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IN VITRO EVALUATION OF SUPPRESSING EFFECT OF SOME MEDICINAL PLANT LEAF EXTRACTS ON RADIAL MYCELIA GROWTH OF *Fusarium oxysporum* f.sp. *lycopersici*

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Abstract: The experiment was conducted to evaluate the effect of six medicinal plant extracts namely *Azadiracta indica* (Neem), *Ocimum tenuiflorum* (Tulshi), *Cajanus cajan* (Pigeon pea), *Cynodon dactylon* (Durba grass), *Lawsonia inermis* (Mehandi) and *Cliotoria ternatea* (Thunkuni) at different concentrations (10, 50, 100 and 200 mg/L) on the radial mycelial growth of *Fusarium oxysporum* f.sp. *lycopersici*. The experiment was conducted following Completely Randomized Design (CRD) with five replications. Radial mycelial growth, inhibition percentages and colony characters were recorded. The radial mycelial growth was maximum (90mm) of Neem leaf extracts at 10 mg/L which was statistically similar with Tulshi at 10 mg/L and control treatment. Lowest radial mycelial growth was found in Pigeon pea (18.80 mm) leaf extracts at 200 mg/L. The highest radial mycelia growth inhibition (79.11%) was observed on Pigeon pea at 200 mg/L.

Keywords: *Fusarium oxysporum* f.sp. *lycopersici*, medicinal plant extracts, radial mycelia growth.

Introduction

Tomato is a very popular vegetable because of its taste, colour and high nutritive value and also for its diversified use (Bose and Som, 2004). It is widely grown almost all over the world due to its adaptability to wide range of soil and climate (Ahmad, 2005). The demand of Tomato is increasing day by day in the Agro and food industries of Bangladesh. Thus, it is now considered as a cash crop in this country. According to recent statistics, the total production of tomato in Bangladesh was about 137000 tons from 17900 hectares of land with an average yield of 7.65 t ha^{-1} (BBS, 2008) which is very low as compared to the other leading tomato producing countries (FAO, 2008). However, the tomato crop is usually attacked by many kinds of diseases such as *Fusarium* wilt, bacterial wilt, and early blight. Among these diseases, *Fusarium* wilt is one of the most serious that can result in tremendous economic losses. It is caused by *Fusarium oxysporum* f.sp. *lycopersici* (Sacc.). In general, this pathogenic fungus is a limiting factor in the production of many crops and accounts for 10 – 20 % yield losses annually which can reach as high as 100 % (USDA, 2008). Several chemical fungicides such as Bavistin etc. are used to suppress the disease but these chemicals have a negative impact on human health and are hazardous to the environment. A better alternative to chemicals are the soil microbes such as *Trichoderma*, *Penicillium*, etc. residing in the rhizosphere of crop plants that have the ability to suppress the

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pathogens (Hyakumachi *et al.* 1994) and stimulate plant growth by the production of phytohormones (Hasan, 2002) and/or degradation of complex substrates (Altmore *et al*. 1999). The chemical fungicide is non biodegradable and its residual effect remains for a long time (Brady, 1984). In this context, use of environmental friendly plant extracts is an alternative for controlling plant diseases. However it is a recent approach of plant disease control. Medicinal plants are affordable by low income farmers and they have the potentiality for use in agriculture and ensure the sound ecology and friendly environment without any pollution (Bajwa and Schaefers, 1998). Fowcett and Spenser (1970) found that F*usarium* wilt of Brinjal was prevented by the use of medicinal plant extracts and thereby increasing their yield. Ravichandar R. (1987) that Neem leaf extracts are also known to reduce the viability of *Fusarium oxysporum* and mycelia growth considerably *in vitro*. Ramos *et al*., (2007) demonestrated that significant (65%) growth reduction of mycelia of *Fusarium* spp. occurred with neem extracts. Basak and Lee (2001b) observed that *Azadiracta indica* and *Cynodon dactylon* are capable of suppressing conidial germination and mycelial growth of *F. oxysporum* f. sp. *Cucumerinum* causing Fusarium wilt of the cucumber. Mothana & Lindequist, (2005) investigated that many medicinal plants are sources of diverse nutrient and non nutrient molecules, many of which showed antioxidant and antimicrobial properties which can protect them against pathogens. Thus the present research endeavors to find out the suppressing effect of some medicinal plant extracts on the radial mycelia growth of *Fusarium oxysporum* f.sp. *lycopersici.*

Materials and methods

The experiment was conducted in the Plant Protection Laboratory of Agrotechnology Discipline, Khulna University, A pathogenic strain of *Fusarium oxysporum f.sp. lycopersici* was collected from Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur.

At first 200 g potato was cut into slice and then boiled in 1000 ml water. After boiling, it was sieved and 15 g dextrose was mixed with it. After few minutes 20 g agar was mixed with it in a water bath and then sterilized in autoclaved at 121°C temperature for about 20 minutes. Then it was used as medium. The fungal isolate was multiplied on PDA medium describe by **Tuite (1969)** for further use. Multiplication procedure was conducted in aseptic condition.

Media were prepared by using a newly adapted method following the Poison Food Technique (Grover and Moore, 1962). The leaves of medicinal plants were clean and grinded using electric grinder to make paste. Then the material was weighted by an electric balance at 10mg, 50mg, 100mg, and 200mg for each plant and were added in PDA separately in different concentrations than the volume of medicinal plant extract amended medium were level upto 1000ml. After that the medium was autoclaved at a temperature of 121°C for 20 minutes.

Fresh leaves of *Azadiracta indica* (Neem), *Ocimum tenuiflorum* (Tulshi), *Cajanus cajan* (Pigeon pea), *Cynodon dactylon* (Durba grass), *Lawsonia inermis* (Mehandi) and *Cliotoria ternatea* (Thunkuni) were collected from different locations of Khulna

Table 1: The identity of plants, parts and concentrations as extracts used.

Name of the Medicinal Plants	Scientific Name	Family Name	Plant Parts	Concentrations (mg/L)
Neem	*Azadiracta indica*	Meliaceae	Leaves	10, 50, 100 and 200
Tulshi	*Ocimum tenuiflorum*	Lamiaceae	Leaves	10, 50, 100 and 200
Durba grass	*Cynodon dactylon*	Poaceae	Leaves	10, 50, 100 and 200
Pigeon pea	*Cajanus cajan*	Leguminosae	Leaves	10, 50, 100 and 200
Mehandi	*Lawsonia inermis*	Lythraceae	Leaves	10, 50, 100 and 200
Thunkuni	*Cliotoria ternatea*	Leguminosae	Leaves	10, 50, 100 and 200

Twenty (20) ml sterilized medium of medicinal plant leaf extracts was poured in each 5 replicated petridishes of every concentrations. Then petridishes were kept for solidification of the media.

Advanced hyphae of 6 days old culture grown on PDA were cut by flame sterilized 5 mm cork borer. Each circle of PDA containing hyphae was placed upside down on PDA at the centre of the plates containing different concentration of medicinal plant leaf extracts medium by an inoculation needle. The inoculated petridishes were kept in the growth chamber at room temperature (25 ± 1°C) for 6 days.

After 6 days of incubation, radial growth (mm) of *Fusarium oxysporum f.sp. lycopersici* in petridishes was recorded. Radial growth (mm) was measured by taking average of the two diameters taken at right angles for each colony. Mean radial mycelial growth of each plant diffusates was recorded and data were subjected to statistical analysis.

Inhibition of radial growth was computed based on colony diameter on control plate using standard formula (Naz *et al*., 2006):

$$\% \text{ Inhibition} = \frac{X - Y}{X} \times 100$$

Where,

X = Average growth (mm) of *Fusarium oxysporum* f.sp. *lycopersici* in control plate.

Y = Average growth (mm) of *Fusarium oxysporum* f.sp. *lycopersici* in each plant extract treated petridish.

The experiment was conducted under CRD and five replicated plates were used for each treatment. The data were analyzed statistically for ANOVA using MSTAT-C computer program. The significant difference, if any, among the means were compared by Duncan's Multiple Range Test (DMRT).

Results

Radial mycelia growth of Fusarium oxysporum* f.sp. *lycopersici: Effect of different medicinal plant extracts on radial mycelia growth of *Fusarium oxysporum* f.sp. *lycopersici* is shown in Table 2. The radial mycelia growth was substantially affected and differed significantly ($p<0.01$) by different doses of medicinal plant extract. The radial mycelia growth was maximum (90 mm) in case of neem and tulshi at 10 mg/L which was statistically similar to that control treatment (Table 2). Lowest radial mycelial growth was found in Pigeon pea (18.80 mm) at 200 mg/L.

Table 2: Effect of different medicinal plant extracts at different concentrations **on** radial mycelial growth of *Fusarium oxysporum* f.sp. *lycopersici.*

Medicinal plants	Concentrations (mg/L)	Radial mycelial growth (mm)
Control (no treatment)	–	90.00 a
Neem	10	90.00 a
	50	51.80 h
	100	43.20 k
	200	33.20 l
Tulshi	10	89.20 a
	50	52.40 h
	100	47.00 j
	200	47.00 j
Durba grass	10	75.60 b
	50	73.00 c
	100	66.20 e
	200	66.20 e
Pigeon Pea	10	47.60 j
	50	26.20 m
	100	21.00 n
	200	18.80 o
Mehandi	10	71.40 c
	50	58.20 g
	100	49.60 i
	200	44.00 k
Thunkuni	10	68.00 d
	50	64.00 f
	100	62.60 f
	200	52.00 h
Level of significance		**

**=1% level of significance

Inhibitory effect of different medicinal plant extracts on radial mycelial growth (mm) of Fusarium oxysporum f.sp. lycopersici: The different medicinal plant extracts inhibited the mycelia growth *of Fusarium oxysporum f.sp. lycopersici* significantly ($p<0.01$). In Fig. 1 (control treatment) represents no inhibition. The highest radial mycelia growth inhibition (79.11%) was observed on Pigeon pea at 200 mg/L that means among the six using medicinal plant Pigeon pea gave the satisfactory result and lowest growth inhibition (16%) was observed in case of durba grass at 10 mg/L in (Fig.1).

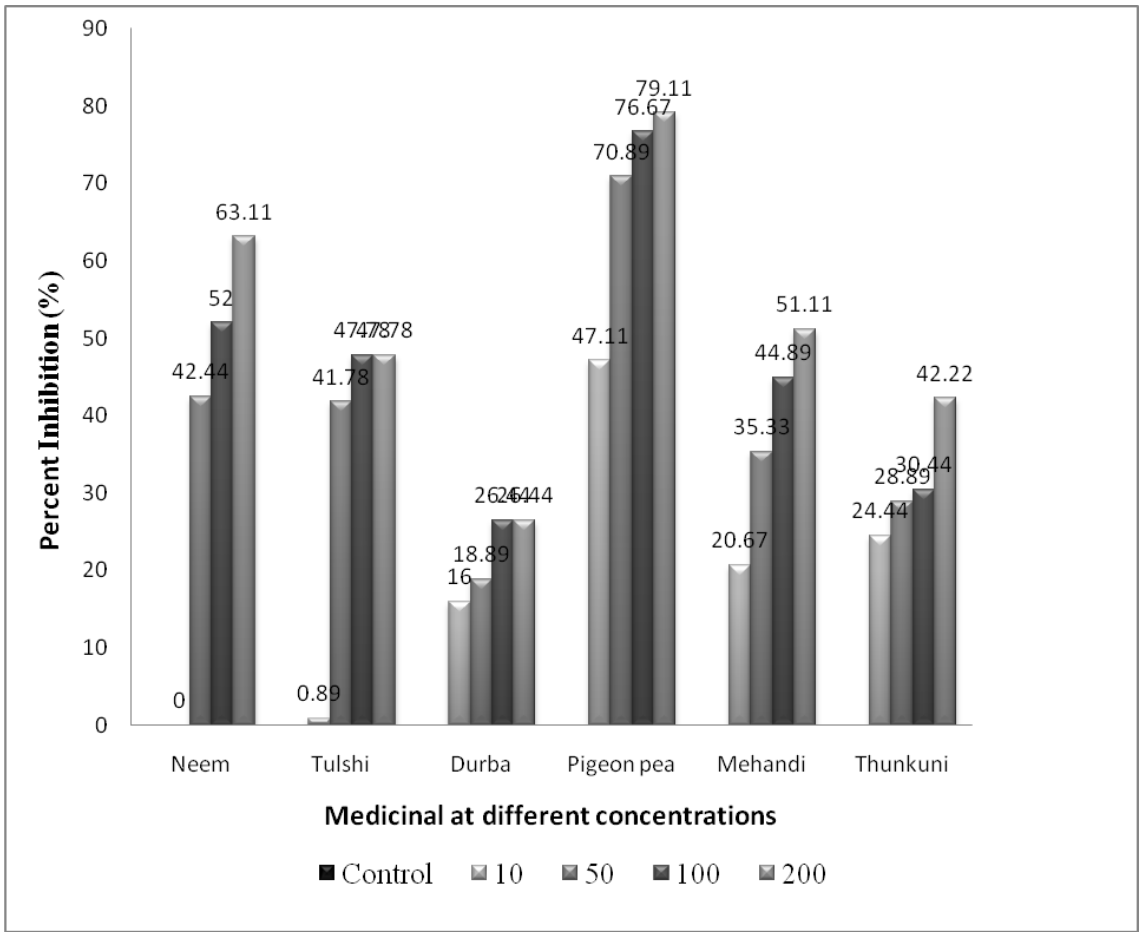

Fig 1: Percent inhibition of different medicinal plant extracts in different concentrations on mycelia growth (mm) of *Fusarium oxysporum f.sp. lycopersici.* *=1% level of significance

Colony characteristics of Fusarium oxysporum f.sp. lycopersici in different medicinal plant extract amended medium: Effects of different medicinal plants on the colony characteristics of *Fusarium oxysporum f.sp. lycopersici* are shown in Table 3. There was no difference in the surface colony margin of Neem and Pigeon pea. Irregular margin was found in case of Tulshi, Durba grass, Mehandi and Thunkuni. Variation was also found in the colony texture and hyphal thickness at all concentration of medicinal plant extracts amended medium. In case of neem colony texture was compact (10 mg/L) and loose (50, 100 and 200 mg/L). In case of Tulshi extracts colony texture was compact (10 and 50 mg/L) and loose (100 and 200 mg/L). In case of Durba grass the colony texture was loose (10, 50, 100 and 200mg/L). In case of Pigeon pea colony texture was loose (10 mg/L) and compact (50, 100 and 200 mg/L). In case of Mehandi colony texture was loose (10, 50, 100 and 200 mg/L). In case of Thunkuni amended medium the colony texture was compacts (10, 50 and 100 mg/L) and loose (200 mg/L). In case of Neem, thick hyphal thickness was found extracts (10 mg/L) and thin hyphal thickness was found in case of Neem extracts (50, 100 and 200 mg/L). In case of Tulshi, thick hyphal thickness was found (10 mg/L) and thin hyphal thickness was found in 50, 100 and 200 mg/L concentrations. In case of Durba grass, thick hyphal thickness was found 200 mg/L and thin hyphal thickness was found in

10, 50 and 100 mg/L concentrations. In case of Pigeon pea thick hyphal thickness was found 50, 100 and 200 mg/L and thin hyphal thickness was found in 10mg/L concentration. In case of Mehandi, thick hyphal thickness was found 10, 50 and 100 mg/L and thin hyphal thickness was found in 200mg/L concentration. In case of Thunkuni, thick hyphal thickness was found 10, 50 and 100 mg/L and thin hyphal thickness was found in 200mg/L concentration.

Table 3: Effect of different medicinal plant extracts on colony characteristics of *Fusarium oxysporum* f.sp. *lycopersici* at different concentrations

Medicinal plants	Concentrations (mg/L)	colony characteristics		
		Margin	Texture	Hyphal Thicness
Control (no treatment)	_	Regular	Compact	Thick
Neem	10	Regular	Compact	Thick
	50	Regular	Loose	Thin
	100	Regular	Loose	Thin
	100	Regular	Loose	Thin
Tulshi	10	Irregular	Compact	Thick
	50	Irregular	Compact	Thin
	100	Irregular	Loose	Thin
	200	Irregular	Loose	Thin
Durba Grass	10	Irregular	Loose	Thin
	50	Irregular	Loose	Thin
	100	Irregular	Loose	Thin
	200	Irregular	Loose	Thin
Pigeon Pea	10	Regular	Loose	Thin
	50	Regular	Compact	Thick
	100	Regular	Compact	Thick
	200	Regular	Compact	Thick
Mehandi	10	Irregular	Loose	Thick
	50	Irregular	Loose	Thick
	100	Irregular	Loose	Thick
	200	Irregular	Loose	Thin
Thunkuni	10	Irregular	Compact	Thick
	50	Irregular	Compact	Thick
	100	Irregular	Compact	Thick
	200	Irregular	Loose	Thin

Discussion

Medicinal plant extracts have positive effect on radial mycelial growth of *Fusarium oxysporum* f.sp. *lycopersici.* Table 2 revealed that medicinal plant extracts increase microbial activity and decrease pathogenic potentiality thereby suppressed the growth of *Fusarium oxysporum* (Lumsden *et al.,* 1986). In this experiment using all medicinal plant extracts was reduced radial mycelial growth of *Fusarium oxysporum* f.sp. *lycopersici* but maximum inhibition was found in Pigeon pea extract amended PDA at 200 mg/L concentration. Application of medicinal plant extracts against controlling pathogen has positive effects on environment. The positive effects can be attributed mainly to the increased nutrient capacity due to the long term application on

medicinal plant extracts. Though the use of large amount of fungicides and pesticides increase costs to growers and the effects of pesticide usage must be seen also in the context of soil pollution and sustainability of the agro-ecosystem. Increasing pesticide usage in agriculture adds to the rise in concern for the environmental contamination (Zhu *et al*, 2004). The chemical fungicide is non biodegradable and its residual effects remain for a long time which causes health *hazard* and environmental pollution. So use of medicinal plant extract for controlling this pathogen might be a good alternative.

Medicinal plant extracts may inhibit to the growth of the soil borne fungus (*Fusarium oxysporum f.sp. lycopersici*). Findings of present experiment agreed with the findings of Day *et al.* (2006) and Chattopadhyay *et al.* (2003). So, result of the study might be considered as new information about the negative effect of medicinal plant extracts on the growth of *Fusarium oxysporum f.sp. lycopersici.*

Conclusion

Growth of *Fusarium oxysporum* f.sp. *lycopersici* was detrimentally affected at all concentrations of using medicinal plant extracts in this experiment. In higher concentrations Pigeon pea leaf extract was very effective in mycelial growth inhibition of *Fusarium oxysporum* f.sp. *lycopersici*. Thus, from this experiment it was found that Pigeon pea leaf extract at 200 mg/L concentrations was given satisfactory result for mycelial growth inhibition of *Fusarium oxysporum* f.sp. *lycopersici*.

In case of using all medicinal plant extracts can reduce mycelial growth of *Fusarium oxysporum* f.sp. *lycopersici*. It is necessary to conduct further experiment from the isolation of noble compound of the medicinal plant extract because mycelial growth inhibition occurred due to released allelochemicals from used medicinal plant extract.

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