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Scientific Research

Antifungal Activity of Selected Medicinal Plant Extract on *Fusarium oxysporum* Schlechtthe Causal Agent of Fusarium Wilt Disease in Tomato

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Abstract

The antifungal activity of ethanol and acetone extract of leaves of nine medicinal plants: *Piper betel*, *Lowsonia inermis*, *Psidium guajava*, *Carica papaya*, *Moringa oleifera*, *Mimosa pudica*, *Catharanthus roseus*, *Adhatoda vasica* and *Andrographis paniculata* against *Fusarium oxysporum* the causal agent of Fusarium wilt in tomato was assessed. All the extracts inhibited mycellial growth at various levels. Among them the superior inhibition (100%) was found in 15% concentration of ethanol extract of *Lowsonia inermis* and *Psidium guajava* against *Fusarium*. In all plant extract there were no significant differences between 20% and 25% concentration, except *Piper betel*, *Carica papaya*, *Andrographis paniculata* and *Lawsonia inermis*. Analysis of variance results on mycelial growth in different concentration shows that the item was highly significant.

Keywords

Medicinal Plants, Acetone Extract, Ethanol Extract, Inhibitory Effect, Disease Management

1. Introduction

Tomato (*Lycopersicon esculentum* Mill.) is economically the most important and popular vegetables throughout the world including Bangladesh. Successful cultivation of tomato is hindered by various diseases caused by

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plant pathogens. Fusarium wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* is one of the most wide spread and destructive diseases, causing infection and losses to crop growers [1]. It is now a major concern not only in Bangladesh, but also in other regions of the world [2].

Use of synthetic fungicides is mainly practiced for management of wilt disease [3]. This measure may cause adverse effects on the environment and human health [4]. In addition the residue of fungicides remaining in the fruit may decrease the quality of the product. The increased awareness of the environmental problems associated with fungicides has led to the search for non-conventional chemicals of biological origin for the management of this disease. Therefore, it is challenge of time to search an alternative control measure of plant diseases by using natural plant materials.

The antimicrobial activity of medicinal plant has been recognized for many years. Leaf extract of *Lawsonia inermis*, *Achyranthes aspera*, *Mimosa pudica*, has been reported significant antimicrobial activities [5] [6]. Plant extract may represent an ideal solution to the problem; it could be easily tested *in vitro* [7]. The effects of neem products have been reported to have significant controlling effects against fungal spore germination [8]. Extract of *Terminalia chebula*, *Mangifera indica* and *Eucalyptus citriodora* and their essential oils have been reported to be effective antimicrobials against bacteria and fungi [9]. The chloroform extract of *Piper betle* L. was found to be effective in controlling *Fusarium* spp. [10] [11]. Aqueous extracts of 46 plants against *Fusarium* spp. revealed that 12 plants have recorded significant antifungal activity and these plants could be exploited for eco-friendly management [10]. However, the use of plant products for control of Fusarium wilt of tomato in Bangladesh is rather limited. In this study, we reported the effectiveness of ethanol and acetone extract of some common medicinal plants in reducing populations of *F. oxysporum* the causal agent of Fusarium wilt in tomato *in vitro*.

2. Materials and Methods

2.1. Preparation of Fungal Media (Potato Dextrose Agar)

The composition of Potato Dextrose is 4 gm/L of potato extract and 20 gm/L of dextrose. For solidification 20 gm/L of agar was added. Final pH of this media is 5.6 ± 0.2 at 25˚C. These constituents were mixed, autoclaved and poured into Petri plates for solidification. These plates were used for antifungal studies.

2.2. Isolation and Identification of the Pathogen

The infected parts of tomato plant were collected in the polythene bags, which were made airtight. Collected materials were labeled properly and then brought to the Laboratory of Plant Pathology, Mycology and Microbiology, Department of Botany, University of Rajshahi, Bangladesh. The pathogen was isolated on potato dextrose agar (PDA) medium [12]. The infected parts of tomato plant were cut into small pieces. The pieces were then washed in running tap water, sterilized in 0.1 percent mercuric chloride solution and washed repeatedly for several times in sterilized distilled water to remove mercuric chloride solution. Three pieces were transferred to PDA plate. Plates were incubated at 25˚C ± 2˚C for 15 days for recovery of pathogen. *Fusarium oxysporum* was purified by single spore method and according to morphological characteristics; identification was done with the help of standard keys [13] [14]. Pathogenicity tests were done on potted tomato plants according to Koch's postulate [12].

2.3. Preparation of Plant Materials

Fresh leaves of nine medicinal plants were collected from the surrounding areas of Motihar, Rajshahi, Bangladesh (**Table 1**). Plant specimens were brought to the Laboratory of Plant Pathology, Mycology and Microbiology, Department of Botany, University of Rajshahi. These plants were authenticated by Taxonomist Dr. A. H. M. Mahbubur Rahman, Associate Professor, Department of Botany, University of Rajshahi, and a voucher specimen was deposited at the Herbarium of the Department.

Leaves of plants were washed thoroughly under running tap water and soaked in 2% solution of sodium hypochlorite for 20 min, rinsed thoroughly with sterilized distilled water and air dried at room temperature. The dried plant materials were milled. 100 g of each finely ground plant materials were used for extraction in 100 ml of ethanol and acetone solvents, respectively. The solution was kept overnight at room temperature and filtered using filter papers (Whatman filter paper No. 1).

Table 1. List of plant materials used in this study.

Local name	Scientific name	Family
Pan	*Piper betel* L.	Liliaceae
Mehedi	*Lowsonia inermis* L.	Lythraceae
Payara	*Psidium guajava* L.	Myrtaceae
Pepe	*Carica papaya* L.	Caricaceae
Sojina	*Moringa oleifera* Lam.	Moringaceae
Lojjaboty	*Mimosa pudica* L.	Fabaceae
Nayontara	*Catharanthus roseus* (L.) G Don	Aacanthaceae
Basok	*Adhatoda vasica* L.	Aacanthaceae
Kalomegh	*Andrographis paniculata* (Burm.f.) Wall. Ex Nees	Aacanthaceae

2.4. Effect of Different Concentrations of Extracts on Radial Growth of *Fusarium* Strain

Plant extracts which could suppress the fungal growth were tested for their efficiency against the pathogen by using an agar dilution technique. Five ml of each extract concentration was added with 95 ml of molten PDA. Thus obtained concentration of 5%, 10%, 15%, 20% and 25% extract to a PDA medium. After the solidification of the medium, 1 cm diameter of mycelia block from 7-day-old colony of *F. oxysporum* was inoculated in the center of each Petri plate and incubated at ±25˚C. The colony diameter of *F. oxysporum* was measured after 7 days of incubation. Three replicates in a completely randomized design were used within each treatment. The media amended with ethanol and acetone was considered as negative and recommended fungicide sulcox was considered as positive control, respectively. The efficacy of medicinal plant products was expressed and percent of radial mycelial growth over the control which was calculated by using the following formula [7]:

$$\text{Inhibition (\%)} = [(C - T)/C] \times 100$$

where, C and T represent the diameter of control and treated colony, respectively. Data on mycelial growth at 3, 6, 9 and 12 days after inoculation (DAI) were recorded. To avoid bacterial contamination 0.5 g of antibacterial streptomycin was added to 1 L of PDA medium.

2.5. Statistical Analysis

The effect of ethanol and acetone extract of plants at different concentrations on the radial growth of *F. oxysporum* evaluated by a one way analysis of variance (ANOVA). Mean differences between treatments or concentration levels of the plant extracts were separated by Fisher's [15] test significant difference (LSD) at 5% significant probability level. The ANOVA was computation by R programming language.

3. Result and Discussion

For eco-friendly and sustainable management of Fusarium wilt disease, the present study tested the antifungal activity of ethanol and acetone extracts and their respective dilutions of nine selected medicinal plants based on their availability in Rajshahi and their traditional use against fungal pathogen. It was found that at 25% concentration; almost all plant extracts were effective in reducing the mycelial growth of *Fusarium* strain (**Table 2**). Effect of different plant extracts on radial mycelial growth of *Fusarium* sp. on 12 days of incubation (DAI) is presented in **Table 3** and **Table 4**. Hundred percent inhibition at 12 DAI and 25% concentration were observed on almost all plant extract except ethanol extract of *Andrographis paniculata* (91%), *Carica papaya* (89.41%), *Moringa oleifera* (90.59%) and on acetone extract of *Andrographis paniculata* (85%), *Catharanthus roseus* (90.76%) and *Psidium guajava* (87.5%). Maximum inhibition was found at 15% concentration of ethanol extract of *Lowsonia inermis* and *Psidium guajava* against studied fungi. Representative figure was showed in **Figure 1**. Plant extract of *Piper betel*, *Carica papaya*, *Andrographis paniculata* and *Lawsonia inermis* did not show significant difference between 20% and 25% concentrations. Result of analysis of variance on mycelial growth in different concentration showed that the item was highly significant. LSD value also indicates significant in different concentrations of plant extract and radial growth (**Table 3** and **Table 4**).

Table 2. Effect of different plant extracts in two solvents at 25% concentration on growth of *Fusarium* sp.

Plant material	% of inhibition	
	Ethanol	Acetone
P. betel	100	100
L. inermis	100	100
P. guajava	100	87.5
C. papaya	89.41	100
M. oleifera	90.59	100
C. roseus	100	90.76
M. pudica	100	100
A. vasica	100	100
A. paniculata	91	85

Table 3. Effect of various concentration of ethanol extracts on the radial mycelial growth (average mm) on *Fusarium* sp. at 12 DAI.

	Cconcentration of ethanol extract					
Plant material	**5%**	**10%**	**15%**	**20%**	**25%**	**LSD**
P. betel	30a*	14.76b	12.33c	0d	0d	2.101
L. inermis	6.67a	6b	0c	0c	0c	0.469
P. guajava	39.67a	9.33b	0c	0c	0c	1.558
C. papaya	48.67a	32.33b	18.33c	15.67d	8.33e	1.758
M. oleifera	34.33a	17.67b	11.67c	7.67d	7.67d	1.937
C. roseus	43.33a	14.67b	6c	0d	0d	1.558
M. pudica	37.33a	15.33b	10.33c	0d	0d	1.819
A. vasica	41a	6b	6b	0c	0c	1.409
A. paniculata	26.67a	18.67b	10c	9.67cd	8.33d	1.485

*Each treatment was replicated 3 times means followed by the same letter within a row do not differ significantly at $p > 0.05$ according to LSD test by R programming language.

Table 4. Effect of various concentration of acetone extracts on the radial mycelial growth (average mm) on *Fusarium* sp. at 12 DAI.

	Concentration of acetone extract					
Plant material	**5%**	**10%**	**15%**	**20%**	**25%**	**LSD**
P. betel	23.67a*	9.67b	6c	0d	0d	2.253
L. inermis	18a	6b	6b	0c	0c	0.814
P. guajava	28a	22.33b	16c	11d	8.67e	1.558
C. papaya	36.33a	20.33b	6c	0d	0d	1.329
M. oleifera	18.33a	13.33b	8.33c	6d	0e	1.993
C. roseus	42.33a	33b	29.67c	17.67d	6e	1.993
M. pudica	16.33a	8.67b	8.33b	6.33c	0d	1.243
A. vasica	35.33a	18.33b	6c	6c	0d	2.395
A. paniculata	30.33a	24.67b	17.33c	14.33c	14	3.845

*Each treatment was replicated 3 times means followed by the same letter within a row do not differ significantly at $p > 0.05$ according to LSD test by R programming language.

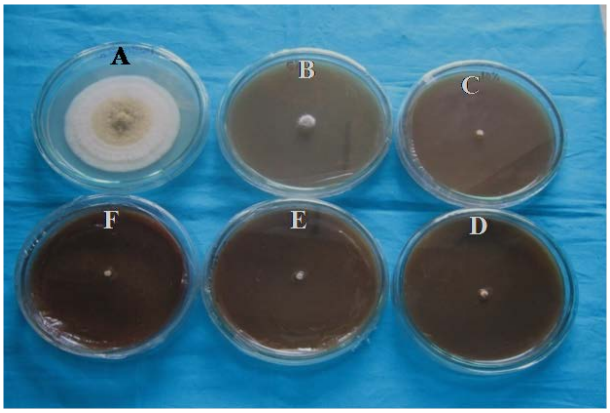

Figure 1. Superior inhibition of ethanol extract of *Psidium guajava* against *Fusarium* strain. Control (A); 5% (B); 10% (C); 15% (D); 20% (E) and 25% (F) of concentration.

From the investigation, it was observed that leaf extract of *Piper betel* in ethanol and acetone at 20% concentrations effectively suppressed the mycelial growth of the *F. oxysporum*. Inhibition observed by *P. betel* may be due to the presence of the essential oils which contained phenolic compounds [16] [17]. There have been reports on the antifungal activities of *P. betle* [18]. Singha *et al.* [11] reported the control of fusarium wilt of tomato caused by *Fusarium oxysporum* using chloroform extract of *P. betle* leaf. They observed that it was efficient in reducing *Fusarium* population in soil than carbendazim. These facts suggested that betel leaf extracts exhibit significant fungicidal properties that inhibit the mycelia growth of *Fusarium* strain.

The inhibitory effect of leaf of *Lawsonia inermis* was effective in 15% ethanol than acetone solution at 12 DAI. It is not surprising because *Lawsonia inermis* Linn. or henna has been used since the earliest times as a herbal medicine. Antifungal activity study with different concentrations of ethyl acetate and ethanol extract of flower and leaf of *Lawsonia inermis* was dose dependent and was found to be effective even at 1 mg/100µl against *Penicillium notatum*, *Fusarium oxysporum*, *Colletotrichum gloeosporioides* except *Rhizopus stolonifer* [19]. The fungicidal property of *L. inermis* might be due to the presence of Lawsone 2-hydroxyl-1, 4-Naphthaquino [20].

The ethanol extract of *Psidium guajava* and acetone extract of *Carica papaya* displayed inhibitory activities, respectively. Few reports are available on these plants for developing commercial formulations for applications against fungal pathogen. Previous study demonstrated that the acetone extracts of leaves of above plants showed moderate to strong activity against *Aspergillus niger*, *Fusarium* sp. and *Candida albicans* [21], whereas we have found that the ethanol extract of *P. guajava* showed strong inhibition at 15% concentration and acetone extract of *C. papaya* were at 20% concentration against *Fusarium* sp., respectively. The results indicated that the two plant extracts are good antifungal agents but *P. guajava* is more effective than *C. papaya*. It is therefore, encouraging to identify and characterize the active principle of these two plants.

Moringa oleifera is an exceptionally nutritious vegetable tree with a variety of potential uses [22]. The plant was reported to contain various amino acids, fatty acids, vitamins, and nutrients [22]. The seed oil has physical and chemical properties equivalent to that of olive oil and contains a large quantity of tocopherols [23]. Many reports described the antifungal and antibacterial activities of *M. oleifera* [24]. Ethanol extracts of *M. oleifera* seeds and leaves showed anti-fungal activities *in vitro* against dermatophytes. Furthermore, 44 chemical compounds were found in essential oil from leaves of *M. oleifera* [25]. The fruit extract of *M. oleifera* showed a broad-spectrum antifungal activity against *Colletotrichum*, *Curvularia*, *Fusarium* and *Alternaria* at different concentrations of methanol extract [22]. In this investigation, we have also found the antifungal activity of *M. oleifera* leaves was effective against *Fusarium* sp. at 25% concentration of acetone than ethanol solvent. This result is noteworthy because this extracts showed a positive relationship with the other findings.

Catharanthus roseus is well known for its pharmacological significance [26]. Antifungal potential in crude extracts of *C. roseus* against clinically significant fungal strains (*Candida albicans*, *Aspergillus fumigatus*, *Aspergillus niger*, *Fusarium moniliforme*) has been reported and ethanol solvent was found to be better extraction solvent as compared to acetone [26]. In this study, we have also found that ethanol extracts of leaf of *C. roseus*

were more effective than acetone extract and reduced the mycelia growth of *F. oxysporum*. It showed 100% inhibition of the test fungus at 20% ethanol solution. These data depicts that the pattern of inhibition largely depend upon extraction solvent.

Ethanol and acetone leaf extract of *Mimosa pudica* and *Adhatoda vasica* at 25% concentration 100% inhibition were recorded. Methanolic extract of *M. pudica* exhibited least activity against *Pithyum debaryanum* [27]. Siva *et al.* [28] reported that ethanol and acetone leaves extract of *Adhatoda vasica* showed 100% inhibition against *F. oxysporuim* at 40% concentration. Present result showed that ethanol and acetone leaf extract of *M. pudica* and *A. vasica* were more effective than the observation of Ambikapathy *et al.* [27] and Siva *et al.* [28].

Radial mycelial growth was recorded at all concentration of leaf extract of *Andrographis paniculata*. The lowest radial mycelial growth was measured in 25% concentration of leaf extract of *A. paniculata* at 12 DAI 8.33 mm in ethanol as solvent and 14 mm in acetone extract. Yasmin *et al.* [29] was first reported on antifungal properties of leaf extract of *A. paniculata* resulted 33.53% growth inhibition of *Fusarium moniliforme*. Present study also supported the findings of Yasmin *et al.* [29] that *A. paniculata*may serve as candidates of plant species for their exploitation as potent fungitoxicants for controlling Fusarium wilt of tomato.

4. Conclusion

In conclusion, the present study laboratory screening of plant extracts has given encouraging results, indicating their potential use in the management of Fusarium wilt in tomato caused by *Fusarium oxysporum*. Further field trial and photochemical analysis of the active compounds of those plants would give a strong antifungal activity comparable to synthetic fungicides. The plant materials used in this study are abundant and commonly found in Rajshahi, Bangladesh.

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