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; Benkeblia, 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; Bhawardj 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 esculentus (Abubakar et al., 2004) and aphids and pickleworms in watermelon cultivation (Burubai and Eribo, 2012). It is observed that cow urine has inhibitory effect against several plant pathogens such as Sclerotinia sclerotiorum (Basak et al., 2002a), Fusarium solani.
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.

Table 1: Plants used in the study.

<table>
<thead>
<tr>
<th>Name of the plant</th>
<th>Family</th>
<th>Part used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artocarpus lakoocha Roxb.</td>
<td>Moraceae</td>
<td>Leaf</td>
</tr>
<tr>
<td>Maesa indica (Roxb.) Walliich</td>
<td>Myrsinaceae</td>
<td>Leaf</td>
</tr>
<tr>
<td>Polyaltha longifolia Thw.</td>
<td>Annonaceae</td>
<td>Leaf</td>
</tr>
<tr>
<td>Hemedesmus indicus R. Br</td>
<td>Asclepiadaceae</td>
<td>Root</td>
</tr>
<tr>
<td>Swertia chirata (Roxb. ex Fleming) H. Karst.</td>
<td>Gentianaceae</td>
<td>Whole plant</td>
</tr>
<tr>
<td>Croton roxburghii Balak.</td>
<td>Euphorbiaceae</td>
<td>Leaf</td>
</tr>
<tr>
<td>Elaeagnus kologa Schlecht</td>
<td>Elaeagnaceae</td>
<td>Leaf</td>
</tr>
<tr>
<td>Gnidia glauca (Fresen.) Gilg</td>
<td>Thymelaeaceae</td>
<td>Leaf</td>
</tr>
<tr>
<td>Fahrenheitia zeylanica (Thw.)</td>
<td>Euphorbiaceae</td>
<td>Leaf</td>
</tr>
</tbody>
</table>

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 R. solanacearum f. sp. zingiberi and P. aphidermatum 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 two times 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:

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

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 using sterile cotton swabs. 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.
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.

<table>
<thead>
<tr>
<th>Cow Urine Extract</th>
<th>Colony diameter in cm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>P. aphanidermatum</em></td>
</tr>
<tr>
<td>Control</td>
<td>2.8</td>
</tr>
<tr>
<td><em>A. lakoocha</em></td>
<td>1.1</td>
</tr>
<tr>
<td><em>H. indicus</em></td>
<td>1.6</td>
</tr>
<tr>
<td><em>E. kologa</em></td>
<td>1.0</td>
</tr>
<tr>
<td><em>G. glauca</em></td>
<td>1.8</td>
</tr>
<tr>
<td><em>P. longifolia</em></td>
<td>1.9</td>
</tr>
<tr>
<td><em>C. roxburghii</em></td>
<td>1.5</td>
</tr>
<tr>
<td><em>F. zeylanica</em></td>
<td>1.1</td>
</tr>
<tr>
<td><em>S. chirata</em></td>
<td>1.8</td>
</tr>
<tr>
<td><em>M. indica</em></td>
<td>1.7</td>
</tr>
</tbody>
</table>

Figure 1: Inhibition of test fungi (%) by Cow urine extracts of selected plants.

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).
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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 Rhyzoctonia 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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