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"POTENTIAL OF COW URINE AS PLANT GROWTH ENHANCER, ITS ANTIMICROBIAL ACTIVITY & PRESENCE OF CELLULOLYTIC & LIPOLYTIC ACTIVITY"

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ABSTRACT

The present study was conducted to determine the efficacy of cow urine as plant growth enhancer by treating methi plant with different concentrations of cow urine i.e. 1%, 2%, 3%, 4% & 5% and blank was maintained using tap water. By following this procedure protein & chlorophyll content was estimated. Among these concentrations 5% was showing higher protein & chlorophyll content. Along with this the cellulolytic activity of cow urine was also determined by observing clearance around the colony on CMC agar plate. The lipase activity of cow urine was estimated by performing lipase assay which is titrimetric analysis. Both showed positive results for cellulase & lipase activity.

Key words: cow urine, cellulase enzyme, lipase enzyme

1. INTRODUCTION

Cow urine is one of the components of panchagavya (urine, dung, milk, curd, and ghee) which is used as a medicine from ancient time. It is one of the most effective secretions of animal origin with innumerable therapeutic values. Cowpathy is a treatment based on the products obtained from cows, used in Ayurvedic medicine. In ancient Ayurveda cow urine has been greatly mentioned for its pharmacological importance. Okra contains nutrients that may confer a number of health advantages, including a decreased risk of several serious medical problems. Methi is one of the oldest medicinal herbs; ongoing research in India and abroad is currently uncovering new possibilities for its potential role in the treatment of diabetes and high cholesterol levels associated with coronary heart disease, both of which plague many industrial societies. CU contains 95% water, 2.5% urea, minerals, 24 types of salts, hormones, and 2.5% enzymes. It also contains iron, calcium, phosphorus, carbonic acid, potash, nitrogen, ammonia, manganese, iron, sulfur, phosphates, potassium, urea, uric acid, amino acids, enzymes, cytokine and lactose. Cow urine is very useful in agricultural operations as biofertilizer and biopesticide. Cow urine in combination with plant extracts is used to prepare disinfectant which is biodegradable and ecofriendly with good antibacterial action. With an approximate shelf life of around 5 years, this can prove to be the most effective natural antiseptic and disinfectant, when compared to the synthetic chemicals that are currently available to the consumers. A **lipase** is an enzyme that catalyzes the hydrolysis of fats (lipids). **Lipases** perform essential roles in the digestion, transport and processing of dietary lipids. Whereas Cellulase is an enzyme which has ability to breakdown cellulose and some related polysaccharides. Presence of lipase & cellulase in cow urine makes it more beneficial.

2. MATERIALS AND METHODS

2.1 Collection of Cow Urine: Fresh cow urine was collected in a sterile container from a local variety of cow. The urine was filtered through Whatman No. 1 filter paper to get rid of debris.

2.2 Pot culture experiment

The methi seeds were soaked overnight in water & sowed into 6 different pots. The garden soil was sterilized in an autoclave at 15 lbs pressure for half an hour. Each pot was irrigated twice a day with different concentrations (1%, 2%, 3%, 4%, and 5% (v/v)) of cow urine. In control pots, the seeds were irrigated with tap water instead of cow urine. The procedure repeated for 25 days. After completing treatment seedlings were collected from each pot to measure different parameters such as plant height, shoot and root length, number of leaves and branches, and to determine the chlorophyll & protein content.

2.2 a) Estimation of Protein content

10 mg of coomassie brilliant blue G250 was mixed with 10mL of 88% phosphoric acid and 45mL of absolute alcohol. Then the mixture was diluted to 100mL with distilled water. 1gm of fresh germinated seedlings (test seedlings) was ground in 20mL of distilled water. It was filtered and filtrate was made upto 20mL.Then 0.1mL of filtrated solution was added with 0.9mL of water to which 2mL of coomassie blue was added. The absorbance was read

at 530 nm. Same procedure was repeated for the seedlings that were treated with water as control.

2.2 b) Estimation of Chlorophyll content

Fresh methi leaves were collected from control plant and test plant respectively and 1gm of leaves was weighed. The collected leaves were cut into small pieces and homogenized in a mortar and pestle with excess of acetone and then filtered using WhatmanNo.1 filter paper to get filtrate. The filtrate was collected and made up to 100mL with acetone. 5mL of extract was transferred into 50mL volumetric flask and diluted to 50mL with 80% acetone. Absorbance was read at 645nm and 663nm using spectrophotometer. The quantity of chlorophyll a, chlorophyll b, and total chlorophyll was calculated using the following formulae:

Chlorophyll a (mg/g)

$$= 12.7\,(A663) - 2.69\,(A645) \times \frac{V}{1000 \times w}$$

Chlorophyll b (mg/g)

$$= 22.9\,(A645) - 4.68\,(A663) \times \frac{V}{1000 \times w}$$

Total chlorophyll (mg/g)

$$= 20.2\,(A645) + 8.02\,(A663) \times \frac{V}{1000 \times w}$$

2.3 Antimicrobial activity

a) Isolation of bacterial pathogens: *Salmonella typhimurium, Staphylococcus aureus, Klebsiella pneumonia, Pseudomonas aureuginosa, Escherichia coli, Bacillus subtilis* were enriched into Brain Heart Infusion broth by incubating it for 24 hrs on shaker respectively.

Preparation of aqueous neem extract: neem leaves were dried and powder is made. The powder was added in distilled water and kept on shaker for 2-3 days.

b) Antimicrobial activity against selected strains: The antimicrobial activity of Fresh cow urine, Aqueous Neem extract added in fresh cow urine, Aqueous Neem extract & Distilled water was performed using disc method. The cultures were swabbed on Mueller Hinton agar plates directly from enriched cultures by using sterile cotton swab. Discs containing respective samples were placed on culture containing plates. The plates were incubated for 24 hrs at 37 degree Celsius. The plates were observed for zone of inhibition (zone of clearance) & the zones were measured.

c) Antimicrobial activity against skin pathogens: The effect of sterile cow urine & sterile aqueous neem extract mixed with cow urine was checked. Hand swab was taken & suspension was made. The hand swab suspension was treated with sterile cow urine & sterile mixture of aqueous neem extract with cow urine separately. The sterile saline with suspension was used as a control. Then o.1 ml sample was spread on plates with the help of sterile glass spreader. Viable count method was done.

2.4 Cellulase activity of cow urine: the cow was directly spot inoculated onto carboxymethyl cellulose agar for detecting cellulase-producing micro-organisms. The plate was incubated at 37 degree Celsius for 24 hrs. The grams iodine was added for

getting the zone of clearance. The appearance of zone of clearance indicate the presence of cellulytic activity.

2.5 Lipase activity of cow urine

The lipase activity of cow urine was determined by titration method. the reaction mixture containing 5ml of olive oil emulsion, 5ml of 0.1 M Tris-Hydrochloride (Tris-HCl) buffer at pH 8.5, and 1.0 ml of the filtered urine sample was prepared & incubated at 30°C for 20min, centrifuged at 120rpm. The reaction was stopped by the adding 10ml of acetone and the liberated free fatty acids were titrated with 0.05N Sodium Hydroxide (NaOH) in the presence of phenolphthalein as an indicator. The blank assay was performed by adding the extract just after the addition of the acetone solution to the flask.

3. RESULTS:

3.1 Pot culture experiment

Fresh cow Urine sample was collected in the small container from sanyas ashram located in Vile-Parle (W). The collected cow urine was filtered to remove cell debris with whatman filter paper to get it in the clear form.

In present studies various aspects of cow urine was examined. Pot experiment was carried out to determine the efficacy of cow urine as plant growth enhancer. Parameters such as plant height, root & shoot length, no. of leaves etc. were observed in the experimental and control plants. It is clear from the results that plant height of Methi increased with increase in concentration of cow urine and duration of time. Maximum plant height of Methi was recorded as 9 cm with maximum concentration of 5% of cow urine. This is followed by 8 cm, 7.5 cm, 7 cm, 6cm for concentrations of 4%, 3%, 2% & 1% respectively.

Plant height of blank was 8.5 cm. The maximum root length recorded as 4 cm for 5% of cow urine which is followed by 3 cm, 2.7 cm, 2.5 cm, 2 cm for concentrations of 4%, 3%, 2% & 1% respectively. Root length for blank was 3.7 cm. The experiment showed increase in protein & chlorophyll content as treated with increasing concentration of cow urine and duration of time. (Table 1 & 2 respect.)

Figure1. Effect of different concentrations (1%, 2%, 3%, 4%, and 5%) of cow urine on Trigonellafoenum - graecum (Methi) after 25 days.

Table 1. Protein estimation of Trigonellafoenum - graecum (Methi)

Serial no.	Conc. Of cow urine (v/v%)	Protein content (mg/mL)
1.	1%	12
2.	2%	22
3.	3%	32
4.	4%	40
5.	5%	50
6.	Control pot	20

Table 2. Chlorophyll estimation of Trigonellafoenum - graecum (Methi)

Conc. Of cow urine (%)	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total Chlorophyll content (mg/g)
1	0.75	0.38	1.13
2	1.06	0.45	1.52
3	0.98	0.40	1.38
4	1.27	0.53	1.80
5	1.63	0.97	2.10
6 (control)	0.79	0.66	1.48

3.2 Antimicrobial activity of cow urine by disc diffusion method

From the observations, the mixture of fresh cow urine & aqueous Neem extract was showing pronounced effect on *Salmonella typhimurium* as it was showing 11mm zone of inhibition which was greater amongst all. Similarly both fresh cow urine & aqueous Neem extract was alone showing greater zone of inhibition of *Bacillus subtilis* (9mm)& *E. coli* (10mm) respectively. The *Klebsiella pneumonia* was not showing zone of inhibition in any case. Hence it was resisting to the inhibitory action of all the solutions used. The remaining cultures are showing more or less similar results. (Table 3)

Figure2. Antimicrobial activity of mixture of fresh cow urine & aqueous Neem extract, fresh cow urine, aqueous Neem extract & distilled water

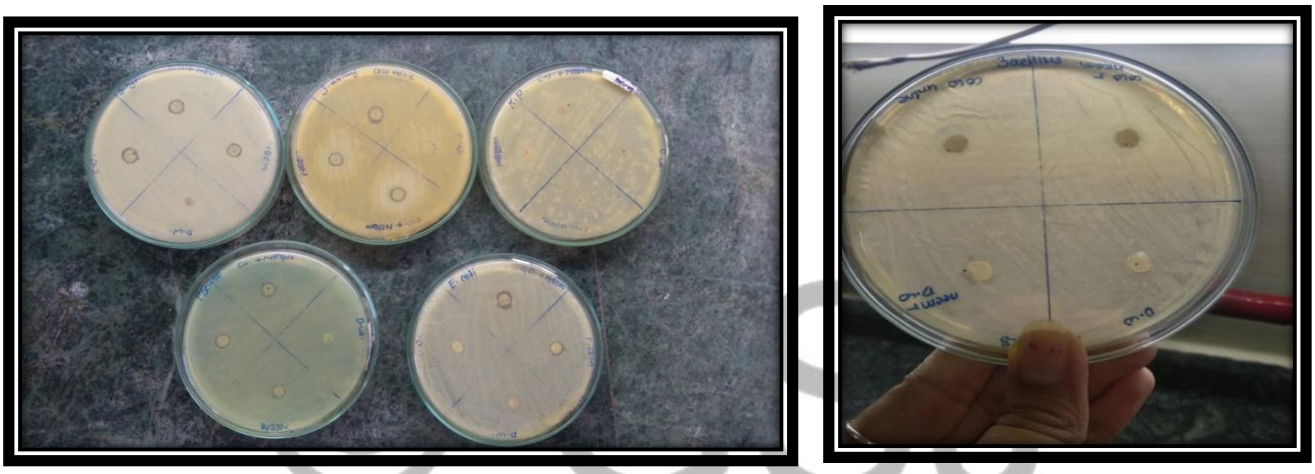

Table3. Antimicrobial potential of different solutions used

Culture used	**Zone of inhibition (mm)**			
	Cow urine + aqueous Neem extract	Fresh Cow urine	Aqueous Neem extract	Distilled water (control)
E. coli	8 mm	7mm	10 mm	-
Klebsiella pneumonia	-	-	_	_
Salmonella typhimurium	11 mm	7 mm	9 mm	_
Staphylococcus aureus	9mm	8mm	7 mm	_
Pseudomonas aeruginosa	8mm	7mm	7mm	_
bacillus subtilis	7mm	9mm	7mm	_

3.3 Antimicrobial activity of cow urine by viable count method

From the observations, there is a drastic elimination was observed in the plate of hand swabbed suspension treated with sterile mixture of fresh cow urine & aqueous Neem extract. The plate of hand swabbed treated with sterile saline was showing highest number of colonies as it was a control. The calculations are shown in the table 4

Figure3. Antimicrobial activity determined by viable count method

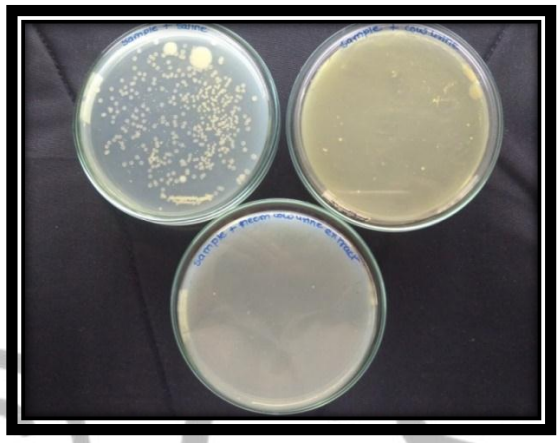

Table4. Antimicrobial activity determined by viable count method

	Dilution used	**Cfu/0.1 ml**	**Cfu/ml**	**Average cfu/ml**
Sample + saline	10^1	TNTC (> 300)	_	_
Sample + sterile cow urine	10^1	$55 * 10^1$	$55 * 10^2$	$5.5 * 10^3$ cfu/ml
Sample + sterile mixture of fresh cow urine & aqueous neem extract	10^1	TFTC (<30)	_	_

3.4 Cellulase activity

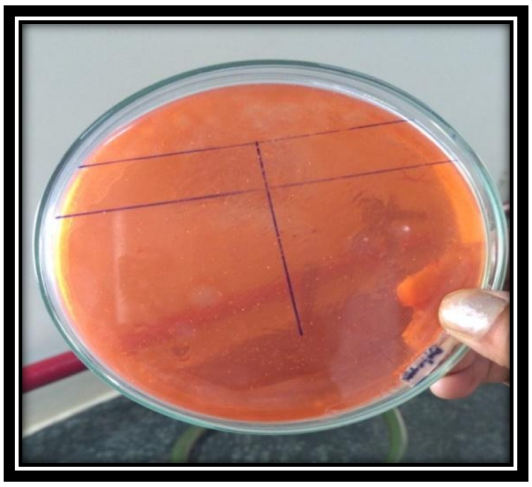

Figure4. The colonies showing zone of clearance on Carboxymethyl cellulose agar plate

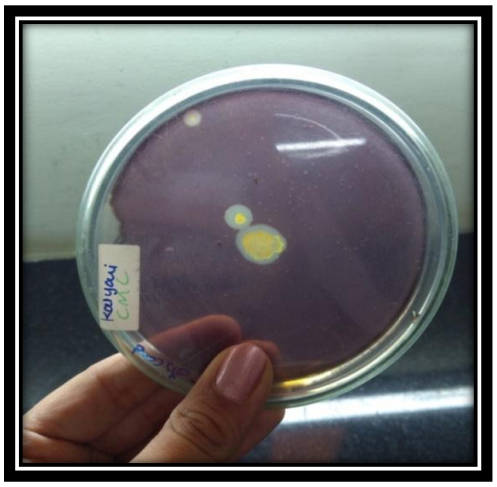

Figure5. Spot inoculation on CMC agar plate

3.5 Lipase Assay –Titrimetric Analysis

In the present study, the fresh cow urine was also checked for its lipase activity by titrimetric assay. Lipase activity was determined by incubating a reaction mixture containing 5ml of olive oil emulsion, 5ml of 0.1 M Tris-Hydrochloride (Tris-HCl) buffer at pH 8.5, and 1.0 ml of the filtered urine sample at 30°C for 20min, centrifuged at 120rpm. After incubation, the reaction was stopped by the addition of 10ml of acetone and the liberated free fatty acids were titrated with 0.05N Sodium Hydroxide (NaOH) in the presence of phenolphthalein as an indicator. The blank assay was also performed. The results are mentioned in the following table:

Table5. Lipase assay

Solution	Titration value
Test Solution Against 50mM NaOH	6.5
Blank Solution Against 50mM NaOH	5.2
Result	
Lipase Activity in Cow's Urine (in units)	130 units/ml

Figure6. Lipase activity by titrimetric assay

CONCLUSION

Cow urine is used for various therapeutic purposes in traditional Indian medicine, Ayurveda. In the current study, various claimed advantages were checked by performing different experiments. The pot culture experiment was carried out to determine the efficacy of cow urine as plant growth enhancer. It is clear that the plant height of Methi increased with increasing concentration of cow urine i.e. 1%, 2%, 3%, 4%, and 5% and duration of time. The maximus results were obtained in case of pot irrigated with 5% conc. of cow urine. The cow urine was also checked for its antimicrobial activity from both the aspects of crops & that of humans. *Salmonella typhimurium* was showing susceptibility to the synergistic action of cow urine &aqueous Neem extract. The *klebsiella pneumoniae* is resistant to the inhibitory activity of cow urine. The organisms from hand swabbed suspension were drastically reduced in numbers when allowed to stay in contact with the sterile mixture of cow urine & aqueous Neem extract. The Lipase Activity in Cow's Urine determined was 130units/ml. The zone of clearance was also observed around the colony indicating the cellulase activity.

DISCUSSION

The cow urine is well known for its therapeutic value from the ancient time. The cow urine contents various essential components which enhanced its activity. Different fractions of cow urine possess antimicrobial activity due to the presence of certain components like volatile and nonvolatile ones. Presence of urea, creatinine, swarnkshar (aurum hydroxide), carbolic acid, phenols, calcium, and manganese has strongly explained the antimicrobial and germicidal properties of cow urine. Presence of amino acids and urinary peptides may enhance the bactericidal effect by increasing the bacterial cell surface hydrophobicity. The present study reveal that the cow urine has efficiently act as a plant growth enhancer & it can be a major source of lipase & cellulase enzymes.

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