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Original Research

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.

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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; 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 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*

f.sp. *cucurbitae* (Basak *et al*., 2002b), *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; Rajapandiyn *et al*., 2011; Tiwari & Das, 2011).

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 1/3rd 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 appears 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* f.sp. *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.

Table 1: Plants used in the study.

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

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 *F. 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 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:

Mycelial growth inhibition (%) = (C–T/ C) × 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^{o}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^{o}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.

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

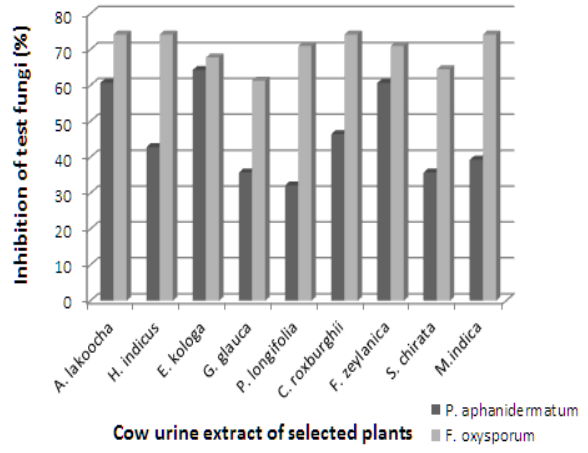

Figure 1: Inhibition of test fungi (%) by Cow urine extracts of selected plants.

The efficacy of cow urine extracts of plants to inhibit phytopathogenic bacterium *R. solanacearum* was evaluated by agar well diffusion method. The presence of zone of inhibition around the well was considered positive for antibacterial activity. It was found that the bacterium was susceptible to all extracts. Among extracts, extract of *A. lakoocha* caused maximum inhibition followed by *H. indicus*, *E. kologa* and others. Least inhibition of the bacterium was recorded in case of *S. chirata* and *M. indica* (Table 3).

Table 3: Antibacterial activity Cow urine extracts against *R. solanacearum.*

Cow Urine Extract	Zone of Inhibition (cm)
A. lakoocha	2.5
H. indicus	2.0
E. kologa	1.9
G. glauca	1.4
P. longifolia	1.2
C. roxburghii	1.8
F. zeylanica	1.2
S. chirata	0.8
M.indica	0.8
Streptomycin	3.2

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. aphanidematum* & *F. solani* isolated from rhizome rot specimen of ginger. It was found that *Azadirachta indica* and *Ferula asafeotida* 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).

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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