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Gowsik Raja S
Department of Plant Pathology, AC & RI, Killikulam, Tamil Nadu, India

Kannan R
Department of Plant Pathology, AC & RI, Killikulam, Tamil Nadu, India

Rajinimala N
Department of Plant Pathology, AC & RI, Killikulam, Tamil Nadu, India

Sabarinathan KG
Department of Plant Pathology, AC & RI, Killikulam, Tamil Nadu, India

Correspondence
Kannan R
Department of Plant Pathology, AC & RI, Killikulam, Tamil Nadu, India

In vitro evaluation of plant extracts against early blight of tomato caused by *Alternaria solan*

Gowsik Raja S, Kannan R, Rajinimala N and Sabarinathan KG

Abstract
Early blight of tomato (Lycopersicon esculentum Mill) caused by *Alternaria solani* (Ellis & Martin Sorauer) is the most serious and destructive disease in worldwide. In the present study, ten plant extracts *viz*; Ashwagandha (*Withania somnifera*), Nithya Kalyani (*Cathranthus roseus*), Tulasi *(Ocimum tenuiflorum*), rhizome of Ginger (*Zingiber officinale*), bulbs of Garlic (*Allium sativum*), Coleus (*Plectranthus scutelarioides*), Bougainville (*Bougainvillea glabura*), Eruku (*Calotropis gigantea*), red and green coloured sea weeds were used against this disease. Experiment was laid out in Completely Randomized Design (CRD) with three replications by poisoned food technique. Mycelial growth of *A. solani* and percentage inhibition was recorded after 5, 7 and 9 days after incubation. All the tested plant extracts significantly inhibited the mycelial growth of the pathogen when compared with control. However, among all ten tested plant extracts ten percent of Garlic extract was found superior over other treatments followed by Bougainville, Eruku and Coleus by recording 77.4, 57.4 and 55.2 percent inhibition over control respectively. Least inhibition was observed in Ashwagandha (24.2%) and sea weeds (0%). Overall results revealed that all the tested concentrations of Garlic (*Allium sativum*) were found significantly effective against early blight of tomato.

Keywords: Tomato, *Alternaria solani*, plant extracts, Inhibition percentage

1. Introduction
Tomato is one of the most important vegetable crop and is known as protective food both because of its special nutritive value and wide spread production. Tomato is said to be the native of tropical America. From tropical America it spreads to other parts of the world in the 16th century and it became popular in India within the last six decades. It is the world's largest vegetable crop after potato and sweet potato. Total world production of 152.9 million ton with value of $74.1 billion. (FAOSTAT database, 2009). The area under tomato in India is about 4.97 lakh hectares and is about 7.3% of the total cropped land under vegetables with a production of about 86 lakh tons (NHB database 2010). Tomato production has increased by almost 15 times, from a mere 0.54 million tons in 1961 to about 8.6 million tons in 2005. (FAO, 2007) and in Tamil nadu tomato is grown under the area of 38,000 ha with the production of 8 lakh million tons in 2016-17(Horti statistics-17). Early blight is a three-phase disease, which produce leaf spots, stem canker and fruit rot, but the foliar phase is the most common and destructive part of the disease (Maiero and Barksdale,1989), responsible for significant economic losses sustained by Tomato producer each year. *A. solani* can cause extensive defoliation leading to a reduction of economic fruit yield (Spletzer and enyedi, 1999). Control of early blight disease has been accomplished primarily by the application of chemical fungicides (Jones *et al*., 1991). Several effective pesticides have been recommended for use against *A. solani*, but are not considered to be long-term solutions due to concerns of expense, exposure risks, fungicide residues and other health and environmental hazards. It is necessary to adopt such 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. The objective of the present study is to evaluate the antifungal activity of ten plant extracts through poisoned food technique against *A. solani* under *in vitro* conditions.

2. Materials and Methods
The pathogenic isolates of *A. solani* was isolated from tomato leaves showing typical symptoms of early blight using potato dextrose agar (PDA) medium and identified *as A. solani* according to Simmsons (2007).

2.1 Collection of plant materials
Fresh healthy disease free botanical plant parts including *viz*., leaves, inflorescence, bulbs and seeds of ten plants were collected from the orchard, garden and medicinal field of Agriculture college and Research institute, Killikulam and seashore of Tuticorin district. (Table-1)

Table 1: Botanical data of the plant species selected for study

Common name	Scientific name	family	Plant parts used
Ashwagandha	*Withania somnifera*	Solanaceae	Leaves
Nithya Kalyani	*Cathranthus roseus*	Apocynaceae	Leaves
Tulasi	*Ocimum tenuiflorum*	Lamiaceae	Leaves
Ginger	*Zingiber officinale*	Zingiberaceae	Rhizome
Garlic	*Allium sativum*	Amaryllidaceae	Bulbs
coleus	*Plectranthus scutelarioides*	Lamiaceae	Leaves
Bougainville	*Bougainvillea glabura*	Nyctaginaceae	Leaves
Calotropis	*Calotropis gigantea*	Asclepiadaceae	Leaves
Sea weed (red)	Rhodophyta	Algae	Leaves
Sea weed (green)	Chlorophyta	Algae	Leaves

2.2 Preparation of plant extracts

Leaves of all ten plant extracts were washed separately with distilled water and then surface sterilized with 1 percent sodium hypochlorite solution. Plant materials were chopped aseptically and homogenized in mixer grinder using sterile distilled water at the rate of 1:1 ratio (*i.e*.100g of plant material in 100 ml of sterile distilled water). The homogenized extracts were filtered through double layered muslin cloth. For preparing of fresh garlic (*Allium sativum*) and ginger (*Zingiber officinale*) 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 extracts were filtered with bacterial filter in order to avoid bacterial contamination. These sterilized crude extracts were considered as representative to 100% concentration and subsequently, serial dilutions (5 and 10 %) were prepared using sterilized distilled water.

2.3 *In vitro* screening of aqueous plant extracts by poisoned food technique

All the selected plants (Table-1) were used to carry out poisoned food technique (Manmohan and Govindaiah, 2012) to evaluate the efficacy against *A. solani* at different concentrations of 5 and 10 percent with 3 replications. Potato dextrose agar (PDA) was used as nutrient medium and required quantity of each plant extract was added separately so as to get a required concentration of the plant extract. The plant extracts were carefully mixed by stirring and about 15 ml of 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 centre of each petri plate containing the poisoned medium. Potato dextrose agar (PDA) plates without the plant extracts were used as control. The plates were incubated at 26±1°C for seven days and the colony diameter was recorded.

2.4 Statistical analysis

Data regarding plant extracts concentrations against pathogen growth were analysed 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 [7]. Percent inhibition of mycelial growth compared to control was calculated. Percent inhibition over control calculated by formula: I=100*(C-T)/C [4].

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

3. Results and Discussion

3.1 *In vitro* evaluation of plant extracts

Ten 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 two concentrations (5 and 10%) each by poisoned food technique. The results indicated that there was significant difference among tested plant extracts for inhibiting the mycelial growth of the pathogen.

The plant extracts were evaluated after nine days of colony growth by taking average mycelial growth and inhibition percentage (Table 2). The results revealed that all plant extracts at all tested concentrations were significantly ($P \leq 0.01$) reduced linear growth and increased inhibition percentage compared to control. Among the tested plant extracts, Garlic (*A. sativum*) was the most effective in decreasing the colony growth and increasing the inhibition percentage of *A. solani* (20.33mm and 77.4%) followed by Bougainville (*Bougainvillea glabura*) (38.33mm and 57.4%), Eruku (*Calotropis gigantea*) and Coleus (*Plectranthus scutelarioides*) (40.33mm and 55.2%). The least inhibition of mycelial growth of pathogen was observed in both sea weeds of red and green (90mm and 0% PDI) followed by Nithya Kalyani at 5 percent (72.33 mm and 19.6%) and Ashwagandha at 5 and 10 percent concentrations (69.66 & 68.2 mm with 22.6% and 24.2%) respectively

Table 2: Mycelial growth (mm) and inhibition percentage after 9 days colony of *A. solani* on PDA influenced by different concentrations of plant extracts

Plant extract		Concentration %		
	Linear area	5%	10%	mean
Ashwagandha	CG.	69.66^{g}	68.21^{g}	68.9
	IP.	22.6	24.2	23.4
Nithya Kalyani	CG.	72.33^{g}	64.3^{g}	68.3
	IP.	19.6	28.5	24.05
Tulasi	CG.	65.33^{f}	60.03^{f}	62.68
	IP.	27.4	33.3	30.35
Ginger	CG.	47.33^{d}	41.0^{d}	44.21
	IP.	47.3	54.4	50.85
Garlic	CG.	23.66^{a}	20.33^{a}	21.99
	IP.	73.7	77.4	75.55
coleus	CG.	51.0^{e}	40.33^{e}	45.66
	IP.	43.33	55.2	49.26
Bougainville	CG.	43.66^{b}	38.33^{b}	40.99
	IP.	51.5	57.4	54.45
Calotropis	CG.	45.66^{c}	40.01^{c}	42.83
	IP.	43.3	55.2	49.25
Sea weed (red)	CG.	90.00^{h}	90.00^{h}	90.00
	IP.	0.0	0.0	0.0
Sea weed(green)	CG.	90.00^{h}	90.00^{h}	90.00
	IP.	0.0	0.0	0.0
Control	CG.	90.00^{h}	90.00^{h}	90.00
	IP.	0.0	0.0	0.0

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

4. Discussion

At this study selected plant extracts were tested against early blight of tomato caused by *Alternaria solani.* Number of plants has been reported to own antifungal activity [8-10]. Thus, an effort has been made to explore locally available plant extracts and therefore the effect of ten plant extracts *i.e*. Ashwagandha (*Withania somnifera*), Nithya Kalyani

(*Cathranthus roseus*), Tulasi *(Ocimum tenuiflorum*), rhizome of Ginger (*Zingiber officinale*), bulbs of Garlic (*Allium sativum*), Coleus (*Plectranthus scutelarioides*), Bougainville (*Bougainvillea glabura*), Eruku (*Calotropis gigantea*) and sea weeds of red and green colour at two concentrations (5 and 10 %) were evaluated through poisoned food technique *in vitro* for their inhibitory effect on the mycelial growth of *A. solani*. The efficacy of various extracts belonging to different plant species other than the tested extracts on the growth of the pathogen have been reported by several researchers [11, 12]. In the present study, all tested botanicals significantly reduced mycelial growth of the pathogen. Among all plant extracts used, bulb extracts of Garlic were found highly effective in inhibiting the mycelial growth of *A. solani* (77.4%) at 10% concentration followed by other plant extracts. While no inhibition was observed in extract of both sea weeds at 5 and 10 percent. The extracts of Nithya Kalyani recorded 19.6 percent of inhibition at 5 percent whereas the extracts of Ashwagandha (*Withania somnifera*) recorded 22.6 and 24.2 percent inhibition at 5 and 10 percent concentrations respectively. 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 [14]. Additionally, it might be due to natural bioactive materials presented in these extracts. Satya *et al*. (2005) [14] and Sing *et al*. (1986) [15] reported that extract of *A. sativum* inhibited growth of the pathogen. Because of the presence of antimicrobial compound present in garlic mainly as Allicin which has antifungal activity. Therefore, from the foregoing argument it may be accomplished that, *Allium sativum* a common medicinal and edible plant could be used as the source of an effective biocide that has vast fungitoxic effect against *A. solani* for controlling early blight of tomato.

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