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Short Communication

EVALUATION OF PLANT EXTRACTS AGAINST *FUSARIUM OXYSPORUM F. SP. LYCOPERSICI*, WILT PATHOGEN OF TOMATO

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ABSTRACT
Wilt is an important disease of tomato crop causing significant reduction in yield. In present study, the pathogenic fungus was isolated from infected plant and identified based on morphological and cultural characters as *Fusarium oxysporum f.sp.lycopersici* whose pathogenicity was confirmed by Koch's postulate on tomato seedling. *In vitro* evaluation of extracts of 20 plant species was done by poisoned food technique for evaluating their fungitoxicity against the test pathogen. The extract of *Ageratum conyzoides* exhibited maximum toxicity (95.57%) against the *Fusarium oxysporum f.sp. lycopersici*. Significant results were also observed in extracts of *Ageratum haustonianum, Clerodendrum inermae* and *Terminalia bellirica* showing inhibition of 90.33%, 84.97% and 79.19% respectively. Application of plant extracts which are easily available for controlling plant diseases are non pollutive cost effective non hazardous and do not disturb ecological balance.

Keywords: *Tomato, Fusarium Wilt, Plant Extracts, Antifungal Activity*

INTRODUCTION
Fusarium wilt disease of tomato caused by *Fusarium oxysporum f. sp. lycopersici* is one of the most important and widespread disease of the cultivated tomato. It is a soil borne pathogen in the class Hyphomycetes that causes wilt of tomato as the only host of pathogen (Rai *et al.*, 2011). Singh and Kamal 2012 reported 10-90% loss in yield of tomato in temperate region due to this disease. Healthy plants can become infected by pathogen if the soil in which they are growing is contaminated with the fungus. The fungus can invade a plant with its sporangial germ tube or mycelium by invading the plant's roots. The roots can be infected directly through the root tips, through wounds in the roots or at the formation point of lateral roots. Mycelium enters the xylem vessels branches and produces micro-conidia which are carried upward in the sap stream. Due to the growth of the fungus within the plant's vascular tissue, the plant's water supply is greatly affected. This lack of water induces the leaves stomata to close, the leaves wilt and the plant eventually dies. Infection usually occurred on plants in the form of chlorosis, leaf wilting and browning of the vascular system. Several procedures have been attempted for managing the *Fusarium* wilt disease in the greenhouse and in field. Ioannou (1999) used soil solarization technique to reduce the population density of *Fusarium* species in soil by 88 to 93, and provide effective control *Fusarium* wilt diseases on tomato. Soil amendment by using mustard til and cotton, vermicompost and farmyard manure (Mathur *et al.*, 2003) had proved best for controlling *Fusarium* wilt. Several biological control agents viz. *Trichoderma* species (Anadani and Pithia, 2010; Kirti and Harish, 2009; Shree *et al.*, 2013), *Aspergillus niger* (Mishra and Prasad 2003), *Bacillus* species (Adebayo and Ekpo 2004) have been successfully tested against *Fusarium* wilt. Presently for controlling *Fusarium* wilt, many synthetic chemicals viz. carbendazim, diflotan, benlate, prochloraz, mannitol and various fungicides have been used. These synthetic chemicals that have been commercialized as pesticides are halogenated hydrocarbons with relatively long environmental half lives and more suspects toxicological and carcinogenic. Plant metabolites and plant based pesticides appear to be one of the better alternatives as they are known to have minimal environmental impact and danger to consumers in contrast to synthetic pesticides.The demand of plant based therapeutics is increasing in developing countries as they are natural products easily available and having no harmful effects. The present study was undertaken to evaluate some plant extracts against *Fusarium oxysporum f.sp. lycopersici* causing wilt disease of tomato.

MATERIALS AND METHODS

Fusarium oxysporum f. sp. lycopersici was isolated from the infected roots of wilted tomato plants showing fungal wilt symptoms. The root pieces were surface sterilized by 0.5% sodium hypochlorite solution for 30 sec. and washed twice by distilled water. The sterilized root pieces were dried using filter paper and were aseptically placed on PDA medium. They were incubated at 25±2ºC for 7 days until the fungus mycelium appears. After formation of fungal colony, single-spore isolates were kept on the PDA slant in the refrigerator on 4°C for further use.

Pathogenicity test of the obtained isolate of *Fusarium oxysporum* was carried out during February. Twenty days old tomato transplants were planted into plastic pots (30 cm in diameter) each containing 11 kg of natural soil mixture consisted of clay and sand at rate of 2:1(by weight) at rate of 3 seedlings per pot. Then spore suspension of a particular *Fusarium* isolate of concentration 10^6 spores/ml was poured over stem base at rate of 20 ml/seedling. In control, distilled water was used instead of spore suspension. Pots were irrigated regularly and were observed for symptoms of wilt for 2 months after inoculation. Observations on plant growth parameters (plant height, number of leaves/plant, Fresh weight of leaves, stem fresh weight, root fresh weight, root length, weight of fruit yield) were also investigated after two months.

Fresh and healthy leaves of different plants were collected from various places of Gorakhpur district of U.P., India. 20g of plant leaves were washed by 0.1% $HgCl_2$ followed by sterilization with 70% ethanol. Then the leaves were washed by sterilized water to remove the traces of ethanol and $HgCl_2$.They were then crushed with 20 ml sterile distilled water in a blender and aseptically filtered through double layered muslin cloth. The filterate was used for antifungal testing.

The antifungal activity of plant extracts against *Fusarium oxysporum f.sp.lycopersici* was tested by poisoned food technique of Singh and Tripathi 1994. For treatment 5ml of prepared aqueous extract of each plant species was mixed with 5ml of molten PDA medium in a pre-sterilized petriplates separately and agitated in circular motion in order to mix the extract homogenously. Control sets were prepared similarly using 5ml of sterile distilled water at the place of extract. Fungal disc (6mm diam.) cut from the periphery of 7 days old culture of *Fusarium oxysporum f.sp.lycopersici* was inoculated aseptically in each petriplates of treatment and control sets. Then all the plates were incubated for six days at 25±2ºC.Colony diameter was measured on 7^{th}day.

The inhibition over control was calculated as

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

Where, I = Percent inhibition
C = Radial growth in control
T = Radial growth in treatment

Table 1: Effect of inoculation with isolate of *F.oxysporum f. sp. lycopersici* on growth parameters of tomato cultivar

Tested	PH	NL	FWL	SFW	RFW	RL	WFY
Inoculated	26.56	3	0.51	2.19	1.73	6.2	0.54
Control	33.73	35	2.58	4.95	2.03	7.9	1.81

Table 2: Effect of inoculation with isolate of *F.oxysporum f.sp. lycopersici* on growth parameters reduction % of tomato cultivar

Test	***Reduction % for**						
	PH	NL	FWL	SFW	RFW	RL	WFY
Inoculated	21.25±4.56	91.42±2.08	80.23±0.22	55.5±1.05	14.77±0.61	21.51±1.3	70.16±0.12
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00

$^{*}Reduction(\%) = \frac{Control\ -Treatment}{Control} X100$

PH = plant height (cm), NL = number of leaves, FWL = fresh weight of leaves (g/plant), SFW = stem fresh weight (g/plant), RFW = root fresh weight (g/plant), RL = root length (cm/plant), WFY = weight of fruit yield (g/plant)

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Table 3: Effect of different plant extracts on mycelial inhibition of *Fusarium oxysporum f.sp. lycopersici*

Plant name	Family	Part Used	PMI
Ageratum conyzoides Linn.	Asteraceae	leaf	95.67±0.58
Ageratum haustonianum Mill.	Asteraceae	leaf	90.33±0.76
Allium cepa L.	Amaryllidaceae	leaf	62.39±2.36
Anthocephalus cadamba (Roxb)Miq	Rubiaceae	leaf	33.05±1.00
Azadirachta indica A.Juss	Meliaceae	leaf	67.44±3.61
Carum carvi L.	Apiaceae	leaf	26.94±8.89
Catharanthus roseus (L.)G.Don	Apocynaceae	leaf	24.41±7.28
Citrus aurantifolia (christm)swingle	Rutaceae	leaf	42.00±0.76
Clerodendrum inermae (L)Gaertn	Lamiaceae	leaf	84.97±0.76
Coriandrum sativum L.	Apiaceae	leaf	50.89±4.50
Emblica officinalis Gaertner	Euphorbiaceae	leaf	33.29±2.29
Flacaurtia jangomans (Lour.)Rausch	Flacaurtiaceae	leaf	16.10±0.87
Murraya koengii Spreng.	Rutaceae	leaf	20.12±2.02
Nyctanthes arbortistis Linn	Oleaceae	leaf	67.83±5.03
Phyllanthus amarus Schumach and Thonn	Euphorbiaceae	leaf	45.57±3.51
Piper longum Linn.	Piperaceae	leaf	13.75±6.33
Tecoma capensis (Thunb.)Lindl	Bignoniaceae	leaf	69.33±1.80
Terminalia bellirica Roxb.	Combretaceae	leaf	79.19±0.58
Vitex negundo L.	Verbenaceae	leaf	28.81±6.02
Zingiber officinale Rosc.	Zingiberaceae	leaf	30.06±2.02

PMI percent mycelia inhibition

A

B

C

D

E

Figure 1: A= symptom of disease, B= Pure culture of *Fusarium oxysporum f.sp.lycopersici* C= macroconidia, D= microconidia E= Inhibition of mycelia growth of *Fusarium oxysporum f.sp.lycopersici* by aqueous extract of *Ageratum conyzoides*

RESULTS AND DISCUSSION

The pathogenicity test showed that the earliest symptom of wilt disease on tomato plants is the yellowing of the older leaves. The yellowing gradually affects most of the foliage and is accompanied by wilting of the plant. The wilting becomes more extensive for day to day until the plant collapses and dies. The observation on plant growth characters and reduction % of plant after the inoculation of pathogen are presented in Table 1 and Table 2 respectively. All the parameters were significantly affected and reduce by the pathogen. There is a significant decrease in all parameters including height of plant (26.56), number of leaves (3), fresh weight of leaves (0.51), stem fresh weight (2.19), root fresh weight (1.73), root length (6.2) and weight of fruit yield (0.54) comparing to the uninoculated plants. The reduction % on all growth parameters showed dangerous losses in plant. The result presented in Table 3 also showed that all plant extracts showed more or less inhibitory tendency towards mycelia growth. Of all the samples screened the extract of *Ageratum conyzoides*, belonging to the family Asteraceae showed highest inhibition of mycelia growth (95.65%) against test fungus. The inhibition of mycelia growth varies from family to family and species to species. Significant results were also obtained in *Ageratum haustonianum, Clerodendrum inermae, and Teminalia bellirica* showing inhibition 90.33%, 84.97%, 79.19% respectively.

The present investigation show that the plant extracted with distilled water had strong antifungal activity with significant inhibition on the growth of mycelium of *Fusarium oxysporum f.sp.lycopersici*. Similar effect of other various plant extracts effective against *Fusarium oxysporum f. sp.lycopersici* have been reported by several workers. The results are confirmatory with those reported by Beg *et al.,* (2011) stating that the aqueous extract of *Blumea lacer*a belonging to the family Asteraceae caused 87.00% mycelia growth inhibition of *Fusarium oxysporum f.sp.lycopersici*. Singha *et al.,* (2010) reported *Piper betle* as a effective fungicide against the *Fusarium oxysporum f.sp.lycopersici*. Hadian (2012) reported 98% mycelia inhibition of pathogen by *Azadirachta indica* seed extract. Rinez *et al.,* (2013) studied the antifungal activity of aqueous extract of *Datura metel* against *Fusarium oxysporum f.sp.lycopersici* which shows 69% inhibition in mycelia growth. Adekunle and Nwaoguala (2010) findings showed that *Ocimum gratissimum* scent was most effective in controlling the *Fusarium oxysporum f.sp.lycopersic*i. Agbenin and Marley (2006) reported 100% inhibition of mycelia growth of pathogen by using dry neem seed extract.

It has been concluded from present research that certain plant extracts are a source of cheap and effective fungicides of *Fusarium oxysporum f.sp.lycopersici*, also it doesn't has human and environment health implications.

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