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Inhibitory Effect of Marine Mangrove on the Growth of *Alternaria solani* Causing Tomato Early Blight Disease

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A B S T R A C T

Tomato is one of important vegetable crop cultivated in worldwide. Among the diseases which causing tomato early blight caused by Alternaria solani is serious disease. The present investigation was undertaken to assess the bio-efficacy of mangroves at different concentrations against *Alternaria solani* causing tomato early blight disease under *in vitro* condition. Among the five mangrove species were tested, the higher antifungal activity was observed with 20% of *Rhizophora apiculata* recorded minimum mycelial growth, sporulation and germination of spore (4.82mm, 14.46mm, + and 5.86%) of *Alternaria solani* under poisoned food technique, agar well method and spore germination assay respectively, which was followed by *Rhizophora annamalayana*, *Avecinia officinalis*, *Rhizophora mucronata* and *Avecinia marina*. While the least antifungal activity was observed with the species of *Avicenia marina* recorded (18.23mm, 35.64mm, ++ and 16.23%) Significant inhibitory activity was also observed in 5, 10 and 15% concentration in all the three methods.

Keywords

Tomato, *Alternaria solani*, *Rhizophora apiculata*, *in vitro*

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Introduction

Tomato (*Lycopersicon esculentum* Mill.) is one of the most widely grown vegetable in the world. It is a small annual or short livedperennial herb belonging to the family solanaceae. Tomato ranks second next to potato in world acreage but it ranks first among processing crops. In Tamil Nadu, it is cultivated throughout the year during rainy, winter and summer seasons and occupies an area of 38.73 lakh ha with production of 840.32 million tonnes in 2016-2017 (Indian Horticulture Database, 2017).

Early blight caused by *A. solani* (Ellis and Martin) Jones and Grout, is one of the most catastrophic and frequently occurring disease of the crop (Jones *et al.*, 1991). The yield loss due to this disease ranged between 50 to 86 % in fruit yield (Mathur and Shekhawat, 1986). The antimicrobial compounds in plants are

being used bio-pesticide are eco-friendly nature and a best alternative for minimizing the usage of chemical pesticide in agriculture. Also, it has alkaloids and essential oil was showed antifungal, antibacterial, insecticidal, nematicidal, herbicidal and antiviral activities (Hosein *et al.,* 2010; Chang *et al.,* 2012; Kordali *et al.,* 2013; Kepenekci and Saglam, 2015).

The antifungal activity of some mangrove species has been well documented against plant pathogens *viz*., *Avicennia marina* against *Alternaria citri*, *Avicennia marina* and *Rhizophora mucronata* against *A. alternata*, *Rhizophora apiculata* against *Macrophomina phaseolina* (Mehdi *et al.,* 2000; Muthukumar *et al.,* 2014; Behbahani *et al.,* 2016; Rastegar and Mohsen Gozan, 2016). More than 200 bioactive compounds were identified from mangroves with antibacterial and antifungal properties belong to steroids, triterpenes, saponins, flavonoids, alkaloids, tannins and phenolics (Bandaranayake, 2002; Bose and Bose, 2008; Chandrasekaran *et al.,* 2009; Vengadeshkumar, 2017). Hence, the present study was undertaken to assess the bio-efficacy of certain mangrove species against *A. solani* under *in vitro* condition.

Materials and Methods

In our previous study, an intensive fixed plot survey was conducted in major vegetable growing districts in Tamilnadu, India during 2017 and infected leaf sample was isolated and identified as *A. solani* named as AS_1 to $AS_{10.}$ Further, all the isolates were tested for their growth and ability of virulent, among the isolates tested, isolate AS_5 was found to be most virulent and showed better in their growth under *in vitro* experiments. Hence, based on our previous study the isolate AS_5 was selected and used as test pathogen in the present study.

Collection and authentication of mangrove

The fresh leaves of marine mangroves were collected from Pichavaram Mangrove forest, Tamil Nadu and authenticated in the herbarium of C.A.S. in Marine Biology, Annamalai University, Parangipettai, Tamil Nadu, India. The leaves were carefully examined and healthy leaves were washed, shade dried, coarsely powdered and stored in air tight bottles for further work. The list of mangrove species used in this study was given below:

S.No	Mangroves	Parts used
1.	*Avicenia marina*	Leaves
2.	*Avicenia officinalis*	Leaves
3.	*Rhizophora apiculata*	Leaves
4.	*Rhizophora annamalayana*	Leaves
5.	*Rhizophora mucronata*	Leaves

Preparation of aqueous extract

The freshly collected leaf materials were separately washed with tap water, then with alcohol and finally with repeated changes of sterile distilled water. They were separately ground in sterile distilled water at the rate of one ml/g of leaf tissue in a sterilized pestle and mortar.

The extract was stained through two layer of muslin cloth subsequently filtered through Whatman No:1 filter paper and centrifuged @ 1500 rpm for 10 min. This formed the standard plant extract (100%) solution (Shekhawat and Prasad, 1971). This extract was further diluted to the desired concentration (5, 10, 15 & 20%) by adding requisite quantities of sterile distilled water and tested against pathogen.

In vitro efficacy of marine mangrove

Poisoned food technique (Narender Kumar *et al*., 2017)

Potato dextrose agar PDA medium mixed separately with extracts of different plant species at different concentrations *viz.*, 5, 10, 15 and 20% poured into sterile petri dishes, allowed to cool and solidify. Mycelial disc (9mm) of 15 days old culture of test pathogen placed at the centre of the petri dishes and incubated at 28±2ºC for 10 days. The PDA medium with the same concentration of sterile distilled water alone served as control. Similarly a fungicide *viz.*, mancozeb (0.25% conc.) as also tested against the pathogen for comparison for each treatment. The experiments was replicated thrice and the per cent inhibition of mycelial growth if any was determined by the formula

$$PI = C - T/C \times 100$$

(Where, C = Diameter of *A. solani* in control, T= Diameter of *A. solani* in treated)

Agar well method

The antimicrobial activity of mangroves against *A. solani* was tested by agar well method (Thongson *et al.,* 2004). Spore suspension of the test pathogen was preparedwith sterile distilled water from 15 days old culture. Desired concentrations of the selected plant extracts were prepared. 20ml of PDA medium was seeded with 3ml of spore suspension (1×10^6spore/ml) and poured into a Petri plate and allowed to solidify. Four wells were made equidistantly with the aid of sterile cork borer. Test plant extracts of different concentrations were pipetted out separately poured into each well. Mancozeb @ 0.25% conc. was used for comparison. Suitable control was maintained for each treatment. The plates were incubated at 28±2ºC and the inhibition zone of the fungal growth around each well was recorded.

Spore germination assay

To test the spore germination of pathogen, two drops of each extracts at 5, 10, 15 and 20 per cent concentration along with spores were placed in a cavity slide and incubated at 28±2ºC for 24 hours and thereafter sporulation and germination of spores was assessed and recorded. The most effective mangrove species (*R. apiculata*) is identified from above studies was further tested against the growth of *A. solani* with different solvents by poisoned food technique is already described. Three replications were maintained for each treatment.

Results and Discussion

Antifungal activity of mangroves at different concentration on growth of *A. Solani* AS_5 (Poisoned Food Technique)

Five species of mangroves were tested at different concentration (5, 10, 15 and 20%) against the test pathogen. In general, the inhibition of growth of fungus increased with an increase in concentration of the aqueous extract of the test plants. Among the plant extracts tested, *R. apiculata* at 20% conc. showed significantly the highest reduction on the mycelial growth of test pathogen with 4.82 mm (93.97% of growth reduction) as against 80.00mm in control treatment.

This was followed by twenty percent extract of *R. annamalayana*, *A. officinalis*, *R. mucronata* and *A. marina* with a growth reduction of 88.60, 85.67, 81.36 and 77.21 % in the decreasing order of merit respectively. Mancozeb 75% WP (0.25%) used for comparison completely inhibited the growth and accounted for 100% growth reduction of the test pathogen (Table 1).

Antifungal activity of mangroves at different concentration on growth of *A. solani* AS_5 (agar well method)

All the mangrove extracts at different concentration *viz*., 5, 10, 15 and 20 per cent were found to be effective in against *A. solani*. Among the plant extract tested, aqueous extract of *R. apiculata* at 20% conc. inhibited the growth of pathogen with minimum mycelial growth (14.46mm) which accounting 81.92 % inhibition over control.

This was followed by the extract of *R. annamalayana* (79.61%), *A. officinalis* (73.45%), *R. mucronata* (63.38%) *and A. marina* (55.45%) in the decreasing order of merit. The standard chemical fungicide Mancozeb 75% WP at 0.25% conc. showed 91.92 per cent inhibition of the test pathogen. The control treatment was recorded with mycelial growth of 80.00mm (Table 2).

Antifungal activity of mangroves at different concentration on sporulation and spore germination of *A. Solani* AS_5 (spore germination assay)

With regard to the sporulation and spore germination, *R. apiculata* at 20% concentration showed significantly poor sporulation (+) and highest reduction in spore germination (5.86%) as against excellent sporulation (++++) and maximum spore germination (80.00%) in control.

This was followed by twenty per cent extract of *R. annamalayana* (8.46%), *A. officinalis* (11.17%), *R. mucronata* (15.19%) and *A. marina* (16.23%) in the reduction of spore germination with the decreasing order of merit (Table 3).

The results obtained with 20% conc. of aqueous extract of *R. apiculata* were almost similar with the result obtained with 15% conc. in reducing fungal growth in all the three experiments (Table 1, 2 and 3).

In the present study the mangrove extracts showed varying degree of growth inhibition against *A. solani*. The inhibition of growth of fungus increase with an increase in concentration of the aqueous extract of test plants. Among the mangrove species tested against *A. solani*, *R. apiculata* @ 20% conc. showed significantly highest reduction in the radial growth, biomass production, sporulation and spore germination of the pathogen under *in vitro* condition. *R. annamalayana* is a next best mangrove to inhibit the growth of test pathogen. However, the results obtained with 20% conc. of *R. apiculata* almost similar to the results obtained with 15% conc. of *R. apiculata*.

Similar to the present study, Rasteger and Gozari (2016) reported that *R. Apiculata* exhibited antifungal principles while against *Penicillium* sp. and *A. alternata*. Likewise, the leaf extracts of *R. apiculata* showed antifungal compounds against fungal pathogen *M. phaseolina* (Muthukumar *et al.*, 2014).

The extract of *Ceriops decandra* showed maximum inhibition against *Candida albicans* (Selvam and Kolanjinathan, 2014). Ethanolic extract of mangrove species *A. marina* was found to inhibit the radial growth of *M. phaseolina* under *in vitro* was reported (Mehdi *et al.*, 1999). Various workers reported that antimycotic activity of botanicals *viz*., *Barbeya oleoides, Maerua oblongifolia, A. sativum* and *Mentha arvensis* against *A. solani*, *Botrytis fabae, Fusarium solani, Phytophthora infestans* and *Aspergillus niger* (Baka, 2010; Taskeen-Un-Nisa *et al.*, 2011; Raji and Raveendran, 2013).

Table.1 Antifungal activity of mangroves at different concentration on growth of *A. solani* AS_5 (Poisoned Food Technique)

S. No	Mangroves	Mycelial growth (mm)				Per cent inhibition over control			
		5%	10%	15%	20%	5%	10%	15%	20%
1.	*Avicenia marina*	28.53^{f}	26.71^{f}	19.73^{f}	18.23^{f}	64.33	66.61	75.33	77.21
2.	*Avicenia officinalis*	23.76^{d}	19.94^{d}	13.26^{d}	11.46^{d}	70.30	75.07	83.42	85.67
3.	*Rhizophora apiculata*	19.42^{b}	15.12^{b}	6.42^{b}	4.82^{b}	75.72	81.10	91.97	93.97
4.	*Rhizophora annamalayana*	21.36^{c}	17.62^{c}	10.92^{c}	9.12^{c}	73.30	77.97	86.35	88.60
5.	*Rhizophora mucronata*	25.21^{e}	23.39^{e}	16.42^{e}	14.91^{e}	68.48	70.76	79.47	81.36
6.	Mancozeb 75% WP @ 0.25%	0.0				100			
7.	Control	80.00^{a}				0.0			

Values in the column followed by same letters not differ significantly by DMRT (P=0.05)

Table.2 Antifungal activity of mangroves at different concentration on growth of *A. solani* AS_5 (Agar well method)

S. No	Mangroves	Mycelial growth (mm)				Per cent inhibition over control			
		5%	10%	15%	20%	5%	10%	15%	20%
1.	*Avicenia marina*	42.76^{f}	41.13^{f}	37.24^{f}	35.64^{f}	46.55	48.58	53.45	55.45
2.	*Avicenia officinalis*	28.36^{c}	26.73^{d}	22.84^{d}	21.24^{d}	64.55	66.58	71.45	73.45
3.	*Rhizophora apiculata*	21.58^{a}	19.95^{b}	15.96^{b}	14.46^{b}	73.02	75.06	80.05	81.92
4.	*Rhizophora annamalayana*	23.42^{b}	21.79^{c}	17.81^{c}	16.31^{c}	70.72	72.76	72.76	79.61
5.	*Rhizophora mucronata*	36.41^{e}	34.78^{e}	31.09^{e}	29.29^{4}	54.48	61.13	56.52	63.38
6.	Mancozeb 75% WP @ 0.25%	7.27^{a}				91.92			
7.	Control	80.00				0.0			

Values in the column followed by same letters not differ significantly by DMRT (P=0.05)

Table.3 Antifungal activity of mangroves at different concentration on sporulation and spore germination of *A. solani* AS_5 (Spore Germination Assay)

S. No	Mangroves	Sporulation				Spore germination (%)			
		5%	10%	15%	20%	5%	10%	15%	20%
1.	*Avicenia marina*	+++	+++	++	++	29.52^{e} (32.91)	26.25^{e} (30.82)	21.41^{e} (27.56)	16.23^{e} (23.75)
2.	*Avicenia officinalis*	+++	++	+	+	24.46^{c} (29.64)	21.19^{c} (27.40)	16.35^{c} (23.85)	11.17^{c} (19.52)
3.	*Rhizophora apiculata*	++	++	+	+	19.15^{a} (25.95)	15.88^{a} (23.48)	11.04^{a} (19.40)	5.86^{a} (14.00)
4.	*Rhizophora annamalayana*	++	++	+	+	21.75^{b} (27.79)	18.48^{b} (25.46)	13.64^{b} (21.67)	8.46^{b} (16.90)
5.	*Rhizophora mucronata*	+++	+++	++	++	28.48^{d} (32.25)	25.21^{d} (30.13)	20.37^{d} (26.82)	15.19^{d} (22.93)
6.	Control	++++				80.00^{f}			

Values in the column followed by same letters not differ significantly by DMRT (P=0.05)

Sporulation: excellent- ++++; good-+++; fair-++; poor-+

It has well documented that leaf extract of *D. stramonium*, *Eucalyptus oblique* and *Allium sativum* are effectively inhibits the mycelial growth of *A. solani* under *in vitro* condition (Nashwa *et al.*, 2012; Sadana and Didwania 2015; Pankaj Kumar and Singh 2017).

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