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Antifungal activity of Different Concentrations of Cow urine against *Alternaria porri* (Ellis) Cif. and *Penicillium citrinum* isolated from onion infected with Purple Blotch and Blue Mold disease_131124

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ABSTRACT
This study investigated the antifungal properties of cow urine against two fungal pathogens, Alternaria Porri (Ellis) Cif. and Penicillium citrinum, isolated from Onion plants exhibiting symptoms of Purple Blotch and Blue Mold Disease, respectively. Using the poison food technique, the antifungal activity of cow urine at concentrations of 25%, 50%, 75%, and 100% was evaluated. Results indicated a significant reduction in fungal growth on plates treated with cow urine compared to control plates. Maximum growth suppression was observed with 100% cow urine concentration, particularly against Alternaria Porri (Ellis) Cif. (100%) followed by Penicillium citrinum (97.77%). These findings suggest the potential of cow urine as a natural antifungal agent for controlling fungal diseases in agriculture.
Keywords: *Antifungal, Alternaria Porri (Ellis) Cif. and Penicillium citrinum, Onion, Cow urine.*

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INTRODUCTION

Onion (*Allium cepa* L.) stands as one of the oldest bulb crops within the Amaryllidaceae family. The genus Allium encompasses a vast array of over 700 species distributed across tropical, temperate, and sub-temperate regions worldwide [1]. Known for its nutritional value, onions serve as a rich source of essential minerals, vitamins, and carbohydrates. Analyses have revealed that the bulb contains carbohydrates (11.0 g), proteins (1.2g), fiber (0.6g), moisture (86.8g), and energy (38cal.) [2]. Despite its nutritional significance, onion bulbs are susceptible to various biological factors leading to their deterioration. Factors such as respiration and pathogen attacks render the bulbs unsuitable for marketing [3]. Notably, one of the most prominent diseases affecting onions during the leafy stage is purple blotch, caused by *Alternaria Porri* (Ellis) Cif., first reported in France by Thaxter [4]. This disease has since spread globally, causing significant damage to onion stalks and resulting in substantial losses in seed and bulb production [5]**.** In addition to field diseases, onions in storage are susceptible to various post-harvest diseases, with Blue Mold of Onions being a particularly detrimental condition. Several *Penicillium* species, including *Penicillium expansum*, contribute to post-harvest illnesses, causing blue mold rot in onions and other horticultural vegetables [6]. The predominant strategy for controlling plant diseases has been the use of chemical agents. However, the overuse and misuse of these chemicals have led to various hazardous effects, including environmental contamination, toxicity to non-target organisms, residual issues, and the development of pathogen resistance. Consequently, there is a growing interest in exploring alternative methods for disease control. Natural products, particularly those derived from plants, represent potential candidates for combating phytopathogenic fungi. Unlike synthetic chemicals, these natural agents offer a risk-free approach to disease management. In ancient Ayurveda, cow urine has been extolled for its pharmacological importance.

Cow urine is a constituent of "Panchagavya" (urine, dung, milk, curd, and ghee), renowned for its medicinal properties [7]. It possesses excellent germicidal, antibiotic, and antimicrobial activity, making it effective against a variety of germs and boosting immunity [8]. Furthermore, cow urine has garnered attention for its agricultural utility as a biofertilizer and biopesticide, capable of combating pesticide and herbicide-resistant bacteria, viruses, and fungi [9]. The present study explores the use of different concentrations of cow urine against *Alternaria Porri* (Ellis) Cif. and *Penicillium citrinum*, aiming to evaluate its antifungal efficacy.

MATERIAL AND METHODS

Collection of Cow Urine: Fresh cow urine was acquired from a local variety of cows and collected in sterile containers. Subsequently, the urine was filtered using Whatman No. 1 filter paper to eliminate debris and precipitated material. The filtered urine was then stored in airtight containers at 4°C until further use.

Isolation of Fungal Pathogens: Fungal pathogens, namely *Alternaria Porri* (Ellis) Cif. and *Penicillium citrinum*, were isolated from infected Onion plants displaying symptoms of Purple Blotch and Blue Mold Rot, respectively.

Identification of Fungi: The fungal cultures were identified at the Agarkar Research Institute Pune, and access numbers were provided for *Alternaria Porri* (NFCCI 5023) and *Penicillium citrinum* (NFCCI 5266). Both cultures were maintained in a research laboratory for subsequent studies.

Antifungal Activity: Four concentrations (25%, 50%, 75%, and 100% v/v) of cow urine were prepared. Each concentration was incorporated into potato dextrose agar (PDA) medium by adding 5 mL of cow urine to 15 mL of PDA medium and thoroughly mixed. A control group was established using distilled water instead of cow urine. The prepared medium was then autoclaved and poured into sterilized Petri plates. Fungal discs measuring 5 mm in diameter were obtained from actively growing cultures using a cork borer and transferred aseptically onto the PDA plates containing cow urine. The plates were incubated at a temperature of 27±2°C for 7 days. After the incubation period, the plates were observed, and colony diameters were measured using a ruler [10]. The percentage of inhibition was calculated using the formula proposed by J. M. Vincent [11].

Percent inhibition of mycelial growth (%)

$$I = [(C-T)/C]x100$$

where I is inhibition percentage, C is colony diameter in control plates, and T is colony diameter in poisoned plates.

RESULTS AND DISCUSSION

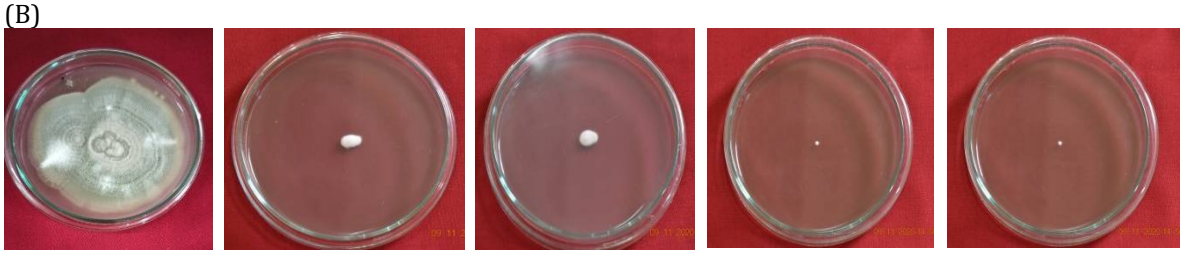
Table 1: Antifungal activity of cow urine by poison food technique.

Sr.No	Fungal pathogen	Concentration (%)	Colony diameter (mm)	Percent of inhibition (%)
1		0 (Control)	90	00
	Alternaria porri	25%	31	65.55
		50%	24	73.33
		75%	2	97.77
		100%	00	100
2		0 (Control)	90	00
		25%	16	82.22.
	Penicllium	50%	12	86.66
		75%	5	94.44
		100%	2	97.77

(A)

Control 25% 50% 75% 100%

(B)



Control 25% 50% 75% 100%

Figure 1: (A) Effect of different concentrations (25%, 50%, 75% and 100%) of cow urine on *Alternaria Porri* (Ellis) Cif. (B) Effect of different concentrations (25%, 50%,75% and 100%) of cow urine on *Penicillium citrinum*.

In this study, two fungal pathogens, *Alternaria Porri* (Ellis) Cif. and *Penicillium citrinum*, were isolated from diseased Onion plants. Data presented in Table 1 demonstrate the effectiveness of all concentrations (25%, 50%, 75%, and 100%) of cow urine against the growth of these fungi, resulting in significant inhibition of their vegetative growth. A clear dose-response relationship was observed, with higher concentrations of cow urine corresponding to greater inhibition of fungal growth. The diameter of fungal colonies on plates treated with cow urine was notably smaller compared to control plates, indicating the antifungal properties of cow urine. Maximum inhibition was observed against *Alternaria Porri* (Ellis) Cif. (100%), followed by *Penicillium citrinum* (97.77%). Conversely, the minimum inhibition was recorded with a 25% concentration of cow urine against *Penicillium citrinum* (82.22%), followed by *Alternaria Porri* (Ellis) Cif. (65.55%). These findings highlight the significant inhibitory effect of cow urine at different concentrations on the vegetative growth of *Alternaria Porri* (Ellis) Cif. and *Penicillium citrinum*. Particularly, the 100% concentration of cow urine exhibited the highest inhibition compared to the control. Similar inhibitory activities of cow urine against fungal pathogens have been reported by various researchers [12-14].

The results underscore the potential of cow urine as an effective antifungal agent against *Alternaria Porri* (Ellis) Cif. and *Penicillium citrinum*, suggesting its utility in fungal disease management. Further investigations are warranted to elucidate the mechanisms underlying its antifungal activity and to explore its practical application in agricultural settings.

CONCLUSION

findings of this study demonstrate the inhibitory effect of cow urine on the growth of both fungal pathogens investigated. This highlights the fungitoxic potential of cow urine against *Alternaria Porri* (Ellis) Cif. and *Penicillium citrinum*, suggesting its efficacy as a biopesticide.The results underscore the viability of cow urine as a natural and eco-friendly alternative for controlling fungal diseases in agricultural settings. Further research and field trials are warranted to evaluate its efficacy under diverse environmental conditions and to explore its integration into sustainable disease management strategies.

In summary, cow urine emerges as a promising biopesticide candidate, offering potential benefits such as safety, sustainability, and effectiveness against fungal pathogens. Its utilization could contribute to the development of environmentally friendly agricultural practices aimed at enhancing crop health and productivity.

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CONSENT FOR PUBLICATION

Not applicable

COMPETING INTEREST

The author declares that they do not have any competing interests.

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