Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(3): 485-493
Received: 27-03-2018
Accepted: 28-04-2018

Tanmay Ghosh
Department of Microbiology, Rabindra Mahavidyalaya, Champadanga, Hooghly, West Bengal, India

MK Biswas
Department of Plant Protection, Palli Siksha Bhavana, Viswa-Bharati, Sriniketan, Birbhum, West Bengal, India

Chiranjib Guin
Department of Microbiology, Rabindra Mahavidyalaya, Champadanga, Hooghly, West Bengal, India

Correspondence
MK Biswas
Department of Plant Protection, Palli Siksha Bhavana, Viswa-Bharati, Sriniketan, Birbhum, West Bengal, India

Efficacy of cow urine in wheat (*Triticum aestivum*) production as plant growth promoter and antifungal agent

Tanmay Ghosh, MK Biswas and Chiranjib Guin

Abstract
Cow urine therapy and all traditional practices from Indian systems of medicine have a strong scientific base. The cow has proved to be a boon in the areas of agriculture, science and technology, industry, energy, medicine etc. for the development of any nation, in addition being eco-friendly in nature. The present study was conducted to determine antifungal activity of different concentrations (20%, 40%, 50%, 70% & 100%) of cow urine against some fungal pathogens (*Aspergillus flavus*, *Aspergillus niger*, *Rhizopus* sp, *Alternaria* sp, *Mucor* sp, *Fusarium* sp, *Penicillium* sp, *Macrophomina* sp) isolated from infected plants of Wheat. The extent of growth of test fungi in plates poisoned with cow urine was lesser when compared with the control plates. Among these concentrations cow urine at 100% concentration was most effective. Finally we concluded that the cow urine has antifungal activities and the inhibitory activity can be used in the control of fungi. The nutritional effect of cow urine on plant growth was also tested with Golden wheat (*Triticum aestivum*).

Keywords: Efficacy, cow urine, wheat (*Triticum aestivum*) production, plant growth promoter, antifungal agent

Introduction

'The cow' is a mobile medical dispensary and cow urine is a panacea of all diseases [1]. The cow urine, one of the ingredients of 'Panchagawya' is capable of treating many curable as well as incurable diseases and has been used extensively in ayurvedic preparations since time immemorial as cited in ancient holy texts like Charaka Samhita, Sushruta Samhita, Vridhabhagabhatt, Atharva Veda, Bhavaprakash, Rajni Ghuntu, Amritasagar, etc [2]. A lots of research has been conducted in Cow Urine Treatment and Research Center, Indore over the past few years and it has been reported that gomutra is capable of curing blood pressure, blockage in arteries, arthritis, diabetes, heart attack, cancer, thyroid, asthma, psoriasis, eczema, prostrate, fits, AIDS, piles, migraine, ulcer, acidity, constipation, gynecological problems, ear and nose problems and several other diseases [3]. The use of cow urine in India can be traced back to the Vedic and probably prevedic period also. Cow urine as such has been most widely referred, used and venerated animal urine owing to its immense therapeutic speciality. While externally it has been used as lotion, ointments and bath, but, internally it has been used in preparation of oral medications and drinks. There is existence of innumerable instances in various ancient medical texts of the curative properties of cow urine for a horde of human ailments. In ancient Indian system of medicine, urine of cow was accepted, used almost as a broad spectrum antibiotic quite akin to that of twenty first century. The cow urine not only used against ailments of diseases as therapeutic agents but also have several other uses as in agriculture and sericulture sectors. So this article attempts to bring forth the diversified use of this heretical potion as was in vogue in ancient Indian system of medicine as gleaned from the ancient medical texts and current scientific findings. In Veda, cow is considered the most valuable animal and is called Mother of all. Different products obtained from cow like urine, dung, milk, ghee and curd are used widely in number of Ayurvedic formulations [4] As per Ayurvedic literatures cow urine possess many medicinal properties and is used in curing number of diseases like skin diseases, kidney problems, epilepsy, anemia, constipation, respiratory disease etc. [5, 6]. Due to its therapeutic values majority of rural population in India use cow urine as a folklore remedy to get rid of various diseases. Nowadays, different preparations of cow urine like urine distillate, photo-activated urine, fresh urine, sterile urine have been marketed with cheap and affordable prices [7] Thus the aim of the present work is to study antibacterial potential of photo-activated cow urine, fresh cow urine, cow's urine Fungi are one among the important aetiological agents of plant diseases. Fungi cause diseases in plants both in field and storage.

The fungal infection of plants results in poor yield of crop and hence results in considerable economic loss. In severe cases, crop losses exceeding >50% can occur due to fungal infections. The management of mycotic diseases of plants mainly focuses the use of chemical agents. The use of synthetic fungicides appears promising but suffers from several drawbacks such as high cost, environmental pollution, adverse effects on non-target organisms and the emergence of resistant strains of pathogenic fungi. Natural products including cow urine and cow urine based formulations have been investigated for antifungal activity and the results appeared promising [8-12]. Cow urine is shown to exhibit potent inhibitory activity against a wide range of pathogenic microbes including phytopathogenic fungi [13-18]. Similarly, formulations based on cow urine and plants have shown to possess inhibitory effect against various pathogenic microorganisms [19-24]. The present study was carried out to investigate antifungal and antibacterial potential of cow urine against 6 phytopathogenic fungi and 3 bacteria.

Methodology

Collection and preparation of cow urine

Cow urine was collected from the well-maintained *Goshala* of local area. The cow selected for this research was a healthy Nagori cow, aged seven years being fed a uniform diet and undergoing regular vaccination schedule. Fresh cow urine was collected in sterile screw cap bottles and brought to the laboratory for testing. It was filtered by ordinary filter paper before being subjected to further testing. Sterile cow urine was prepared by sterilizing the urine sample maintained in an autoclave at a temperature of 121°C and 15 lb/in2 pressure for 15 minutes. Photo activated cow urine was prepared by keeping the urine in transparent sterile bottle for 72 hours in sunlight. Thus purified, fresh and photoactivated cow urine were stored at 4 °C for further future use as per experimental requirements. Different samples of cow urine thus obtained are:

1. Fresh cow urine,
2. Sterile cow urine,
3. Photo activated cow urine,

Fig 1: different types of cow urine

Isolation of fungal pathogens

All bacterial and fungal strains were isolated from different oil and cereal crops seed. Seeds were incubated in Potato dextrose Agar (PDA) and Standard blotter paper for 5 to 7 days. Then the microbial colony on agar and paper plate were isolated and identified. Then they were made slant culture separately. Then they were stored in room temperature for further use.

Medium

PDA plates were used for the growth of fungal strain and MHA plates were used for the growth of Bacterial strain. 3.9 g of Potato dextrose agar and 3.8 g of Mueller Hinton Agar (MHA) was added to 100ml of distilled water in separate conical flask and autoclaved at 121^0c for 15 minutes at 15 lbs and poured in sterile petri plates up to a uniform thickness of approximately 4mm and the agar is allowed to set at ambient temperature and used. A few slants were made for storage of Bacterial and fungal culture.

Microbiological assay

Procedure of determining the efficacy of cow urine as antifungal agent by well diffusion method

The antimicrobial activity of cow urine was tested by preparing 5 different percentage of cow urine. Percentages were made by dissolving cow urine in sterile water. Five percentages 20%, 40%, 50%, 70%, 100% were taken for the analysis of antibacterial activity. A hollow tube was heated and pressed above the inoculated agar plate. It was removed immediately by making a well in the plate; two and three wells on each plate were made.

Inocolums and incubation

0.1mg of bacterial and fungal cultures was transferred to the agar plates. The inoculated plates were allowed to stand for 5 min, before making wells for different percentages to be tested. The percentages of cow urine were loaded at different concentrations in the well on agar plate. Then bacterial and fungal cultures and incubated at normal room temperature for 24-48 hours in an incubator.

Procedure of determining the efficacy of cow urine as antifungal agent by poisoned plate method

The five percentages (20%, 40%, 50%, 70% and 100% v/v) of cow urine were prepared. 5mL of different concentrations of cow urine was amended in 15mL of potato dextrose agar medium and mixed thoroughly by stirring. Control was maintained in which distilled water was used instead of cow urine. The medium was autoclaved and poured into sterilized Petri plates and left. The fungal discs of 5mm diameter were taken from actively growing cultures by using cork borer and the discs were transferred aseptically on PDA plates poisoned with cow urine. Plates were incubated at 28 ± 2∘C temperature in incubator for 7 days. After 7 days plates were observed and colony diameters were measured with the help of ruler. The percent of inhibition was calculated using the following formula given below:

Percent inhibition of mycelia growth (%)

$$I = \frac{100\,(C - T)}{C}$$

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

Statistical analysis

After incubation the diameter of zone of inhibition around the well was measured using zone reader. Corresponding 3 values

of zones of inhibition for each percentage of cow urine were taken. The values so obtained were compared within the group (same percentage of cow urine) and with different groups (different percentage of cow urine) for different bacteria and fungi and statistical analysis was done

Effect of cow urine on plant growth

Collection of seeds

The seeds of Golden wheat *(Triticum aestivum)* were collected from local area of Tarakeswer, Hooghly, West Bengal.

Pot culture experiment

The pot culture study was conducted to find out the effect of various concentrations of cow urine on growth of Golden wheat (*Triticum aestivum*) plants. The seeds were soaked in water over night and then 25 seeds were sown in different pots filled with sterile garden soil. The garden soil was sterilized in an autoclave at 15 lbs pressure for half an hour. The pH of the soil was adjusted to 7. Each pot was irrigated twice a day with different concentrations (20%, 40%, 50%, 70%, 100% v/v) of cow urine. In control pots, the seeds were irrigated with tap water instead of cow urine. When the plants grew randomly 3 seedlings from each treatment were uprooted without disturbing the root system and different parameters such as plant height, root length, leaf length and leaf breadth were measured after 25 days to observe the plant growth.

Fig 2: pot culture method

Result and discussion

Result of determining the efficacy of cow urine as antifungal agent by well diffusion method

The results of antibacterial and antifungal potential of different cow urine preparation are shown in Table 1. The result of the present study shows that antibacterial and antifungal activity of fresh cow urine is more active than photoactivataed urine. These observations are likely to be the result of the presence of certain volatile and non-volatile components present in urine. The antibacterial activity of photoactivated urine may be due to its acidic pH. It may also be due to presence of more cations and formation of nitrosoamines. It was observed that gram positive organisms were more sensitive than gram negative organisms. These observations are likely to be the result of the differences in cell wall structure between gram positive and gram negative bacteria, with gram negative outer membrane acting as a barrier to many environmental substances. In the present studies six fungal species namely *Aspergillus* sp, *Rhizopus* sp, *Mucor* sp, *Penicillium* sp, *Alternaria* sp, *Macrophomina* sp and three bacterial cultures include *Bacillus subtilis*, *Pseudomonas sp*, *Streptococcus sp* are used. All percentagess (20, 40, 50, 70, 100) of cow urine were effective against the growth of the fungus which exhibited the significant inhibition in the growth of fungal plant pathogens. 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 poisoned plates was lesser when compared to control plates and it indicates the antifungal effect of cow urine. The highest zone of inhibition was shown against *Aspergillus* sp while the smallest zone of inhibition was shown against *Macrophomina* sp in fresh cow urine. The highest zone of inhibition was shown against *Aspergillus* sp while the smallest zone of inhibition was shown against *Pseudomonas* sp in photoactivated cow urine. The highest zone of inhibition was shown against *Bacillus subtilis* while the smallest zone of inhibition was shown against *Pseudomonas* sp in sterile cow urine. In our study it was reconfirmed that the cow urine possesses antimicrobial properties. From our study we came to a conclusion that urine from different cows had different level of antimicrobial properties. The difference in level of antimicrobial properties of different cow urine may be because of difference in chemical composition of urine which may arise due to several reasons. We found out in our studies that fresh cow urine was more effective antimicrobial agent than photo activated urine this may be because fresh urine is more acidic in nature. The microbial zones of inhibition values are given in the table below.

Table 1: Zones of inhibition of fungal pathogens by cow urine in agar cup method

Types of cow urine	Percentage of cow urine	Diameter of zone of inhibition (mm)								
		Name of the seed microflora (bacterial and fungal species)								
		Aspergillus sp	*Rhizopus* sp	*Mucor* sp	*Penicillium* sp	*Alternaria* sp	*Macrophomina* sp	*Bacillus subtilis*	*Pseudomonas sp*	*Streptococcus sp.*
Fresh	20%	14.26±1.0	11.22± 3.0	12.12±1.0	12.04± 0.0	12.63±2.0	10.42± 1.0	13.08± 1.0	11.38± 3.0	12.08±3.0
	40%	16.23± 3.0	13.00± 0.0	12.50±3.0	13.48± 1.0	13.13±1.0	12.23± 3.0	15.45±1.0	13.22±1.0	15.07±1.0
	50%	17.63±0.0	14.21±1.0	13.89±1.0	15.78± 3.0	15.24±3.0	13.22± 1.0	17.55± 3.0	15.02±1.0	17.10±1.0
	70%	20.03±1.0	16.05± 0.0	16.05±3.0	17.12± 1.0	17.16±1.0	15.56± 0.0	19.62± 1.0	16.88±3.0	19.11±3.0
	100%	23.42±3.0	19.02± 3.0	19.22±0.0	19.06± 2.0	19.72±1.0	19.66± 1.0	22.28± 1.0	19.72±2.0	22.21±1.0
Photo activated	20%	12.25±1.0	10.28± 1.0	12.57±1.0	11.37± 1.0	11.57±3.0	10.44± 1.0	12.64± 3.0	10.14±2.0	10.54±0.0
	40%	14.35±3.0	12.80± 0.0	13.35±0.0	13.19± 0.0	12.87±0.0	12.00± 3.0	14.31± 0.0	11.01±1.0	14.34±2.0
	50%	15.58±0.0	13.87± 1.0	13.50±1.0	14.25± 3.0	14.34±1.0	13.69± 2.0	15.59± 2.0	13.79±3.0	16.13±1.0
	70%	18.79±1.0	16.69± 3.0	15.94±3.0	17.31± 2.0	16.31±2.0	14.50± 0.0	18.29± 1.0	15.47±2.0	19.51±3.0
	100%	21.93±3.0	18.35± 1.0	18.58±0.0	19.21± 1.0	19.94±3.0	17.49± 1.0	21.19± 3.0	18.40±02	21.42±1.0
Sterile	20%	10.48±1.0	11.29± 2.0	10.28±1.0	11.87± 0.0	11.22±2.0	10.57± 3.0	11.28± 1.0	10.16±3.0	10.11±0.0
	40%	12.25±0.0	12.94± 1.0	11.25±1.0	12.83± 3.0	12.16±1.0	12.47± 0.0	13.42± 2.0	11.31±1.0	13.64±2.0
	50%	13.72±2.0	12.05± 1.0	12.78±3.0	13.90± 0.0	13.05±3.0	13.43± 1.0	15.34± 3.0	12.66±0.0	16.31±3.0
	70%	16.00±1.0	15.35± 0.0	14.34±1.0	16.05± 3.0	17.52±1.0	15.61± 3.0	17.49± 1.0	14.31±1.0	18.97±1.0
	100%	19.94±3.0	17.85± 3.0	17.80±0.0	18.58± 1.0	19.31±3.0	19.54± 1.0	20.38± 3.0	17.84±3.0	21.28±3.0

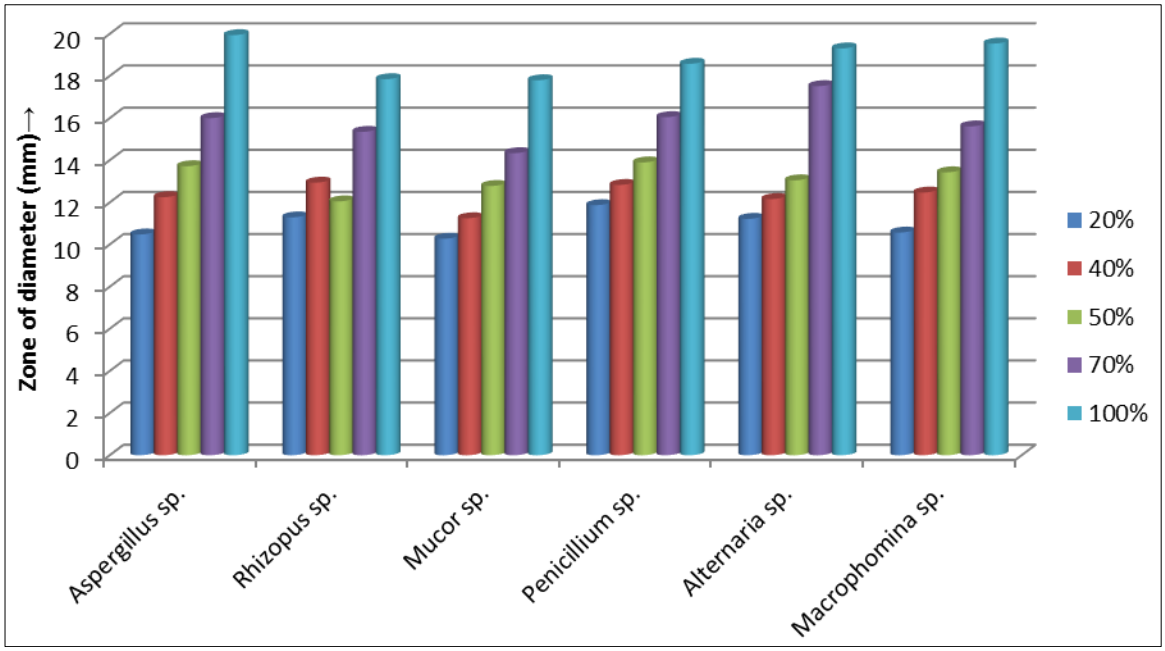

Fig 3(A): Antifungal activity of fresh cow urine

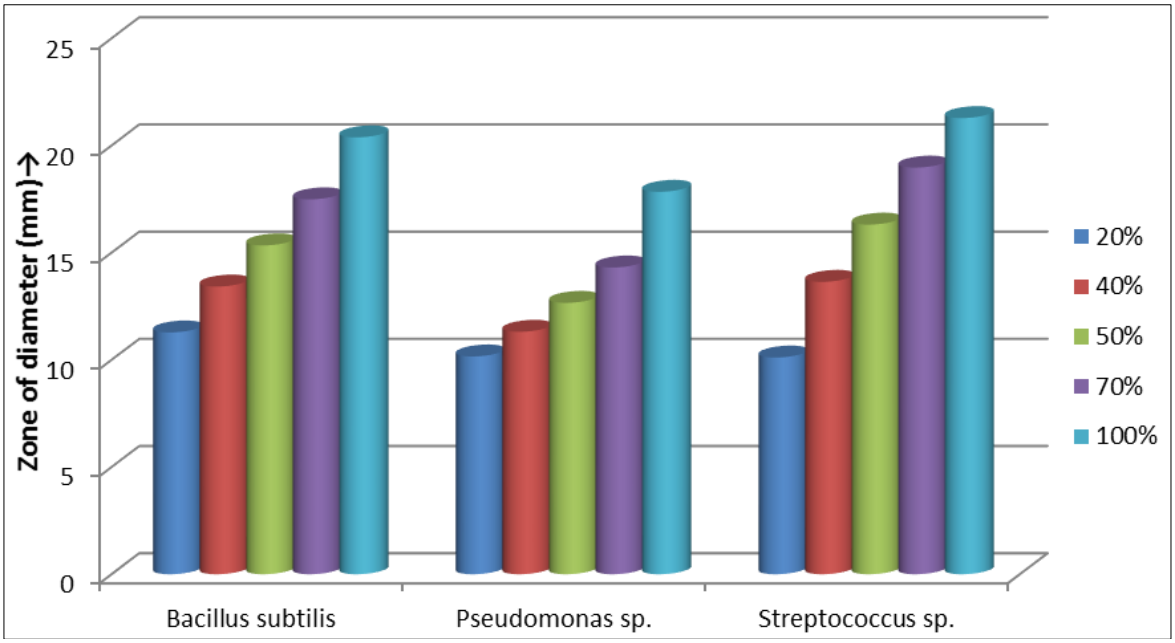

Fig 3(B): Antibacterial activity of fresh cow urine

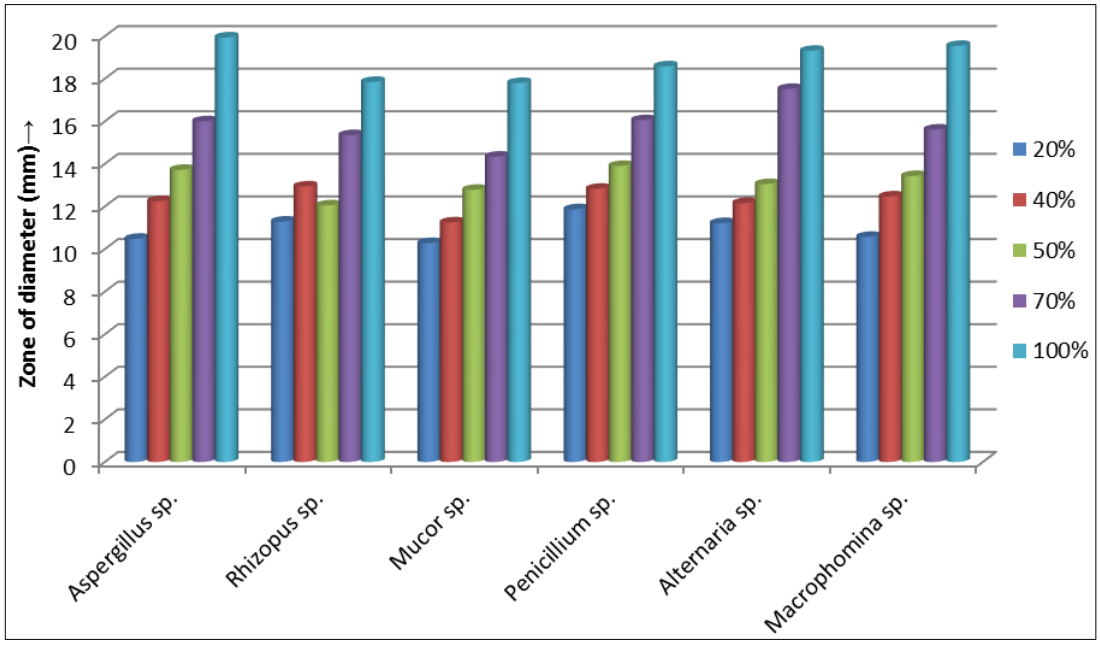

Fig 4(A): Antifungal activity of Photo activated cow urine

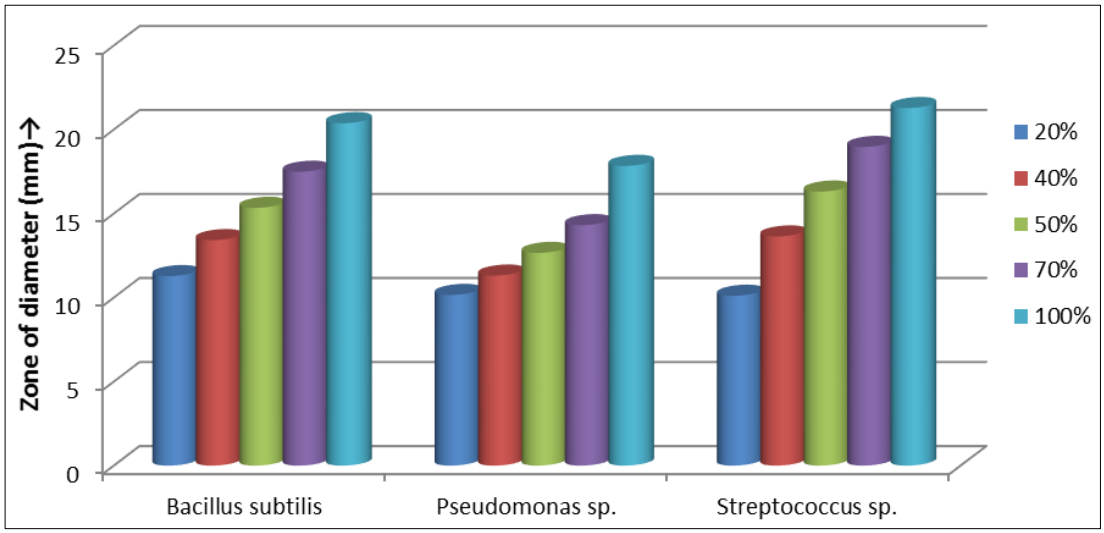

Fig 4(B): Antibacterial activity of Photo activated cow urine

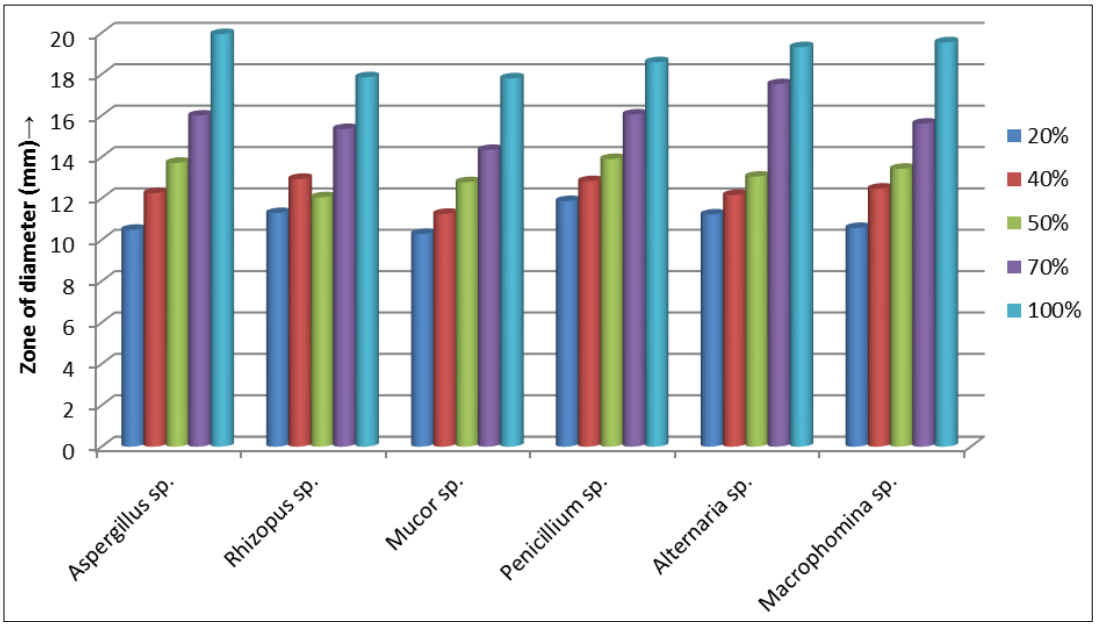

Fig 5(A): Antifungal activity of sterile cow urine

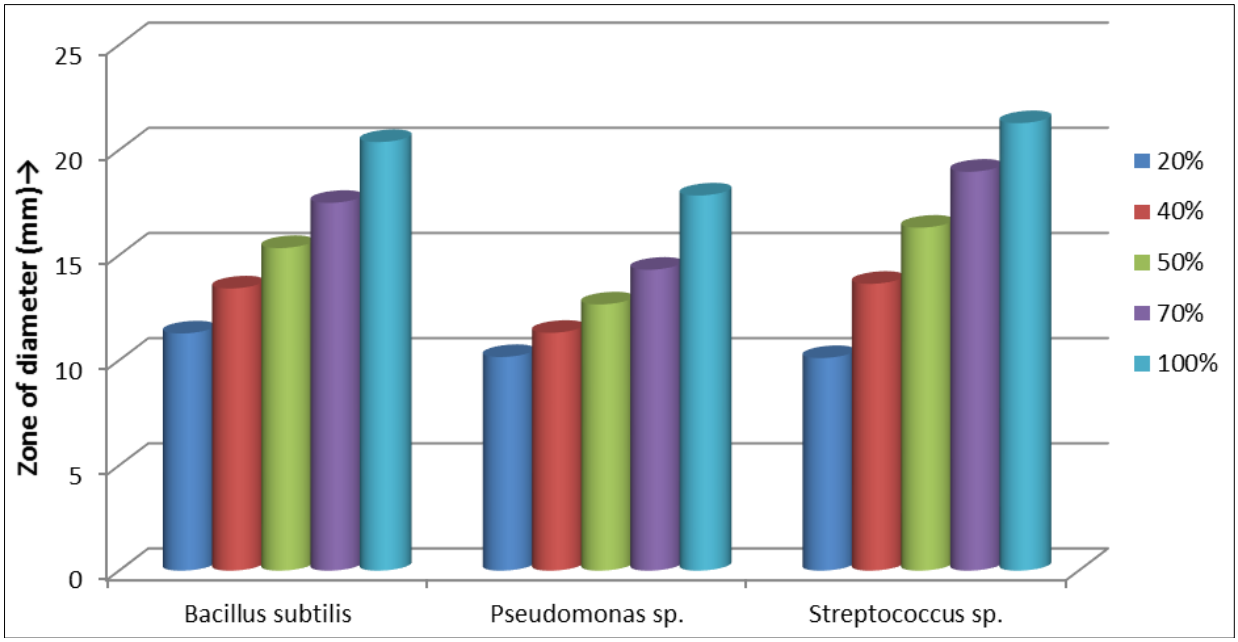

Fig 5(B): Antibacterial activity of sterile cow urine

Result of determining the efficacy of cow urine as antifungal agent by poison plate method

In the present studies *Aspergillus* sp fungal pathogens was isolated from the oil and cereal crop seed. Data presented in Table depicts that all percentages (20%, 40%, 50%, 70% and 100% v/v) of cow urine were effective against the growth of the fungus which exhibited the significant inhibition in the growth of fungal plant pathogens. 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 poisoned plates was lesser when compared to control plates and it indicates the antifungal effect of cow urine. Maximum inhibition was shown in 100% pure cow urine and minimum inhibition was shown in 20% cow urine plate.

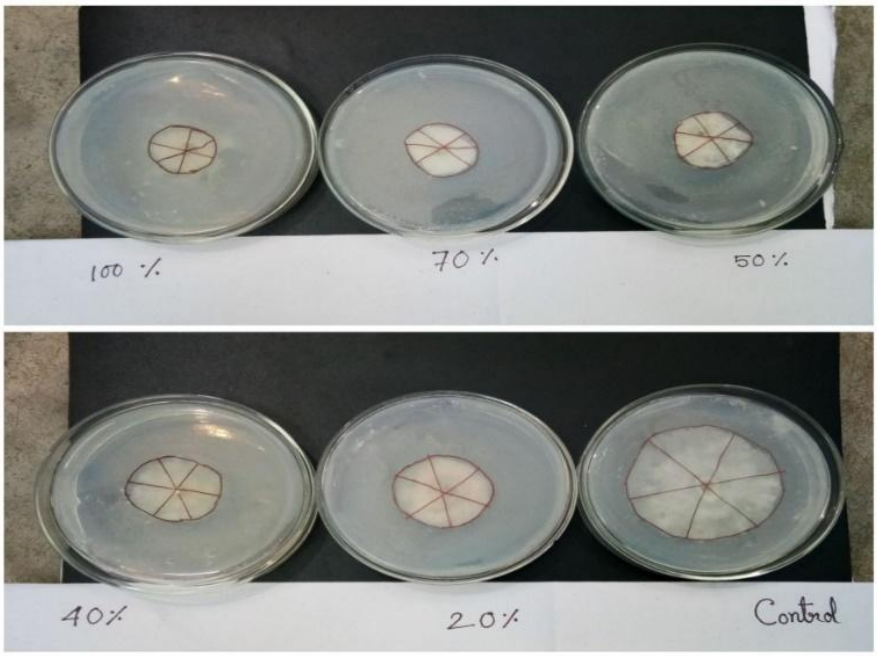

Fig 6: efficacy of cow urine as antifungal agent

Table 2: Cow urine as antifungal agent by poison plate method

S. No.	Percentage Of Cow Urine	Colony Diameter(Mm)	Percentage Of Inhibition of Fungal Growth
1	0%(control)	59.30	0.0
2	20%	37.30	37.09
3	40%	33.70	43.17
4	50%	31.30	47.21
5	70%	28.70	51.60
6	100%	25.00	57.84

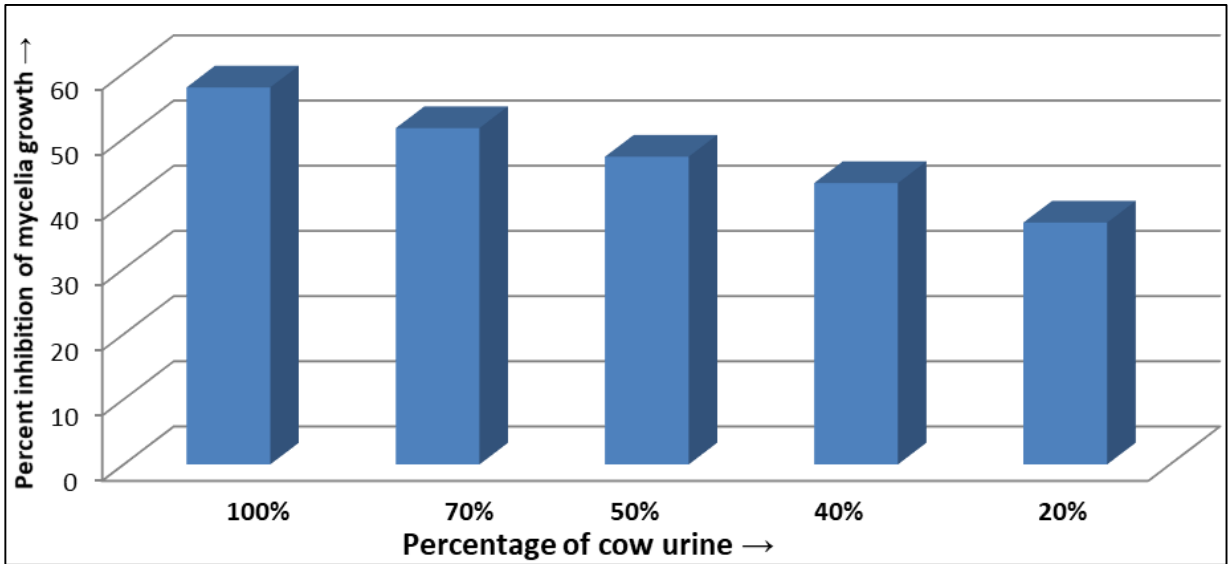

Fig 7: Efficacy of cow urine as antifungal agent

Result of pot culture method

Pot culture studies were carried out to find out the effects of cow urine spray on the phenotypic characters of golden wheat after 25 days. Parameters such as plant height and root length, number of leaves, and leaf length and breadth were observed in the experimental and control plants. It is clear from the results that plant height of Golden wheat increased with increase in concentration of cow urine and duration of time. Maximum plant height of Wheat was 14.30 ± 0.417.00 ± 0.40 cm with maximum concentration, that is, 100% of cow urine. Minimum plant height of Wheat was 15.00 ± 0.75cm with minimum concentration, that is, 20% of cow urine. Maximum root length of Wheat was 15.00 ± 0.35cm with maximum concentration, that is, 100% of cow urine. Minimum root length of Wheat was 10.4 ± 0.50 cm with minimum concentration, that is, 20% of cow urine.

Fig 8: Plant of pot culture experiment

Table 3: Effect of cow urine on exomorphological characters of *Triticum aestivum* (golden wheat) by pot culture experiment after 25 days.

Conc. of cow urine (%)	Plant height (cm)	Root length (cm)	Stem length (cm)	Leaf length (cm)	Leaf breadth (cm)
20	15.00 ± 0.75	10.4 ± 0.50	5.00 ± 0.30	10.00 ± 0.26	0.20 ± 0.15
40	15.40 ± 0.65	12.00 ± 0.40	5.10 ± 0.28	10.30 ± 0.25	0.30 ± 0.12
50	15.70 ± 0.66	13.50 ± 0.36	5.20 ± 0.26	10.50 ± 0.25	0.40 ± 0.35
70	16.50 ± 0.75	13.00 ± 0.31	5.70 ± 0.23	10.80 ± 0.12	0.50 ± 0.38
100	17.00 ± 0.40	15.00 ± 0.35	6.00 ± 0.20	11.00 ± 0.15	0.60 ± 0.21
Control	14.60 ± 0.46	8.50 ± 0.25	4.80 ± 0.15	9.80 ± 0.20	0.10 ± 0.10

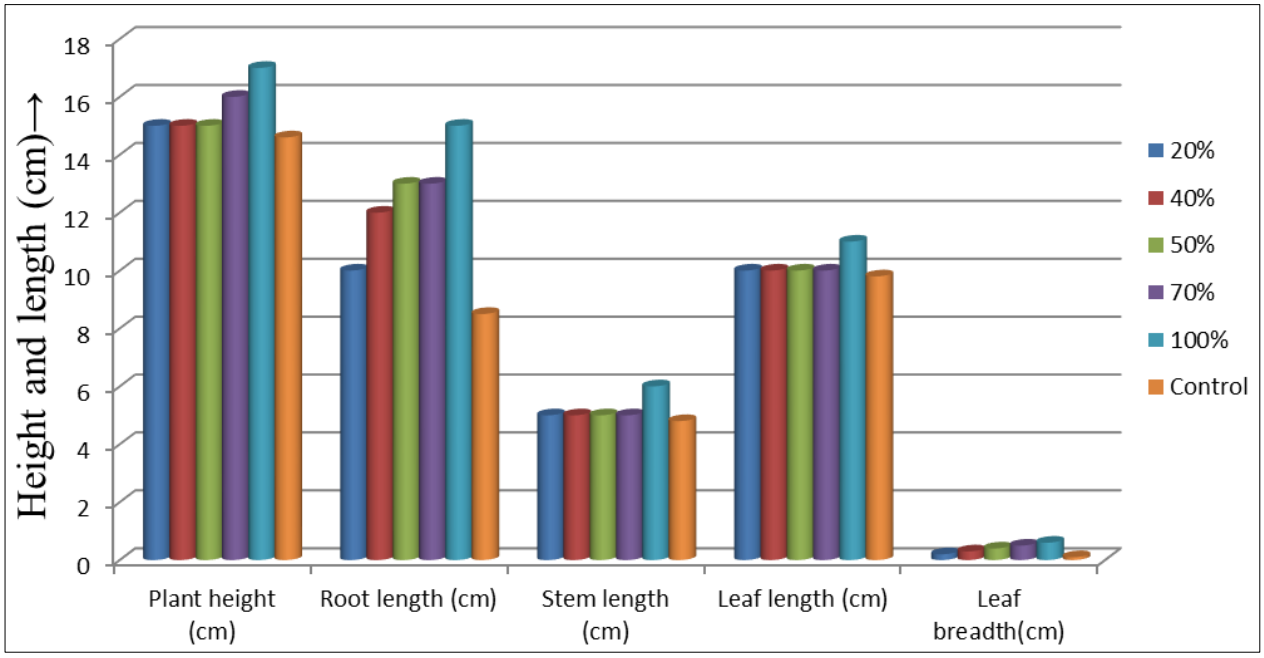

Fig 9: Effect of cow urine on exomorphological characters of *Triticum aestivum* (golden wheat) by pot culture experiment

Table 4: Effect of cow urine on percentage of germination of *Triticum aestivum* (golden wheat) by pot culture experiment after 25 days.

S. No	Concentration of cow urine (%)	Number of germination	Percentage of germination (%)
1	20	16	64
2	40	17	68
3	50	18	72
4	70	21	84
5	100	23	92
6	Control	15	60

Discussion

The study revealed that the cow urine at different concentrations had considerable effect on the vegetative growth of *Aspergillus flavus*, *Aspergillus niger*, *Alternaria* sp., *Mucor* sp., *Fusarium* sp., *Macrophomina* sp. It is clear that the results that 100 percent concentration of cow urine showed maximum inhibition in growth of all the fungal pathogens as compound to control. Inhibitory activity of cow urine against fungal pathogens have been reported by different workers [11, 12, 13]. Pot culture studies revealed that increase in cow urine concentrations increase the performance of all phenotypic character of Golden wheat *(Triticum aestivum)*. Present studies are in accordance with the findings of [14] Oliveira *et al.* 2009 who reported that the increase in cow urine concentrations increased the performance of all lettuce characteristics like fresh and dry leaf mass, fresh and dry stem mass, stem length, fresh root mass, fresh head mass, and commercial yield. The work of [15] Tharmaraj, 2011, reported that growth substances in panchagavya help to bring rapid changes in phenotypes of plants and also improve the growth and productivity. The protein and carbohydrate content found in seedlings sprayed with cow urine was more irrespective of the concentration as compared to the control.

Future aspect

In the present study soil application of cow urine increased the growth and yield of crops. The treatment supplied with 120 kg N along with cow urine showed higher grain and straw yield compared to that obtained through 150 kg N. Also, the treatment in which cow urine was applied showed higher N uptake in grain and straw. Hence, cow urine can be an effective input to improve the nutritional quality of the food grain and fodder also. India is a country of villages and cattle are being domesticated traditionally with the largest cattle population in world. But, because of mis-management and poor handling practices the cow urine is not utilized and losses of N through volatilization occur in the cattle shed. There is an immense scope in increasing the utilization of cow urine in field.

Conclusion

As cow urine showed remarkable antibacterial activity against thepathogenic bacteria, for which it can be selected for further studies to isolate bioactive natural constituents that may address to unmet therapeutic needs. Such screening of various natural compounds and identifying active agents is the needful, because successful prediction of lead molecule and drug like properties at the onset of drug discovery will pay off later in drug development. It was revealed from the study that cow urine caused inhibition in growth of all the three fungal pathogens used in the present studies. This demonstrated fungi toxic potential of cow urine against the *Aspergillus flavus*, *Aspergillus niger*, *Alternaria* sp., *Mucor* sp., *Fusarium* sp., *Macrophomina* sp. pathogenic fungi. The biochemical contents of both the plants increased when sprayed with cow urine. Therefore the use of cow urine provides better alternative to synthetic chemicals which are expensive and pose potential danger to the farmers, marketers, consumers, and environment. The cow urine can be used as biopesticide.

References

1. Pathak ML, Kumar A. Gomutra-descriptive study. Sachitra Ayurveda. 2003; 7:81-84.

2. Pathak ML, Kumar A. Cow praising and importance of Panchyagavya as medicine. Sachitra Ayurveda. 2003; 5:56-59.
3. Pathak ML, Kumar A. Cow praising and importance of Panchyagavya as medicine. Sachitra Ayurveda. 2003; 5:56-59.
4. Chauhan R, Singh BP, Singhal LK. Immunomodulation with kamdhenu Ark in mice. Journal of Immunology and Immunopathology. 2001; 71:89-92.
5. Bhadauria H. Cowurine-amagical therapy. Vishwa Ayurveda Parishad. International Journal of Cow Science. 2002; 1:32-36.
6. Dharma K, Rajesh R, Chauhan RS, Simmi T. Panchgavya (Cowpathy): an overview. International Journal of Cow Science. 2005; 1(1):1-15.
7. Mandavgane SA, Rambhal AK, Mude NK. Development of cow urine based disinfectant, Natural Product Radiance. 2005; 4:410-412.
8. Krishnamurthi K, Dutta D, Sivanesan SD, Chakrabarti T. Protective effect of distillate and redistillate of cow's urine in human polymorphonuclear leukocytes challenged with established genotoxic chemicals, Biomedical and Environmental Sciences. 2004; 17(3):247-256.
9. Rajesh M, Jayakumar K. Changes in morphological, biochemical and yield parameters of *Abelmoschus esculents* (L.) Moench due to panchagavya spray. International Journal of Modern Plant & Animal Sciences. 2013; 1(2):82-95.
10. Rakesh KN, Dileep N, Nawaz NAS, Junaid S, Kekuda PTR. Antifungal activity of cow urine against fungal pathogens causing rhizome rot of ginger, Environment and Ecology. 2013; 31(3):1241-1244.
11. Rajesh M, Jayakumar K. Changes in morphological, biochemical and yield parameters of *Abelmoschus esculents* (L.) Moench due to panchagavya spray. International Journal of Modern Plant & Animal Sciences. 2013; 1(2):82-95.
12. Basak AB, Lee MW, Lee TS. Inhibitive activity of cow urine and cow dung against *Sclerotinia sclerotiorum* of cucumber, *Mycobiology*, 2002; 30(3):175-179.
13. Rakesh KN, Dileep N, Junaid S, Prashith KTR, Vinayaka KS, Noor NAS. Inhibitory effect of cow urine extracts of selected plants against pathogens causing rhizome rot of ginger Science. Technology and Arts Research Journal. 2013; 2(2):92-96.
14. Oliveira NL, Puiatti M, Santos RHS, Cecon PR, PHR. Rodrigues, Soil and leaf fertilization of lettuce crop with cow urine, Horticultura Brasileirae. 2009; 27:102-536.
15. Tharmaraj K, Ganesh P, Suresh Kumar R, Anandan A, Kolanjinathan K. A critical review on Panchagavya-a boon plant growth. International Journal of Pharmaceutical and Biological Archive. 2011; 2(6):1611-1614.