \frac{100\ (C-T)}{C}$

Where I is inhibition percentage, C is colony diameter incontrol plates, and T is colony diameter in poisoned plates.

Testing of cow urine for IAA production

Urine sample was diluted from 10^{-1} to 10^{-4} using with physiologic saline (NaCl 0.85%). A total of 0.1 mL of the mixture was spread on Nutrient Agar (NA) containing 1 mM L-tryptophan and incubated for 24 h at an incubator at 38°C. Bacterial colonies were counted using the total plate count method, purified on NA media containing 1 mM L-tryptophan. A single colony obtained and maintained on slant agar media (Harca *et al.*, 2014) [8].

The bacterial isolates obtained were tested for their ability to produce IAA. For this each isolate was inoculated into Nutrient Broth (NB) containing 1 mM L-tryptophan and incubated in 100 rpm rotary incubator for 8 h at room temperature (± 27 °C), then 2 mL of culture inoculated into 18 mL NB media at Erlenmeyer. The culture was incubated at 100 rpm for 24 h at room temperature and then centrifuged at 10.000 rpm for 10 minutes. A total of 1 mL the supernatant was homogenated with 4 mL of Salkowski reagent and incubated for 15 minutes in the dark. The absorbance was measured by spectrophotometer at 520 nm. IAA concentrations of the bacterial isolate were determined based on the IAA standard curve with a range of 0-100 ppm (Astriani *et al.*, 2016) [1].

The data obtained from various characters under study were analyzed using analysis of variance (ANOVA) for RBD (Panse and Sukhatme, 1967) [20].

Result and Discussion

Nutrient composition of cow urine

pH and EC of cow urine and N, P, K content in percent were presented in Table 1. Cow urine contains 0.59% nitrogen, 0.13% phosphorous and 0.61% potassium. pH of cow urine was 7.53 and EC value was 31.06 (dS m^{-1}). Application of cattle urine helps in improving the fertility of soil and it is essential to achieve the sustainability in terms of ecosystem.

Table 1: Nutrient composition of cow urine

Parameters	Cow urine
pH	7.53
EC (dS m^{-1})	31.06
N (%)	0.59
P (%)	0.13
K (%)	0.61

Antifungal activity test in cow urine

The study found that cow urine at different concentrations (10,

20, and 30%) effectively inhibited the growth of fungal plant pathogen, *Fusarium oxysporum*. With increase in concentration of cow urine there was corresponding increase in the inhibition of vegetative growth of the fungal pathogens. The diameter of the fungal colonies in cow urine included plates was lesser when compared to control plates and it indicates the antifungal effect of cow urine. The maximum inhibition was observed against *Fusarium oxysporum* (53.33%) at 30% concentration, followed by 20% and 10%, and no inhibition at 0% concentration of cow urine. This indicates that cow urine has a significant effect on the vegetative growth of *F. oxysporum*, as reported by various researchers. Inhibitory activity of cow urine against fungal pathogens have been reported by different workers Rajesh and Jayakumar 2013 [22], Rakesh *et al*. 2013 [10].

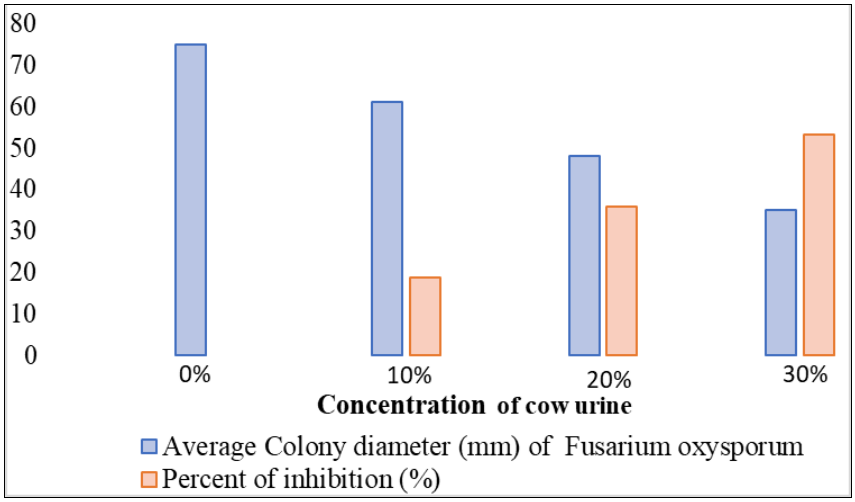

Fig 1: Effect of different concentration of cow urine on *Fusarium oxysporum* growth

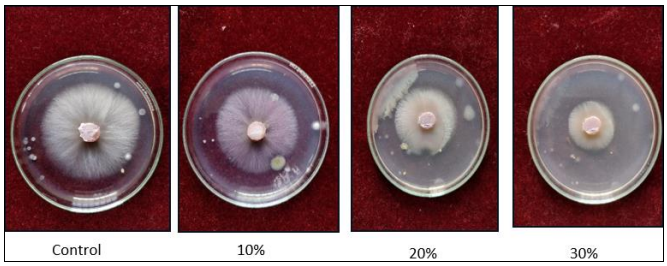

Fig 2: Antifungal activity test in cow urine.

Testing of cow urine for IAA Production

Isolation of IAA producing bacteria from cow urine sample was carried out using nutrient agar (NA) containing 1 mM L-tryptophan. L-tryptophan was known as IAA precursor (Kafrawi *al*., 2014) [11]. There were 04 isolates (FU1, FU2, FU3 and FU4) were obtained from urine sample (Table 3). All isolates except FU2 were quantitatively measured in IAA producing at NB medium containing 1 mM L-tryptophan for 24 h of incubation. (Astriani *et al.*, 2016) [1]. The Concentration of IAA were ranged from 0.274 - 0.389 ppm. The highest IAA production was 0.389 ppm. Ananda (2011) studied characterization of total microbial and fungi population in cow urine and revealed the total bacterial population was 260x104cfu/ml

Table 2: IAA production of bacterial isolates, isolated from cow urine sample

Source	Bacterial population cfu/ml of cow urine	Bacterial isolates	Concentration of IAA (ppm)
Fresh urine of cow	110 x10^5	FU1	0. 312
		FU2	-
		FU3	0.389
		FU4	0.274

Grain yield and straw yield (q ha^{-1})

The grain and straw yields of rice were significantly influenced by various levels of cow urine. T10 [75% RDF + cow urine 30% twice (60 & 75 DAT)] recorded higher grain and straw yields i.e., 6.08 and 9.42 kg/plot, respectively as compared to control (no fertilizer) Also significantly maximum yield was obtained from 100 percent recommended dose of fertilizer applications. While the lowest Grain yield was recorded in T1 Control (no fertilizer)] 20.68 qha^{-1}.

Table 3: Effect of foliar spray of various levels of cow urine along with different levels of fertilization on yield of rice.

Treatment	Treatment details	Yield (q ha^{-1})	
		Grain yield	Straw yield
T 1	Control (no fertilizer)	20.68	23.47
T 2	50% RDF (40:25:15 kg NPK/ha)	24.57	32.06
T 3	75% RDF (60:37.5:22.5 kg NPK/ha)	28.03	44.25
T 4	100% RDF (80:50:30 kg NPK/ha)	29.0	41.76
T 5	50% RDF + cow urine 10% thrice (45, 60 & 75 DAT)	25.96	37.92
T 6	50% RDF + cow urine 20% thrice (45, 60 & 75 DAT)	26.11	36.09
T 7	50% RDF + cow urine 30% thrice (45, 60 & 75 DAT)	26.34	35.61
T 8	75% RDF + cow urine 10% twice (60 & 75 DAT)	27.93	41.90
T 9	75% RDF + cow urine 20% twice (60 & 75 DAT)	28.89	44.25
T 10	75% RDF + cow urine 30% twice (60 & 75 DAT)	29.18	45.21
S.Em±		0.18	0.45
CD (0.05%)		0.51	1.29

Conclusion

The field experiment conducted at IGKV, Raipur, during the 2022-23 kharif season evaluated cow urine's efficacy in rice cultivation. The randomized complete block design experiment with ten treatment combinations, including a control, highlighted the potential of cow urine as a soil amendment. Results showed cow urine's nutrient-rich composition, conducive pH, and electrical conductivity for agricultural use. Antifungal tests against Fusarium oxysporum exhibited significant inhibition, indicating potential disease management benefits. Additionally, cow urine displayed IAA production, promoting plant growth. These findings underscore cow urine's promise in enhancing soil fertility, disease control, and crop productivity, emphasizing its role in sustainable agricultural practices.

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