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ANTIFUNGAL ACTIVITY OF SOME COMMON MEDICINAL PLANT EXTRACTS AGAINST SOIL BORNE PHYTOPATHOGENIC FUNGI *FUSARIUM OXYSPORUM* CAUSING WILT OF TOMATO

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ABSTRACT

Biopesticides are mostly used to control fungal plant diseases because of their ecofriendly nature and their cost effectiveness. The present study focused on antifungal activity of solvent based plant extracts of common medicinal plants *Azadirachta indica* ,*Tinospora cordifolia*, *Oscimum sanctum*, *Justicia adhatoda* , *Catharanthus roseus*, *Aegle marmelos*, *Aloe barbadensis, Tithonia diversifolia*, *Hyptis suaveolens* and *Pongamia pinnata* were observed against soil borne phytopathogenic fungus *Fusarium oxysporum* by modified poisoned food technique. The methanol, ethyl acetate, benzene, acetone and chloroform extracts were evaluated for present study. The extracts of *Azadirachta indica* and *Oscimum sanctum* were most effective against *Fusarium oxysporum*. The present investigation suggests that acetone and chloroform extracts of *Azadirachta indica* and methanol extract of *Oscimum sanctum* acts as stong biopesticides and completely inhibit the growth of pathogen. This study reveals that these extracts contains amazing fungicidal properties and may be used as botanical biopesticides.

INTRODUCTION

A major reason for the application of medicinal plants is their ability to control pests and pathogens in their surrounding environment. So, they could be effective source of antimicrobial agents and their identification is very important to produce ecofriendly and cost effective pesticides. Biopesticides are gaining growing interest because of their ecofriendly attributes (Dwivedi and Singh, 1998; Karnwal and Singh, 2006). Phytopathogenic fungi are the chief infectious agents which causes malfunctioning during developmental stages and also in post-harvest.Now a days, infection due to fungal pathogens has become more common incidence. Tomato (*Lycopersicon esculentum* Mill) is perennial herb and belongs to family Solanaceae. Tomato is the second most important vegetable crop next to potato and generally used in soups and stews. *Fusarium* wilt is most destructing disease of tomato (Singh *et.al*; 1980). The disease is seed and soil born shows yellowing and wilting symptoms. According to Sherf and Macnab, 1986 *Fusarium oxysporum* causes root rot and wilt of tomato. Fungal species of the genera *Fusarium* and *Aspergillus* are major plant pathogens world wide (Gafoor and Khan, 1976; Mirza and Kureshi, 1978).

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Fusarium is very common fungal pathogen which cause wilt and rot symptom in plants. Controlling *Fusarium* wilt is very difficult because it spreads so fast and it is estimated that nearly 80% of the crop damage worldwide is causeddue to this busy fungi (Agrios, 2000). The most effective method of protecting the plants from fungal pathogens is the application of fungicides. The continuous application of any fungicide may lead to develop resistance in target pathogen and such resistance is acquired by the pathogen. There are so many fungicides available in market which are non-biodegradable and they accumulate in the soil which causes lethal effects on human and other organisms in surrounding environment through food chain. Therefore, there is need to use some ecofriendly cost effective substitutes for management of plant diseases. Natural products are very effective solution to the environmental problems caused by the synthetic fungicides and many investigators are trying to know the effective natural products to replace the synthetic pesticides (Kim *et al.,* 2005). The use of botanical biopesticides for the control of disease in plants is accepted as an substitute source to synthetic pesticides due to their lower negative impacts on the surrounding environment. The botanical biofungicides are cheap, easily available, non toxic and biodegradable (Singh *et.al*., 1986; Dubey, 1991; Alam *et al.,* 2002).

Although there is a growing interest in the use of medicinal plants to control the fungal diseases, only about 2,400 plant species among more than 250,000 higher plants have been reported for the phytoactivity (Oluwalana and Adekunle, 1998; Oluwalana *et al.*, 1999; Khafagi and Dewedar, 2000). The present study therefore, performed to obtain efficacy of these common medicinal plant extracts against soil borne tomato wilt causing fungus *Fusarium oxysporum.*

MATERIALS AND METHODS

Plant material

Eight common medicinal plants were collected from local areas of near the P.D.V.P. college Tasgaon. Table- 1 depicts the list of common medicinal plants used in present investigation. All plants were collected in sterile polythene bags and brought to the laboratory.

The fungal culture was maintained at 28^0C on Czapeck-Dox agar (CDA) and Potato dextrose agar (PDA) medium. Spores of the fungus were obtained from 8 day old actively growing mycelia culture of *F. Oxysporum*. The spore suspension was kept at -30 ^{0} C in 10% Glycerol.

Antifungal activity of 10 medicinal plant extracts by modified food poisoned technique

The 10 medicinal plant extracts were evaluated by the modified food poisoning technique. 700 µl of CD broth in 2 ml micro centrifuge tube. 100 µl of each extracted solvent were taken in micropipette. CD broth and plant extracts mixed well separately. 100 µl of pure mycelium were added in SD broth. The centrifuge tubes were incubated for 24 hours at 28^0C. Sterile agar disc of 0.5 mm was dipped in test mycelium suspension (700 µl CD broth + 100 µl plant extract + 100 µl mycelium suspension) in micro centrifuge tube.

Table 1. List of common medicinal plants selected as biopesticides

Sr. no	Botanical name	Vernacular name	Parts used
1	*Azadirachta indica* L.	Kadu limb	Leaves
2	*Tinospora cordifolia* (Thunb.) Miers	Gulvel	Leaves
3	*Hyptis suaveolens* L.	Vilayati tulas	Leaves
4	*Pongamia pinnata* L.	Karanj	Leaves
5	*Oscimum sanctum* L.	Tulas	Leaves
6	*Tithonia diversifolia* A.	Kanakgol	Leaves
7	*Justicia adhatoda* L.	Adulsa	Leaves
8	*Catharanthus roseus* L.	Sadafuli	Leaves
9	*Aegle marmelos* L.	Bel	Leaves
10	*Aloe barbadensis* Mill.	Korfad	stem

Table 2. Antifungal activity of common medicinal plant extracts against soil borne phytopathogenic fungi Fusarium oxysporum

Medicinal plants	Percentage inhibition of mycelial growth				
	Methanol extract	Ethyl acetate extract	Benzene extract	Acetone extract	Chloroform extract
Azadirachta indica	86.77	88.33	80.66	100	100
Tinospora cordifolia	69.55	67.33	76.75	78.88	92.44
Hyptis suaveolens	88.99	69.44	65.77	74.33	69.88
Pongamia pinnata.	68.44	62.77	50.55	61.99	67.79
Oscimum sanctum.	100	92.33	91.66	78.66	87.44
Tithonia diversifolia	60.44	78.99	77.44	74.66	84.66
Justicia adhatoda	96.88	88.66	89.44	94.33	91.44
Catharanthus roseus	92.33	91.33	98.33	90.44	92.33
Aegle marmelos.	79.33	68.66	89.44	73.33	74.22
Aloe barbadensis	77.71	82.33	76.33	69.33	62.44

Preparation of plant extracts

Different plant parts of the collected medicinal plants were shade dried for 15 days. The dried plants were powdered with the help of grinder. One gram separately weighted powder of each plant was then extracted in 10 ml of each five different solvents that is methanol, ethyl acetate, benzene, acetone and chloroform. The plant extracts were filtered through Whatman's filter paper no. 1 and subjected to water bath for temperature 80^0 C to remove solvents from the extract. After end of evaporation 10 ml of di methyl sulfoxide were added in each extract. The resulted plant extracts were stored in refrigerator for further analysis.

Fungal culture

The pure mycelial culture of *Fusarium oxysporum* were obtained from the infected stem of *Lycopersicon esculentum*.

The sterile agar disc were were placed upside down on CDA medium. Plates were incubated at 28^0C for 4 days. The control plates were made by adding DMSO instead of plant extracts. The linear radial growth of mycelium was measured after 4 days of incubation period and compared with control. The mycelia inhibition percentage were calculating by following formula (Alam *et.al.,* 2002)

$$I\% = \frac{C-T \quad X \quad 10}{C}$$

Where;

I= reduced mycelia growth, C= myceliumdiameter in control plates, T= mycelium diameter in treated plates.

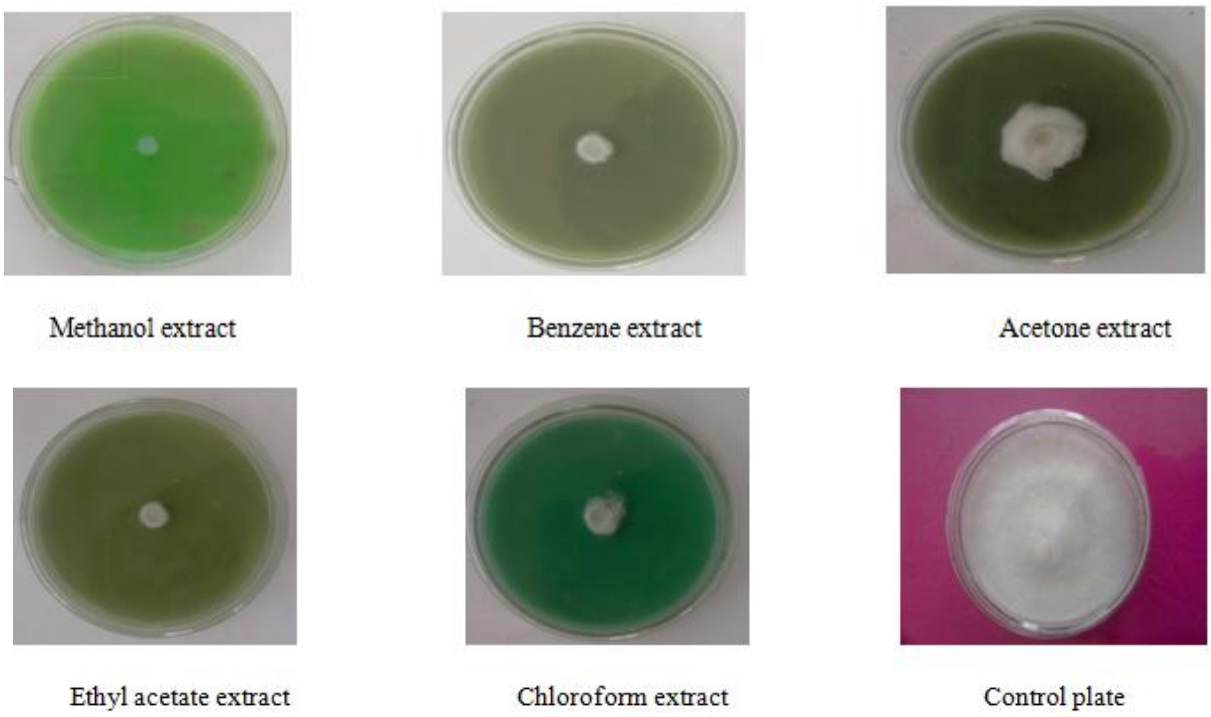

Fig.1. Effect of *oscimum sanctum* based 5 solvent extracts on mycelium of F. oxysporum

Calculation of Minimum Inhibitory Concentration (MIC)

The reduced mycelial growth of the fungus were considered as sensitive to extract, and those plates with full mycelial growth were considered as resistant. For MIC serial dilutions of the extracts were done. Each inoculum was prepared in its respective medium and density was adjusted to 0.5 Mcfarland standards (108 CFU/ml) and diluted to 1: 100 for the broth micro dilution procedure. Microtiter plates were incubated at 100 rpm and 28°C. The MIC was recorded after 4 days. The MIC is the lowest concentration of the compound at which the microorganism tested does not showed mycelial growth.

RESULTS

Ten medicinal plant extracts were screened for their antifungal activity against soil borne phytopathogenic fungi *Fusarium oxysporum* causing wilt of tomato. All the plant extracts showed antifungal activity against *Fusarium oxysporum* and acts as good biopesticides. The extracts of *Azadirachta indica* and *Oscimum sanctum* were most effective against *Fusarium oxysporum* which showed 100 % of inhibition of mycelium on culture plates. acetone and chloroform extracts of *Azadirachta indica* and methanol extract of *Oscimum sanctum* acts as strong biopesticides and completely inhibit the growth of pathogen followed by *Justicia adhatoda* and *Catharanthus roseus* on which fungus showed very slight growth (Table 2).

DISCUSSION

The solvent based extracts of *Azadirachta indica* and *Oscimum sanctum* showed a broad spectrum antifungal activity against soil-borne phytopathogenic fungus *Fusarium oxysporum.*

The methanol extract of *Justicia adhatoda* and benzene extract of *Catharanthus roseus* acts as very effective biopesticides which inhibit the growth of pathogen more than 95 %. The present study is very important part in providing botanical biopesticides which are ecofriendly and cost effective.

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