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Ragupathi KP
Ph.D., Scholar, Department of Seed Science & Technology, TNAU, Coimbatore, Tamil Nadu, India

PR Renganayaki
Professor and Head, Department of Seed Science & Technology, TNAU, Coimbatore, Tamil Nadu, India

S Sundareswaran
Director, Directorate of Seed Center, TNAU, Coimbatore, Tamil Nadu, India

S Mohan Kumar
Director, CPMB & B, TNAU, Coimbatore, Tamil Nadu, India

A Kamalakannan
Professor, Department of Plant Pathology, TNAU, Coimbatore, Tamil Nadu, India

Corresponding Author:
Ragupathi KP
Ph.D., Scholar, Department of Seed Science & Technology, TNAU, Coimbatore, Tamil Nadu, India

Efficacy of botanicals against *Alternaria solani* causing early blight of tomato

Ragupathi KP, PR Renganayaki, S Sundareswaran, S Mohan Kumar and A Kamalakannan

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Abstract
Tomato is one of the commercial vegetable crops, being affected with many diseases, among which early blight caused by *Alternaria solani* resulting in huge yield loss. As an alternative to the agro chemicals, botanicals were widely adopted owing to its eco friendliness. Several botanical extracts were prepared and screened against this seed borne pathogen. All treatment showed the varied antifungal activity against the pathogen. Among the screened botanicals percent growth inhibition of pathogen was higher in zimmu leaf extract (89%). Plant biometric observations *viz.*, seed germination (94%) and seedling vigor (2383) was maximum with lower percent disease index (12%) was recorded in zimmu leaf extract treated seeds and seedlings of tomato cultivar PKM 1.

Keywords: Botanicals, early blight, *Alternaria solani*, Zimmu

Introduction
Tomato (*Solanum lycopersicum* L.) is the second most important vegetable crop considered as "protective food" because of its high nutritive value. Tomato is found to suffer from a variety of disease caused by fungi, bacteria, viruses and nematodes. The important diseases include damping off, early blight, late blight, Fusarium wilt, *Verticillium* wilt, bacterial wilt and tomato mosaic virus. Among the diseases early blight caused by *Alternaria solani* (Ellis and Martin) Jones and Grout is one of the most severe disease in India can cause direct loss by the infection of fruits and indirect loss by reducing plant vigour and incurring loss both at pre and post-harvest stages causing 35 to 78 per cent reduction in yield (Jones *et al.*, 1993) [9]. The effective management strategy of the disease could be through cultural practices, chemical, biological control and use of resistant variety. Wide use of synthetic fungicides can cause environmental hazards and have ill effects on human beings and animals. The chemical fungicides not only develop fungicidal resistance but also accumulate in food and ground water as residues. In order to overcome these problems the development of alternative methods which are safe to the environment, non-toxic to humans and animals and are rapidly bio-degradable, one such strategy is use of botanicals to control fungal plant diseases. Plants are the richest source of organic chemicals and produce wide variety of eco-friendly secondary metabolites with antifungal activities (Riaz *et al.*, 2010) [18]. Use of botanicals in plant disease management assumes special significance by being an ecofriendly and cost effective strategy, which can be used in integration with other strategies for a greater levels of crop protection with sustained crop yields. Objective of the present investigation is to find out the eco-friendly management strategies for early blight of tomato.

Material and Methods
Collection and extraction of botanical extracts
Fresh samples of medicinal plants with antifungal constituents (listed in Table 1) were collected. 100 g of leaves from each botanical was washed thoroughly under running tap water, dried with blotting paper, cut into smaller pieces and ground using a sterile mortar and pestle by adding 100 ml of sterile distilled water. Finally, it was filtered through two layers of cheesecloth and the extract was then centrifuged at 10,000 rpm for 15 min and the supernatant alone was transferred to a fresh tube.

The extract was then sterilized using 0.2 µm disposable syringe filters. The filtrate (100%) was further diluted to required concentrations for further use (Tiwari and Singh, 2005) [20].

Symptomology of *Alternaria solani* on tomato

The first symptom of the early blight disease caused by *Alternaria solani* on tomato appeared as small brown water soaked lesions on the older leaf. The symptoms were oval or angular in shape from 1 to 4 mm diameter and there was a narrow chlorotic zone around the spot. These spots enlarged and covered the entire stem and petioles leading to withering of the plants. Symptoms also developed on calyx and flower buds in the form of minute brown to dark brown spots which enlarged later and spread to sepals and fruits resulting in pre-mature dropping of fruits.

Table 1: Botanicals used for testing their efficacy against *Alternaria solani*

S. No	Botanical name	Common name	Family	Part used
1	*Azadirachta indica Juss*	Neem	Meliaceae	Leaf
2	*Zingiber officinale*	Ginger	Zingiberaceae	Rhizome
3	*Allium sativum L*	Garlic	Liliaceae	Bulb
4	*Allium cepa*	Onion	Lilliaceae	Bulb
5	*Dhatura stramonium L*	Datura	Solanaceae	Leaf
6	*Ociumum tenuiflorum*	Tulsi	Lamiaceae	Leaf
7	*Lawsonia inermis*	Henna	Lythraceae	Leaf
8	*Psoralea corylifolia*	Bakuchi	Fabaceae	Seed
9	*Mentha citrata*	Mint	Lamiaceae	Leaf
10	*Catheranthus roseus*	Periwinkle	Apocynaceae	Leaf
11	Zimmu *(Allium cepa* L. × *Allium sativum* L.*)*	Zimmu	Lilliaceae	Leaf
12	*Aloe vera*	Indian aloe	Asphodelaceae	Leaf
13	*Vitex negundo*	Notchi	Lamiaceae	Leaf
14	*Eucalyptus globulus* Labill.	Eucalyptus	Myrtaceae	Leaf
15	*Nigella sativa L.*	Black cumin	Ranunculaceae	Seed
16	*Calotropis gigentea L*	Calotropis	Asclepiadaceae	Leaf
17	*Coleus forskohlii*	Medicinal coleus	Lamiaceae	Leaf
18	*Curcuma longa* L.	Turmeric	Zingiberaceae	Rhizome

Table 2: Description of disease scale (Datar and Mayee, 1986) [4].

Scale	Description
0	No symptoms on the leaf
1	0-5 per cent leaf area infected and covered by spot, no spot on petiole and branches
2	6-20 per cent leaf area infected and covered by spot, some spots on petiole
3	21-40 per cent leaf area infected and covered by spot, spots also seen on petiole, branches
4	41-70 per cent leaf area infected and covered by spot, spots also seen on petiole, braches, stem
5	>71 per cent leaf area infected and covered by spot, spots also seen on petiole, branch, stem and fruits

Pathogenicity test

The pure culture of *Alternaria solani* was obtained by single spore isolation method and sub culture was used for pathogenicity test by following Koch's postulate. The pathogenicity test was carried by pre-inoculation with spore suspension and homogenized mycelial bits of *A. solani* on foliage of 30 days old plants of PKM 1 cultivar of tomato. After inoculation, the symptoms appeared on inoculated leaves as brown, oval or angular necrotic spots with concentric rings and surrounded by a border of yellow host tissue. The fungus was re-isolated and purified culture from these artificially infected leaves was similar to that of original culture. The plants which were not inoculated with the fungal spore suspension did not show any symptoms of the disease. Thus pathogenicity on tomato was confirmed.

Antifungal activity assay of botanical extracts by using Poison food technique

Plant extracts at 10% concentration from the each stock solution were added in 20 ml of sterilized potato dextrose agar in petri plates. A 5 mm diameter of the actively growing mycelium disc of the pathogen of 6–7 day old culture was placed in the center of the petri dish. Plates without plant extract served as negative control. Plates were incubated at 27 °C. Triplicates were maintained for each treatment. Radial growth of mycelium was measured after seven days of incubation. The results were compared with negative control. The experiment was repeated thrice and mean of three readings was taken for calculations. The percent inhibition of the fungus in treatments was calculated using following formula

$$\text{Percentage of inhibition} = \frac{A - B}{A} \times 100$$

Where,

A= Radius of pathogen in control plate, B = Radius of pathogen in treatment plate.

Evaluation of botanicals against *Alternaria solani* of tomato

Seeds were treated with 10% botanical extracts for 12 h and dried back to original moisture content. Pin prick method of *Alternaria solani* inoculation was followed. The leaves were injured with sterilized pin and the mycelial disc of pathogen was placed over the injured leaf portion and covered with moist cotton and incubated inside the moist chamber. The plants were sprayed frequently with water for 2 days. After 48 h, the plants were sprayed with different botanicals. The plant biometric observations *viz.*, Germination%, Root length, Shoot length, Vigour index was recorded and the per cent disease index (PDI) was calculated by using following formula proposed by Wheeler (1969) [22].

$$PDI = \frac{\text{Sum of the individual disease ratings}}{\text{Number of fruits/ leaves observed}} \times \frac{100}{\text{Maximum disease grade}}$$

Germination (%)

Seeds were soaked in botanical extracts for 12 h and then shade dried. Four replicates of 100 seeds were uniformly placed on standard germination paper roll-towel medium and kept in germination room maintained at 25± 2°C and 90 ±2 per cent relative humidity. After 14 days, the seedlings were evaluated as total number of normal seedlings and germination as percentage. (ISTA, 1993) [8].

Table 3: Effect of various botanicals on growth of *Alternaria solani*

Botanical name	Average Colony diameter (mm)	Per cent Growth Inhibition (%)
Azadirachta indica Juss	36.23	59.74
Zingiber officinale	31.41	65.10
Allium sativum L	18.90	79.00
Allium cepa	25.34	71.84
Dhatura stramonium L	72.23	19.74
Ociumum tenuiflorum	50.98	43.36
Lawsonia inermis	22.29	75.23
Psoralea corylifolia	28.97	67.81
Mentha citrata	47.24	47.51
Catheranthus roseus	74.41	17.32
Zimmu	10.07	88.81
Aloe vera	63.75	29.17
Vitex negundo	59.92	33.42
Eucalyptus globulus Labill.	48.29	46.34
Nigella sativa L.	66.64	25.96
Calotropis gigentea L	57.82	35.76
Coleus forskohlii	34.77	61.37
Curcuma longa L.	32.19	64.23
Control	90.00	_
Mean	45.87	51.76
SEd	0.86	1.29
C D (P = 0.05)	1.78	2.66

Root length and Shoot length

On fourteenth day, ten normal seedlings per replication from roll towel medium were carefully removed at random from each treatment. The root length was measured from the base to the top of the primary root and the shoot length was measured from the base of the shoot to tip of primary leaf and the mean value was calculated and expressed in cm. (ISTA, 1993) [8].

Table 4: Effect of various botanicals on the biometrics of tomato cultivar PKM 1

Botanical name	Germination (%)	Seedling length (cm)	Vigour Index	Percent Disease Index (%)
Azadirachta indica Juss	79	22.61	1786	23.01
Zingiber officinale	84	23.67	1988	18.82
Allium sativum L	91	24.67	2245	14.24
Allium cepa	88	24.09	2120	16.21
Dhatura stramonium L	70	19.68	1378	42.64
Ociumum tenuiflorum	76	21.73	1651	32.72
Lawsonia inermis	89	24.21	2155	16.93
Psoralea corylifolia	86	23.88	2054	17.92
Mentha citrata	77	22.21	1710	26.23
Catheranthus roseus	70	19.43	1360	44.59
Zimmu	94	25.35	2383	12.35
Aloe vera	72	20.01	1441	37.34
Vitex negundo	73	20.56	1501	36.18
Eucalyptus globulus Labill.	77	21.89	1686	30.24
Nigella sativa L.	71	19.8	1406	39.87
Calotropis gigentea L	74	21.11	1562	35.28
Coleus forskohlii	81	22.93	1857	20.9
Curcuma longa L.	83	23.12	1919	19.28
Control	61	18.65	1138	70.46
Mean	78.74	22.08	1739	29.22
SEd	1.80	0.40	45.09	0.46
C D (P = 0.05)	3.70	0.82	93.07	0.94

Vigour index

The Vigour index was calculated and compared by adopting the following formula and expressed as whole number. (Abdul-Baki and Anderson, 1973) [1].

Vigour Index = Germination (%) x Mean total length of seedling (Root+ Shoot length) in cm.

Results and Discussion

Antifungal activity assay of botanical extracts

The result presented in Table 3 revealed that all the treatments were statistically significant and all the treatments performed better as compared to control. Among the botanicals used the minimum *Alternaria solani* colony diameter was recorded with zimmu leaf extract. It also exhibited the higher (89%) growth inhibition of the pathogen. The antifungal components presented in the botanicals is responsible for restricting the pathogen growth. The similar results were reported by Ho *et al*., 2007; Ghosh *et al*., 2002; Kagale *et al*., 2004; Najjaa *et al*., 2007; Lazarevic *et al*., 2011 [6, 5, 10, 16, 12] on various crops. Lazarevic *et al*. (2005) reported that the compounds of zimmu leaf extract which showed strong antifungal activity against *R. solani* were phenolic compounds. Muthukumar *et al*. (2010) [15] identified the presence of 22 compounds in the zimmu leaf extract through GC-MS analysis.

Effect of botanicals on germination and biometrics of tomato

The result presented in Table 4 revealed that all the treatments were statistically significant and all the treatments performed better as compared to control. Among the botanicals used the plant biometrics *viz.*, maximum germination per cent (94%), seedling length (25 cm) and vigour index (2383) was recorded in zimmu leaf extract. Lower percent disease index (12%) was also recorded in zimmu leaf extract treatment. Control recorded minimum germination of 61% with lower vigour and it also had maximum percent disease index of 70% as compared with other treatments. The similar results were reported by Chen *et al*., 2011; Phay *et al*., 1999; Huang *et al*., 2012; Karthikeyan *et al*., 2007; Muthukumar *et al*., 2010; Bowers and Locke, 2000; Thangavelu *et al*., 2013 [3, 2, 17, 7, 11, 15, 19] on various crops.

Conclusions

The present investigation revealed that extracts of many medicinal plants had inhibitory effect against *Alternaria solani*. Phytochemicals liberated from the plant extracts act better against the pathogen. Hence these could be exploited as an alternate management strategy for chemical pesticides in the management of early blight of tomato. The future studies focusing on identification and elucidation of the active ingredients present in these medicinal plants having potential antimicrobial properties will be the need of hour.

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