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EFFECTIVENESS OF EXTRACT OF SOME MEDICINAL PLANTS AGAINST SOIL- BORNE FUSARIA CAUSING DISEASES ON LYCOPERSICON ESCULANTUM AND SOLANUM MELONGENA PLANTS

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ABSTRACT

The antifungal activity of extracts of *Tinospora cordifolia* (leaves), *Moringa oleifera* (bark) and *Trachyspermum ammi* (seeds) at three concentrations *viz.,* 25, 50, 75% (v/v) were evaluated *in vitro* by poisoned food technique against *Fusarium oxysporum* f. sp*. lycopersici* and *Fusarium solani* causing wilt disease on tomato and brinjal plants. The antifungal activity was assessed in terms of percentage of inhibition of mycelial growth of the test fungi. All the plant extracts showed significant inhibition in the mycelial growth of the test pathogens. Among the extracts evaluated, *M. oleifera* against *Fusarium oxysporum* f. sp*. lycopersici and T. cordifolia* against *Fusarium solani* completely inhibited mycelial growth at 75% concentration followed by *T. ammi* seed extract.

KEYWORDS: Plant extracts, antifungal activity, phytopathogenic fungi, wilt disease.

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INTRODUCTION

In recent years the use of eco-friendly technologies especially use of plant extracts are gaining tremendous importance for the control of plant disease in agriculture because they are less harmful to the human health and environment. [7, 10] Botanical pesticides are considered as potential alternatives to chemical agents which can be hazardous to human and animal health.[30] Many plants have been found to possess antifungal properties. [39, 5, 48] The natural compounds provide less phytotoxic, more systemic and easily biodegradable fungitoxic compounds. [41] There has been a growing interest on the research of possible plant diversity for a variety of biologically active chemicals. These chemicals posses a variety of properties *viz.,* antibacterial, antifungal, antiviral, anthelminthic, anticancer, sedative, laxative, cardiotonic, diuretic and others. [34]

Antifungal compounds from plant origins are of the most promise due to their being less toxic and more environmentally compatible by nature. [26] During the past decades, many plants have been extracted and screened for antifungal activities. Essential oils are among the plant derive natural products that have been reported to possess desirable bioactivities including fungicidal activities, [51] bactericidal [9] insecticidal [16] and nematocidal [33] activities. The essential oils can be utilized in different ways as a pest or disease control agent according to their properties and formulation such as spray, dipping solutions or fuming agent like essential oil extracted from mature seeds of ajowan (an aromatic plant) of the family Umbelliferae, as a biofumigant against *Botrytis cinerea*. [65]

Several synthetic drugs have been also reported having serious side effects. [21, 4] Therefore, like other ailments, attention is also being directed to the medicines of herbal origin to find out safer and cheaper drugs for hypolipidaemic activity. [42, 60, 56, 59] *Tinospora cordifolia* Miers, commonly known as 'Guduchi' (family Menispermaceae) is a plant prescribed in Ayurveda, the Indian traditional system of Medicine as a 'Rasayana' or general tonic. [57] Dry barks of *T. cordifolia* has antispasmodic, anti-pyretic [15], anti-allergic [32], anti-inflammatory [36] and anti-leprotic [6], therapeutics [18, 17] properties. The *in vitro* anti-oxidant activity of *T. cordifolia* justifies the ethno medical use of this plant. [66] Moringa is commonly known as 'Horse Radish' (Family Moringaceae) and its roots, flowers, bark, and stem including seeds possess antimicrobial properties.[27, 3] Seed of *Moringa oliefera* are also known for *coagulation* properties for treating water and waste water due to presence of flocculent protein/peptides. [22] Seed extracts of *M. oleifera* have been found to have antimicrobial properties. [23, 19]

Trachyspermum ammi, commonly known as ajowan (Family Apiaceae) is one of the aromatic seed spices which is generally used for medicinal purposes as a digestive stimulant or to treat liver disorders. Thymol, the major phenolic compound present in Ajowan, has been reported to be a germicide, antispasmodic and antifungal agent. [31] The cardiac aliments are directly related to hyperlipidaemia. [24] It has also reported to have platelet aggregation inhibitory action [54], antifungal potency [11] and blood pressure lowering action. [1] Recently, antihyperlipidaemic effect of *T.ammi* seed has been evaluated in albino rabbits. It was assessed that *T. ammi* powder @ 2 g/kg body weight and its equivalent methanol extract were effective lipid lowering agents. [20]

The *in vitro* efficacy of essential oil of ajowan against three distinct phytopathogenic strains of *Fusarium oxysporum* were studied as a part of continuous studies on exploring the hidden potential of indigenous flora of Pakistan. [64, 61, 63, 62] They have also screened the extracts of *T. ammi* (*Apiaceae*) locally known as Ajwain; *Vernonia anthelmintica* (*Asteraceae*) locally known as Kali ziri, *Thuja* occidentalis (Cupressaceae),locally known as Morpankh and Dryopteris chrysocoma (*Dryopteridaceae*) locally known as Sarkhas for their antibacterial and antifungal activities. In the present study, the extracts of the three medicinal plants were tested against

pathogenic *Fusarium solani* f. sp. *melongenae* and *Fusarium oxysporum* f. sp. *Lycopersici* causing wilt of brinjal and tomato respectively.

MATERIALS AND METHODS

(i) Source of crude plant extracts

Tinospora cordifolia (leaves), *Moringa oleifera (*bark) and *Trachyspermum ammi (*seeds) were used for the extraction. Fresh plant materials were washed thoroughly under running tap water followed by sterilized distilled water. The leaves were air dried and then grinded with the help of pestle and mortar. One gram of extract was added in 1 ml distilled water (1:1 w/v) separately for each plant extract; filtered through muslin cloth and 100% plant extract solution was prepared. The extracts were poured in the flasks plugged with cotton to avoid contamination. The extracts were further diluted to different concentrations (25, 50 and 75%) by adding distilled sterile water for further use in the experiment.

(ii) Test fungus

Fusarium solani and Fusarium oxysporum f. sp. *lycopersici* were isolated from the wilt affected brinjal and tomato plants using standard pathological techniques. The media used was Czapek's Dox agar medium and the pure culture of the test fungus was maintained. The fungitoxicity was studied by poisoned food technique. [13]

(iii) Determination of mycelial inhibition by poisoned food technique

Different concentrations (25, 50 and 75%) of ethanolic extracts of *T. cordifolia, M. oleifera* and T. *ammi* were incorporated in to the Czapek's Dox agar medium in sterilized Petri dishes. The Petri dishes containing media devoid of the extracts with same amount of distilled water served as control. The blocks of 4mm diameter of test pathogens, cut from actively growing margin of seven days old culture, were placed at the centre of the Petridishes containing different concentration of the poisoned medium and incubated at 25±2°C for 7 days. The average diameter of the fungal colonies was measured on the 7th day of incubation and percentage of mycelial growth inhibition was calculated using the following formula. [38]

Percentage mycelial growth inhibition $\frac{gc-gt}{gc} \times 100$

Where, gc = growth of mycelial colony in control set after incubation period subtracting the diameter of inoculum disc. gt = growth of mycelial colony in treatment set after incubation period subtracting the diameter of inoculum disc. The Data recorded during the course of investigation has been subjected to two-way classification. The conclusion was drawn on the basis of analysis of variance. The calculated value of F was compared with table value of F at 5% levels of significance for an appropriate degree of freedom.

RESULTS AND DISCUSSION

Comparative analysis of antifungal activity

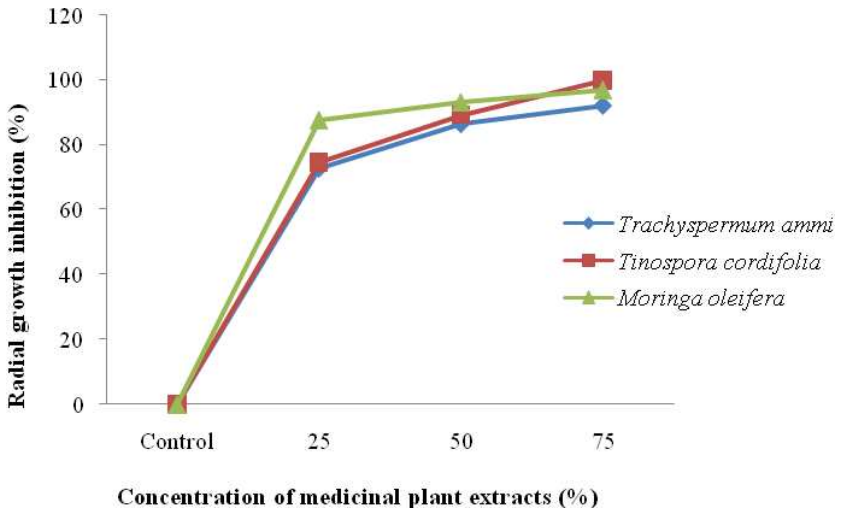
The recorded mean fungal radial growth (mm) for each treatment is presented in Table 1, Table2 and Graph 1 and Graph 2 indicate the inhibition zones of different extracts. All the medicinal plant extracts showed siginificant reduction in the growth of the test pathogens. Among the different extracts, *M. oleifera* against *F. oxysporum* f. sp. *lycopersici* and *T. cordifolia* against *F. solani* were most effective as they completely inhibited the mycelia growth at the concentration of 75%. The growth of *Fusarium solani* was inhibited at 25% concentration of *M. oleifera* (bark), *T. ammi* (seeds) and *T. cordifolia* (leaves) by 87.65, 72.66 and 74.78%, respectively. On the other hand, at 50% concentration of the foresaid medicinal plants inhibited the mycelia growth of *Fusarium solani* by 93.12, 86.33 and 89.37% respectively, where as maximum inhibition i.e. 96.96, 92.02 and 100% was recorded in the case of 75% significant at 5%, among all the medicinal plants tested (Table 1 and Graph 1).

Table 1
Efficacy of medicinal plant extracts against Fusarium solani f. sp. melongena at different concentration (mean diameter of colony in mm)

Medicinal plants	Concentration (%)		
	25	50	75
Trachyspermum ammi (seeds)	22.50±6.51	11.25±1.64	6.56±1.32
Tinospora cordifolia (leaves)	20.75±2.04	8.75±0.00	0.00±0.00
Moringa oleifera (bark)	10.16±1.46	5.66±1.58	2.50±0.00
Control	82.30±0.00	82.30±0.00	82.30±0.00

Values shown are the mean ± SE of 3 replicates, significant at p≤0.05

Factor	***SE***	***CD***
Medicinal Plants	***1.173043***	***2.464562***
Concentration	***1.173043***	***2.464562***
Medicinal Plants× Concentration	***2.031769***	***4.268747***



Graph 1
Radial growth inhibition of F. solani f. sp. melongena through antagonistic medicinal plant extracts 7 days after incubation.

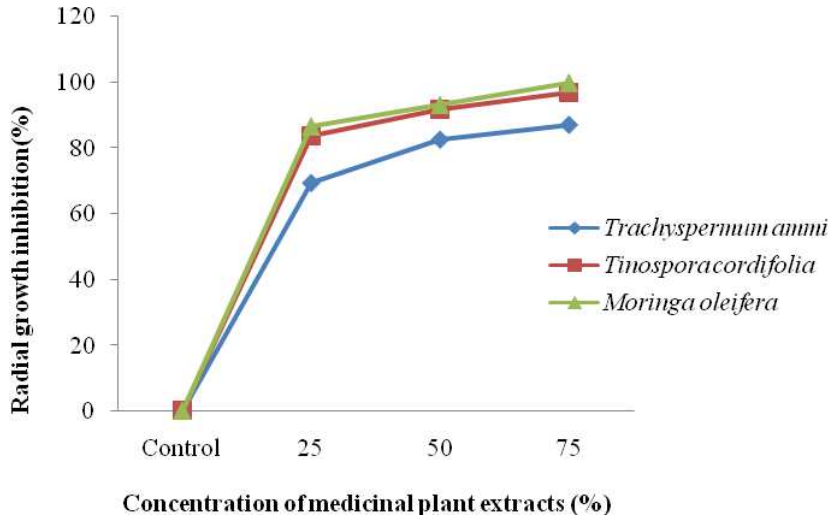
T. ammi, T. cordifolia and *M. oleifera* at 25% concentration inhibited the mycelia growth of *Fusarium oxysporum f. sp. lycopersici* by 69.44, 83.69 and 86.67%, respectively. On the other hand, at 50% concentration of these plants mycelial growth was inhibited by 82.67, 91.69 and 93.23%, respectively. Maximum percentage inhibition i.e. 87.17, 96.92 and 100% was recorded at 75% significant at 5% as indicated in Table 2 and Graph 2.

Table 2
Efficacy of medicinal plant extracts against Fusarium oxysporum f. sp. lycopersici at different concentration (mean diameter of colony in mm)

Medicinal plants	Concentration (%)		
	25	50	75
Trachyspermum ammi (seeds)	24.83±4.88	14.08±3.40	10.04±0.87
Tinospora cordifolia (leaves)	13.25±0.43	6.75±0.00	2.50±0.00
Moringa oleifera (bark)	10.83±1.04	5.50±0.00	0.00±0.00
Control	81.25±0.00	81.25±0.00	81.25±0.00

Values shown are the mean ± SE of 3 replicates, significant at p≤0.05

Factor	***SE***	***CD***
Medicinal Plants	***0.962518***	***2.02225***
Concentration	***0.962518***	***2.02225***
Medicinal Plants× Concentration	***1.66713***	***3.502639***



Graph 2
Radial growth inhibition of F. oxysporum f. sp. lycopersici through antagonistic medicinal plant extracts 7 days after incubation.

The effect of extracts (leaves, bark and seeds) of *Tinospora cordifolia* against *Fusarium solani* and *Moringa oleifera* against *Fusarium oxysporum* f. sp. *lycopersici* indicate that they possess high antifungal activity. The antimicrobial potential of plant essential oil has long been recognized and numerous research papers have reported that aromatic medicinal plants are good sources of essential oil. The major and minor components present in the extract, *i.e.* phenolic and non-phenolic alcohols, are considered to be mostly antifungal agents. [25, 46] The antifungal activities have been reported in Cymbopogan, Ajowan, and Dill oils against *Colletotrichum lindemuthianum*.

[52] The essential oil of ajowan has been reported to inhibit some of the dermatophytes and are fungistatic towards *A. niger* and *T. violaceam*. [58] The aqueous extract of ajowan seeds contain aflatoxin inactivation factor. An approximately 80% reduction in total aflatoxin content over the control was observed. Moreover, it was observed that toxin decontamination in spiked corn samples could be achieved by using the aflatoxin inactivation factor. [44] The investigation of chemical constituents and antifungal effects of ajwain essential oil, *Trachyspermum ammi* (L.) Sprague (Apiaceae). [47]

The earlier researches have elucidated that Ajowan oil constitutes of thymol as the major component, *ca.* 40-60 % of its whole composition. [35, 43] The antimicrobial activity of essential oils is affected by their composition**.** Thus, it is highly possible that thymol is responsible for the antifungal activity of the oil. Ajowan oil has been also reported to possess nematocidal [50] and insecticidal [35] activities. However, reports on the antifungal activity of ajowan oil are relatively rare, especially against phytopathogenic strains of *F. oxysporum*. *T. cordifolia* was tested for their antifungal potential against eight important species of *Aspergillus* such as *A. candidus*, *A. columnaris*, *A. flavipes, A. flavus*, *A. fumigatus, A. niger, A. ochraceus,* and *A. tamari*. [40] *T. cordifolia* exhibited antibacterial activity against both Gram-positive and Gram-negative bacteria. [53] Pre-treatment with *T. cordifolia* was to impart protection against mortality induced by intra-abdominal sepsis following coecal ligation in rats. [49]

Moringa is native to India but has been planted and naturalised in many areas around the world. [8] The use of plant extracts and biocontrol agents have been seen as a viable method for controlling plant diseases. [55, 45, 14, 29] The findings from the present study indicate that Moringa bark extracts was effective against mycelial growth of *F. solani* and *F. lycopersici*. No growth was recorded at concentration of 75%. The results indicate that the plant extract affected the mycelial growth and no growth was observed. This confirms the antifungal activities exerted by Moringa extracts against fungal pathogen mycelium. [55, 45]

The fungicidal effect of Moringa leaf extracts on some soil-borne fungi such as *Rhizoctonia, Pythium* and *Fusarium*. [55] *Moringa oleifera* provides a rich and rare combination of zeatin, quercetin, b-sitsterol, caffeoylquinic acid and kaempferol which have antifungal and antibacterial activities. [2, 37] The co-ordinance of the chemical literature finding resistant strains of organism plant biodiversity may lead to unexpected research findings. [28] Exploitation of naturally available chemicals from plants, which retards the reproduction of undesirable microorganisms, would be a more realistic and ecologically sound method for plant protection and will have a prominent role in the development of future commercial pesticides for crop protection strategies with special reference to the management of plant diseases. [12]

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