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Evaluation of antifungal activities of Xerophytes against plant pathogenic fungi considering their radial mycelial growth inhibition at different concentrations

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ABSTRACT

Bangladesh is the land of many indigenous herbal medicinal plants. These plants have the potential for antimicrobial activities because of having a wide variety of secondary metabolites. Extracts of leaves of four selected xeric plants were taken to evaluate the antifungal activities against plant pathogenic organisms considering their radial mycelial growth inhibition at different concentrations. Extracts of the selected xerophyte plants (Vacuellia nilotica, Calotropis gigantea, Ziziphus mauritiana and Cassia fistula) with different concentrations were found effective against tested three Phytopathogens like Bipolaris sorokiniana, Bipolaris oryzae and Alternaria padwicki. This experiment was conducted following CRD method with five replications. The inhibitory performances of xerophytes were higher at 25 % concentration against all tested Phytopathogens. Calotropis gigantea showed a significant (P<0.01) effect on radial mycelial growth of Bipolaris sorokiniana and Alternaria padwicki at all concentrations, but there was no significant effect on Bipolaris oryzae. Cassia fistula showed a momentous effect on radial mycelial growth at all concentrations, but a maximum inhibitory effect was observed in Bipolaris oryzae at 25% concentration. The inhibitory performance of Ziziphus mauritiana and Vacellia nilotica was higher on Bipolaris sorokiniana than the other tested phytopathogens. The inhibition percentage was increased with the concentrations of different xerophytic leaf extracts.

Key Words: *Antifungal Activities, Phytopathogens and Xerophytes.*

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I. Introduction

Bangladesh is the land of many indigenous herbal medicinal plants (Khan et al., 2006). These plants have the potential for antimicrobial activities because they have a wide variety of secondary metabolites such as tannins, terpenoids, alkaloids, flavonoids, glycosides, etc. (Hassan and Khalid, 1992; Gupta et al., 2003). The easiest way to control plant diseases is by synthetic chemicals. Using synthetic chemicals to manage plant diseases undoubtedly increases crop protection but deteriorates

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environmental quality and human health (Cutler and Cutler, 1999). Pathogenic resistance to chemicals and increased infection rates are intense concerns that demand exploring potential alternative drugs from various sources, such as medicinal plants (Cordell, 2000; Shahid et al., 2008). Natural products obtained from higher plants may give a new source of antimicrobial agents with possibly novel mechanisms of action (Kirtikar and Basu, 1935; Matalawska et al., 2002). A large number of plants have been reported to possess toxic properties against fungal plant pathogens, which could be exploited commercially with practically no residual or toxic effect on the ecosystem (Kumar et al., 2008). Antifungal activity of leaf extract of *Cassia fistula, Ziziphus mauritiana* and *Vachellia nilotica* was reported by many researchers (Mazumder et al., 1998; Sartorelli et al., 2007). In order to investigate new sources of antimicrobial agents in the xeric plant of *Calotropis gigantea, Cassia fistula, Ziziphus mauritiana, Vachellia nilotica* have been selected. The present study evaluated the antifungal activities of four selected xeric plants against some selected Phytopathogens.

II. Materials and Methods

Leaves of xerophytic plants (*Vacuellia nilotica, Calotropis gigantea, Ziziphus mauritiana and Cassia fistula)* were collected from Khulna University campus (Table 01). The fungal isolates of *Bipolaris oryzae*, *Bipolaris sorokiniana* and *Alternaria padwicki* were collected from plant protection laboratory of Agrotechnology discipline, Khulna University, Khulna.

Table 01. List of xerophytic plant materials that were used in the experiment

Local name	Botanical name	Family	Used parts	Concentration (%)
Akondo	*Calotropis gigantea*	Apocynaceae	Leaf	5%, 10%, 15%, 20% and 25%
Badorlathi	*Cassia fistula*	Caesalpiniaceae	Leaf	5%, 10%, 15%, 20% and 25%
Boroi	*Ziziphus mauritiana*	Rhamnaceae	Leaf	5%, 10%, 15%, 20% and 25%
Babla	*Vachellia nilotica*	Fabaceae	Leaf	5%, 10%, 15%, 20% and 25%

Toxic chemicals have been found in xerophytic plants (Kumar et al. 2008). Different xerophytic plants were evaluated in vitro conditions following poison food technique (Dhingra and Sinclaier, 1985). Xerophytic leaf extracts were prepared following Masih et al. (2014) protocol (Table 01). Inoculation and incubation were done under aseptic conditions for observation of the growth of mycelium. The radial growth of mycelium in each plate was recorded after seven days of inoculation. Antifungal activity of Medicinal plant extract against plant pathogens by measuring radial mycelial growth and inhibition percentages

The inhibition percentages of radial mycelial growth over the control were calculated using the following formula (Vincent, 1947).

$$I = \frac{C-T}{C} \times 100$$

Where,

I : Percent of inhibition
C : Average radial growth of tested fungi in control (PDA) petridishes
T : Average radial growth of tested fungi in xerophitic leaf extract in treated petridishes

The experiment was laid out in Completely Randomized Design (CRD) with five replications. The data were analyzed using statistical program STAR (statistical tools for agricultural research program, version-02, IRRI, Los Baños, Philippines) for comparing mean.

III. Results and Discussion

Evaluation of the efficacy of *Calotropis gigantea* against different phytopathogen

Calotropis gigantea showed a significant effect ($P< 0.01$) on radial mycelial growth of the tested phytopathogen such as *Bipolaris sorokiniana, Bipolaris oryzae* and *Alternaria padwicki* (Table 02). In the case of *Bipolaris sorokiniana, Alternaria padwicki* minimum mycelial growth was observed at 25% concentration (48.80 mm and 67.60 mm). Contrarily, *Bipolaris oryzae* was not significantly affected by *Calotropis gigantea.*

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Table 02. Effect of *Calotropis gigantea* on radial mycelial growth of *Bipolaris sorokiniana, Bipolaris oryzae* and *Alternaria padwicki*

Concentrations (%)	Radial mycelial growth of *Bipolaris sorokiniana* (mm)	Radial mycelial growth of *Bipolaris oryzae* (mm)	Radial mycelial growth of *Alternaria padwicki* (mm)
Control (0)	79.20[a]	75.00	75.40[a]
5	74.20[ab]	74.40	71.20[ab]
10	70.40[b]	74.60	70.60[ab]
15	72.80[b]	72.80	70.20[ab]
20	54.80[c]	72.60	67.80[b]
25	48.80[d]	72.00	67.60[b]
CV (%)	3.93	2.66	3.20
Level of sig.	**	NS	**

CV= Coefficient of Variation, NS=Non Significant, **= p<0.01

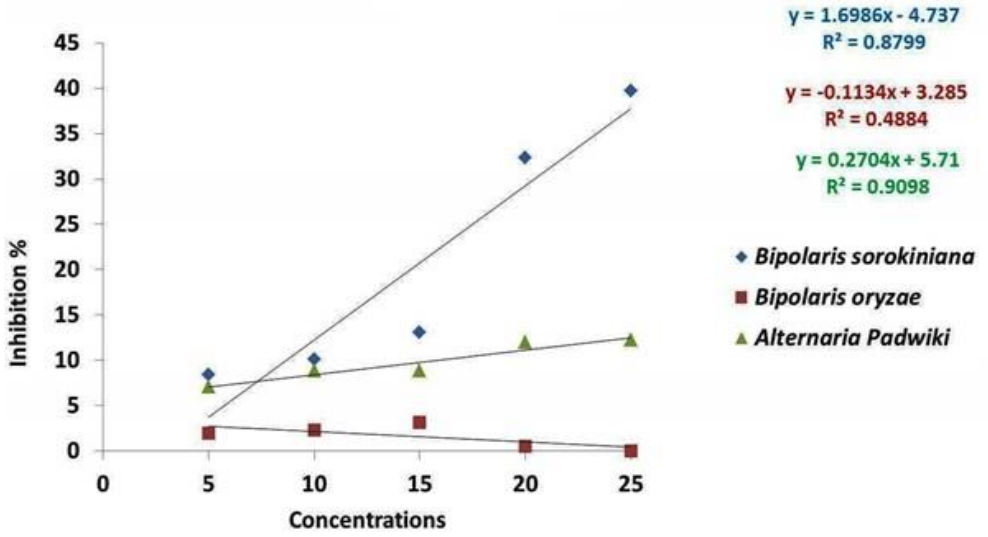

Figure 01. Functional relationship between concentration and mycelial growth inhibition of tested fungi against *Calotropis gigantea* at different concentrations

Radial mycelial growth inhibition of *Bipolaris sorokiniana*, *Bipolaris oryzae* and *Alternaria padwicki* against *Calotropis gigantea* at various concentrations were correlated functionally with different concentrations (Figure 01). These functional relationships showed that the increase in concentration could account for more than 88%, 49% and 90% of the change in mycelial inhibition percentage of *Bipolaris sorokiniana, Bipolaris oryzae* and *Alternaria padwicki.*

Evaluation of the efficacy of *Cassia fistula* against different phytopathogen

Cassia fistula showed significant effect (*P*<0.01) on radial mycelial growth of the tested phytopathogen like *Bipolaris sorokiniana, Bipolaris oryzae* and *Alternaria padwicki* (Table 03). In the case of *Bipolaris sorokiniana, Alternaria padwicki* and *Bipolaris oryzae* minimum mycelial growth was observed at 25 % concentration (62 mm, 23.40 mm). Over *Cassia fistula* leaf extracts have a significant inhibition percentage against *Bipolaris oryzae* at 25% concentration.

Table 03. Effect of *Cassia fistula* on radial mycelial growth of *Bipolaris sorokiniana, Bipolaris oryzae* and *Alternaria padwicki*

Concentrations (%)	Radial mycelial growth of *Bipolaris sorokiniana* (mm)	Radial mycelial growth of *Bipolaris oryzae* (mm)	Radial mycelial growth of *Alternaria Padwiki* (mm)
Control (0)	69.00[a]	80.20[a]	69.00[a]
5	68.40[a]	74.20[b]	67.40[ab]
10	67.40[ab]	62.40[c]	66.60[ab]
15	65.20[ab]	43.40[d]	65.20[abc]
20	64.00[ab]	34.80[e]	64.0[bc]
25	62.00[b]	23.40[f]	62.00[c]
CV %	3.37	3.61	3.42
Level of sig.	**	**	**

CV= Coefficient of Variation, **= *p*<0.01

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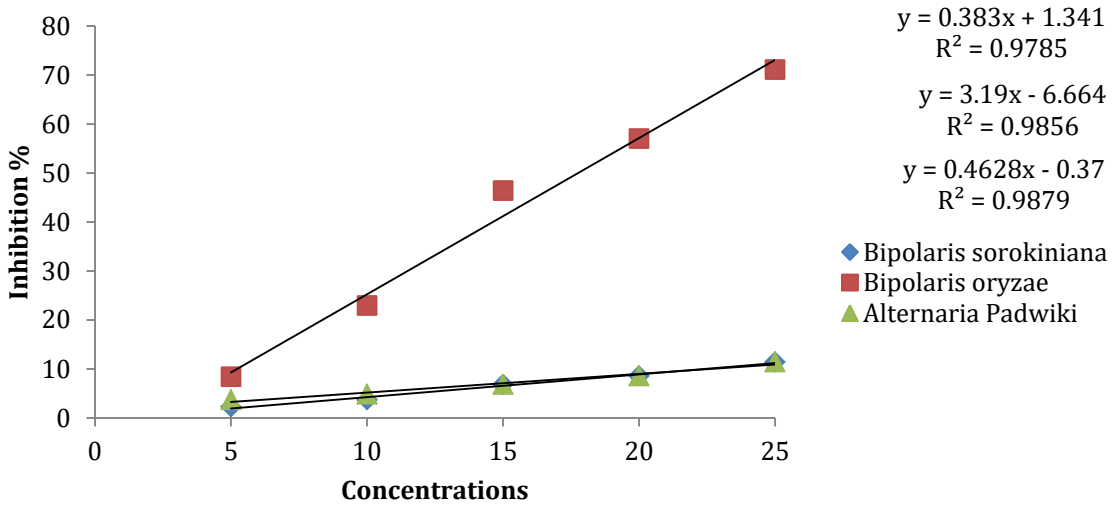

Figure 02. Functional relationship between concentration and mycelial growth inhibition of tested fungi against *Cassia fistula* at different

In the case of *Bipolaris sorokiniana, Bipolaris oryzae* and *Alternaria padwicki*, from regression equation (Figure 02) between different concentrations of *Cassia fistula* extracts and mycelial growth inhibition percentage revealed that more than 99%, 99% and 98%of the variation in inhibition percentage could be explained by the increase of concentration.

Evaluation of the efficacy of *Ziziphus mauritiana* against different Phytopathogen

Ziziphus mauritiana showed significant effect ($P< 0.01$) on radial mycelial growth of the tested phytopathogen like *Bipolaris sorokiniana, Bipolaris oryzae* and *Alternaria padwicki* (Table 04). In the case of *Bipolaris sorokiniana, Bipolaris oryzae* and *Alternaria padwicki,* minimum mycelial growth was observed at 25 % concentration (15.40 mm, 26.40 mm and 23.80 mm).

Table 04. Effect of *Ziziphus mauritiana* on radial mycelial growth of *Bipolaris sorokiniana, Bipolaris oryzae* and *Alternaria padwicki*

Concentrations (%)	Radial mycelial growth of *Bipolaris sorokiniana* (mm)	Radial mycelial growth of *Bipolaris oryzae* (mm)	Radial mycelial growth of *Alternaria Padwiki* (mm)
Control (0)	71.40 ^{a}	73.20^{a}	75.00 ^{a}
5	27.20^{b}	41.80 ^{b}	25.80^{b}
10	26.80^{b}	33.00^{c}	24.20^{b}
15	16.40 ^{c}	30.80^{cd}	24.00^{b}
20	16.60^{c}	32.80^{cd}	23.40^{b}
25	15.40 ^{c}	26.40^{d}	23.80^{b}
CV %	3.74	8.27	6.94
Level of sig.	**	**	**

CV= Coefficient of Variation, **= p<0.01

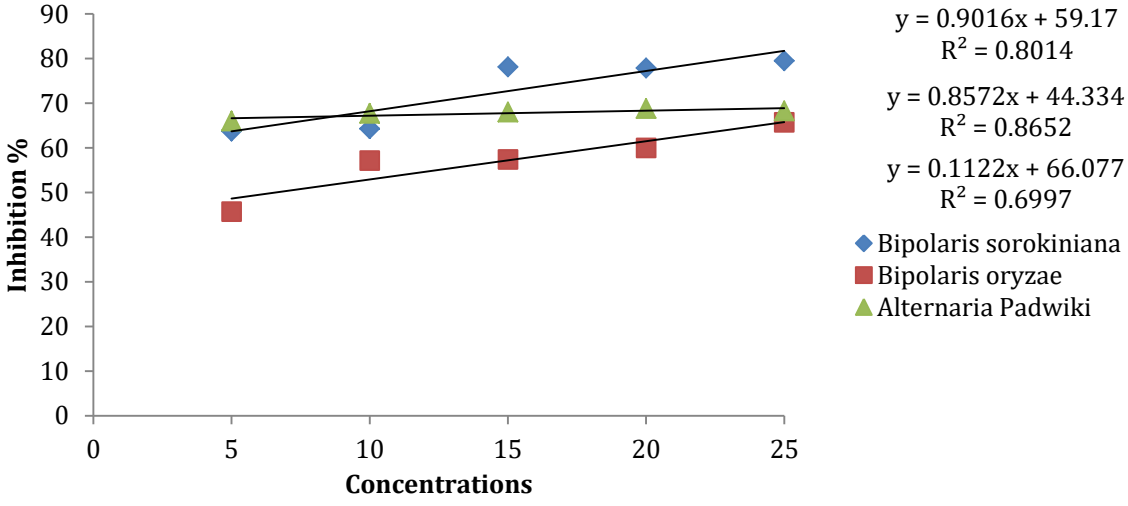

Figure 03. Functional relationship between concentration and mycelial growth inhibition of tested fungi against *Ziziphus mauritiana* at different concentrations

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In the case of *Bipolaris sorokiniana*, *Bipolaris oryzae* and *Alternaria padwicki* from regression equation (Figure 03) between different concentrations of *Ziziphus mauritiana* extracts and mycelial growth inhibition percentage demonstrated that the increase in concentration may account for more than 80%, 87% and 70% of the variation in the inhibition percentage.

Evaluation of the efficacy of *Vacellia nilotica* against different Phytopathogen

Vacellia nilotica exhibited a significant effect ($P < 0.01$) on radial mycelial growth of the tested phytopathogen like *Bipolaris sorokiniana, Bipolaris oryzae and Alternaria padwicki* (Table 05). In the case of *Bipolaris sorokiniana, Bipolaris oryzae* and *Alternaria padwicki,* minimum mycelial growth was observed at 25 % concentration (38.60 mm, 60.40 mm and 39.00 mm).

Table 05. Effect of *Vacellia nilotica* on radial mycelial growth of *Bipolaris sorokiniana, Bipolaris oryzae* and *Alternaria padwicki*

Concentrations (%)	Radial mycelial growth of *Bipolaris sorokiniana* (mm)	Radial mycelial growth of *Bipolaris oryzae* (mm)	Radial mycelial growth of *Alternaria Padwiki* (mm)
Control (0)	75.80^{a}	74.80^{a}	75.60^{a}
5	64.80^{b}	73.20^{ab}	74.20 ^{a}
10	61.20^{bc}	71.40^{ab}	73.80^{a}
15	58.00 ^{c}	72.20^{ab}	53.20 ^{b}
20	52.00 ^{d}	65.60^{c}	48.60^{c}
25	38.60^{e}	60.40^{d}	39.00^{d}
CV (%)	4.99	2.42	3.60
Level of sig.	**	**	**

CV= Coefficient of Variation, **= p<0.01

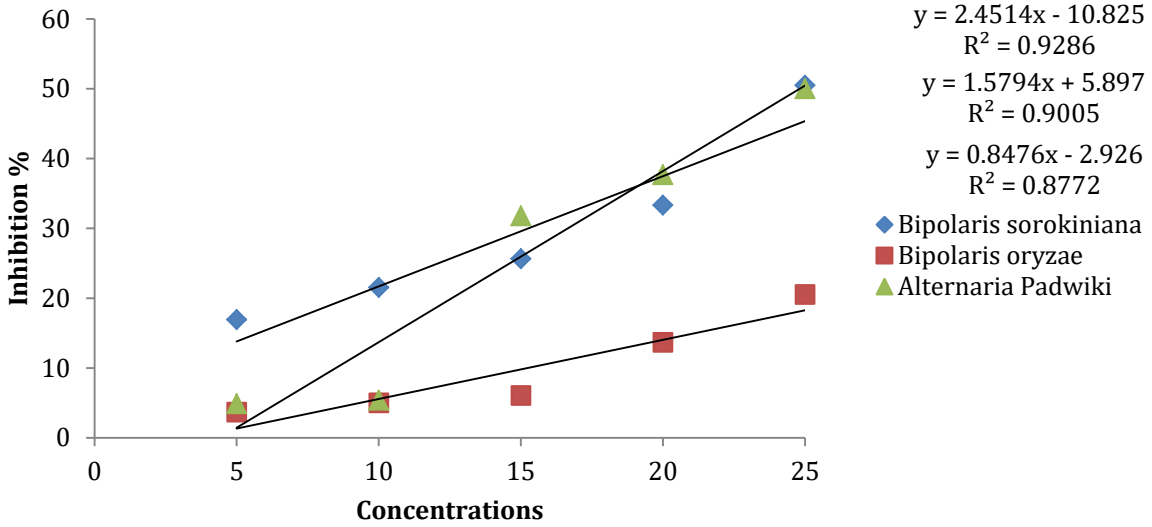

Figure 04. Functional relationship between concentration and mycelial growth inhibition of tested fungi against *Vacellia nilotica* at different concentrations

In the case of *Bipolaris sorokiniana*, *Bipolaris oryzae* and *Alternaria padwicki*, from regression equation (Figure 04) between different concentrations of *Vacellia nilotica* extracts and mycelial growth inhibition percentages revealed that more than 90%, 88% and 93% of the variation in inhibition percentage could be explained by the increase of concentration.

IV. Discussion

The most potent xerophytic plant extracts at higher concentrations had an inhibitory effect on mycelial growth in vitro when tested against phytopathogens. The findings showed that these leaf extracts inhibit the development of phytopathogens. Zain et al. (2012) along with Khan and Manzoor Rashid (2006), observed a similar result. They found methanolic extracts in xeric plant extract. They also observed antifungal activities against *Aspergillus niger* and *Aspergillus flavus*. Sharma et al. (2015.) also conveyed that ethanolic extract of *Calotropis gigentica* significantly inhibited fungi at 20% concentration. Khan et al. (2009) reported that *V. nilotica* is more effective against multidrug-resistant fungus causing community-acquired infections. Considering the leaf extract of *Cassia fistula*, Nayan et

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al. (2011) found that *Cassia fistula* plays a significant role against three fungal strains *Aspergillus niger, Aspergillus clavatus* and *Candida albicans* at 5, 25, 50, 100, 250 µg/ml concentrations. They found remarkable inhibition against the tested organisms. These findings agreed with the present result. Sarfaraz et al. (2002) also reported that *Ziziphus spp* has antifungal activity against *Candida albicans, C. tropicalis, Aspergillus flavus, A. niger* and *Malassezia furfur.* Similar findings were also found by Tapiwa et al. (2015). Khan (2011) reported that *Ziziphus spina-christi* biologically active against the two root rot pathogens *Drechslera biseptata* and *Fusarium solani* in vitro.

V. Conclusion

Calotropis gigantea has a higher inhibiting capacity against *Bipolaris sorokiniana* and *Alternaria padwicki* at 25% concentration but has no significant inhibitory effect on *Bipolaris oryzae. Cassia fistula* and *Vacellia nilotica* have a higher inhibiting capacity at 25% concentration against *Bipolaris sorokiniana, Bipolaris oryzae* and *Alternaria padwicki*. *Ziziphus mauritiana* has an uncountable inhibitory effect on *Bipolaris sorokiniana* and *Bipolaris oryzae* at 25% concentration. *Ziziphus mauritiana* has no significant variation in radial mycelial growth of Alternaria padwicki at different concentrations. Colony characteristics like color, texture, margin and hyphal thickness of *Bipolaris sorokiniana, Bipolaris oryzae* and *Alternaria padwicki* varied at different concentrations of xerophytic leaf extract.

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