Cow Urine: Plant Growth Enhancer and Antimicrobial Agent

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Abstract. The cow is considered a divine animal in the Hindu culture. The milk, cow dung, urine of the cow is used for various purposes. The benefits of cow urine have been depicted in ancient Hindu texts, Vedas. The present study aimed at using cow urine as a plant growth enhancer and antimicrobial agent. The plants chosen for this study were *Zea mays* (maize), *Triticum aestivum* (wheat), *Cymbopogon citratus* (grass), *Ocimum basilicum* (tukmaria) and *Trigonella foenum graecum* (methi). The plants were grown for 30 days using different cow urine concentrations i.e 0%(control), 5%, 15%, 25%. The various parameters such as plant height, shoot, and root length, number of leaves, the mass of the root etc were observed. The antibacterial test using different cow urine concentrations i.e 0%(control), 5%, 15%, 25% was conducted on *Escherichia coli* using disc diffusion method. The fungus was screened and isolated from raw coconut and was grown on YPD media to obtain the mother culture. Using Slide culture technique, the fungi were identified as *Aspergillus*. The media was poisoned using different cow urine concentrations i.e 0%(control), 5%, 15%, 25% and the fungi culture was inoculated. GCMS analysis was conducted to identify the compounds present in the cow urine. Among the concentrations, 5% cow urine concentration showed maximum growth when compared to other concentrations whereas 25% concentration showed more antibacterial and antifungal activity when compared to others. In GCMS Analysis, 16 compounds have been identified, in which, 6 compounds were antifungal, 3 compounds were antibacterial, and 2 compounds as plant growth enhancers. This study concludes that cow urine can be used as a plant growth enhancer and it possesses antimicrobial characteristics.

Introduction

The urine of Cow is commonly used to prevent and cure diseases especially by the rural people in India. Cow is considered sacred among the Hindu religion in India since ages. The use of Cow urine diminished when the western medicine has been developed, although it is still practiced as a treatment in some parts of the world. Currently, the use of Cow Urine as a therapeutic agent is being practiced to treat diseases and prevent the spread of diseases. Nonetheless, not much of the composition of the Cow Urine and its functions are known.

In Vedas (Ancient Hindu texts), Cow Urine has been given a lot of significance. It was also called as Amrita, the beverage of immortality. The Cow Urine from the Indian cow breed has medicinal properties unlike the crossbred or exotic cows. Recent studies have showed that Cow Urine has an effect on the immune system by increasing the phagocytic activity of macrophages. It was also shown that it has anti-bacterial properties [1]. Some medicinal properties of Cow Urine include reduction of weight, treating kidney problems, treating indigestion and edema [2]. The practitioners of Buddhist culture in Myanmar still use Cow Urine as a traditional medicine [3].

The constituents of the Cow Urine in right proportions are the reason to cure diseases. The analysis of Cow Urine showed that it is made up of all the essential Salts, Metals, Minerals, Vitamins, Enzymes and other entities in small quantities [4].

In this study, we tried to identify if there is any effect on the Plant growth in the presence of Cow Urine. The Antimicrobial activity of the Cow Urine was also studied. The Cow Urine was also analyzed using Gas Chromatography-Mass Spectroscopy (GCMS) to determine its constituents and its effects.

Materials and Methods

Cow urine collection

The cow urine was collected in a nearby organic farm and was filtered using filter paper. The urine was collected in urine sample kits and was stored at 4 Celsius. The soil was also collected from the organic farm.

Seed collection

5 types of seeds were collected i.e

1. Zea mays – Maize
2. Triticum aestivum – Wheat
3. Trigonella foenum graecum – Methi
4. Ocimum basilicum - Tukmaria (Sabja)
5. Cymbopogon citratus - Lemon grass

Preparation of different cow urine concentrations

Various concentrations of cow urine were prepared i.e 0%, 5%, 15%, 25%.

5% cow urine was prepared by mixing 5ml in 100ml distilled water.

15% cow urine was prepared by mixing 15ml in 100 ml distilled water.

25% cow urine was prepared by mixing 25ml in 100ml distilled water.

Sowing of seeds

The seeds were sown in pots and were labeled using small flags which depict the name of the seed and the concentration of the cow urine used as shown in the Fig. 1. The plants were irrigated using different concentrations of cow urine (10ml) for 30 days and the growth was observed as shown in Fig. 1.

Figure 1. Seeds sown in their respective blocks

Parameters

Various parameters of the plants were observed. The parameters include

1. Total chlorophyll [5]:
Chlorophyll was extracted using acetone and spectrophotometer was used to measure the absorbance at specific wavelengths i.e 663nm and 645nm.
Chlorophyll a ($\mu\text{g/ml}$) = $12.7 (A_{663}) - 2.69 (A_{645})$
Chlorophyll b ($\mu\text{g/ml}$) = $22.9 (A_{645}) - 4.68 (A_{663})$
Total Chlorophyll ($\mu\text{g/ml}$) = $20.2 (A_{645}) - 8.02 (A_{663})$
2. Plant height: Measured using scale in cms.

3. Root Length: Measured from the shoot ending to the root tip using scale.
4. Shoot Length: Measured shoot using scale in cms.
5. Leaf Length: The leaf with highest length was considered.
6. Leaf breadth: The breadth of the highest leaf length was considered.
7. No of branches
8. Root weight: dried weight(gm) of the root is considered.

Soil analysis

The soil samples is given for analysis in ECOICONS, ECIL, to determine what changes have been made by the cow urine in the soil. Various parameters and methods used for soil analysis are tabulated in Table 1.

Table 1. The parameters measured in the soil analysis

S.No	Parameters	Method	Units
1	pH	IS:2720 (Part-26):2011	-
2	Electrical Conductivity	Method Manual, Soil Testing, Ministry of Agriculture, Govt of India	micro mhos
3	Bulk Density	IS:2720 (Part-28):2011	g/cc
4	Moisture content	IS:2720 (Part-18):2002	%
5	Soil Texture-Sand	Sieve Method	%
6	Soil Texture-Silt	Sieve Method	%
7	Soil Texture-Clay	Sieve Method	%
8	Nitrogen	-	kg/ha
9	Phosphorus as P ₂ O ₅	Method Manual, Soil Testing, Ministry of Agriculture, Govt of India	kg/ha
10	Potassium as K ₂ O	Method Manual, Soil Testing, Ministry of Agriculture, Govt of India	kg/ha
11	Sodium as Na ₂ O	Method Manual, Soil Testing, Ministry of Agriculture, Govt of India	mg/kg
12	Calcium as Ca	Method Manual, Soil Testing, Ministry of Agriculture, Govt of India	mg/kg
13	Magnesium as Mg	Method Manual, Soil Testing, Ministry of Agriculture, Govt of India	mg/kg
14	Chlorides as Cl	Method Manual, Soil Testing, Ministry of Agriculture, Govt of India	mg/kg
15	Sulphates as SO ₄	Method Manual, Soil Testing, Ministry of Agriculture, Govt of India	mg/kg
16	Copper as Cu	USEPA:3050B, & Copper by Flame AAS	mg/kg
17	Manganese	USEPA:3050B, & Manganese by Flame AAS	mg/kg
18	Molybdenum	Method Manual, Soil Testing, Ministry of Agriculture, Govt of India	mg/kg
19	Boran	Method Manual, Soil Testing, Ministry of Agriculture, Govt of India	mg/kg
20	Zinc as Zn	USEPA:3050B, & Zinc by Flame AAS	mg/kg
21	Chromium as Cr	USEPA:3050B, & Chromium by Flame AAS	mg/kg
22	Iron as Fe	USEPA:3050B, & Iron by UV-Vis spectrophotometer	mg/kg
23	Lead as Pb	USEPA:3050B, & Lead by Flame AAS	mg/kg
24	Organic Carbon	-	%

Antimicrobial analysis

Bacteria

- E. coli bacteria was obtained from the SNIST Biotechnology Department.
- E. coli was grown on the nutrient medium to obtain the mother culture.
- Antibacterial activity was conducted using various cow urine concentrations (5%, 15%, 25%) using well diffusion method.

Fungi

- A fungal species was isolated from a raw coconut. Based on its morphological character, it was tentatively identified as *Aspergillus*.
- Fungi was grown on the YPD medium to obtain the mother culture.
- Antifungal activity was conducted using various cow urine concentrations (5%, 15%, 25%).

Gas chromatography mass spectroscopy analysis

Gas chromatography-mass spectroscopy analysis was conducted to determine the compounds present in the cow urine which may have made the changes in the plant growth, soil and antimicrobial properties.

1. The urine sample was extracted in dichloromethane using the principle of liquid-liquid extraction.
2. The cow urine was mixed with dichloromethane in 1:1 ratio and was left in shaker for 3hrs.
3. The difference in their densities resulted in a two-layer liquid i.e top layer-cow urine, bottom layer-dichloromethane.
4. The dichloromethane was extracted using a pipette and was transferred into 2ml vials.

The protocol for GCMS [6]:

The GC-MS system started with the initial oven temperature of 60 °C for 5 min, increasing to 240 °C at a rate of 15 °C for 5 min, and then to 300 °C at a rate of 15 °C for 5 min. The injector temperature was maintained at 200 °C. The interface temperature was 250 °C. Helium was used as a mobile phase at a flow rate of 1.0 mL/min. Mass spectral detection was carried out in electron ionization mode by scanning at 40–600 (m/z).

The GCMS analysis was performed at IICT, Tarnaka.

Results

Growth observation

The plants were placed on white sheet of paper and the parameters were measured as per protocol mentioned in the methods. The growth observation of various plants is shown in Fig. 2, Fig. 3, Fig. 4, Fig. 5.

Figure 2. Growth of Zea Mays (MAIZE) in various concentrations

Figure 3. Growth of *Cymbopogon citrates* (GRASS) in various concentrations

Figure 4. Growth of *Triticum aestivum* (WHEAT) in various concentrations

Figure 5. Growth of *Trigonella foenum graecum* (METHI) in various concentrations

There was no growth observed in (*Ocimum basilicum*)Tukmaria seeds.

The following seeds have not grown at particular cow urine concentrations

- (*Ocimum basilicum*) Tukmaria seeds- control
- (*Ocimum basilicum*)Tukmaria seeds-5% concentration
- (*Ocimum basilicum*)Tukmaria seeds-15% concentration
- ((*Ocimum basilicum*)Tukmaria seeds-25% concentration
- (*Trigonella foenum graecum*) Methi seeds-15% concentration
- (*Trigonella foenum graecum*) Methi seeds-25% concentration

The following seeds have shown poor growth

- (*Zea mays*) Maize seeds-15% concentration
- (*Zea mays*) Maize seeds-25% concentration
- (*Triticum aestivum*) Wheat seeds-25% concentration

Physical parameters comparison

The plants have shown different physical changes with different cow urine concentrations. This shows that cow urine has effect on the growth of the plants.

The data from the physical parameters was tabulated in Table 2, Table 3, Table 4, Table 5.

Table 2. Data of physical parameters of Maize (*Zea mays*)

Maize	Control	5% Concentration	15% Concentration	15% Concentration
Chlorophyll A	0.8711	0.2005	-0.168	0.2304
Chlorophyll B	0.5051	0.804	-0.2105	0.4813
Total Chlorophyll	0.4481	0.7145	-0.02276	0.4471
Plant height-cm	31.5	41.6	18.3	13.6
Root length-cm	9	13.1	10.5	5
Shoot length-cm	22.5	28.5	7.8	8.6
Leaf length-cm	21.2	21.7	0	0
Leaf breadth-cm	1.7	1.6	0	0
No. Of Branches	4	4	0	0
Root weight-gm	0.98	2.16	1.58	0.11

Table 3. Data of physical parameters of Lemon grass (*Cymbopogon citratus*)

Grass	Control	5% Concentration	15% Concentration	15% Concentration
Chlorophyll A	0.3787	0.7829	0.1587	0.1272
Chlorophyll B	1.3882	2.3316	0.5284	0.1443
Total Chlorophyll	1.2351	2.0794	0.4854	0.1687
Plant height-cm	34.7	43.1	16.5	10.3
Root length-cm	9.5	11.3	5.5	2.9
Shoot length-cm	25.2	31.8	11	7.4
Leaf length-cm	19.3	24.8	7.9	5
Leaf breadth-cm	0.4	0.6	0.2	0.1
No. Of Branches	4	4	3	3
Root weight-gm	0.06	0.11	0.04	0.03

Table 4. Data of physical parameters of Wheat (*Triticum aestivum*)

Wheat	Control	5% Concentration	15% Concentration	15% Concentration
Chlorophyll A	1.5038	0.4236	0.6342	-1.163
Chlorophyll B	0.8055	0.9345	0.6788	-1.9297
Total Chlorophyll	0.8189	0.8356	0.6139	-2.7919
Plant height-cm	28.9	30.2	17.8	3
Root length-cm	12.4	8.3	4.6	0.2
Shoot length-cm	16.5	21.9	13.2	2.8
Leaf length-cm	12.6	16.2	8.9	1.9
Leaf breadth-cm	0.4	0.3	0.3	0.1
No. Of Branches	4	5	4	2
Root weight-gm	0.03	0.05	0.01	0

Table 5. Data of physical parameters of Methi (*Trigonella foenum graecum*)

Methi	Control	5% Concentration	15% Concentration	15% Concentration
Chlorophyll A	0.3069	2.962	0	0
Chlorophyll B	0.6915	5.929	0	0
Total Chlorophyll	0.6159	5.2519	0	0
Plant height-cm	10.8	17.9	0	0
Root length-cm	5.6	9.2	0	0
Shoot length-cm	5.2	8.7	0	0
Leaf length-cm	1.4	2.2	0	0
Leaf breadth-cm	0.6	0.9	0	0
No. Of Branches	5	7	0	0
Root weight-gm	0.03	0.01	0	0

The graphs were plotted for each of the plant under various concentrations. The graphs are depicted in Fig. 6-Maize, Fig. 7-Grass, Fig. 8-Wheat, Fig. 9-Methi.

Figure 6. Graphical representation of physical parameters of (*Zea mays*) MAIZE on various concentrations

Figure 7. Graphical representation of physical parameters of (*Cymbopogon citrates*) GRASS on various concentrations

Figure 8. Graphical representation of physical parameters of (*Triticum aestivum*) WHEAT on various concentrations

Figure 9. Graphical representation of physical parameters of (*Trigonella foenum graecum*) METHI on various concentrations

The observations in the plant physical features depict that 5% cow urine concentration has shown enhanced growth in the following plants:

- (Zea mays) Maize
- (Triticum aestivum) Wheat
- (Cymbopogon citrates) Grass
- (Trigonella foenum graecum) Methi

Data from soil analysis

The data from the soil analysis was tabulated in Table 6.

Table 6. Data from soil analysis

Parameters	Control	5% cow urine	15%cow urine	25%cow urine
pH	8.75	8.58	8.38	7.85
Electrical Conductivity	113.5	134.2	156.6	174.7
Bulk Density	1.6	1.91	2.16	2.31
Moisture content	3.41	7.82	12.81	15.6
Soil Texture-Sand	10	7	8	13
Soil Texture-Silt	28	12	12	14
Soil Texture-Clay	62	81	80	73
Nitrogen	91.05	95.42	93.28	91.08
Phosphorus as P ₂ O ₅	20.31	18.27	17.99	18.76
Potassium as K ₂ O	87.17	85.01	88.64	91.11
Sodium as Na ₂ O	91.33	93.64	90.07	97.63
Calcium as Ca	32.06	60.12	48.09	40.08
Magnesium as Mg	16.98	4.83	4.92	16.98
Chlorides as Cl	269.9	304.9	274.9	289.9
Sulphates as SO ₄	16.44	18.13	19.18	18.21
Copper as Cu	<1.0	<1.0	<1.0	<1.0
Manganese	<1.0	<1.0	<1.0	<1.0
Molybdenum	<1.0	<1.0	<1.0	<1.0
Boran	<1.0	<1.0	<1.0	<1.0
Zinc as Zn	1.92	2.32	2.24	2.19
Chromium as Cr	<1.0	<1.0	<1.0	<1.0
Iron as Fe	<2.0	<2.0	<2.0	<2.0
Lead as Pb	<2.0	<2.0	<2.0	<2.0
Organic Carbon	0.34	0.37	0.35	0.39

The following parameters have shown increase in content with increase in cow urine concentration:

- Moisture content
- Electrical conductivity
- Bulk density
- pH

Antibacterial tests

The antibacterial tests were conducted using disc diffusion method and the results are depicted in Fig.10.

Figure 10. Antibacterial Activity of Cow urine on various concentrations: a) 0% concentration b) 5% concentration c) 15% concentration d) 25% concentration

The data from the antibacterial tests are shown in Table 7.

Table 7. Diameter of the inhibition zone in various concentrations

Concentration	Diameter of the inhibition zone
0% cow urine concentration	0.0cm
5% cow urine concentration	0.6cm
15% cow urine concentration	1.4cm
25% cow urine concentration	2.1cm

From the data, it can be inferred that the diameter of the inhibition zone increases with increase in cow urine concentration. This depicts that cow urine acts as an antibacterial agent with increase in concentration.

Antifungal tests

The fungal sample was collected from a raw coconut (Fig. 11) and was inoculated in YPD medium which supports the growth of fungi.

Figure 11. Growth of fungi on raw coconut

The mother culture was obtained by growing the fungi on YPD media-Fig. 12.

Figure 12. Fungi grown on YPD medium for mother culture

Using slide culture technique (Fig. 13), the fungus was identified as *Aspergillus* based on its morphological characteristics.

Figure 13. Slide culture technique for fungus

The antifungal tests were conducted by poisoning the media with cow urine. The cow urine concentrations i.e 5%-Fig. 14, 15%-Fig 15, 25%-Fig 16, was added into the YPD media and was sterilized using autoclave. The fungi were inoculated on the media and was kept in an incubator for 6 days at a temperature of 30 Celsius.

Figure 14. Antifungal test using 5% concentration

Figure 15. Antifungal test using 15% concentration

Figure 16. Antifungal test using 25% concentration

The results are shown in Table 8.

Table 8. Diameter of fungi culture in various concentrations

Concentration	Diameter of the fungi culture
0% cow urine concentration	6cm
5% cow urine concentration	5.5cm
15% cow urine concentration	4.9cm
25% cow urine concentration	3.1cm

The growth of fungi was inhibited with the increase in cow urine concentration.

Gas chromatography and mass spectroscopy analysis

The GCMS analysis was conducted as per the protocol mentioned in the methods and the graphs obtained.

The compounds identified in the analysis were,

- 1H-Indole, 1-Methyl-2-Phenyl (**Pubchem ID-77095**)
- Isophthalaldehyde (**Pubchem ID-34777**)
- Octane, 6-ethyl-2-methyl (**Pubchem ID-537768**)
- 7-Methyl-Z-8,10-dodecadienal (**Pubchem ID-5363533**)
- 1(3H)-Isobenzofuranone,3a,4,5,7a-tetrahydro-4-hydroxy-3a,7a-dimethyl-, (3a.alpha.,4.beta.,7a.alpha.) - (.+/-.) (**Pubchem ID- Not found**)
- Phenol, 2,4-bis(1,1-dimethylethyl) (**Pubchem ID-528937**)
- Triacontane (**Pubchem ID-12535**)
- L-leucine, N-(N-acetylglucyl)-butylester (**Pubchem ID-Not found**)
- Tetradecanoic acid, trimethylsilylester (**Pubchem ID-519592**)
- 1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester (**Pubchem ID-6818**)
- Tridecane, 7-propyl (**Pubchem ID-Not found**)
- N-Hexadecanoic acid (**Pubchem ID-540086**) fungicide
- Dibutyl phthalate (**Pubchem ID-3026**) *Drug*
- Hexadecanoic acid, trimethylsilyl ester (**Pubchem ID- 521638**)
- 4-(p-methoxyphenyl)-3-buten-2-one, thiosemicarbazone (**Pubchem ID-9603450**)
- 1-cyclohexyldimethylsilyloxy-3,5-dimethyl benzene (**Pubchem ID-532617**)

The following compounds (16) were identified and their properties are mentioned in the Table 9.

Table 9. Pubchem ID, Properties and References of the identified compounds

Pubchem ID	Name of compound	Properties	Reference
77095	1H-Indole, 1-Methyl-2-Phenyl	Antifungal	https://doi.org/10.14233/ajchem.2017.20423 [7]
34777	Isophthalaldehyde	Antifungal, Antibacterial	https://www.ncbi.nlm.nih.gov/pubmed/1953927 [8]
537768	Octane, 6-ethyl-2-methyl	-	No information available about this compound
5363533	7-Methyl-Z-8,10-dodecadienal	-	No information available about this compound
Not found	1(3H)-Isobenzofuranone,3a,4,5,7a-tetrahydro-4-hydroxy-3a,7a-dimethyl-,(3a.alpha.,4.b eta.,7a.alpha.)-(./-.)	-	No information available about this compound
528937	Phenol, 2,4-bis(1,1-dimethylethyl)	Antifungal	https://doi.org/10.1016/j.jplph.2013.07.004 [9]
12535	Triacontane	Plant growth regulator	https://doi.org/10.1080/17429145.2011.619281 [10]
Not found	L-leucine, N-(N-acetylglucyl)-butylester	-	No information available about this compound
519592	Tetradecanoic acid, trimethylsilylester	Antifungal, Antibacterial	https://doi.org/10.1002/ardp.19963291102 [11]
6818	1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester	Antifungal	http://shodhganga.inflibnet.ac.in/bitstream/10603/184541/19/6_abstract.pdf
246301	Tridecane, 7-propyl	-	No information available about this compound
540086	N-Hexadecanoic acid	Antifungal, Antibacterial	https://doi.org/10.7164/antibiotics.39.888 [12] https://doi.org/10.1111/j.1747-0285.2012.01418.x [13]
3206	Dibutyl phthalate	Medicinal oil	https://doi.org/10.1186/s40199-014-0078-1 [14]
521638	Hexadecanoic acid, trimethylsilyl ester	-	No information available about this component
9603450	4-(p-methoxyphenyl)-3-buten-2-one,	Plant growth regulator	http://agris.fao.org/agris-search/search.do?recordID=XB8320803 [15]
532617	1-cyclohexyldimethylsilyl oxy-3,5-dimethyl benzene	-	No information available about this compound

Out of the following compounds, 6 compounds were identified as antifungal compounds, 3 were identified as antibacterial compounds and 2 compounds were identified as plant growth regulators.

Discussion

The plants were grown using different concentrations (control (0%), 5%, 15%, 25%) of cow urine for 30 days and the growth was observed. The plants showed varied growth at different concentrations and the physical parameters were measured and tabulated. According to the measurements, the 5% cow urine concentration showed more growth when compared to other concentrations. The soil was sent for soil analysis to check for the changes made to the soil by cow urine. The various parameters of the soil were analysed. The parameters i.e pH, Bulk density, Electrical conductivity, Moisture content were increased with increase in cow urine concentrations. The antimicrobial properties of the cow urine were examined. The antibacterial test was conducted on *E. coli* using disc diffusion method. The inhibition zone increased with increase in cow urine concentration indicating the presence of antibacterial compounds in cow urine. The fungus was isolated from raw coconut and slide culture technique was used to determine its morphological features. Based on its morphological features, it was identified as *Aspergillus*. The antifungal tests were conducted by poisoning the media with various cow urine concentrations and inoculating the fungi. The diameter of the fungi culture decreased with increase in cow urine concentration indicating the presence of antifungal agents. Gas chromatography mass spectroscopy analysis was performed to identify the compounds in the cow urine. The cow urine was extracted by liquid-liquid phase extraction using dichloromethane and was sent for analysis. 16 compounds were identified in the sample and their respective pubchem IDs were searched. 2 compounds didn't have any pubchem ID indicating the presence of new compounds. The compounds were searched for their properties using literature review. Out of 16 compounds, 6 compounds were identified as antifungal, 3 compounds were identified as antibacterial, and 2 compounds as plant growth enhancers.

From the above study, we can conclude that cow urine has the properties of plant growth enhancer and antimicrobial properties. Most of the compounds identified were produced synthetically. This can be avoided by extracting the selected compounds from the cow urine which can save money as well as protect our environment by avoiding the synthetic means of production.

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Conflict of interest

The authors declare that they don't have any conflict of interest.

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