

Prevalence of tomato early blight disease in Tamilnadu and morpho-cultural characterization of *Alternaria solani* isolates

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ABSTRACT

The present study was conducted with an objective to assess the prevalence and incidence of tomato early blight disease at different locations in five districts of Tamilnadu, India during 2016. The growth characters and pathogenic variability among the isolates of *Alternaria solani* were recorded. The survey revealed that the per cent disease index ranged from 12.52 to 34.72 per cent among five tomato growing districts. Amongst the collected isolates AS₅ showed maximum radial growth (88.56mm), conidial size (265.63µm x 23.28µm) and beak length (147.71 µm). The same isolate was also found as most aggressive with maximum per cent disease incidence (69.46%) on artificial inoculation. The pigmentation, sporulation, growth margin and zonation were also found to vary amongst different isolates. AS₅ was found most aggressive with maximum per cent disease index (69.46%) followed by AS₂, AS₉ and AS₆ with a PDI of 65.42, 56.74 and 52.41 per cent, respectively. The least PDI (24.38%) was recorded with isolate AS₈.

Key words: *Alternaria solani*, early blight, survey, tomato

Tomato early blight caused by *Alternaria solani* is one of the most destructive and wide-spread disease in temperate, tropical and subtropical regions of the world which causes reduction in both quality and quantity of tomato (Hijmans *et al.*, 2000). Kamble *et al.* (2009) reported an overall early blight incidence of 35.12 to 55.75 per cent from Thane district of Maharashtra. Likewise, Soni *et al.* (2017) also reported the incidence of early blight disease from southern Rajasthan ranging between 35.14 to 51.98 per cent. The disease is reported to cause about 50-80% yield loss by incurring damage on foliage and fruits and its severity varied between 15 to 90 per cent (Tewari and Vishnavat, 2012; Joseph *et al.*, 2016). The characteristic variation in *A. solani* isolates *viz.* production of long beaked and obclavate conidia, with 9 to 11 transverse septa and size ranging between 257 to 285 µm x 23.2 to 24.4 µm have also been reported (Simmons, 2007; Kemmitt, 2013; Loganathan *et al.*, 2016; Pachori and Sharma, 2016). The understanding of morphological, cultural and

pathogenic variability of different *A. solani* isolates will aid in understanding of pathogenic behaviour and thus formulation of disease management strategy, henceforth, the study was undertaken.

MATERIALS AND METHODS

Intensive fixed plot surveys were conducted to record the occurrence of tomato early blight disease in five districts of Tamil Nadu *viz.*, Cuddalore, Thiruvannamali, Erode, Dharmapuri and Madurai during July to October-2017. In each district two regions were selected for survey and in each region five fields were surveyed randomly. The disease intensity was recorded using disease score chart (Plate 2) suggested by Pandey *et al.* (2003) and the formula suggested by Wheeler (1969).

Disease score chart

Grade	Description
0	No lesions
1	< 10% surface area covering leaf, stem and fruit
2	11-25% foliage of plant covered with a few scattered spots
3	26-50% many spots coalesce on the leaves, covering surface area of plant

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- 4 51-75% area of plant infected, fruit also infected at apical end, defoliation and blighting started. Sunken lesions with prominent concentric rings on stem, petioles and fruit
- 5 >75% area of plant part blighted, severe lesion on stem, and fruit rotting on apical end

$$PDI = \frac{\text{Sum of disease rating}}{\text{Total number of leaves observed}} \times \frac{100}{\text{Maximum grade}}$$

The leaves were microscopically examined to confirm the presence of the fungal conidia and isolations were made following standard tissue isolation technique (Naik *et al.*, 2010). The infected leaves (Plate 1) were cut into small bits (2mm), surface sterilized with 1% sodium hypochlorite solution for 1min and washed thrice with sterile distilled water. Further, a piece of specimen was transferred to Petri dish containing Potato Dextrose Agar (PDA) medium. The plates were incubated at room temperature ($28 \pm 2^\circ\text{C}$) for 7 days and the isolates were purified by hyphal tip method (Plate 3). The culture was maintained on PDA slant and used for further studies. A nine mm disc of all the ten isolates of *A. solani* was taken from 15 day old culture plate and aseptically subcultured on PDA.

Three replications of each isolate was maintained and incubated at $28 \pm 2^\circ\text{C}$. The radial growth, appearance, colony color, sporulation, size of conidia, number of septa and beak length of conidia were observed and recorded after the incubation period.

Conidia were harvested from 20 days old PDA culture by flooding the plates with 20ml of sterile distilled water followed by scrapping of culture with a sterile glass rod. The dislodged conidia were filtered through thin layers of cheese cloth to remove mycelial fragments. The conidial suspension of the pathogen was prepared according to the method given by Sutton and Shane (1983). The surface of the culture was scrapped and the inoculum load was adjusted to 5×10^6 conidia per ml of sterile distilled water. One ml teepol AG (alkyl benzene sodium salt) was added to 100ml conidial suspension to increase the spreading ability of the suspension.

The Koch's postulates were proved to test the pathogenicity of *A. solani* isolates on susceptible tomato cultivar PKM1. Twenty days old seedlings were transplanted in earthen pots filled with potting mixture comprising sand, red soil and farmyard manure @ 1:1:1 ratio. Three plants were maintained



Plate 1. Symptom of early blight disease

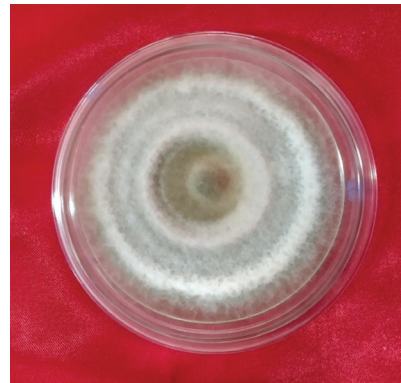


Plate 3. Axenic culture of *A. solani*

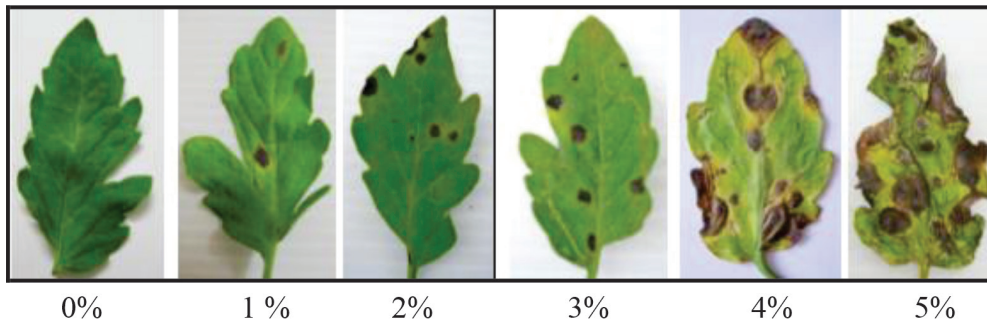


Plate 2. Disease index

Table 1. Prevalence of tomato early blight disease in five districts of Tamilnadu during 2017

Locality	Village	Soil type	Variety	Crop stage (days)	PDI (%)
Cuddalore	Sivapuri	Clay	Local vty.	>40	16.63 ^h (24.07)
	Panruti	Red	PKM1	40-60	32.63 ^b (34.83)
Thiruvannamalai	Sozhavaram	Red	CO1	<40	18.69 ^g (25.60)
	Thandarampattu	Clay	Local vty.	40-60	14.61 ⁱ (22.46)
Dharmapuri	Rayakottai	Red	PKM 1	>60	34.72 ^a (36.10)
	Chinnamampattu	Sandy loam	CO 1	>40	26.81 ^d (31.18)
Erode	Gobichettipalayam	Red loamy	CO1	<40	21.58 ^f (27.67)
	Poonatchi	Sandy loam	Local vty.	40-60	12.52 ^j (20.72)
Madurai	Kalamedu	Sandy loam	PKM 1	<40	29.74 ^c (33.04)
	Kalarkovil	Clay	CO 1	>60	23.67 ^e (29.11)

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

in each pot. Ten days after transplantation leaves were injured with sand paper and spray inoculated with conidial suspension of *A. solani* @ 5×10^6 conidia per ml of sterile distilled water. The inoculated plants were maintained in the greenhouse and observed for symptom development to confirm pathogenicity after 7 days (Pandey and Pandey, 2002).

RESULTS AND DISCUSSION

The data presented in Table 1 on the fixed plot survey conducted in major tomato growing districts viz., Cuddalore, Thiruvannamali, Erode, Dharmapuri and Madurai during July-October 2017 indicated that the plant disease index (PDI) ranged between 12.52 to 34.72 per cent. The maximum PDI of 34.72 per cent was recorded in Rayakottai village of Dharmapuri district, followed by Panruti village of Cuddalore district (32.63 %), Kalamedu village of Madurai district (29.74), Chinamampattu village of Dharmapuri district (26.81). The minimum PDI of 12.52 per cent was observed in Poonatchi village of Dharmapuri district. In general, early blight disease incidence was found more in cultivar PKM as compared to other cultivars. Similar to the present observations, Patel (2012) reported that the per cent disease incidence of

tomato early blight in Chhattisgarh ranged between 44.24 to 51.31 per cent. Further, Kamble *et al.* (2009) reported the endemic nature of tomato early blight in Konkan region of Maharashtra. Soni *et al.* (2017) reported the per cent disease incidence of tomato early blight disease ranged between 35.14 to 51.88 per cent in open field condition and 33.94 to 69.28 per cent under in protected conditions in southern parts of Rajasthan.

All the ten isolates of *A. solani* exhibited variability with respect to mycelial growth, colony colour and sporulation. After 14 days of incubation, the isolate AS₅ showed maximum radial growth (88.56mm) followed by AS₂ (82.35mm) and AS₉ (78.83mm) isolates. The minimum growth was observed in the isolate AS₈ (62.63mm). Also, the same isolates exhibited the cultural characters viz., circular, smooth with concentric zonation, brownish black in colour and excellent sporulation (++++). Whereas, the isolates AS₆, AS₇ and AS₁₀ appeared as irregular smooth with concentric zonation, olivaceous black with good sporulation (+++). The fair sporulation observed with the isolate AS₈ (++) (Table 2). The outcomes of present study were similar to the findings of Nikam *et al.* (2015). Whereas, Rahmatzai *et al.*,

Table 2. Cultural characteristics of *A. solani* isolates

Isolate	Radial growth (mm)	Appearance	Colony colour	Sporulation
AS ₁	70.85 ^b	Irregular, rough, without zonation	Light grey	++
AS ₂	82.35 ^b	Circular, smooth with concentric zonation	Brownish black	++++
AS ₃	74.37 ^g	Circular, smooth without zonation	Black	++
AS ₄	64.64 ⁱ	Irregular, smooth, without zonation	Brownish black	++
AS ₅	88.56 ^a	Circular, smooth with concentric zonation	Brownish black	++++
AS ₆	78.36 ^d	Irregular, smooth, with concentric zonation	Olivaceous black	+++
AS ₇	74.82 ^f	Irregular, smooth with concentric zonation	Black	+++
AS ₈	62.63 ^j	Irregular, smooth, without zonation	Olivaceous black	++
AS ₉	78.83 ^c	Circular, smooth, concentric zonation	Dark greyish	+++
AS ₁₀	76.36 ^c	Irregular, smooth with concentric zonation	Creamy white	+++

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

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

Table 3. Morphological characteristics of collected *A. solani* isolates

Isolate	Size of conidia (µm)		Septa in conidia		Length of beak (µm)
	Length	Breadth	Horizontal	Vertical	
AS ₁	111.93	14.21	3-4	1-2	56.86
AS ₂	259.79	22.46	5-7	1-4	142.28
AS ₃	132.26	14.19	3-4	1-2	57.42
AS ₄	110.46	12.24	2-3	1-2	54.98
AS ₅	265.63	23.28	5-7	1-4	147.71
AS ₆	205.62	16.42	5-6	1-3	111.57
AS ₇	145.74	16.27	5-6	1-3	98.46
AS ₈	100.84	12.45	2-3	1-2	47.91
AS ₉	218.46	17.27	5-7	1-4	138.28
AS ₁₀	194.61	18.29	5-6	1-3	106.63

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

Table 4. Per cent disease index produced *A. solani* isolates on artificial inoculation over PKM-1 cultivar

Isolate number	Per Disease Index (PDI)
AS ₁	33.18 ^b (35.17)
AS ₂	65.42 ^b (53.98)
AS ₃	40.67 ^g (39.61)
AS ₄	26.21 ⁱ (30.79)
AS ₅	69.46 ^a (56.45)
AS ₆	52.41 ^d (46.38)
AS ₇	42.75 ^f (40.82)
AS ₈	24.38 ^j (29.58)
AS ₉	56.74 ^c (48.89)
AS ₁₀	46.34 ^e (42.90)

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

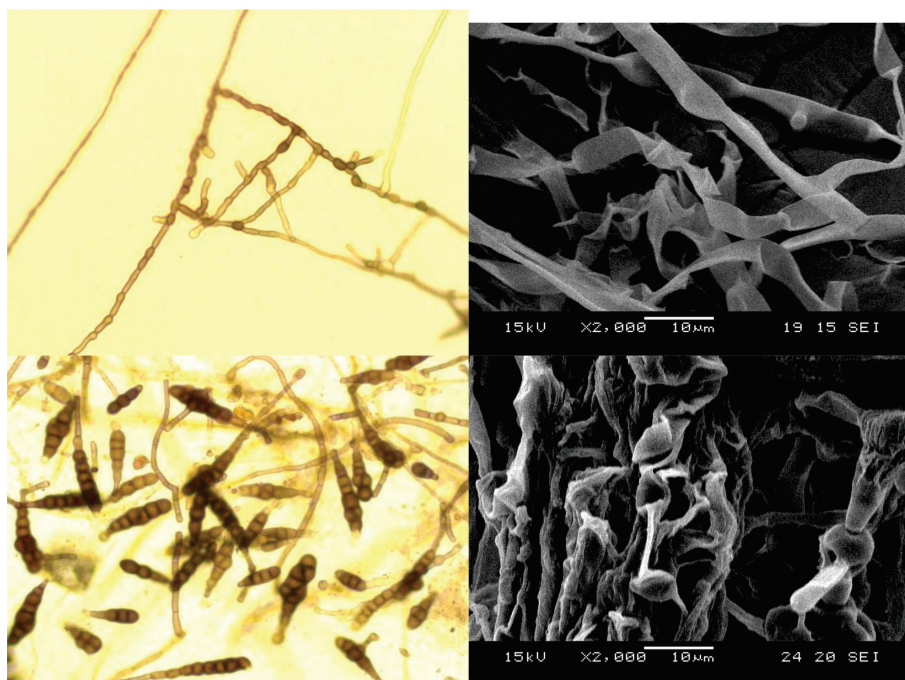


Plate 4. Morphological and cultural characters of isolate AS₅



Plate 5. Pathogenicity test

(2016) observed that the fungus produced cottony growth, dark grey to black with tints of brown or olive nature of mycelium. Also, the cultural variability among the isolates of *A. solani* grown on PDA medium was reported earlier by different workers (Kaul and Saxena, 1988; Tong *et al.*, 1994; Kumar *et al.*, 2008; Naik *et al.*, 2010).

All the isolates of *A. solani* showed slight morphological variations in terms of size of conidia,

number of septa and beak length of conidia (Plate 4). The isolate AS₅ showed maximum conidial size (265.63µm × 23.28µm) and beak length (147.71 µm) followed by AS₂ and AS₉. The same isolates produced similar character with respect to number of septa (5-7 horizontal and 1-4 vertical septa). The least conidial size (100.84µm × 12.45µm), number of septa (2-3 horizontal & 1-2 vertical) and beak length (47.91µm) was observed in AS₈ isolate (Table 3).

The present study goes inline with the findings of Pachori *et al.* (2016). Also, such variation on morphology of *A. solani* isolates is well reported (Nikam *et al.*, 2015; Rahmatzai *et al.*, 2016).

The data pertaining to the pathogenicity of *A. solani* isolates is presented in Table 4 and Plate 5. Among the ten isolates tested, isolate AS₅ was found most aggressive with maximum per cent disease index (69.46%) followed by AS₂, AS₉ and AS₆ with a PDI of 65.42, 56.74 and 52.41 per cent, respectively. The least PDI (24.38%) was recorded with isolate AS₈. The results of the pot culture experiment conducted by artificial inoculation of the pathogen revealed varied levels of pathogenicity with different isolates. Among the isolates of *A. solani* collected from different conventionally tomato growing districts of Tamilnadu, the isolate collected from Rayakottai (AS₃) village was found to be the most aggressive, recording the highest PDI (69.46%) and the isolate AS₈ collected from Poonatchi was the least aggressive. Tippetwamy *et al.* (2010) and Rahmatzai *et al.* (2016) also reported the similar finding.

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