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In vitro studies of inhibitory activity of plant extracts and cow urine on mycelial growth of stem rot, *Sclerotim oryzae* of rice

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Abstract: Soil borne phytopathogen *Sclerotium oryzae* significantly affect rice production. To reduce load of chemical pesticides, antifungal activity of plant extracts and cow urine against mycelial growth of *S.oryzae* were tested using poisoned food technique under *in vitro* condition. Plant extracts of 2.5%, 5.0%, 7.5% and 10% concentration was prepared from *Allium cepa, Azadirachta indica, A. sativum, Ricinus communis* and *Syzygium cumini.* Inhibition of mycelial growth of *S.oryzae* was recorded only in case of *A. sativum* and *A. cepa* while *Azadirachta indica, Ricinus communis* and *Syzygium cumini* did not show any inhibition of mycelial growth as compared to control. *A.sativum* plant extracts showed maximum inhibition of mycelia growth of 68.88% at concentration 10% followed by 32.96%, 22.96% and 18.88% at concentration 7.5%, 5.0% and 2.5% resepectively. 22.60%, 19.62%, 17.77% and 8.88% inhibition of mycelial growth as compared to control was recorded at 10%, 7.5%, 5.0% and 2.5% concentration of plant extracts of *A.cepa* All the concentration of cow urine inhibited the mycelial growth of *S. oryzae.* Cow urine at the concentration 5, 7.5 and 10.0 per cent resulted in 100 per cent inhibition of mycelia growth of test pathogen as compared to control. Maximum inhibition of 98.14 per cent was observed at 2.5 per cent concentration followed by 1.25 per cent (63.7%) concentration. This study showed that *A.sativum and A.cepa* and cow urine possess antifungal activity under *in vitro* condition. It can also be tested for antifungal activity under *in vivo* condition.

Keywords: Cow urine, Plant extracts, *Sclerotium oryzae*

INTRODUCTION

Stem rot caused by *S. oryzae* Catt. (*Magnaporthe salvinii* (Catt.) Krause and Webster) is one of the major diseases of rice in India. In India it causes 30-80% yield loss (Rice Knowledge Management Portal (rkmp), 2011). The use of synthetic fungicides to control disease pose various problems such as residue in feed and food, pathogen resistance (Deising *et al.,* 2008), toxicity to non-target organisms, environmental pollution (Arcury and Quandt, 2003). Hence it has become necessary to use eco-friendly formulations which can fit into integrated pest management.

Various plant products like plant extracts, essential oils, gum, resins etc. were shown to exert biological activity *in vitro* and *in vivo* and are used as bio-fungicidal compounds (Pawar and Thaker, 2006; El-Mougy and Alhabeb 2009; Fawzi *et al*., 2009, Sealey *et al.* 2007 and Satish *et al.,* 2007). Basak and Lee (2001b, 2002a, 2002b) observed that cow dung and cow urine were capable of suppressing conidial germination and mycelial growth of *F. oxysporum f.sp. cucumerinum* (causing Fusarium wilt of crop). Plant extracts and Cow urine have inhibitory effect on *Rhizoctonia spp.* (Aye and Matsumoto, 2011; Tiwari and Das, 2011; Al askar and Rashad, 2010; Seema *et al*., 2011), *S. rolfsii* (Amin *et al.,* 2013), *S. hydrophyllum* (Aye and Matsumoto, 2011), *Sclerotinia sclerotiorum* (Basak *et al.*, 2002a), *Colletotrichum capsici* (Rahman *et al.,* 2011 and Kekuda *et al.*, 2014) and *Bipolaris sorokiniana* (Akhter *et al.,* 2006). Seed borne pathogens like *Alternaria alternata*, *F. oxysporium*, *Colletotrichum capsici* and *Curvularia lunata,* are also suppressed by Cow dung and cow urine (Sharma *et al.,* 2010). Post harvest pathogens (Mogle, 2013) and plant pathogenic bacteria (Kebede, 2013) are also inhibited by cow urine and plant extracts. Inhibitory effect of cow urine is also observed in case of clinical pathogens (Sathasivam *et al.,* 2010 and Rana and De, 2013) Such compounds, being biodegraded and selective in their toxicity are considered valuable for controlling different plant diseases. The present study was under taken to study the inhibitory effect of plants extracts and cow urine on mycelial growth of *S. oryzae* under *in vitro* condition.

MATERIALS AND METHODS

***In vitro* efficacy of plant extracts against *S. oryzae*:** Onion (*A. cepa*), neem (*Azadirachta indica*), garlic (*A. sativum*), castor (*Ricinus communis*) and Jamun (*Syzygium cumini*) were used for present investigation. Plant sample were collected from campus area of G.B.

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Pant University of Agriculture and Technology, Pantnagar. Fresh leaves were washed through under tap water followed by sterilized water the leaves were air dried and were grinded with the help of pestle and mortar by taking (1:1 w/v) one gram of extract added in 1 ml distilled water separately for each plant extract. The extracts were clarified by passing through two layers of cheese cloth, a Whatman no. 1 filter paper. The extracts were poured in the flasks plugged with cotton and heated at 100°C for 10 minutes to avoid contamination (Madavi and Singh, 2005). The sterilized extracts were quoted in the study as 100 per cent extract. The appropriate amount of plant extract was mixed in sterilized distilled water to make the desired concentration (v/v) for experiments. For bioassay, concentrations of botanicals were prepared by dissolving 1.5, 3.0, 4.5 and 6.0 ml of plant extract in 60 ml of sterilized PDA, respectively to get the final concentration of 2.5, 5.0, 7.5 and 10 per cent. In control plate, no plant extracts was mixed.

***In vitro* bioassay of botanicals:** Poisoned food technique (plant extract amended PDA medium) was used to screen different plant extracts *in vitro*. Different concentrations (2.5, 5.0, 7.5, 10 per cent and control) of plant extracts were incorporated to PDA medium for inoculation of the test pathogen in sterilized Petri dishes. The isolated pathogen was grown on PDA medium. Five mm mycelial disc cut from the margin of actively growing colony was placed at the center of Petri dishes containing different concentration of the poisoned medium and incubated at 28±1°C for 4 days. Radial growth of fungus was measured in millimeter (mm) after 4 days of inoculation.

***In vitro* study of the antifungal activity cow urine against *S. oryzae*:** Cow urine taken freshly, was filtered with the help Whatman filter paper No. 1 under aseptic conditions in the laminar air flow chamber. The filtrate obtained was then mixed with sterilized water to have the final concentrations viz. 0.625, 1.25, 2.50, 5.0, 7.5 10.0% and control respectively. These concentrations were ready to be used to check the efficacy of cow urine against *S. oryzae.* In control plate, no cow urine was mixed.

***In vitro* bioassay of cow urine:** Effect of cow urine at different concentrations on the radial growth of the test fungus was evaluated by poisoned food technique on potato dextrose agar (PDA) medium. Different concentrations of cow urine were taken viz. 0.625, 1.25, 2.50, 5.0, 7.5 and 10.0%. The desired amount of the cow urine suspension were added in the medium and mixed thoroughly before plating. Different medium toxicated with cow urine was poured in each Petri plate. Subsequently a 5 mm mycelial disc of 4 days old culture of *S. oryzae* was cut with sterile cork borer and placed in centre of each Petri plate. The plates were incubated at 27^0C. The diameter of the fungal colony was measured after 4 days of incubation. After 4 days of incubation percent inhibition was calculated by using the following formula:

$$\text{Percent inhibition} = \frac{X-Y}{X} \times 100$$

Where,
X = colony diameter in check,
Y = colony diameter on amended medium

RESULTS AND DISCUSSION

Effect of plant extracts on radial growth of *S. oryzae*: Table 1 shows the effect of certain botanicals at different concentrations on the mycelial growth of *S. oryzae* in *in vitro* condition. Inhibition of mycelial growth varied significantly with different botanicals and their concentrations. The observations of the experiment were taken 4 days after inoculation. The effect on plant extracts on inhibition of mycelial growth of *S.oryzae* is presented in table 1. Out of five plant extract (viz., *A. cepa, Azadirachta indica, A. sativum, Ricinus communis* and *Syzygium cumini*), *A. cepa* and *A. sativum* showed inhibitory effect on mycelial growth of *S.oryzae. Azadirachta indica, Ricinus communis* and *Syzygium cumini* did not show any inhibitory effect on mycelial growth of *S.oyzae.* In case of *A.cepa* at concentration of 2.5, 5.0, 7.5 and 10 per cent, radial growth of mycelium of *S.oryzae* were 82.00, 74.00, 72.33 and 69.66 mm recorded. In case of *A.cepa,* per cent inhibition of mycelial growth of *S.oryzae* of 8.88, 17.77, 19.62 and 22.60 per cent respectively, was recorded as compared to control. At concentration of 2.5, 5.0, 7.5 and 10 per cent in case of *A.sativum,* 73.00, 69.33, 60.33 and 28.00 mm respectively, mycelial growth was recorded. In case of *A.sativum,*per cent inhibition of mycelial growth of *S.oryzae* of 18.88, 22.96, 32.96 and 68.88 per cent was recorded as compared to control. Per cent inhibition at all concentration were statistically significant from control. Per cent inhibition at each concentration were also statistically significant different from each other. Per cent of mycelial growth is more in *A. sativum* than *A.cepa.*Seema *et al.*(2011) reported that out of 10 plant extracts viz., *Thevetia peruviana*, *Ocimum basilicum, Piper betel*, *Murraya koenigii*, *Chrysanthemum coronarium*, *Polyalthia longifolia*, *Catharanthus roseus, Pelargonium graveolens, Moringa officinalis and Lawsonia inermis,* plant extracts of only four plant (*Lawsonia inermis*, *Piper betel*, *Polyalthia longifolia* and *Pelargonium graveolens*) showed antifungal activity against *R.solani* (infecting Tobacco). Seema *et al.* (2011) also reported that with the increase in concentration of plant extracts inhibition of mycelial growth also increases. As in present investigation also, out of five plant (*A. cepa, Azadirachta indica, A. sativum, Ricinus communis* and *Syzygium cumini),* plant extracts of only two plant viz., *A. cepa* and *A. sativum* showed inhibitory effect on mycelial growth of *S.oryzae.* In case of *Allium cepa* and *Allium sativum*,

Table 1. Effect of plant extracts on radial growth of *S. oryzae* on PDA at 28±1°C.

Plant extracts	Concentrations (%)								
	Radial growth* (mm)					Per cent inhibition			
	2.5	5.0	7.5	10.0	Mean	2.5	5.0	7.5	10.0
Allium cepa	82.00	74.00	72.33	69.66	74.50	8.88	17.77	19.62	22.60
Azadirachta indica	90.00	90.00	90.00	90.00	90.00	00.00	00.00	00.00	00.00
Allium sativum	73.00	69.33	60.33	28.00	57.66	18.88	22.96	32.96	68.88
Ricinus communis	90.00	90.00	90.00	90.00	90.00	00.00	00.00	00.00	00.00
Syzygium cumini	90.00	90.00	90.00	90.00	90.00	00.00	00.00	00.00	00.00
Control	90.00	90.00	90.00	90.00	90.00	00.00	00.00	00.00	00.00
Mean	85.83	83.88	82.11	76.27	82.02				
CD at 5%	A (dose) = 1.79								
	B (treatment) = 2.19								
	A×B = 4.39								

with the increase in concentration of plant extracts, inhibiton of mycelial growth increases. Onion broth (Plant extracts of *A.cepa*) successfully control seedlings damping off caused by *R.solani* and *S. rolfsii* (Rivera, 2013). Plant extracts of *A.sativum* (Garlic) showed inhibitory affect on broad range of soil borne of plant pathogen (viz., *Pythium aphanidermatum, P. irregulare, P. ultimum, Phytophthora cinnomomi, Phytophthora nicotianae, R. solani, F. oxysporum,* and *Thielaviopsis basicoli*). (Sealy *et al*., 2007). Hence these findings of *A.cepa* and *A.sativum* is in support of findings of this investigation. Amin *et al.* (2013) found that among different plant extracts (rhizome of turmeric, rhizome ginger, neem leaf, tobacco leaf, tobacco leaf extract in water, tobacco leaf extract in cow's urine,), Plant extracts of neem (*A.indica*) showed least inhibition of mycelial growth and sclerotia formation in case of *S. rolfsi.* The findings about *A.indica* is similar with Amin *et al*. (2013). While *A.indica* plant extracts also have inhibitory affect on conidial germinaton and germ tube formation of *Colletotrichum capsici* (Rahman, 2011). It also has inhibitory affect on soil borne rice phytopathogens *Rhizoctonia spp* and *S. hydrophyllum* (Aye and Matsumoto, 2011). *A.indica* plant extracts in combination with cow dung has 100 per cent inhibitory affect on conidial germination of *Bipolaris sorokiniana* (Akhter *et al.,* 2006). This report of *A.indica* is contradictory with the present finding where *A.indica* did not show any inhibitory affect on mycelia growth of *S.oryzae.* Aqueous extract of *Syzygium cumini* showed inhibitory affect on seed borne *Aspergillus spp.*(Satish *et al.,* 2007). This finding is also contradictory with the present findings.

Effect of cow urine on the radial growth of *S. oryzae*: Inhibition of mycelial growth varied significantly with different concentrations of cow urine. The observations of the experiment were taken 3 days after inoculation. Data graphical representation regarding antifungal activity of cow urine against test fungus are presented in table 2. At concentration 2.5, 1.5 and 0.62 per cent, radial growth of mycelium of *S. oryza* was 1.66, 32.66 and 50.33 mm recorded respectively. No mycelial growth was recorded at the concentrations of 5.0, 7.5 and 10.0 per cent. Cow urine at the concentrations of 5.0, 7.5 and 10.0 per cent were found highly effective against *S. oryzae*, whereas, cow urine at the concentrations of 2.5, 1.5 and 0.62 per cent resulted in 98.0, 63.7 and 44.07 per cent inhibition, respectively. Per cent inhibiton at the concentration of 2.5, 1.5 and 0.62 per cent are statistically significantly different from control as well as they are also significantly different from each other. Per cent inhibition at concentration 2.5, 5.0, 7.5 and 10.0 per cent are statistically at par with each other. Per cent inhibition of mycelial growth is more in cow urine as compared to *A. cepa* and *A. sativum.*

Table 2. Effect of Cow urine on radial growth of *S. oryzae* on PDA at 28±1°C.

Cow urine Concentrations (%)	Radial growth (mm)	Per cent inhibition
0.625	50.33	44.07
1.25	32.66	63.70
2.50	1.66	98.14
5.00	0.00	100.00
7.50	0.00	100.00
10.00	0.00	100.00
Control	90.00	0.00
CD at 5%	9.92	

Cow urine and cow dung has inhibitory affect on mycelial growth and suppressive affect on sclerotial germination of *Sclerotinia sclrotiorum* (Basak *et al*., 2002a) and *F. solani fsp. cucurbitae* (Basak *et al.,* 2002b). In case of *F. latinerum* causing Fusarium bark disease of coffee, conidial germination, germ tube length, mycelial growth rate and sporulation are suppressed by cow urine (Gotora *et al.,* 2014). 40, 50, 60 and 70 per cent concentration of cow urine suppress sclerotial germination and mycelial growth of *S.rolfsii* causing foot and root rot of Betel Vine (Amin *et al.,* 2013). Conidial germination of *Alternaria alternata*, *F. oxysporium*, *Colletotrichum capsici* and *Curvularia lunata* (Sharma *et al.,* 2010) as well as *Bipolaris sorokiniana* (Akhter *et al.,* 2006) is suppressed by cow urine and cow dung. Plant extracts prepared from cow urine has more antifungal activity. Cow urine based plant extracts has more inhibitory affect on bell pepper

pathogens (*R. solani, Sclerotinia sclerotiorum, S. rolfsii, Phytophthora nicotianae, F. oxysporum* f. sp. *capsici, F. solani* and *Colletotrichum capsici*.) (Ashlesha *et al.,* 2013), anthracnose of chilli (*Colletotrichum capsici*) (Kekuda *et al.,* 2014) and sheath blight of rice (*R. solani*) (Tiwari and Das, 2011). Apart from this, cow urine also has antimicrobial affect on clinical pathogens like *Bacillus subtilis, Bacillus cereus, Staphylococcus aureus, Staphylococcus epidermidis, Escherichia coli*, *Enterobacter aerogenes, Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus vulgaris*, S*almonella typhi* and two molds-*Aspergillus flavus* and *Candida albicans* (Rana and De, 2013; and Sathasivam *et al.,* 2010). All these reports of cow urine is in congruency with the findings of present investigation.

Conclusion

Out of five plant extracts of *Allium cepa, Azadirachta indica, Allium sativum, Ricinus communis* and *Syzygium cumini,* only *A. cepa* and *A. sativum* showed inhibitory effect on mycelial growth of *S.oryzae.* Plant extracts of *A. indica, R. communis* and *S. cumini* did not have inhibitory affect on *S.oryzae* but these plant extracts may have antifungal activity against other plant pathogens like *S. rolfsi* (causing foot and root rot of betel vine)*, Colletotrichum capsici* (causing anthracnose of chilli) soil borne rice phytopathogens *Rhizoctonia spp* and *S. hydrophyllum* and on conidial germination of *Bipolaris sorokiniana*. Cow urine was inhibitory to mycelial growth of *S.oryzae* at all concentrations. At high concentration of cow urine, no mycelial growth was recorded. Hence this investigation clearly showed antifungal potential of *A. cepa* and *A. sativum* and cow urine against *S.oryzae.* Antifungal potential was highest in cow urine followed by *A. sativum* and *A. cepa.*

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