



INHIBITORY EFFECT OF DIFFERENT PLANT EXTRACTS, COW DUNG AND COW URINE ON CONIDIAL GERMINATION OF *BIPOLARIS SOROKINIANA*

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Abstract

Inhibition of conidial germination of *Bipolaris sorokiniana* was tested using eight ethanolic plant extracts, ten aqueous plant extracts in combination with cow dung and five aqueous plant extracts in combination with cow urine. Hundred percent of conidial germination was inhibited with the application of ethanolic extracts of *Adhatoda vasica* (leaf) and *Zingiber officinale* (rhizome) at 2.5% concentration. After the treatment with *Vinca rosea*, *Piper betle* and *Azadirachta indica* extracts in combination with cow dung suspension showed 100% inhibition of conidial germination; where the lowest was noted in case of *Rauwolfia serpentina* (30%) extract at same concentration. At 2.5% concentration of *Calotropis procera* extracts in combination with cow urine, 91% inhibition of conidial germination was observed. In most cases, *Ocimum sanctum* extract exhibited less inhibitory effect against *B. sorokiniana*.

Key words: *Bipolaris sorokiniana*, Leaf blight, Plant extracts, *Triticum aestivum*, Cow dung, Cow urine.

Introduction

Wheat (*Triticum aestivum* L.) is the second staple food crop next to rice in Bangladesh. It covers almost 1,379,900 acres of land with a total production of 975,985 metric tones (Akhter *et al.* 2005); where the world wheat production is 618.8 million tones (FAO 2005). The crop is vulnerable to many diseases (Rangwasami 1979) of which leaf blight caused by *Bipolaris sorokiniana* is economically most important one (Ahmed and Hossain 2005). The severity of *Bipolaris* leaf blight has been increasing in an alarming proportion in Bangladesh (Alam *et al.* 1993). The pathogen *B. sorokiniana* caused leaf spot of wheat and reduced to 88.7% grain/ear (Hossain *et al.* 1998). Zhang *et al.* (1999) assessed the loss of yield of wheat in the field in China and found that the grain weight of wheat was reduced significantly by *Bipolaris* leaf blight. Control of plant disease by chemicals cause environmental pollution and it is hazardous both for plants and animals. Therefore, alternate means and ways need to be found out to control of the disease. Several higher plants and their constituents have shown success in plant disease control (Ashrafuzzaman and Hossain 1992, Hossain and Ashrafuzzaman 1994). The extracts of plants exhibited marked effect on germination of fungal spores as well (Singh *et al.* 1990, Dubey 1991), and it inhibited the fungal growth (Khair *et al.* 1995). Present study has been made to observe the effects of different plant extracts alone and in combination with cow dung and cow urine on the inhibition of conidial germination of *B. sorokiniana*.

Materials and Methods

Organism used: *B. sorokiniana* (Sacc.) was isolated from wheat leaf, which caused leaf blight disease of wheat. Fungus was cultured on host-extracted medium and the conidia were taken for the experiment from 12 days old culture.

Preparation of plant extracts: Extraction of different plants in alcohol and water was done following the method described by Mahadevan and Sridhar (1982). Five gram tissue was cut in pieces and immediately

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plunged in to boiling 80% ethyl alcohol in a beaker and allowed to boil for 5-10 minutes, using 5-10 ml of alcohol for each gram tissue. The extraction was carried out on top of a steam bath. It was cooled in a pan of cold water. The tissues were crushed thoroughly in a mortar with a pestle and then passed through two layers of cheese cloth and re-extracted the ground tissues in hot 80% alcohol for 3 minutes, using 2-3 ml of alcohol for per gram of tissues. Extracts were cooled and passed through cheese clothe and filtered through Whatman No. 1 filter paper. The volume of the extract was evaporated on a steam bath to dryness. The dried plant extracts were mixed with sterile distilled water at a concentration of 2.5%. In case of water extract, the dried plant extracts were mixed with sterile distilled water at concentrations of 1.0, 1.5, 2.0 and 2.5%, and finally mixed with cow dung and cow urine to obtain 1:15 w/v and 1:10 ml, respectively.

Preparation of conidial suspension

Conidia of *B. sorokiniana* was taken from 12 days old culture and conidial suspension (10^3 conidia/ml) were made separately in different concentrations of ethanolic and aqueous plant extracts in combination with cow dung and cow urine. Conidial suspensions were taken in small Petri plates (65 mm) and were kept at 28°C. A drop of treated conidial suspension (12×10^3 conidia/ml) was taken on grove slides in a moisture chamber for 24 hours of incubation. Then a drop of lactophenol cotton blue was added to conidial suspension on the slides. The slides were examined under high power microscope ($\times 400$) for observing the inhibition percentage of conidial germination using the following formula:

$$\text{Inhibition (\% of conidial germination)} = \frac{\text{Total No. of conidia} - \text{No. of germinated conidia}}{\text{Total no. of conidia}} \times 100$$

Statistical analysis of data expressed as percentage was carried out from angular transform software. Least of significant difference (LSD) were determined, wherever, the calculated 'F' value were significant at 5% level (Snedecor and Cochran 1980).

Results and Discussion

Inhibition of conidial germination of *B. sorokiniana* was tested against eight ethanolic plant extracts and the results are presented in Table 1. Among the plant extracts tested, *Adhatoda vasica* (leaf) and *Zingiber officinale* (rhizome) extracts showed complete (100%) inhibition of conidial germination of *Bipolaris sorokiniana* at 2.5% of concentrations after 4 to 24 hours of incubation. The lowest (48%) conidial germination was inhibited with the *Ocimum sanctum* extract at 2.5% concentration after 24 hours of incubation. Among the plant extracts tested, the most promising fungi toxic effect was recorded using the extracts of *A. sativum*, *A. cepa*, *T. erecta* and *P. orientale* at 2.5% concentration after 24 hours of incubation, which corresponded to 98, 93, 73 and 67% inhibition of conidial germination, respectively. In this study, *O. sanctum extract* seemed to be less inhibitory against the pathogen. It was observed, the rate of inhibition of conidial germination increased with the increase of incubation period. Alam *et al.* (2004) tested five plant extracts against conidial germination of *Fusarium oxysporum* and reported that the extract of *Calotropis procera* showed high inhibitory effect. Islam *et al.* (2003) examined ten plant extracts (ethanolic) on conidial germination, mycelial growth and sporulation of *Aspergillus flavus*, *A. niger* and *A. fumigatus* and found that *Lowsonia inermis* inhibited conidial germination of *A. flavus* and *A. fumigatus*, while *A. niger* was mostly inhibited by *A. indica*. Alam *et al.* (2002) tested the effect of ten plant extracts on conidial germination of *C. gloeosporioide* and recorded that *Tagates erecta* (leaf) and *Azadirachta. indica* (bark) extracts were most

effective in inhibition of conidial germination at 5:1.5 (w/v) concentration. The fungi toxic effect of *Vinca rosea* and *A. indica* has been reported earlier by several investigations against *B. sorokiniana*, *F. oxysporium* f. sp. *vasinfectum*, *Rhizopus artocarp* (Alam 2002) and *Alternaria tenuis* (Alam et al. 1999). Bhatti (1988) found that extracts of *Acacia nilotica*, *Calotropis procera*, *Datura stramonium*, *Dodonaea niseosa* and *Rhazya stricta* effectively controlled the rust on detached leaved of wheat. Khan and Kumar (1990) observed antifungal activity of leaf extracts of *Azadirachta indica* on wheat seeds mycoflora. Suratuzzaman (1995) observed excellent inhibitory effect of *Allium sativum* and *Zingiber officinale* to control seed-borne fungi *Colletotrichum dematium* var. *truncatum*, *Macrophomina phaseolina* and *Colletotrichum kikucchii* of soybean seed.

Table 1. Effect of different plant extracts on the inhibition of conidial germination of *B. sorokiniana* after 24 hr of incubation at 2.5% concentration.

Plant	Plant part used	Inhibition of conidial germination ^a (%) after various incubation period					
		2 h	4 h	6 h	8 h	12h	24 h
<i>Tagetes erecta</i>	Leaf	38 ^a	49	45	46	55	73
<i>Ocimum sanctum</i>	Leaf	21	22	43	45	47	50
<i>Piper betle</i>	Leaf	25	35	36	40	43	48
<i>Adhatoda vasica</i>	Leaf	100	100	100	100	100	100
<i>Allium sativum</i>	Bulb	84	87	90	95	97	98
<i>Allium cepa</i>	Bulb	80	82	87	90	91	93
<i>Zingiber officinale</i>	Rhizome	100	100	100	100	100	100
<i>Polygonum oriental</i>	Leaf	40	50	53	55	60	67
LSD (0.05)		0.6271	0.5775	0.6007	0.5662	0.7017	1.1714

^aMean of three replications

Ten aqueous plant extracts in combination with cow dung suspension were tested against the conidial germination of *B. sorokiniana* and results are summarized in Table 2. Hundred percent conidial germination was inhibited with the application of *Vinca rosea* and *Piper betle* extracts at 2.0 and 2.5% concentrations, respectively. In case of *A. indica* extracts combined with cow dung suspension, 100% conidial germination was inhibited at 2.5% concentration of the extract. No inhibition of conidial germination was observed at the concentrations of 1.0 to 1.5% after treatment with *R. serpentina*, *A. indica*, *D. metel* and *S. macrophylla* extracts. The lowest conidial germination was inhibited with the treatment of *R. serpentina* extract and 30% conidial germination inhibition counted only at 2.5% concentration after 24 hours of incubation. Good inhibition of conidial germination of *B. sorokiniana* occurred with the treatment of *A. vasica* and *P. longum* extracts at 1.0 to 2.5% concentrations after 24 hours of incubation. Rest of the plant extracts showed moderate to poor inhibitory effect against conidial germination of *B. sorokiniana*. In this study, it was observed that the rate of conidial germination inhibition was increased with increase of concentrations of plant extracts. Basak and Lee (2001, 2002) reported that fresh cow urine and cow dung has positive response in suppression of mycelial growth of *F. solani*, *F. oxysporum* and *S. sclerotiorum*.

Five aqueous plant extracts were tested in combination with cow urine (1:10 ml). The highest inhibition of conidial germination (91%) of *B. sorokiniana* was observed with the extract of *C. procera* at 2.5%

concentration, which was followed by the leaf extracts of *L. camara* (87%), *A. indica* (85%), *V. rosea* (83%), *O. sanctum* (80%) and flower extract of *O. sanctum* (59%) at 2.5% concentration with the same dilution (1:10ml) of urine.

Table 2. Effect of different concentrations of plant extracts in combination with cow dung on the inhibition of conidial germination of *B. sorokiniana* after 24 hr of incubation at 28°C.

Plant	Ratio (w/v) of plant extract to cow dung	Inhibition of conidial germination ^a (%) at different concentrations of plant extracts in combination with cow dung			
		1.0 ^b	1.5	2.0	2.5
<i>Piper betle</i>	1:15	38 ^a	48	87	100
<i>Rauwolfia serpentina</i>	1:15	0	22	26	30
<i>Datura metel</i>	1:15	0	0	33	35
<i>Vinca rosea</i>	1:15	95	97	100	100
<i>Adhatoda vasica</i>	1:15	78	80	83	90
<i>Azadirachta indica</i>	1:15	0	75	80	100
<i>Ocimum sanctum</i>	1:15	26	40	49	55
<i>Tagetes erecta</i>	1:15	33	36	45	50
<i>Smilax macrophylla</i>	1:15	0	14	50	52
<i>Piper longum</i>	1:15	56	59	62	71
LSD (0.05)		0.4572	0.6550	1.0645	0.3560

^aMean of three replications, ^bConcentrations

Table 3. Effect of different concentrations of plant extracts in combination with cow urine on the inhibition of conidial germination of *B. sorokiniana* after 24 hr of incubation at 28°C

Plant	Ratio (ml) of plant extract to cow urine	Inhibition of conidial germination ^a (%) at different concentrations of plant extracts in combination with cow urine			
		1.0 ^b	1.5	2.0	2.5
<i>Ocimum sanctum</i> (leaf)	1:10	72 ^a	77	80	85
<i>Ocimum sanctum</i> (flower)	1:10	34	43	50	59
<i>Lantana camara</i> (leaf)	1:10	78	80	83	87
<i>Calotropis procera</i> (leaf)	1:10	77	78	89	91
<i>Azadirachta indica</i> (leaf)	1:10	74	78	82	85
<i>Vinca rosea</i> (leaf)	1:10	64	71	78	83
LSD (0.05)		0.2641	0.7108	0.4238	0.4249

^a Mean of three replications, ^b Concentrations

Table 3 also showed that the lowest inhibition of conidial germination (34%) recorded at 1.0% concentration after the application of *O. sanctum* flower extract in combination with the same concentration of cow urine

(1:10 ml) and the highest inhibition of conidial germination (77%) was recorded against 1.0% concentration of *C. procera* extract in combination with 1:10 ml concentration of cow urine after 24 hours of incubation. In this study, all the concentrations of *C. procera* extract in combination with cow urine showed the highest inhibition of conidial germination. *O. sanctum* (flower extract) had less inhibitory effect against the pathogen. It is clear from the results that the rate of conidial germination inhibition increased with the increase of concentration of the extracts. Jabin (2003) reported that cow urine has inhibitory activity against *F. semitectum* and cow urine mixed with leaf extracts of *C. procera*, *V. negundo* and *C. alata* completely (100%) inhibited the mycelial growth of the pathogen. Cow urine has been proved to be inhibitory to the mycelial growth of *F. oxysporum* f. sp. *cucumerinum*, *F. solani* f. sp. *cucurbitae* and *S. sclerotiorum* that cause disease in cucumber (Basak and Lee 2002).

In conclusion, the present study demonstrated that the extracts of *A. vasica* and *Z. officinalis*; *P. betle*, *V. rosea* and *A. indica* extracts in combination with cow dung, and *C. procera* extract in combination with cow urine possess high ability to inhibit conidial germination of *B. sorokiniana*, which might be used for controlling phytopathogens of crop plants.

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