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DETECTION OF ANTIFUNGAL ACTIVITY OF VARIOUS PLANT EXTRACTS AGAINST *ALTERNARIA SOLANI*, THE CAUSE OF EARLY BLIGHT OF TOMATO

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ABSTRACT

Alternaria solani, the cause of early blight of tomato, is an important plant pathogen from economic standpoint. An attempt was made to select locally occurring plant species with fungicidal potential. Fifteen plant species were selected from local flora and gardens for evaluation of their antifungal potential using poison food technique against *Alternaria solani* in aqueous extracts at 4% concentration in Potato Dextrose Agar (PDA). ANOVA disclosed that 8 plants out of 15 significantly reduced the mycelial growth of *Alternaria solani*. The following species inhibited the radial growth of *Alternaria solani* by more than 60 percent. *Tephrosia purpurea* (72%), *Capsicum annum* (70%), *Gliricidia sepium* (stem) (70%), *Cleome viscosa* (69%), *Caesalpinia bonduc* (67%), *Cassia fistula* (fruit) (63%), *Azadirachta indica* (62%) and *Cassia alata* (62%). Farming of these plants in local gardens should be practiced to obtain sufficient quantities of these non-hazardous and eco-friendly potent fungicides.

Key-words: Antifungal activity, plant extract, *Alternaria solani*, tomato, early blight.

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) belongs to economically one of the most important family (Solanaceae) that includes a number of vegetables that are used directly in homes and also used as processed food (Canene-Adams *et al*., 2005) .Tomato is affected by many plant pathogens but early blight is the most catastrophic disease on tomato caused by the fungus *Alternaria solani* causing heavy economic losses (Mathur and Shekhawat, 1986). Herriot *et al*., (1986) stated that use of fungicides is the most effective control measure but are not economically feasible since they involve health risks in case of edible products such as tomato. The use of resistant varieties is an environmental friendly method against early blight may be desirable but resistant varieties are either not available or expensive to develop and require huge investment and technical knowledge. In these circumstances, the current investigation is aimed to develop eco-friendly management method against early blight. The use of herbal extract in plant disease management is most significant by being an eco-friendly and cost-effective strategy. The potential involvement of antimicrobial plant metabolites in plant defense system against microbial invaders is one of the longest studied plant responses to pathogens (Link *et al*. 1929). Many researchers have shown that plant extracts effectively control various plant pathogens *in vitro* (Mishra *et al*. 2009; Sankara Subramanian *et al*. 2008; Yanar *et al*. 2011; Talibi *et al*. 2012). Use of plant extract *in vivo* to control airborne plant pathogenic microbes by inactivating or killing the pathogen spores as they land on plant surface has been recommended (Leksomboon *et al*. 2001;Govindappa *et al*. 2011). Essential oils and plant extracts showed antifungal activity against a wide range of fungi (Grane & Ahmad, 1988; Wilson *et al*., 1997; Abd-Alla *et al*., 2001). Alkhail (2005) observed that aqueous extracts of plants viz., *Cymbopogon proxims, Allium sativum, Carum carvi, Eugenia caryophyllus* and *Azadirachta indica* showed strong antifungal activity against fungi such as *Botrytis cinerea, Fusarium oxysporum* and *Rhizoctonia solani.* Plants are the richest resource of drugs in traditional systems of medicine, food supplements, folk medicines, modern medicines, nutraceuticals, pharmaceutical intermediates and chemical entities for synthetic drugs (Hammer *et al.*, 1999).The major aim behind the present study was to use plants from local natural flora and gardens to screen plants whose extracts have antipathogenic potential against *Alternaria solani.* These may in turn be developed as effective fungicides.

MATERIAL AND METHODS

1. Collection, isolation and identification of *Alternaria solani*

Diseased samples like leaf and fruits were collected from field of Federal Urdu University, Karachi. To isolate the pathogen, infected portions of leaf and fruit samples were surface sterilized (70% ethanol and 0.1% mercuric chloride) and cultured on Potato Dextrose Agar (PDA). Plates were incubated for seven days at 25 °C. Cultured fungus was identified based on morphological characters of spores and mycelium.

2. Collection of Plant Material

Plants were selected from local flora usually on the basis of the reported presence of antimicrobial properties in accordance with the available literature. After collection, the plant material was washed with tap water. The plant material was surface sterilized with 1% sodium hypochlorite solution and subsequently oven dried at 40 °C and grinded to form powder.

3. Screening Bioassays Preparation of Plants Extract

16 g of each plant was macerated with 40mL of distilled water. For exudation of biochemicals the macerated tissue was kept overnight and biomass was filtered using Whatman No. 1 filter paper. The filtered extracts were sterilized and stored at 4 °C as 40% stock solutions.

Table 1. List of plants.

S. NO	Name of Plants	Part used
1	*Anona squamosa* L.	Young Leaf
2	*Anona squamosa* L.	Young Stem
3	*Aloe perfoliata var. vera* L.	Young leaf
4	*Azadirachta indica* A. Juss.	Young twigs
5	*Capsicum annum* L.	Young fruits
6	*Cassia alata* L.	Young twigs
7	*Caesalpinea bonduc* (L.) Roxb.	Young leaf
8	*Cassia fistula* L.	Young fruits
9	*Cathranthus roseases* L.	Young twigs, flowers
10	*Cleome viscosa* L.	Young leaf
11	*Gilricidia sepium* L.	Young Stem
12	*Gilricidia sepium* L.	Young leaf
13	*Mentha arvensis* L.	Young twigs
14	*Ocimum basilicum* L.	Young twigs, flowers
15	*Tephrosia purpurea* L.	Young twigs
16	*Thespesia pupulnea* L.	Young twigs
17	*Trigonella foenum-graecum* L.	Young twigs

4. Determination of the efficacy of plant extracts against *A. solani* by poison food technique

By the help of poison food technique efficacy of plant extracts against *A. solani in vitro* was determined.2.2mL of Plant extract from the each stock solution (40% concentration) was added to 20mL of sterilized PDA medium in a conical flask, sterilized in autoclave and poured4% of extract in Petri plates. 5mm (diameter) disc of 7 day old culture of *Alternaria solani* was placed in the centre of the Petri plates. Plates containing medium with fungicide (Allette 0.2%) served as a positive control and for the negative control plates with medium added with 2.2mL distilled water. Three replicates were maintained for each treatment and Plates were incubated at 28 °C. After six days of inoculation radial growth of mycelium was measured. Results were compared with negative control and mean of observations was taken, experiment was repeated twice. Using following formula for the percent inhibition of the fungus in treatments was calculated.

$$\text{Germination inhibition (\%)} = \frac{\text{Growth in control} - \text{Growth in treatment}}{\text{Growth in control}} \times 100$$

5. Statistical Analysis

Data on inhibition of mycelia growth of *A. solani* were subjected to one-way analysis of variance (ANOVA). The follow-up of ANOVA included Fisher's least significant test (LSD) at p=0.05 and Duncan's multiple range test.

RESULTS

Efficacy, as determined by poisoned food technique, of different plants extracts *in vitro* against *A. solani* was evaluated and efficacy of test botanicals in inhibiting the mycelial growth of *A. solani* is presented in Table 2. The data revealed that all the test plants significantly inhibited mycelial growth of *A. solani* at 4% concentrations.

Table 2. Antifungal activity of aqueous plant extracts at 4% concentration.

S NO	Name of Plants	Part used	Colony diameter	Significance *	% of inhibition
1	Control		6 ± 0	a	0
2	Fungicide		0 ± 0	h	100
3	*Anona squamosa*	Young Leaf	4.56 ± 0.29	bc	24
4	*Anona squamosa*	Young Stem	5.83 ± 0.12	a	17
5	*Aloe perfoliata var. vera*	Young leaf	5.03 ± 0.29	b	16.1
6	*Azadirachta indica* .	Young twigs	2.26 ± 0.14	fg	62.3
7	*Capsicum annum*	Young fruits	1.8 ± 0.11	g	70
8	*Cassia alata*	Young twigs	2.36 ± 0.38	fg	60.6
9	*Caesalpinia bonduc*	Young leaf	1.93 ± 0.42	fg	67.8
10	*Cassia fistula*	Young fruits	2.2 ± 0.15	fg	63.3
11	*Cathranthus roseases*	Young twigs, flowers	3.46 ± 0.20	de	42.3
12	*Cleome viscosa*	Young leaf	1.83 ± 0.14	g	69.5
13	*Gilricidia sepium*	Young Stem	2.86 ± 0.91	e	52.3
14	*Gilricidia sepium*	Young leaf	1.8 ± 0.15	g	70
15	*Mentha arvensis*	Young twigs	4.46 ± 0.26	bc	25.6
16	*Oscimum basilicum*	Young twigs, flowers	4.2 ± 0.20	bcd	30
17	*Tephrosia purpurea*	Young twigs	1.66 ± 0.06	g	72.3
18	*Thespesia pupulnea*	Young twigs	4.06 ± 0.52	cd	32.3
19	*Trigonella foenum-graecum*	Young twigs	2.93 ± 0.26	ef	51.1

$LSD_{0.05}$= 0.7381 * Means sharing a common letter are not significantly different at $p \leq 0.05$.

Results given in Table 2 showed that 8 plants out of 15 significantly reduced the mycelial growth of the *Alternaria solani* by more than 60 percent which included: *Tephrosia purpurea* (72%), *Capsicum annum* (70%), *Gliricidia sepium* (stem) (70%), *Cleome viscosa* (69%), *Caesalpinia bonduc* (67%), *Cassia fistula* (fruit)(63%), *Azadirachta indica* (62%) and *Cassia alata* (62%). The result of ANOVA disclosed that the treatments were highly significant ($p<0.001$).

Table 3.Results of analysis of variance (ANOVA).

Source of Variation	SS	df	Ms	F	P
Treatment	185.73	18	10.318	37.96	< 0.001
Error	15.99	57	0.271	-	
Total	201.22	75	-	-	

DISCUSSION

Efforts were made to use different plant extracts or their products to manage plant diseases. Numerous plants have been reported to possess antifungal activity (Shekhawat and Prasada, 1971; Dixit and Tripathi, 1975; Neeraj, 2010; Derbalah *et al*., 2011). Some have employed leaf extracts of datura and mentha or bulb extracts of garlic (Shivpuri *et al*., 1997; Shivpuri and Gupta, 2001; Chattopadhyay *et al*., 2002; Singh *et al*., 2003). Numerous reports have been made to control pathogenic fungi and plant diseases by the use of plant extracts. Chen and Dai (2012) stated that extract of *Cinnamomum camphora* effectively inhibited *Colletotrichum lagenariumin vitro*. Gomes de Saravia and Gaylardeb (1998) observed that aqueous extract of *Brassica nigra* showed antimicrobial activity against the bacteria *Pseudomonas* spp. Goussous *et al.* (2012) observed that extracts of *Rosmarinus officinalis* and *Salvia fructicosa* effectively inhibit *Sclerotinia sclerotiorum*. Kagale *et al*. (2004) observed that leaf extract of *Datura metel* effectively inhibited the growth of *Rhizoctonia solani* and *Xanthomon as*. Skinner (1955) explained that presence of antibiotic in the form of resinous, phenolic, non-volatile and gummy substances. Amonkar and Banergi (1971) stated such antimicrobial properties are due to presence of fixed oils in *D. stramonium* and diallyl-disulphide and diallyl-tri-sulphide in *Allium sativum*. Roopa (2012) found similar results on the efficacy of different plant extracts against *Alternaria* spp. Several researchers have reported almost similar finding on *in vitro* effect of different plant extracts on *Fusarium solani* and other *Fusarium* spp. (Vimala *et al*., 1993; Arya *et al*., 1995; Lolpuri, 2002). Anonymous (1972) has attributed the presence of essential oils in tulsi (*Ocimum sanctum*) and garlic (*Allium sativum*) and fixed oils in datura (*D. alba*) and canabinol in cannabis (*Canabis sativa*) are also responsible for such inhibition. Vanitha (2010) stated that green oil (EC formulation) exhibited 100% inhibition of mycelial growth of *Alternaria chlamydospora*. Similar results on the efficacy of different plant extracts against *A. alternate* have also been reported by, Zaker and Mosallanejad (2010) and Raghavendra *et al.* (2009). Suleiman (2010) reported that pawpaw leaves showed highest mycelial growth inhibition against *A. solani.* Ravi Kumar and Raj Kumar (2013) observed that 13 plant extracts significantly reduced the mycelial growth of *A. solani.* All the antifungal plants produce antifungal principles that provide the biocontrol mechanism. According to Castillo *et al*., (2012) the application of bioactive phytochemicals derived from plants with antifungal properties represent an attractive alternative to inhibit the growth of several fungal pathogens instead of using hazardous chemical fungicides. These bioactive compounds are naturally produced in certain plants called as secondary metabolites. The principal groups that exhibit antifungal activity are phenolics, terpenes, tannins, flavonoids, essential oil, alkaloids, lecithin and polypeptides. Such groups of compounds play a significant role in the physiology of plants contributing properties that confer resistance against various types of pathogens as well as stresses such as heat shock, heavy metal and UV-B exposure.

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