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Effect of Buffalo Urine Treatment on Plant Growth and Antimicrobial Activity on *Gossypium hirsutum* L.

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Abstract
Indian traditional system of medicine, especially *Ayurvedic* system, uses animal urine as a source of drug. In *Ayurveda*, the properties of the urine of eight different animals along with the human urine and its uses are described. The milk, dung, urine of the buffalo is used for various purposes. The present study was done using buffalo urine as plant growth enhancer and antifungal agent. The plant chosen for this study is *Gossypium hirsutum* L. *(*cotton). The plant was grown for 90 days using different buffalo urine concentration that is 0% (control) 1:10, 1:5, 1:1. The various parameters such as plant height, root length, shoot length, leaf area, and biomass of the plant were observed. The antifungal activity was studied by using different buffalo urine concentrations on (*Alternaria macrospora*) using agar diffusion method. Among all the concentrations, the 1:1 concentration showed maximum growth compared to other two concentrations. Whereas 1:5, concentrations showed more antifungal activity. This study concluded that buffalo urine can be used as plant growth enhancer, and it possess antifungal characteristics.

Keywords
Buffalo urine, antimicrobial activity, plant growth Enhancer, *Alternaria macrospora*.

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INTRODUCTION
Indian traditional system of medicine, especially *Ayurvedic* system, uses animal urine as a source of drug. In *Ayurveda*, the properties of the urine of eight different animals along with the human urine and its uses are described. Basically, Buffalo's urine (BU) is used mainly for the treatment of various diseases in *Ayurveda.* Apart from urine of the other animals such as goat, sheep, elephant, horse, camel, and donkey were also used as remedies for the treatment of different diseases. (1) Urine may act as a good biomarker for the health status of livestock animals. No such studies were conducted on the antioxidative properties of dairy cow, buffalo and goat urine.

Cattle urine has antibacterial activity as well as effective as a fungicide (2). Cow urine distillate serves as a potential tool as an antioxidant reported that the cow urine treated groups of imidacloprid-treated white leghorn cockerels had shown a significant difference in antioxidative parameters as lipid peroxidation and reduced glutathione level as compared to groups without cow urine treatment. Thus, it may be concluded that it serves as a source of antioxidative defense system by either decreasing

the level of lipid peroxide generation or by enhancing the glutathione action to counteract the generated peroxide free radicals use of cow urine in today's society is not only for the prevention of dangerous diseases but also in the agricultural and sericulture areas for the antioxidative properties of urine (3). Urine may act as a good biomarker for the health status of livestock animals. No such studies were conducted on the antioxidative properties of dairy cow, buffalo, and goat urine. In this context, this study was conducted to determine the physics-chemical and antioxidative properties of dairy cow, buffalo and goat urine, which may establish urine as a strong biomarker to know the health status of livestock animals in Himachal Pradesh, India.

Gossypium hirsutum L. is a plant under the family Malvacae. It is cultivated primarily for its vegetable, seed, and fiber. It is also used in large volumes as a raw material in textile industries. The bulk of the commercial cottons come from this species (4). Several species of Malvaceae family have been used in traditional medicine (5), for several infectious diseases however, their active principle(s) has not been elucidated. It is possible that compounds other than gossypol could be responsible for the anti-parasitic activity (6).The pharmacological characteristics of Gossypol, a compound initially isolated from *Gossypium hirsutum L.* (Malvaceae) seeds have been studied mainly in relation to its reversible ant fertility effects in men (7), effect on diverse pathogenic agents, such Trypanosoma cruzi (8), Plasmodium telpemerum (9), Edwardsiella ictaluri (10). Gossypol not only inhibits the growth of numerous parasitic organisms but also shows antiviral activity against a number of enveloped viruses, including the AIDS virus (11). The study is aimed at determining the Buffalo urine can be used as plant growth enhancer and it possess antifungal characteristics on *Gossypium hirsutum L.* plant

MATERIALS AND METHODS

Collection of Buffalo Urine

From a local variety of Buffalo, fresh urine was collected in a sterile container. The urine was filtered through Whatmann No. 1 filter paper to get rid of debris and precipitated material and was stored in airtight container at 4°C before use.

Preparation of different Concentrations of buffalo Urine

Various Concentrations of buffalo urine was prepared i.e. 1:10, 1:5, 1:1% concentrations were prepare by mixing of Buffalo urine in distilled water.

Collection of Seeds

The seeds of *Gossypium hirsutum L.* (Cotton) of WGCV-48 variety were obtained at Regional Agricultural Research Station, Warangal, Telangana.

Seed treatment

Before sowing seeds, they were treated with Buffalo urine solution.

Sowing of seeds by Pot Culture Experiment

The Pot culture method was adapted to the present study to find the effect of various concentrations of buffalo urine on the growth of cotton plants. 5 seeds of cotton were sown each in different pots which were filled with sterile garden black soil. For about half an hour, it was sterilized in an autoclave at 15 lbs pressure with the pH adjusted to 7. Each pot was irrigated twice a day with different concentrations (1:10, 1:5, 1:1. v/v)) of buffalo urine. In control pots, tap water was used for the irrigation of seeds. When the plants grew randomly, 3 seedlings from each treatment were uprooted without disturbing the root system. Different parameters such as plant height, shoot and root length, number of leaves and branches, and leaf area were measured after 30 and 60 days to observe the plant growth. Shown in Fig.1 and Fig.2.

Fig.1. Cotton plant 30 days Pot experiment

Fig.2. Cotton plant 60 days Pot experiment

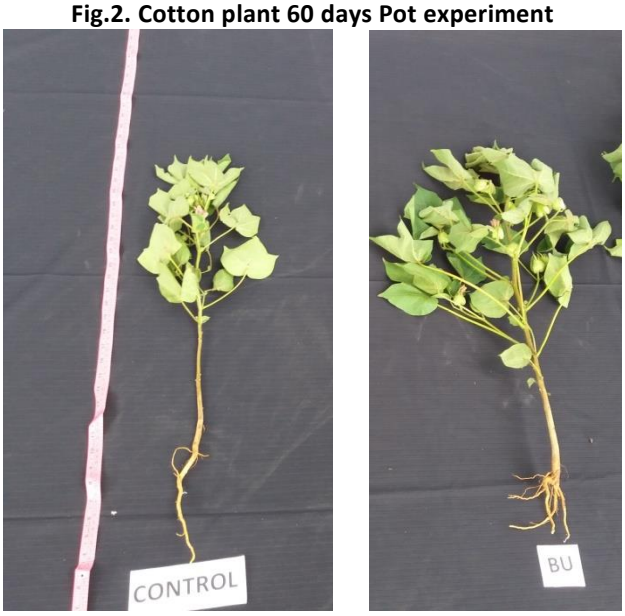


Fig.3. Cotton plant 90 days Pot experiment

Parameters

Various parameters of the plants were observed. The parameters include

1. Root Length: Measured from the shoot ending to the root tip using scale.
2. Shoot Length: Measured shoot using scale in cms.
3. Plant height: Measured using scale in cms.
4. Leaf area: The leaf with highest area was considered by Graphical method in cm.
5. Plant biomass: As an index of growth character increase in biomass (w) was expressed in terms of dry weight (12)

$$W = W_2 - W_1$$

Where the subscripts 1 and 2 indicate values of *W* on two occasions.

Soil analysis

The treatment given to the soil samples are estimated of the changes made by the cow urine in the soil. For soil analysis, various parameters and methods were used.

N- nitrogen: 0.782ppm/mg

P- Prosperous: 0.331ppm/mg

K-Potassium: 0.291ppm/mg

Isolation of Fungal Pathogens

Alternaria macrospora isolated from infected plants of *Gossypium hirsutum L.* that showed symptoms of damping off and wilting

Antifungal Activity

The three concentrations (10%, 5%, and 1% v/v) of buffalo urine were prepared. 10 ml of different concentrations of buffalo urine was amended in 10 ml of potato dextrose agar medium and mixed thoroughly by stirring. Distilled water was used to maintain the control. The medium was treated in autoclave. The content was poured into sterilized Petri plates and was left. From the actively growing cultures, the fungal discs of 5mm diameter were

taken by using cork borer. Then the discs were transferred aseptically on PDA plates duly poisoned with cow urine. Plates were incubated at 28 ± 2°C temperature in incubator for 7 days. After 7 days, the plates were observed, and colony diameters were measured with the help of ruler. The percent of inhibition was calculated by using the following formula.

Percent inhibition of mycelia growth (1%)

$$I = 100(C-T)/C$$

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

RESULTS

Growth observation

The plants were placed on white sheet of paper and the parameters were measured as per protocol mentioned in the methods.

Physical parameters comparison

The plant has shown different physical changes with different buffalo urine concentrations. This shows that urine has effect on the growth of the plant.

The data from physical parameters for 30 days, 60 days and 90 days was tabulated in table 1,2 &3.

Table 1: Effect of Buffalo urine on plant growth parameters for 30 days Pot experiment:

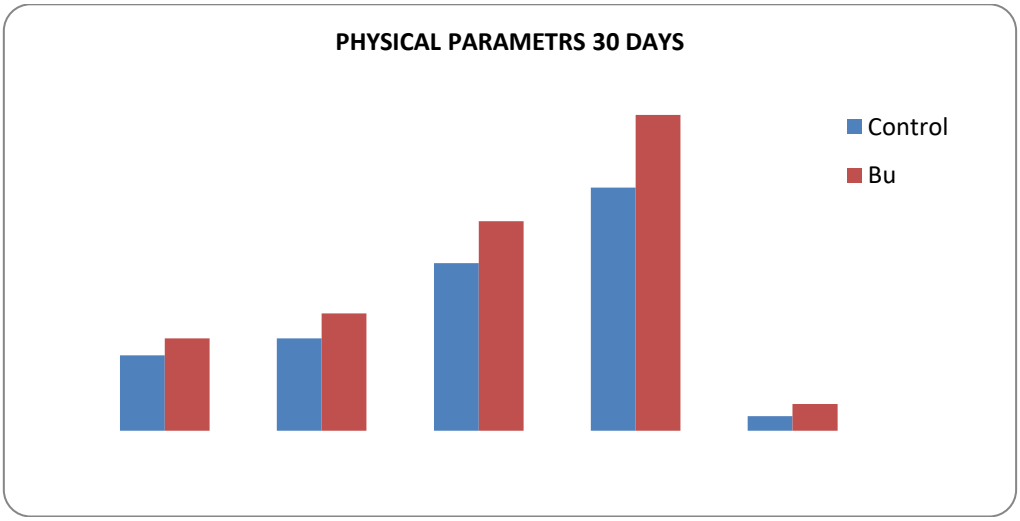
Sample	Root length cm.	Shoot length cm.	Plant length cm.	Leaf area cm.	Plant biomass gms.
control	9	11	20	28.98	2.72
Buffalo urine	11	14	25	37.37	3.2

Table 2: Effect of Buffalo urine on plant growth parameters for 60 days Pot experiment.

Sample	Root length cm.	Shoot length cm.	Plant length cm.	Leaf area cm.	Plant biomass gms.
control	14.96	30	44.96	247	26
cow urine	27.46	49,66	77.12	263	33.31

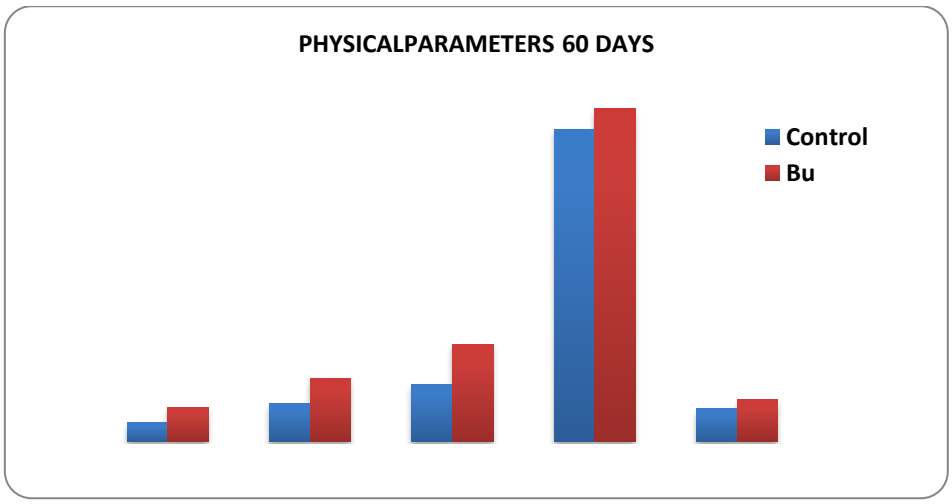
Table 3: Effect of Buffalo urine on plant growth parameters for 90 days Pot experimen

Sample	Root length cm.	Shoot length cm.	Plant length cm.	Leaf area cm.	Plant biomass gms.
control	27.33	48.33	76.66	248.9	69.37
cow urine	33	61	94	275.24	94.46



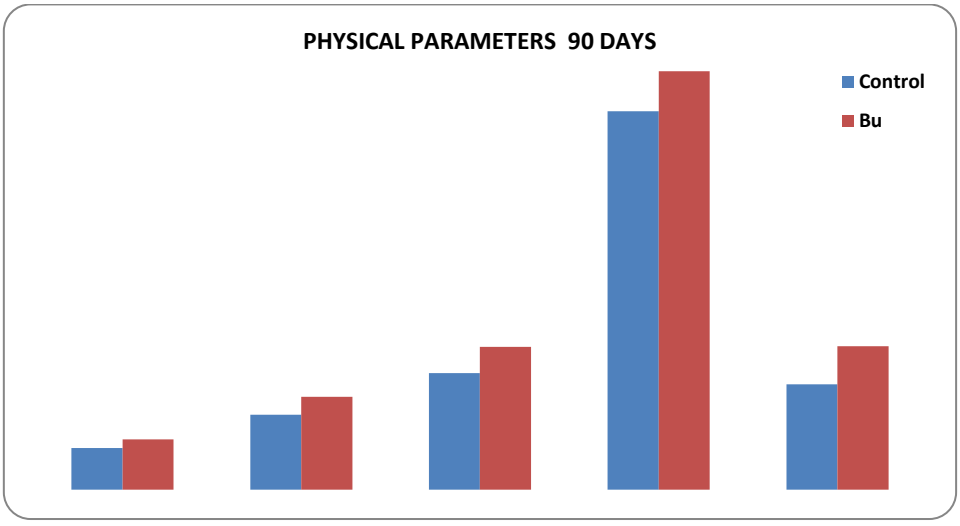
X- Axis physical parameter, Y- Axis result, BU-Buffalo urine

Figure 4: Graphical representation of physical parameters of (*Gossypium hirsutum L.*) 30 days cotton plant with Buffalo urine treatment



X- Axis physical parameters, Y- axis results, BU-Buffalo urine.

Figure 5: Graphical representation of physical parameters of (*Gossypium hirsutum L.*) 60 days cotton plant with Buffalo urine treatment



X- Axis physical parameters, Y- Axis results, BU- Buffalo urine

Figure 6: Graphical representation of physical parameters of (*Gossypium hirsutum L.*) 90 days cotton plant with Buffalo urine treatment.

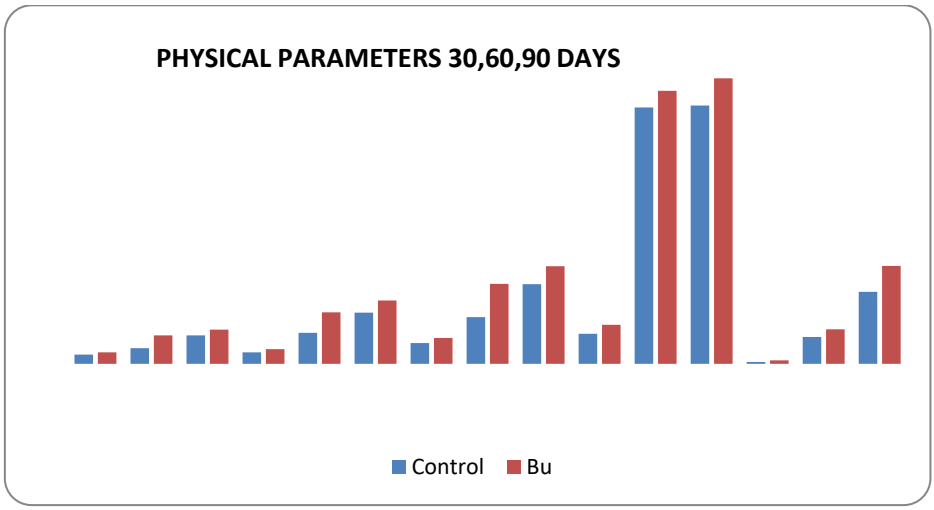


Figure 7: Graphical representation of total physical parameters of (*Gossypium hirsutum L.*) 30,60,90 days cotton plant with Buffalo urine treatment

Antifungal Activity

The anti-fungal activity was conducted using disc diffusion method and the result are depicted in figure

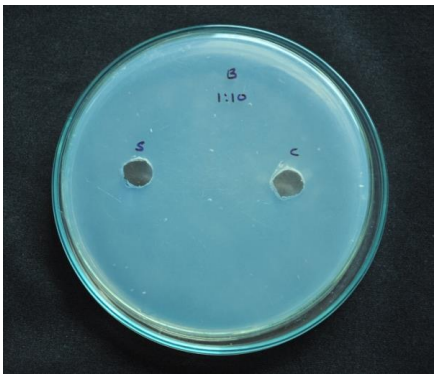


Fig A

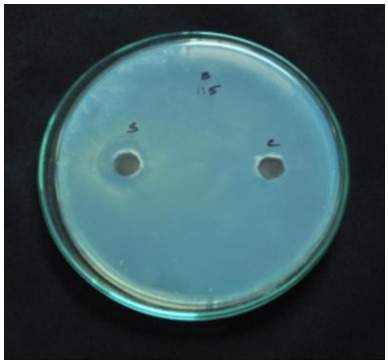


Fig. B

Fig.C

Figure7: Antifungal Activity of Buffalo urine on various concentrations:
a) 1:10% concentration b) 1:05% concentration c) 1:01% concentration

Table 4. Diameter of the inhibition zone in various concentrations.

Sample name	1:10% Conc.	1:05% Conc.	1:01%Conc.
Control	NA	NA	NA
Buffalo urine	NA	11	16

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

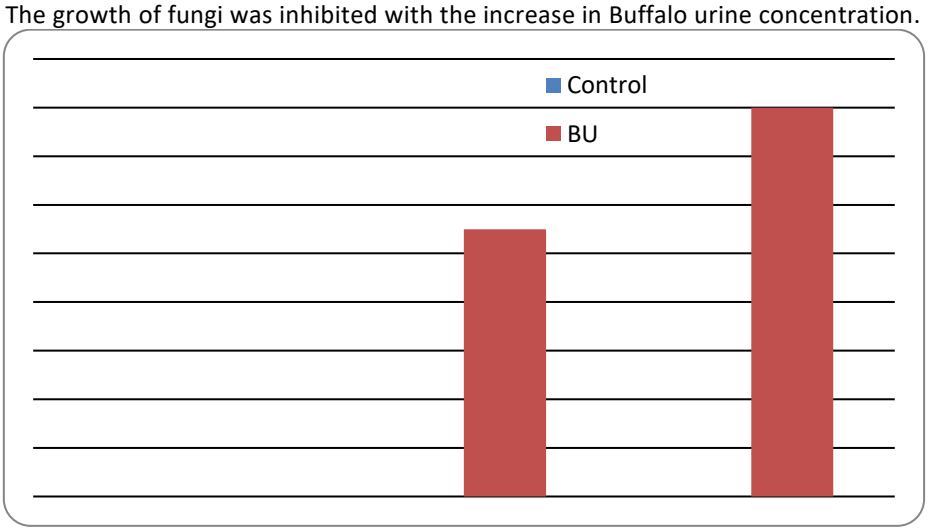


Figure 8. Graphical representation antifungal activity of cow urine various concentration

DISCUSSION

The plant *Gossypium hirsutum L.* was grown using different concentrations (Control, 1:10%, 1:05% &1:01%) of buffalo urine for 30 days, 60days and 90 days. In 90 days the plant growth was observed. The plant showed varied growth at different concentrations and parameters were measured and tabulated. According to the measured observations the 1:01% buffalo urine concentration showed more growth when compared with other concentrations. Soil was sent for soil analysis to check the changes made to the soil by buffalo urine. The various parameters of the soil were analyzed. The antifungal activity was conducted by using disc diffusion method then measured zone of inhibition. The zone of inhibition was increased with increased buffalo urine concentration it is indicating the presence of antifungal agents in buffalo urine.

CONCLUSION

From the above study we can conclude that cow urine has the properties of plant growth enhancer and antimicrobial properties. This can be avoided by extracting compounds from the cow urine which can be protecting our environment by avoiding the synthetic means of production

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