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 citratum (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.

Parameter
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(µg/ml) =12.7 (A_{663}) – 2.69 (A_{645})
   Chlorophyll b(µg/ml) =22.9 (A_{645}) – 4.68 (A_{663})
   Total Chlorophyll (µg/ml) =20.2 (A_{645}) – 8.02 (A_{663})

Figure 1. Seeds sown in their respective blocks
3. Root Length: Measured from the shoot ending to the root tip using scale.
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>
<thead>
<tr>
<th>S.No</th>
<th>Parameters</th>
<th>Method</th>
<th>Units</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>pH</td>
<td>IS:2720 (Part-26):2011</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Electrical Conductivity</td>
<td>Method Manual, Soil Testing, Ministry of Agriculture, Govt of India</td>
<td>micro mhos</td>
</tr>
<tr>
<td>3</td>
<td>Bulk Density</td>
<td>IS:2720 (Part-28):2011</td>
<td>g/cc</td>
</tr>
<tr>
<td>4</td>
<td>Moisture content</td>
<td>IS:2720 (Part-18):2002</td>
<td>%</td>
</tr>
<tr>
<td>5</td>
<td>Soil Texture-Sand</td>
<td>Sieve Method</td>
<td>%</td>
</tr>
<tr>
<td>6</td>
<td>Soil Texture-Silt</td>
<td>Sieve Method</td>
<td>%</td>
</tr>
<tr>
<td>7</td>
<td>Soil Texture-Clay</td>
<td>Sieve Method</td>
<td>%</td>
</tr>
<tr>
<td>8</td>
<td>Nitrogen</td>
<td>-</td>
<td>kg/ha</td>
</tr>
<tr>
<td>9</td>
<td>Phosphorus as P2O5</td>
<td>Method Manual, Soil Testing, Ministry of Agriculture, Govt of India</td>
<td>kg/ha</td>
</tr>
<tr>
<td>10</td>
<td>Potassium as K2O</td>
<td>Method Manual, Soil Testing, Ministry of Agriculture, Govt of India</td>
<td>kg/ha</td>
</tr>
<tr>
<td>11</td>
<td>Sodium as Na2O</td>
<td>Method Manual, Soil Testing, Ministry of Agriculture, Govt of India</td>
<td>mg/kg</td>
</tr>
<tr>
<td>12</td>
<td>Calcium as Ca</td>
<td>Method Manual, Soil Testing, Ministry of Agriculture, Govt of India</td>
<td>mg/kg</td>
</tr>
<tr>
<td>13</td>
<td>Magnesium as Mg</td>
<td>Method Manual, Soil Testing, Ministry of Agriculture, Govt of India</td>
<td>mg/kg</td>
</tr>
<tr>
<td>14</td>
<td>Chlorides as Cl</td>
<td>Method Manual, Soil Testing, Ministry of Agriculture, Govt of India</td>
<td>mg/kg</td>
</tr>
<tr>
<td>15</td>
<td>Sulphates as SO4</td>
<td>Method Manual, Soil Testing, Ministry of Agriculture, Govt of India</td>
<td>mg/kg</td>
</tr>
<tr>
<td>16</td>
<td>Copper as Cu</td>
<td>USEPA:3050B, &amp; Copper by Flame AAS</td>
<td>mg/kg</td>
</tr>
<tr>
<td>17</td>
<td>Manganese</td>
<td>USEPA:3050B, &amp; Manganese by Flame AAS</td>
<td>mg/kg</td>
</tr>
<tr>
<td>18</td>
<td>Molybdenum</td>
<td>Method Manual, Soil Testing, Ministry of Agriculture, Govt of India</td>
<td>mg/kg</td>
</tr>
<tr>
<td>19</td>
<td>Boran</td>
<td>Method Manual, Soil Testing, Ministry of Agriculture, Govt of India</td>
<td>mg/kg</td>
</tr>
<tr>
<td>20</td>
<td>Zinc as Zn</td>
<td>USEPA:3050B, &amp; Zinc by Flame AAS</td>
<td>mg/kg</td>
</tr>
<tr>
<td>21</td>
<td>Chromium as Cr</td>
<td>USEPA:3050B, &amp; Chromium by Flame AAS</td>
<td>mg/kg</td>
</tr>
<tr>
<td>22</td>
<td>Iron as Fe</td>
<td>USEPA:3050B, &amp; Iron by UV-Vis spectrophotometer</td>
<td>mg/kg</td>
</tr>
<tr>
<td>23</td>
<td>Lead as Pb</td>
<td>USEPA:3050B, &amp; Lead by Flame AAS</td>
<td>mg/kg</td>
</tr>
<tr>
<td>24</td>
<td>Organic Carbon</td>
<td>-</td>
<td>%</td>
</tr>
</tbody>
</table>
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.
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)

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

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

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>5% Concentration</th>
<th>15% Concentration</th>
<th>15% Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorophyll A</td>
<td>0.3787</td>
<td>0.7829</td>
<td>0.1587</td>
<td>0.1272</td>
</tr>
<tr>
<td>Chlorophyll B</td>
<td>1.3882</td>
<td>2.3316</td>
<td>0.5284</td>
<td>0.1443</td>
</tr>
<tr>
<td>Total Chlorophyll</td>
<td>1.2351</td>
<td>2.0794</td>
<td>0.4854</td>
<td>0.1687</td>
</tr>
<tr>
<td>Plant height-cm</td>
<td>34.7</td>
<td>43.1</td>
<td>16.5</td>
<td>10.3</td>
</tr>
<tr>
<td>Root length-cm</td>
<td>9.5</td>
<td>11.3</td>
<td>5.5</td>
<td>2.9</td>
</tr>
<tr>
<td>Shoot length-cm</td>
<td>25.2</td>
<td>31.8</td>
<td>11</td>
<td>7.4</td>
</tr>
<tr>
<td>Leaf length-cm</td>
<td>19.3</td>
<td>24.8</td>
<td>7.9</td>
<td>5</td>
</tr>
<tr>
<td>Leaf breadth-cm</td>
<td>0.4</td>
<td>0.6</td>
<td>0.2</td>
<td>0.1</td>
</tr>
<tr>
<td>No. Of Branches</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Root weight-gm</td>
<td>0.06</td>
<td>0.11</td>
<td>0.04</td>
<td>0.03</td>
</tr>
</tbody>
</table>
Table 4. Data of physical parameters of Wheat (Triticum aestivum)

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

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

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

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**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>5% cow urine</th>
<th>15% cow urine</th>
<th>25% cow urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>8.75</td>
<td>8.58</td>
<td>8.38</td>
<td>7.85</td>
</tr>
<tr>
<td>Electrical Conductivity</td>
<td>113.5</td>
<td>134.2</td>
<td>156.6</td>
<td>174.7</td>
</tr>
<tr>
<td>Bulk Density</td>
<td>1.6</td>
<td>1.91</td>
<td>2.16</td>
<td>2.31</td>
</tr>
<tr>
<td>Moisture content</td>
<td>3.41</td>
<td>7.82</td>
<td>12.81</td>
<td>15.6</td>
</tr>
<tr>
<td>Soil Texture-Sand</td>
<td>10</td>
<td>7</td>
<td>8</td>
<td>13</td>
</tr>
<tr>
<td>Soil Texture-Silt</td>
<td>28</td>
<td>12</td>
<td>12</td>
<td>14</td>
</tr>
<tr>
<td>Soil Texture-Clay</td>
<td>62</td>
<td>81</td>
<td>80</td>
<td>73</td>
</tr>
<tr>
<td>Nitrogen</td>
<td>91.05</td>
<td>95.42</td>
<td>93.28</td>
<td>91.08</td>
</tr>
<tr>
<td>Phosphorus as P2O5</td>
<td>20.31</td>
<td>18.27</td>
<td>17.99</td>
<td>18.76</td>
</tr>
<tr>
<td>Potassium as K2O</td>
<td>87.17</td>
<td>85.01</td>
<td>88.64</td>
<td>91.11</td>
</tr>
<tr>
<td>Sodium as Na2O</td>
<td>91.33</td>
<td>93.64</td>
<td>90.07</td>
<td>97.63</td>
</tr>
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<td>Calcium as Ca</td>
<td>32.06</td>
<td>60.12</td>
<td>48.09</td>
<td>40.08</td>
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<tr>
<td>Magnesium as Mg</td>
<td>16.98</td>
<td>4.83</td>
<td>4.92</td>
<td>16.98</td>
</tr>
<tr>
<td>Chlorides as Cl</td>
<td>269.9</td>
<td>304.9</td>
<td>274.9</td>
<td>289.9</td>
</tr>
<tr>
<td>Sulphates as SO4</td>
<td>16.44</td>
<td>18.13</td>
<td>19.18</td>
<td>18.21</td>
</tr>
<tr>
<td>Copper as Cu</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>Manganese</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>Molybdenum</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>Boron</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>Zinc as Zn</td>
<td>1.92</td>
<td>2.32</td>
<td>2.24</td>
<td>2.19</td>
</tr>
<tr>
<td>Chromium as Cr</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
<td>&lt;1.0</td>
</tr>
<tr>
<td>Iron as Fe</td>
<td>&lt;2.0</td>
<td>&lt;2.0</td>
<td>&lt;2.0</td>
<td>&lt;2.0</td>
</tr>
<tr>
<td>Lead as Pb</td>
<td>&lt;2.0</td>
<td>&lt;2.0</td>
<td>&lt;2.0</td>
<td>&lt;2.0</td>
</tr>
<tr>
<td>Organic Carbon</td>
<td>0.34</td>
<td>0.37</td>
<td>0.35</td>
<td>0.39</td>
</tr>
</tbody>
</table>
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.

![Antibacterial Activity of Cow urine on various concentrations](image)

**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**

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Diameter of the inhibition zone</th>
</tr>
</thead>
<tbody>
<tr>
<td>0% cow urine concentration</td>
<td>0.0cm</td>
</tr>
<tr>
<td>5% cow urine concentration</td>
<td>0.6cm</td>
</tr>
<tr>
<td>15% cow urine concentration</td>
<td>1.4cm</td>
</tr>
<tr>
<td>25% cow urine concentration</td>
<td>2.1cm</td>
</tr>
</tbody>
</table>

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

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

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**

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Diameter of the fungi culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>0% cow urine concentration</td>
<td>6cm</td>
</tr>
<tr>
<td>5% cow urine concentration</td>
<td>5.5cm</td>
</tr>
<tr>
<td>15% cow urine concentration</td>
<td>4.9cm</td>
</tr>
<tr>
<td>25% cow urine concentration</td>
<td>3.1cm</td>
</tr>
</tbody>
</table>

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,

1. 1H-Indole, 1-Methyl-2-Phenyl (Pubchem ID-77095)
2. Isophthaladehyde (Pubchem ID-34777)
3. Octane, 6-ethyl-2-methyl (Pubchem ID-537768)
4. 7-Methyl-Z-8,10-dodecadienal (Pubchem ID-5363533)
5. 1(3H)-Isobenzofuranone,3a,4,5,7a-tetrahydro-4-hydroxy-3a,7a-dimethyl-,(3a,alpha.,4.beta.,7a.alpha.) - (+/-.) (Pubchem ID- Not found)
6. Phenol, 2,4-bis(1,1-dimethyllethyl) (Pubchem ID-528937)
7. Triacontane (Pubchem ID-12535)
8. L-leucine, N-(N-acetylglycyl)-butylester (Pubchem ID-Not found)
9. Tetradecanoic acid, trimethylsilylester (Pubchem ID-519592)
10. 1,2-Benzendicarboxylic acid, butyl 2-ethylhexyl ester (Pubchem ID-6818)
11. Tridecane, 7-propyl (Pubchem ID-Not found)
12. N-Hexadecanoic acid (Pubchem ID-540086) fungicide
13. Dibutyl phthalate (Pubchem ID-3026) *Drug*
14. Hexadeconic acid, trimethylsilyl ester (Pubchem ID-521638)
15. 4-(p-methoxyphenyl)-3-buten-2-one, thiosemicarbazone (Pubchem ID-9603450)
16. 1-cyclohexylidimethylsilyloxy-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

<table>
<thead>
<tr>
<th>Pubchem ID</th>
<th>Name of compound</th>
<th>Properties</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>537768</td>
<td>Octane, 6-ethyl-2-methyl</td>
<td>-</td>
<td>No information available about this compound</td>
</tr>
<tr>
<td>5363533</td>
<td>7-Methyl-Z-8,10-dodecadienal</td>
<td>-</td>
<td>No information available about this compound</td>
</tr>
<tr>
<td>Not found</td>
<td>1(3H)-Isobenzofuranone,3a,4,5,7a-tetrahydro-4-hydroxy-5a,7a-dimethyl-(3a.alpha.,4,b eta.,7a.alpha.)(+/-)</td>
<td>-</td>
<td>No information available about this compound</td>
</tr>
<tr>
<td>528937</td>
<td>Phenol, 2,4-bis(1,1-dimethylethyl)</td>
<td>Antifungal</td>
<td><a href="https://doi.org/10.1016/j.jplph.2013.07.004">https://doi.org/10.1016/j.jplph.2013.07.004</a> [9]</td>
</tr>
<tr>
<td>Not found</td>
<td>L-leucine, N-(N-acetylglycyl)-butylester</td>
<td>-</td>
<td>No information available about this compound</td>
</tr>
<tr>
<td>6818</td>
<td>1,2-Benzenedicarboxylic acid, butyl 2-ethylhexyl ester</td>
<td>Antifungal</td>
<td><a href="http://shodhganga.inflibnet.ac.in/bitstream/10603/184541/19/6_abstract.pdf">http://shodhganga.inflibnet.ac.in/bitstream/10603/184541/19/6_abstract.pdf</a></td>
</tr>
<tr>
<td>246301</td>
<td>Tridecane, 7-propyl</td>
<td>-</td>
<td>No information available about this compound</td>
</tr>
<tr>
<td>540086</td>
<td>N-Hexadecanoic acid</td>
<td>Antifungal, Antibacterial</td>
<td><a href="https://doi.org/10.7164/antibiotics.39.888">https://doi.org/10.7164/antibiotics.39.888</a> [12]</td>
</tr>
<tr>
<td>3206</td>
<td>Dibutyl phthalate</td>
<td>Medicinal oil</td>
<td><a href="https://doi.org/10.1186/s40199-014-0078-1">https://doi.org/10.1186/s40199-014-0078-1</a> [14]</td>
</tr>
<tr>
<td>521638</td>
<td>Hexadeconic acid, trimethylsilyl ester</td>
<td>-</td>
<td>No information available about this component</td>
</tr>
<tr>
<td>532617</td>
<td>1-cyclohexyldimethylsilyl oxy-3,5-dimethyl benzene</td>
<td>-</td>
<td>No information available about this compound</td>
</tr>
</tbody>
</table>

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 antimicrobial, 3 compounds were identified as antifungal, 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.

Acknowledgement

This research was conducted in Sreenidhi Institute of Science and Technology, as a part major project. I would like to thank, Dr Bishwambhar Mishra for guiding us. I thank IICT, Tarnaka for allowing us to use their resources i.e Gas chromatography and mass spectroscopy and ECOICONS, ECIL for soil analysis.

Conflict of interest

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

References


