Inhibitory Effect of Cow Urine Extracts of Selected Plants against Pathogens Causing Rhizome Rot of Ginger

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Abstract
The present study was carried out to investigate the inhibitory effect of cow urine extracts of nine plants against two fungi viz., Fusarium oxysporum f.sp. zingiberi, Pythium aphanidermatum and a bacterium Ralstonia solanacearum that are known to cause rhizome rot of ginger. Antifungal and antibacterial activity of cow urine extracts was investigated by poison food technique and agar well diffusion method respectively. The extent of growth of test fungi in plates poisoned with extracts was lesser when compared with the control plates. Among fungi, high susceptibility was recorded in case of F. oxysporum. Cow urine extract of Elaegnus kologa caused high inhibition of P. aphanidermatum whereas cow urine extract of Artocarpus lakoocha, Hemidesmus indicus, Croton roxburghii and Maesa indica caused high inhibition of F. oxysporum. All extracts caused inhibition of R. solanacearum. Extract of A. lakoocha caused maximum inhibition followed by H. indicus, E. kologa and others. Overall, cow urine extracts of plants selected in this study caused varied inhibition of test microbes. These extracts may find a possible application in agriculture against phytopathogenic microorganisms.

Keywords: Rhizome rot of ginger, Cow urine extract, Poison food technique, Agar well diffusion.

INTRODUCTION
Microorganisms cause a number of diseases in crops than any other pathogens and results in major crop losses. The harvest losses of crops are much higher in developing countries. Pathogens such as species of Fusarium, Alternaria, Pythium, Sclerotium, Phytophthora, Curvularia, Botrytis, Ralstonia, Xanthomonas etc., cause severe damages to agricultural crops before and after harvesting. The plant diseases caused by microorganisms are usually controlled by the use of chemicals. However, the use of synthetic compounds to control phytopathogens suffers from two main drawbacks viz., potential development of resistance in pathogens and the risk of toxicity. Due to this, research focused on compounds derived from natural sources such as plant extracts and their possible application in agriculture is being intensified. Many natural products, including plant extracts, have been shown to possess marked inhibitory activity against a variety of pathogens (Ojala et al., 2000; Benkebla, 2004; Bhai et al., 2005; Bajpai et al., 2008; Paret et al., 2010; Ranaware et al., 2010; Zhao et al., 2011; Tiwari and Das, 2011; Bhardwaj et al., 2011; De Britto et al., 2011).

From the ancient period in India, cow urine has been used for several medicinal purposes and the description on its use has been in several classical Ayurveda texts like Charaka samhita and Shushruta samhita. Cow is believed to be a sacred animal in India its urine is known to cure several diseases. In Veda, cow urine is compared with the nectar (Krishnamurthi et al., 2004; Gururaja et al., 2011). Cow urine has got applications in agriculture. It has been found that cow urine has potential to control Meloidogyne incognita in Lycopersicon esculentum (Abubakar et al., 2004) and aphids and pickleworms in watermelon cultivation (Burubai and Erbo, 2012). It is observed that cow urine has inhibitory effect against several plant pathogens such as Sclerotinia sclerotiorum (Basak et al., 2002a), Fusarium solani.
Ginger (Zingiber officinale Rosc., Zingiberaceae) is an important commercial crop grown for its aromatic rhizomes being used as spice and medicine. India is the largest producer of ginger and accounts for about $1/3$ of total world output. Ginger is grown in Kerala, Karnataka, West Bengal, Andhra Pradesh, Orissa, Arunachal Pradesh, Sikkim and other parts of India (Kumar et al., 2008; Sharma et al., 2010). The production of ginger is influenced largely by a number of diseases caused by bacteria, fungi, viruses, mycoplasma and nematodes. Main diseases of ginger are bacterial wilt caused by Ralstonia solanacearum, rhizome rot caused by Pythium species, Fusarium species, Sclerotium species, Pseudomonas species and others (Dake and Edison, 1989; Senapati and Ghose, 2005; Paret et al., 2010; Sharma et al., 2010; Kavyashree, 2009). Soft rot is a serious disease and has drastic effects on crop and eventually leads to rhizome loss. It is manifested initially by foliar yellowing and later water soaked lesions appear on the collar of the pseudostem which extend to rhizomes and leaves resulting in rotting of the entire plant. The disease is both seed and soil-borne (Bhai et al., 2005). In the present study, we have determined the inhibitory activity of cow urine extracts of selected plants against the pathogens viz., Fusarium oxysporum fsp. zingiberi, Pythium aphanidermatum and Ralstonia solanacearum causing rhizome rot of ginger.

**MATERIALS AND METHODS**

**Collection of Cow Urine**

Urine was collected in a sterile container from a local cow variety called Malnad gidda at early morning 6:30am. The urine was filtered through Whatman No. 1 and stored in airtight container.

**Preparation of Cow Urine Extract of Selected Plants**

Table 1 represents the plants used in the present study. The plants were shade dried, powdered mechanically and used for preparation of extract. A known quantity (10g) of powdered plant material was added to 100ml of cow urine and left for 15 days. Later, the contents were filtered through muslin cloth followed by Whatman no. 1 and the filtrates were stored in refrigerator until use.

**Antifungal Activity**

Poisoned food technique was employed to screen the antifungal efficacy of cow urine extracts of selected plants (Dileep et al., 2013). In brief, Potato dextrose agar (HiMedia, Mumbai) media amended with cow urine extracts (10%) were autoclaved and poured into sterile petriplates. Fungal discs of 5mm diameter were cut with the help of sterile cork borer from the periphery of 5 days old culture of P. oxysporum f. sp. zingiberi and P. aphanidermatum and the discs were transferred aseptically on PDA plates poisoned with cow urine extracts and incubated for 5 days at 28°C. Colony diameters in mutual perpendicular directions were measured on the 5th day with the help of a ruler. The experiment was repeated twice and average colony diameter was noted. Antifungal activity of cow urine extracts was recorded in terms of inhibition of mycelial growth (%) and was calculated using the formula:

\[
\text{Myceial growth inhibition} (\%) = \frac{(C-T)}{C} \times 100
\]

where ‘C’ is average colony diameter in control plates and ‘T’ is average colony diameter in poisoned plates.

**Antibacterial Activity**

In order to assess antibacterial activity of cow urine extracts against R. solanacearum, we have employed Agar well diffusion method (Kekuda et al., 2012). The bacterium was inoculated into sterile Nutrient broth (HiMedia, Mumbai) tubes and incubated for 24 hours at 37°C. The broth culture was swabbed on sterile Nutrient agar (HiMedia, Mumbai) plates amended with cow urine extracts (10%) were autoclaved and poured into sterile petriplates. With the help of a sterile cork borer, wells of 0.6cm diameter were punched in the inoculated plates and cow urine extracts and standard (Streptomycin, 1mg/ml) were transferred into respectively labeled wells. The plates were incubated at 37°C for 24 hours and the zone of inhibition formed around the wells was measured. The experiment was repeated twice and the average value was recorded.

| Name of the plant          | Family       | Part used |
|----------------------------|--------------|-----------|
| Artocarpus lakoocha Roxb.  | Moraceae     | Leaf      |
| Maesa indica (Roxb.) Walliç | Myrsinaceae  | Leaf      |
| Polyalthia longifolia Thw. | Annonaceae   | Leaf      |
| Hemedesmus indicus R. Br   | Asclepiadaceae | Root    |
| Swertia chirata            | Gentianaceae | Whole plant |
| (Roxb. ex Fleming) H. Karst.|             |           |
| Croton roxburghii Balak    | Euphorbiaceae | Leaf      |
| Elaeagnus kologa Schlecht  | Elaeagnaceae | Leaf      |
| Gnidia glauca (Fresen.) Gilg  | Thymelaeaceae | Leaf      |
| Fahrenheitia zeylanica (Thw.) | Euphorbiaceae | Leaf      |

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Rakesh et al., 2002. Bipolaris sorokiniana (Akhter et al., 2006) and Xanthomonas oryzae pv. oryzae (Murugan et al., 2012). It has been shown that cow urine extract of certain plants as well as cow urine in combination with certain plant extracts are found to possess marked inhibitory effect on human pathogens as well as plant pathogens (Akhter et al., 2006; Yadav et al., 2008; Rajapandiy et al., 2011; Tiwari & Das, 2011).

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RESULTS

The result of inhibitory effect of cow urine extracts of selected plants against *F. oxysporum* and *P. aphanidermatum* is presented in Table 2 and Figure 1. The growth of test fungi, in terms of diameter of the fungal colony in poisoned plates, was measured and compared with the control plates. The colony diameter of test fungi was lesser in poisoned plates in comparison with that of colony diameter in control plates indicating antifungal potential of cow urine extract of plants. The test fungi were found to be sensitive to all the extracts. Among fungi, high susceptibility was recorded in case of *F. oxysporum* with growth inhibition of >50% produced by all extracts. Only 3 extracts caused >50% inhibition of *P. aphanidermatum*. Cow urine extract of *E. kologa* & *P. longifolia* caused high and least inhibition of *P. aphanidermatum* respectively. In case of *F. oxysporum*, higher inhibition was produced by *A. lakoocha*, *H. indicus*, *C. roxburghii* and *M. indica*.

Table 2: Antifungal activity of Cow urine extracts of selected plants.

| Cow Urine Extract   | Colony diameter in cm |
|---------------------|-----------------------|
|                     | *P. aphanidermatum* | *F. oxysporum* |
| Control             | 2.8                  | 3.1            |
| *A. lakoocha*       | 1.1                  | 0.8            |
| *H. indicus*        | 1.6                  | 0.8            |
| *E. kologa*         | 1.0                  | 1.0            |
| *G. glauca*         | 1.8                  | 1.2            |
| *P. longifolia*     | 1.9                  | 0.9            |
| *C. roxburghii*     | 1.5                  | 0.8            |
| *F. zeylanica* leaf| 1.1                  | 0.9            |
| *S. chirata*        | 1.8                  | 1.1            |
| *M. indica*         | 1.7                  | 0.8            |
| Streptomycin        |                      |                |

DISCUSSION

The term rhizome rot of ginger is accepted generally for soft rot and yellow disease complex as soft rot and yellows are generally found together affecting the plants and symptoms often mixed up. Soft rot is a serious disease leading to drastic effects on crop (Bhai *et al*., 2005; Senapati and Ghose, 2005). The rhizome rot disease management involves cultural, biological and chemical approaches for suppression of the pathogens. However, the control of the disease by the use of chemical agents is not so beneficial due to high cost, breakdown of resistance, residual problem and deleterious effect on non-target organisms including humans. This has necessitated search for alternatives for controlling the rhizome rot of ginger (Bhai *et al*., 2005; Pandey *et al*., 2010). Plants have been shown to possess inhibitory effect against fungi causing rhizome rot of ginger. Sagar *et al.* (2007) showed the fungitoxic efficacy of some plant extracts against *P. aphanidermatum* & *F. solani* isolated from rhizome rot specimen of ginger. It was found that *Azadirachta indica* and *Ferula asafoetida* showed maximum inhibition of mycelial growth of *P. aphanidermatum* and *F. solani* respectively. In an earlier study, we have shown the potential of ripe and unripe pericarp extract of *Polyalthia longifolia* against *P. aphanidermatum* and *F. solani* isolated from ginger rhizome rot (Dileep *et al*., 2013).
Rakesh et al.,

It has been shown that cow urine based extracts of plants have been reported to possess marked antibacterial and antifungal activity. The extract of Calotropis procera, in combination with cow urine, has shown 91% inhibition of conidial germination of Bipolaris sorokiniana, causative agent of leaf blight of wheat (Akhter et al., 2006). Tiwari and Das (2011) found in vitro and in vivo inhibitory efficacy of some medicinal plant extracts prepared in cow urine against Rhizoctonia solani, causal agent of sheath blight of rice. Murugan et al. (2012) showed the efficacy of cow urine and cow urine with Pongamia pinnata seed against bacterial leaf blight of paddy caused by Xanthomonas oryzae pv. oryzae. In the present study, we have evaluated the inhibitory effect of cow urine extract of 9 plants against P. aphanidermatum and F. oxysporum by poison food technique. Reduction of colony diameter of test fungi was considered as antifungal effect of the extracts. It has been observed that the susceptibility to cow urine extracts of plants was higher in case of F. oxysporum. The extracts were also effective against R. solanacearum.

CONCLUSION

A marked inhibition of rhizome rot pathogens by cow urine extracts of selected plants was observed in this study. The extracts may find a possible use in agriculture as potent agents against pathogens. Further studies involving field trials is needed to justify the results of the present study.

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