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Efficacy of aqueous plant extracts against *Alternaria solani*, the causative agent of early blight of tomato

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Abstract
Aqueous extracts of 25 plants selected from local flora were evaluated for antifungal potential against *Alternaria solani* by Poisoned Food Technique and Dry Mycelial weight method at five different concentrations viz.10%, 20%, 30%, 40% and 50%. Maximum Inhibition activity was observed in Poisoned Food Technique, *Prosopis juliflora* recorded 88.9% inhibition at 50% concentration followed by *Carica papaya* (77.84%), *Ricinus communis* (70.5%) and *Polyalthia longifolia* (63%). Dry Mycelial weight method revealed 90.7% inhibition by *Prosopis juliflora*, followed by *Carica papaya* (89.47%), *Ricinus communis* (86.1%) and *Polyalthia longifolia* (79.2%). Significant inhibitory activity was also observed in 10, 20, 30 and 40% concentration in both the methods. Among the 25 plants tested *Prosopis juliflora, Carica papaya, Ricinus communis* and *Polyalthia longifolia* plant extracts significantly reduced the mycelial growth of the pathogen in both the tests conducted.

Keywords: Plant extracts; *Prosopis juliflora*; antifungal; *Alternaria solani*; Early blight of Tomato

1. Introduction
In recent years there has been a tremendous improvement in the cultivation and consumption of Tomato (*Lycopersicon esculentum*. Mill, Family Solanaceae) world over as an important vegetable and as an important raw material for many processed products. Processed products of tomato have become an important dietary component of the developed world and also developing countries Early blight caused by *Alternaria solani* is one of the most destructive diseases of tomatos in India. Every 1% increase in intensity reduces yield by 1.36%, and complete crop failure can occur when the disease is most severe [1]. Yield losses of up to 79% have been reported in the U.S.A of which 20-40% is due to seedling losses in the field [2]. *A. solani* is one of numerous tomato pathogens that is controlled with the same products, accurately estimating the total economic loss and the total expenditure on fungicides for the control of early blight is difficult. Best estimates suggest that total annual global expenditures on fungicide control of *A. solani* is approximately $77 million of which $32 million is for tomatoes [3].
One of the environment friendly methods is the use of resistant varieties against early blight but majority of the resistant varieties are not durable. In addition, the method is costly to develop and requires huge investment with great technical knowledge. Development of herbal formulations for management of plant diseases is an important eco-friendly and cost effective approach. In the present study, higher plants were screened for antifungal activity against *Alternaria solani* to select the potent aqueous plant extract that could be exploited for the disease management.

2. Materials and methods
2.1 Collection, isolation and identification of the pathogen
Different parts of the diseased plant materials like stem, leaf and fruits of tomato were collected from different fields in and around Mysore, Karnataka, India. Plant materials were thoroughly washed under running tap water and surface sterilized with 0.2% sodium hypochlorite for 2-3 minutes followed by repeated washing with sterile distilled water. Infected parts were dissected and placed on Petri plates lined with moistened sterile blotter discs and incubated at 28 ± 2° C for 24 - 48hrs. After 24hrs of incubation, the plates were examined under stereomicroscope. Fungal colonies showing typical sporulating structures were picked with a sterile inoculation needle and transferred on to PDA plates and incubated at 28 ± 2 °C for 7 days. After incubation identification of fungi was done based on the morphological and cultural characters. Pure culture of *Alternaria solani* was sub cultured on PDA plates and maintained at 4 °C.

2.2 Preparation of plant extracts

Apparently healthy leaves of all plant materials (Table 1) were collected and washed thoroughly in running tap water. Hundred gm of fresh material were macerated with 200ml of distilled water in a waring blender and filtered through three layered muslin cloth, then centrifuged at 8000rpm for 25min. The supernatant was filtered through whatman No. 1 filter paper and sterilized at 120^0C. The extracts were preserved aseptically in sterile brown bottle at 4 °C until further use.

2.3 Antifungal activity of plant extracts

a. Poison food technique

Potato dextrose agar plates with desired concentration of extracts viz., 10%, 20%, 30%, 40% and 50% were prepared and 8mm diameter disc of 7-day old fungal culture of *Alternaria solani* was inoculated at the centre of the plate and incubated at 28±2 °C for 7 days [4]. Triplicates were maintained for each concentration. Radial growth of mycelia was measured in mm after 7 days of incubation and percentage of growth inhibition was calculated using the formula,

$$\% \text{ of growth inhibition} = \frac{gc\text{-}gt \times 100}{gc}$$

Where, gc= Average increase in mycelial growth in control.
gt= Average increase in mycelial growth in treatment.

b. Dry mycelial weight method

Potato Dextrose broth with different concentrations of plant extracts viz., 10%, 20%, 30%, 40% and 50% was prepared. Eight mm diameter mycelia discs of 7 days old culture of *Alternaria solani* was inoculated to the medium. Triplicates were maintained for each treatment. Inoculated flasks were incubated at 28±2°C for 7 days. After the incubation period, the contents of each flask was filtered through a pre weighed Whattman no. 1 filter paper. The filter papers with the mycelial mats were dried in an oven at 60°C until constant weights were obtained. The dry mycelial weight was determined by subtracting the weight of the filter paper from the total weight. Appropriate controls were maintained [5].

c. Spore germination inhibition activity by Cavity slide method

Plant extracts of desired concentration and volume were added to the cavity of a cavity slide. Known volume of spore suspension of test fungi was spread over the film and stirred. Slides were then placed on a glass rod in Petri dish under moistened conditions and incubated for 28±2 °C for two days. After the incubation period, slides were observed for germination of spores [6]. Distilled water served as control. Percentage of spore germination was calculated according to the following formula.

% Spore Germination = [Number of spores germinated/ Total number of Spores] X 100

Table 1: List of Test Plants.

S. No	Plants Used	Family
1.	*Achyranthes aspera* L.	Amaranthaceae
2.	*Brugmansia suaveolens* Willd.	Solanaceae
3.	*Carica papaya* L.	Caricaceae
4.	*Catharanthus roseus* (L.) G Don	Apocynaceae
5.	*Cycas circinalis* L.	Cycadaceae
6.	*Delonix regia* Linn	Fabaceae
7.	*Filicium decipens* Thw.	Sapindaceae
8.	*Lantana camara* L.	Verbenaceae
9.	*Leucas aspera* (Willd)	Lamiaceae
10.	*Manilkara zapota* L.	Sapotaceae
11.	*Mirabilis jalapa* L.	Nyctaginaceae
12.	*Nerium oleander* L	Apocynaceae
13.	*Peltophorum inerme* (Roxb.)	Fabaceae
14.	*Polyalthia longifolia* Hook.f. & Thom	Annonaceae
15.	*Pongamia pinnata* L.	Fabaceae
16.	*Prosopis juliflora* (Sw.) DC	Fabaceae
17.	*Ricinus communis* L.	Euphorbiaceae
18.	*Senna siamea* (Lam.)	Fabaceae
19.	*Swietenia macrophylla* King	Meliaceae
20.	*Synadenium grantii* Hook	Euphorbiaceae
21.	*Tecoma argentea* Britton	Bignoniacea
22.	*Thevetia peruviana* (Pres.) K. Schum	Apocynaceae
23.	*Thunbergia fragrans* Roxb.	Acanthaceae
24.	*Tribulus terrestris* L	Zygophyllaceae

3. Results & Discussion

a. Poisoned food technique

Among the 25 plants tested *Prosopis juliflora* recorded maximum inhibition of 88.92% at 50% concentration, *Carica papaya* leaf extract showed 77.84% at 50%concentration and 72.68% inhibition at 40%concentration (Table-2). Significant inhibitory activity was also observed at 10%, 20% and 30% concentration.

In Case of *Ricinus communis*, 70.53% inhibition was recorded at 50% concentration, 61.31% at 40% concentration and 49.72% inhibition at 30% concentration. 63.03% inhibition at 50% concentration and 51.86% inhibition at 40% concentration in *Polyalthia longifolia*. Complete mycelial growth inhibition was observed when treated with Mancozeb at recommended dosage (2g/l).

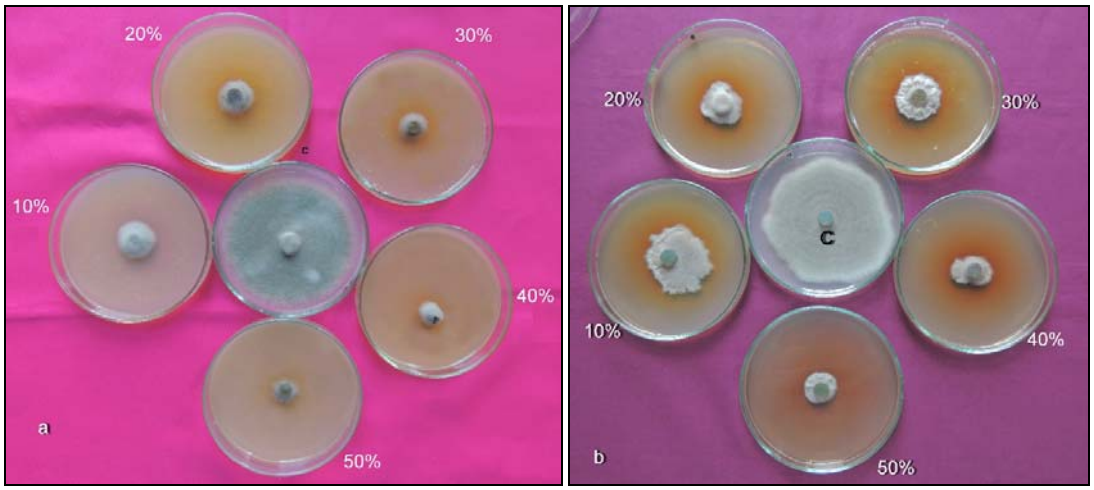

Fig 1: a. Inhibition of Mycelial growth by *Prosopis juliflora* extract b. Inhibition of Mycelial growth by *Carica papaya* extract.

b. Dry mycelial weight method

Antifungal activity by dry weight method also revealed 90.70% mycelial growth inhibition in *Prosopis juliflora* extract followed by *Carica papaya* (89.47%) and 79.24% in *Polyalthia longifolia* extract at 50% concentration (Table 3). Significant activity was also observed in 10%, 20%, 30% and 40% concentration in both the extracts. When compared to synthetic fungicide plant extracts have equally shown significant activity. 100% Mycelial growth inhibition was observed when treated with Mancozeb at recommended dosage (2g/l).

c. Spore germination inhibition activity by Cavity slide method

Highest spore germination inhibition percentage of 92% was observed in *Prosopis juliflora* extract followed by *Carica papaya* extract (86%). The present study indicated that the inhibitory effect of the plant extracts of *Prosopis juliflora, Carica papaya, Polyalthia longifolia* and *Ricinus communis* on *Alternaria solani* spore germination which might be credited to the presence of some partially effective antifungal ingredients.

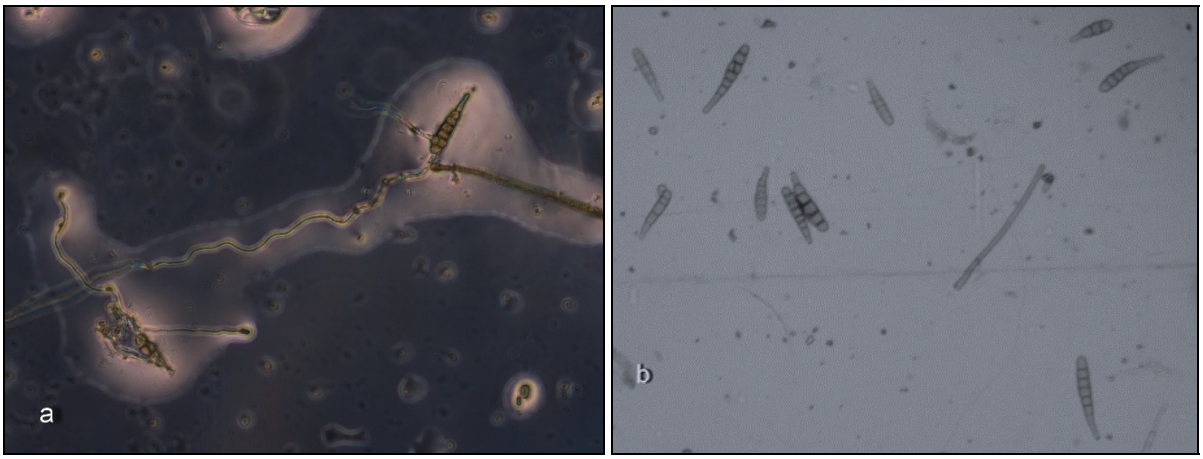

Fig 2: a. Spore germination in sterile water, b. Inhibition of spore germination in *Prosopis juliflora* extract

The Plants world is a rich storehouse of natural chemicals that could be exploited foe use as pesticides and fungicides. The total number of plant chemicals may exceed 4,000,000 and of these 10,000 are reported to be found secondary metabolites whose play a major role in the plants is reportedly defensive [7]. The increasing demand of production and regulations on the use of agrochemicals and the emergence of pathogens resistant to the products employed, justifies the search for novel active molecules and new control strategies.

Formulated plant extracts and oils have been investigated for control of diseases caused by *Phytophthora* spp. [8] Soil infested with chlamydospores of *Phytophthora nicotianae* was treated by incorporating 1, 5 and 10% aqueous emulsions of formulations containing clove oil, neem oil, pepper extract, mustard oil, cassia extract, synthetic cinnamon oil and the fungicide metalaxyl. In the greenhouse after 35 days, 10% aqueous emulsions of pepper extract-mustard oil formulation, cassia extract and the synthetic cinnamon oil formulation suppressed disease development in periwinkle 93.0 to 96.7% compared with the non-treated infested soil. The observed reduction in the pathogen population and significantly healthier plants in the greenhouse has indicated that these formulations of plant extracts and oils could have important roles in biologically based management strategies for control of diseases caused by pathogens.

Table 2: Mycelial growth of inhibition of *Alternaria solani* by different concentrations of Test plant extracts by Poisoned Food Technique.

S. No.	Plant Name	Concentration of Test plant extracts				
		10%	20%	30%	40%	50%
1	*Achyranthes aspera* L.	04.26 ± 0.87	5.20 ± 0.84	10.53 ± 0.25	9.60 ± 0.68	15.07± 2.67
2	*Brugmansia suaveolens* Willd.	01.78 ± 0.35	0.40 ± 1.09	4.39 ± 0.58	3.83± 0.74	4.80 ± 0.48
3	*Carica papaya* L.	54.20 ± 0.56	68.53 ± .66	68.98 ± 0.67	72.68 ± 0.86	77.84 ± 0.74
4	*Catharanthus roseus* (L.) G Don	02.00 ± 0.34	4.60 ± 0.44	10.70 ± 0.77	16.50± 0.20	20.50 ± 0.23
5	*Cycas circinalis* L.	00.00 ± 0.00	0.00 ± 0.00	3.41± 0.92	2.45 ± 1.30	0.80 ± 1.077
6	*Delonix regia* Linn	11.00 ± 0.50	7.60 ± 0.35	10.50 ± 0.93	15.00 ± 0.57	13.00 ± 0.51
7	*Filicium decipens* Thw.	01.22 ± 0.62	5.29 ± 0.58	6.24 ± 0.49	2.97 ± 2.47	0.80 ± 1.01
8	*Lantana camara* L.	03.96 ± 0.81	12.68 ± 0.73	22.38 ± 0.69	24.35 ± 0.90	25.76 ± 0.83
9	*Leucas aspera* (Willd)	14.54 ± 0.22	13.02 ± 0.26	16.9 ± 0.37	18.29 ± 2.73	15.24 ± 2.65
10	*Manilkara zapota* L.	10.60 ± 0.36	14.64 ± 0.48	23.85 ± 0.61	31.80 ± 0.14	38.36 ± 0.43
11	*Mirabilis jalapa* L.	0.00± 0.00	0.00±0.00	3.42 ± 0.95	2.59 ±1.19	0.94 ± 0.98
12	*Nerium oleander* L	07.49 ± 1.41	13.45± 0.77	19.21 ± 1.51	26.47± 2.22	33.11 ± 1.20
13	*Peltophorum inerme* (Roxb.)	18.00 ± 0.15	31.00 ± 0.48	34.00 ± 0.27	38.70 ± 2.06	43.00 ± 0.83
14	*Polyalthia longifolia* Hook.f. & Thom	16.94± 0.75	28.63 ± 0.62	50.84 ± 1.70	51.86 ± 0.56	63.03 ± 0.46
15	*Pongamia pinnata* L.	0.97±0.37	0.83±0.24	1.11±0.60	0.83±0.64	1.53±0.13
16	*Prosopis juliflora* (Sw.) DC	65.67± 0.43	75.24 ± 0.24	82.76 ± 0.09	85.91 ± 0.52	88.92 ± 0.40
17	*Ricinus communis* L.	24.16± 0.16	44.83 ± 0.27	49.72 ± 0.31	61.31 ± 0.07	70.53 ± 0.21
18	*Senna siamea* (Lam.)	07.60± 0.52	9.81± 0.82	17.70 ± 0.78	18.80 ± 1.20	28.51 ± 0.57
19	*Swietenia macrophylla* King	10.72±0.37	14.91±0.54	24.23±0.39	31.75±0.17	38.30±0.40
20	*Synadenium grantii* Hook	03.85 ± 0.52	7.97 ± 0.91	15.83 ± 0.50	25.06 ± 0.98	30.43± 0.71
21	*Tecoma argentea* Britton	01.10 ± 1.71	9.97 ± 1.54	6.53 ± 2.43	7.60 ± 2.79	13.32± 1.06
22	*Thevetia peruviana* (Pres.) K. Schum	00.27±0.27	00.27±0.49	2.88±0.69	2.60±0.343	3.027±0.15
23	*Thunbergia fragrans* Roxb.	02.33 ± 0.15	04.39 ± 0.5	7.26 ± 0.47	10.68 ± 0.23	15.62 ± 0.18
24	*Tribulus terrestris* L	01.50 ± 0.59	02.32 ± 0.48	1.09 ± 0.36	0.81± 0.71	0.54 ± 1.06
25	*Tridax procumbens* (Linn)	03.10±0.35	24.87±0.29	20.68±0.24	33.11±0.37	14.46±0.26
26	Mancozeb (2g/l)	100.00±0.00				

*Values are the mean of three replicates ± standard error.

Table 3: Mycelial growth of inhibition of *Alternaria solani* by different concentrations of Test plant extracts by Dry Mycelial Weight Method and Spore Germination Percentage in different plant extracts.

S. No.	Plant Name	Concentrations of Test Plant Extracts					Spore Germination Inhibition (%)
		10%	20%	30%	40%	50%	
1	*Achyranthes aspera* L.	7.35 ± 2.14	7.72 ± 0.55	11.92 ± 2.16	11.71 ± 4.40	18.88 ± 4.72	07
2	*Brugmansia suaveolens* Willd.	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	13
3	*Carica papaya* L.	79.36 ± 0.47	82.37 ± 0.41 0.406 0.406	86.61 ± 0.22	88.10 ± 0.74	89.47 ± 0.18	86
4	*Catharanthus roseus* (L.) G Don	0.00 ± 0.00	6.60 ± 3.80	3.00 ± 1.76	20.70 ± 2.40	23.10 ± 3.95	47
5	*Cycas circinalis* L.	0.00 ± 0.00	0.00 ± 0.00	6.78 ± 1.28	5.05 ± 0.94	7.15 ± 1.12	04
6	*Delonix regia* Linn	0.00 ± 0.00	6.10 ± 0.74	8.20 ± 0.70	19.30 ± 1.64	26.70 ± 0.90	15
7	*Filicium decipens* Thw.	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	05
8	*Lantana camara* L.	5.20 ± 0.34	7.82 ± 0.26	8.15 ± 0.32	11.90 ± 2.09	12.08 ± 2.49	41
9	*Leucas aspera* (Willd)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	14
10	*Manilkara zapota* L.	4.79 ± 0.59	8.14 ± 0.07	8.01 ± 0.38	8.50 ± 0.37	13.19 ± 5.23	29
11	*Mirabilis jalapa* L.	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	03
12	*Nerium oleander* L	9.48 ± 0.28	25.41 ± 0.19	30.20 ± 5.74	32.14 ± 2.04	49.95 ± 1.98	49
13	*Peltophorum inerme* (Roxb.)	9.00 ± 1.45	18.90 ± 0.28	28.70 ± 0.51	34.80±0.69	38.60 ± 0.59	68
14	*Polyalthia longifolia* Hook.f. & Thom	59.70 ± 2.30	64.26 ± 1.13	63.59 ± 1.30	74.19 ± 0.35	79.24 ± 0.55	79
15	*Pongamia pinnata* L.	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	15
16	*Prosopis juliflora* (Sw.) DC	83.00 ± 0.18	83.90 ± 0.23	86.80 ± 0.53	92.10 ± 0.54	90.70 ± 0.89	92
17	*Ricinus communis* L.	31.01 ± 1.09	70.59 ± 0.22	73.95 ± 0.34	79.12 ± 0.36	86.07 ± 0.36	86
18	*Senna siamea (Lam.)*	3.60 ± 0.45	7.00 ± 0.28	11.00 ± 1.86	17.00 ± 2.62	24.00 ± 0.16	33
19	*Swietenia macrophylla* King	6.73 ±02.65	11.46±03.01	20.51±01.32	27.10±0.68	30.98±0.40	30
20	*Synadenium grantii* Hook	0.00 ± 0.00	0.00 ± 0.00	4.38 ± 0.65	6.25 ± 0.50	7.35 ± 0.326	12
21	*Tecoma argentea* Britton	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	07
22	*Thevetia peruviana* (Pres.) K. Schum	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	12
23	*Thunbergia fragrans* Roxb.	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	2.423±0.265	4.796±0.468	09
24	*Tribulus terrestris* L	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	08
25	*Tridax procumbens* (Linn)	0.00 ± 0.00	1.19 ± 0.46	2.45 ± 0.48	5.05 ± 0.56	10.33 ± 0.60	20
26	Mancozeb (2g/l)	100.00 ± 0.00					100

*Values are the mean of three replicates ± standard error.

4. Conclusions

Many reports are available on the *in vitro* screening of plant extracts for antifungal and antibacterial activity. None of the early workers have screened the efficacy of aqueous plant extracts for the antifungal activity against *A. solani* the causal agent of early blight ot Tomato. The results of the present investigation clearly suggest that the plant extracts of *Prosopis juliflora, Carica papaya, Polyalthia longifolia* and *Ricinus communis* are important candidate plants for further experiments to develop formulations for the management of *A. solani.*

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