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Management of early blight of tomato through the use of plant extracts

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Abstract
Early blight of tomato caused by *Alternaria solani* (Ellis & Martin) Sorauer is the most serious and destructive disease worldwide. In the present study, five plant extracts were used viz; *Azadirachta indica* (Neem), *Allium sativum* (Garlic), *Parthenium hysterophorus* (Chatak Chandni), *Datura stramonium* (Datura) and *Eucalyptus camaldulensis* (Safeda) against this disease. Experiment was laid out in Completely Randomized Design (CRD) with three replications by poisoned food technique. Mycelial growth and inhibition percentage of *A. solani* were recorded after 3, 5 and 7 days post application. All the tested plant extracts significantly inhibited the mycelial growth of the pathogen when compared with control. However, among all five tested plant extracts *Azadirachta indica* (69.65%) was significantly superior over other treatments followed by *Allium sativum* (66.15%), *Parthenium hysterophorus* (59.94%) and *Datura stramonium* (49.46%). Least inhibition was observed in *Eucalyptus camaldulensis* (49.31%). Overall results demonstrated that all the tested concentrations of *Azadirachta indica* were found significantly effective for controlling early blight of tomato.

Keywords: *Alternaria solani*. Colony growth. Inhibition percentage. *Solanum lycopersicum* L

1. Introduction
Tomato (*Solanum lycopersicum* L.) is an obligatory enthusiastic vegetable and fruit crop belongs to Solanaceae family. It is a most versatile plant used as in both forms, natural (raw material) and as element of other products. In Pakistan, yield of tomato is low as compared to other developed countries due to attack of several diseases caused by fungi, bacteria, viruses, nematodes. Among the fungal diseases early blight is caused by *Alternaria solani* (Ellis and Martin) [1-3]. During all stages of plant development, *A. solani* causes disease (leaf blight, stem rot, fruit lesions) and results in severe damage across the country [4]. Symptoms include small to irregular brown spots that give "bull eye" appearance on older plant leaves. These spots enlarge in diameter lead to concentric rings surrounded by yellow halo under suitable environmental conditions. Seedling, stem, blossom blight and fruit drop symptoms also produced by this pathogen [5].

The disease is managed by the use of several conventional fungicides [6] but due to development of resistance in most common pathogenic fungi against fungicides and also the factor of exposure risks, fungicide residues and human health hazards have given a push for obtaining alternatives to control *A. solani*. It is necessary to adopt suchs control measures that are ecologically sound and environmentally safe. In this regard natural products are considered to be the best as alternative to synthetic chemicals due to less negative environmental impact. Many botanicals used for this purpose for reducing spores production of foliar pathogens and also controlling disease development [7, 8]. Plant extracts also have antimicrobial activity for controlling early blight and other plant diseases both *in vitro* as well as *in vivo* [9, 10]. Aqueous extract of *Decalepis hamiltonii* showed antifungal activity against different species of *Fusarium*, *Aspergillus*, *Penicillium*, *Drechslera* and *Alternaria* [11]. Seed and leaf extracts of neem exhibited anti-fungal property and also used as insecticide for controlling agricultural insect pests [12]. The objective of the present study is to evaluate the antifungal activity of five plant extracts through food poison technique against *A. solani* under *in vitro* conditions.

2. Materials and Methods
A comparative study for the evaluation of plant extracts (Table 1) i.e., Leaves of *Azadirachta indica*, *Allium sativum*, *Parthenium hysterophorus*, *Datura stramonium* and *Eucalyptus camaldulensis* at 5, 10 and 15% concentrations was carried out at laboratory of plant pathology, University College of Agriculture, University of Sargodha, Sargodha, Pakistan by poisoned food technique [13] to evaluate the significant better plant extract for the control of *A. solani* associated with early blight of tomato.

Table 1: Plants used for antifungal activity assay

Botanical name	Common name	Family
Azadirachta indica	Neem	Meliaceae
Allium sativum	Garlic	Liliaceae
Parthenium hysterophorus	Chatak Chandni	Asteraceae
Datura stramonium	Datura	Solanaceae
Eucalyptus camaldulensis	Safeda	Myrtaceae

2.1 Preparation of plant extracts
Leaves of *A. indica*, *A. sativum*, *P. hysterophorus*, *D. stramonium* and *E. camaldulensis* were washed with distilled water and then surface sterilized with 1% sodium hypochlorite solution. Leaves were homogenized in sterile distilled water at 1:1 (w/v) and filtered through a muslin cloth to produce a 100% crude extract. For preparing of fresh garlic extract the outer, dry peel of cloves was first removed, surface-sterilized

for 2 min in ethanol and washed thrice in sterile distilled water. Cloves were crushed into a pulp and filtered through a muslin cloth. All the prepared plant extracts were heated for 10 to 15 minutes to avoid contamination. These sterilized crude extracts were considered as representative to 100% concentration, and serial dilutions (5, 10 and 15%) were prepared using sterilized distilled water.

2.2 Preparation of extracts concentration

The required quantity of each plant extract was added separately so as to get a requisite concentration by using potato dextrose agar which was used as nutrient medium. The plant extracts were carefully mixed by stirring and about 15 ml poisoned medium was poured to each of the 9 cm petri dishes and allowed for solidification.

The actively growing periphery of the seven days old culture of *A. solani* was carefully cut using a gel cutter and transferred aseptically to the center of each petri plate containing the poisoned medium. Potato dextrose agar (PDA) plates without the plant extracts used as control. The plates were incubated at 26±1°C for seven days and the colonies diameter were recorded.

2.3 Statistical analysis

Data regarding plant extracts concentrations on pathogen growth were analyzed statistically by using R-software (Ri386 2.15.3) program and their means were separated by the test of least significant difference (LSD) at the 0.05% of the probability level [14]. Percent inhibition of mycelial growth compared to control was calculated. Percent inhibition over control calculated by formula: I=100*(C-T)/C [15].

Whereas,

I = Inhibition percentage, C = Control (check) T = Treatment

3. Results and Discussion

3.1 *In vitro* evaluation of plant extracts

Five plant extracts, belonging to the different families were selected and evaluated for antifungal activity in laboratory for their effectiveness against *A. solani*, causative agent of early blight of tomato. Plant extracts tested at three concentrations (5, 10 and 15%) each by poisoned food technique. The results indicated that there were significant difference among tested plants extract for inhibiting the mycelial growth of the pathogen.

The plant extracts evaluated after seven days of colony growth by taking average mycelial growth and inhibition percentage (Table 4). Results revealed that all tested extracts at all tested concentrations were significantly ($P \leq 0.01$) reduced linear growth and increased inhibition percentage compared to control. Among tested plant extracts, *A. indica* was the most effective in decreasing the linear growth and increasing the inhibition percentage of *A. solani* (42.13 mm and 42.44%) followed by *A. sativum* (43.77 mm and 40.20%), *P. hysterophorus* (46.94 mm and 35.88%) and *D. stramonium* (51.16 mm and 30.11%) respectively while, *E. camaldulensis* was the least effective extract with 53.88 mm mycelial growth an-d 26.40% inhibition. Data (Table 2-4) showed individual performance of plant extracts (all tested doses) at third, fifth and seventh day of colony growth. Among the different plant extracts tested, *A. indica* at 15 % concentration (69.65%) was very effective in controlling the pathogen growth followed by *A. sativum*, *P. hysterophorus*, *D. stramonium* (66.15%, 59.94%, 49.46%, 49.31%) respectively. The least inhibition of mycelial growth of pathogen was observed in *E. camaldulensis* (21.85%) at 5 % concentration after *D. stramonium* (25.03%), *P. hysterophorus* (28.53%), *A. sativum* (32.92%) and *A. indica* (35.65%).

Table 2: Mycelium growth (mm) and inhibition percentage after 3 days colony of *A. solani* on PDA influenced by different concentrations of different plant extracts

Plant extract	Concentration %					
	Linear area	Control	5%	10%	15%	Mean
Azadirachta indica	CG.	29.67±0.50^{a}	7.56±0.52^{b}	4.33±0.5^{c}	3.22±0.44^{d}	11.9
	IP.	00	74.51	85.40	89.14	62.28
Allium sativum	CG.	29.67±0.50^{a}	10.67±0.5^{b}	5.44±0.52^{c}	5.44±0.52^{d}	12.80
	IP.	00	64.03	81.66	81.66	56.83
Parthenium hysterophorus	CG.	29.67±0.50^{a}	9.33±0.50^{b}	5.44± 0.52^{c}	3.44±0.52^{d}	11.97
	IP.	00	68.55	81.66	88.40	59.65
Datura stramonium	CG.	29.67±0.50^{a}	8.56±0.52^{b}	6.56± 0.52^{c}	4.22±0.44^{d}	12.25
	IP.	00	71.14	77.89	85.77	58.7
Eucalyptus camaldulensis	CG.	29.67±0.50^{a}	10.4±0.52^{b}	9.22± 0.66^{c}	7.67±0.50^{d}	14.25
	IP.	00	64.81	68.92	74.14	51.96

CG= Colony growth IP=Inhibition percentage Means within the column with same letters are statistically non-significant

Table 3: Mycelium growth (mm) and inhibition percentage after 5 days colony of *A. solani* on PDA influenced by different concentrations of different plant extracts

Plant extract	Concentration %					
	Linear area	Control	5%	10%	15%	Mean
Azadirachta indica	CG.	54.00±0.70^{a}	22.670±.5^{b}	10.89±0.70^{c}	9.44± 0.52^{d}	24.25
	IP.	00	58.01	79.83	82.51	55.08
Allium sativum	CG.	54.00±0.70^{a}	24.89±0.7^{b}	9.89± 0.78^{c}	9.44± 0.72^{c}	24.55
	IP.	00	53.90	81.65	82.51	54.51
Parthenium hysterophorus	CG.	54.00±0.70^{a}	24.89±0.7^{b}	14.00±0.72^{c}	10.56±0.52^{d}	25.86
	IP.	00	53.90	74.07	80.44	52.10
						29.66
Datura stramonium	CG.	54.00±0.70^{a}	26.00±0.7^{b}	21.1±10.7^{c}	17.56±0.52^{d}	
	IP.	00	51.85	60.90	67.48	45.05
Eucalyptus camaldulensis	CG.	54.00±0.70^{a}	27.89±0.6^{b}	23.78±0.83^{c}	18.33±0.70^{d}	31
	IP.	00	48.35	55.96	66.05	42.59

CG= Colony growth IP=Inhibition percentage Means within the column with same letters are statistically non-significant

Table 4: Mycelium growth (mm) and inhibition percentage after 7 days colony of *A. solani* on PDA influenced by different concentrations of different plant extracts

Plant extract	Concentration %					
	Linear area	Control	5%	10%	15%	Mean
Azadirachta indica	CG.	73.22±0.83^{a}	47.11±0.78^{b}	26.00±0.70^{c}	22.22±0.66^{d}	42.13
	IP.	00	35.65	64.49	69.65	42.44
Allium sativum	CG.	73.22±0.83^{a}	49.11±0.60^{b}	28.00±0.70^{c}	24.78±0.83^{d}	43.77
	IP.	00	32.92	61.75	66.15	40.20
Parthenium hysterophorus	CG.	73.22±0.83^{a}	52.33±0.50^{b}	32.89±0.78^{c}	29.33±0.70^{d}	46.94
	IP.	00	28.53	55.08	59.94	35.88
Datura stramonium	CG.	73.22±0.83^{a}	54.89±0.78^{b}	39.56±0.52^{c}	37.00±0.70^{d}	51.16
	IP.	00	25.03	45.97	49.46	30.11
Eucalyptus camaldulensis	CG.	73.22±0.83^{a}	57.22±0.83^{b}	48.00±0.70^{c}	37.11±0.78^{d}	53.88
	IP.	00	21.85	34.44	49.31	26.40

CG= Colony growth IP=Inhibition percentage Means within the column with same letters are statistically non-significant

4. Discussion

Selected plant extracts are used against early blight of tomato for management of this disease. A number of plants have been reported to own antifungal activity [16-18]. Thus, an effort has been made to explore locally available plant extracts and therefore the effect of five plant extracts i.e. *A. indica*, *A.* sativum, *P. hysterophorus*, *D. stramonium* and *E. camaldulensis* at three concentrations (5, 10 and 15%) were evaluated through poisoned food technique *in vitro* for their inhibitory effect on the linear growth of *A. solani*. The efficacy of different extracts belongs to different plant species other than the tested extracts on the growth of the pathogen have been reported by several researchers [19-23]. In present study, all tested botanicals significantly reduced mycelial growth of the pathogen. Among all plant extracts used, leaf extracts of *A. indica* were highly effective in inhibiting the linear growth of *A. solani* (69.65%) at 15% concentration followed by other plant extracts. While least inhibition was observed in extract of *E. camaldulensis* (21.85%) at 5%. The inhibitory effect of these extracts may be due to their direct lethal effect on the pathogen growth or antimicrobial activity against fungal pathogens under *in-vitro* and *in-vivo* conditions [24]. Additionally, it is might be due to natural bioactive materials presented in these extracts [25]. The leaf extract of *Azadirachta indica* inhibited the mycelial growth of *A. solani* under *in vitro* conditions [26]. Antifungal activity of *A. indica* (neem) is due to different types of tetra terpenoids and phenolics compounds present which inhibited the mycelial production of *A. solani* [21]. Naz *et al.* [15] also reported antifungal activity of *A. indica* against *Rhizoctonia solani*. Hadian [27] reported that 98% inhibition of mycelial growth observed from *A. indica* extract against *Fusarium oxysporum* while 96% against *Rhizoctonia solani.* Similarly, Satya *et al.* [28] and Sing *et al.* [29] reported that extract of *A. sativum* inhibited growth of the pathogen. This might be due to presence of antimicrobial compound that was described mainly as Allicin which has antifungal activity. Antifungal activity of *D. stramonium* was due to the presence of phytochemicals reported by Eftekhar *et al.* [30]. Similar observations were reported by Amadioha [31], Joseph *et al.* [32] and Jabeen *et al.* [33], in which *A. indica* leaf extract gave significant results over others plant extracts when compared with control. Therefore, from the foregoing argument it may be accomplished that *A. indica*, a common medicinal plant could be used as the source of an effective biocide that has vast fungi toxic effect to several fungal pathogens including *A. solani* for controlling early blight of tomato.

5. References

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