



Surveillance and assessment of disease severity of twister blight of onion in Tamilnadu

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Abstract

Onion productivity is affected by the diseases like Colletotrichum (twister) blight, Purple blotch, Stemphylium blight and basal rot. Since Onion twister blight cause heavy loss this investigation was carried out to assess the prevalence of disease in different districts of Tamilnadu to report the disease severity in terms of PDI (Percent disease index) along with AUDPC (Area under disease progress curve) value. Based on the AUDPC mean values, the districts surveyed were classified into four divisions. Amongst, Perambalur hold under severity division. Chettikulam village in Perambalur district recorded the highest AUDPC value and the highest PDI (1908.52, 52.6 percent) and Pavitram village in Trichy district recorded the lowest PDI (31.30 percent) among the districts surveyed, with a AUDPC score of 1117.35. During the Rabi (September-November) season, different isolates in pot culture was assessed with variety Co -5. The greatest PDI was observed from Perambalur district, Chettikulam village isolate 51.3%, followed by Rasipuram 48.9% PDI from Nammakal, and the lowest PDI was recorded from comibatore district, Pollachi village isolate 26.9% and Chettikulam with highest AUDPC value 1269 and Pollachi with lowest AUDPC value 809.25. During the Kharif (Feb-May) season the greatest PDI was observed from Perambalur district, Chettikulam village isolate 25.8%, followed by odichattiram 23.3% PDI from Nammakal, and the lowest PDI was recorded from comibatore district, Pollachi village isolate 15.9%, Chettikulam with highest AUDPC value 801.75 and Pollachi with lowest AUDPC value 578.25. These also revealed size diversity, with conidia sizes ranging from (9.52 x 3.85 µm to 23.20 x 5.15 µm). The smallest conidial size is 9.52 x 3.85 µm in isolate from V.P kovil village, and the largest conidial size is 23.20 x 5.15 µm in isolate from tiruppur village.

Keywords: survey, twister blight, AUDPC, PDI

Introduction

The onion (*Allium cepa* L.) is a prominent bulbous vegetable crop that is grown all over India. It is also known as the "queen of kitchen." It is a member of the Alliaceae family. Sulfur, potassium, vitamin B6, iron, vitamin C, anthocyanin, and antioxidants, particularly quercetin, are all abundant in it (Cramer 2000) [3]. Diseases such as Twister blight, purple blotch, Stemphylium blight, and base rot are among the factors that contribute to lower onion output and productivity. Onion twister blight is one of the epidemic disease that has caused significant crop loss. Twister disease of onion (*Allium cepa* L.) was originally recorded by (Ebenebe 1980) [2] near Zaria, northern Nigeria in 1969. In the Kalpitiya Peninsula in Sri Lanka's North Western Province, (Kuruppu 1999) [9] reported a disease on shallot onions, *Allium cepa* var. ascalonicum, that caused output losses of up to 30%. This pathogen wreaked havoc on red onions in a number of Indonesian onion crop centres in 2005-06 (Wiyono, 2007) [15]. According to (Hegde *et al.* 2012) [5], twisting of onion leaves, stems, and bulbs has posed a severe challenge to farming, with losses of 40-60% predicted. The infection was described by (Nitaya *et al.* 1987) as curling, twisting, and chlorosis of the leaves. In all growing areas of India, disease incidence was lower in the kharif season than in the rabi season. Yield loss can occur in the presence of favourable environmental conditions such as high relative humidity (80 to 90 percent) and optimum temperature (24+2°C). Weather parameters, rainfall contributed more on incidence and severity for the

disease development. To combat the twister blight disease of onions, fungicides have been used, however they have failed to limit the pathogen due to the formation of new pathogen races.

This investigation was carried out in five districts of central Tamilnadu, India, to determine the amount of the disease's incidence and express the damage in terms of percent disease index (PDI) and AUDPC. To analyse the cultural, morphological, and pathogenic heterogeneity among. isolates, it was also planned to isolate the pathogen strains from the district with the highest prevalence.

Identification of symptoms

The following are the symptoms of the disease that were observed on the leaves, neck, flowering stalk, inflorescence, and bulbs, twiddling, curling, and chlorosis were noticed. A zone of discoloured tissue grew around the spots, which were round to oval and water-soaked. In the shallow sunken necrotic areas that were black and resembled anthracnose symptoms, clusters of acervuli formed in concentric rings. Additionally, in severely afflicted plants, dieback symptoms were seen. Elongated neck with narrow bulb that was unusually twisted.

Root knots, discolouration of the roots, and an underdeveloped root system are all signs of a problem with the root system. Root galling at the tip and intercalary, which were yellowish in the early stages, was seen by (suresh patil 2013) [13].

Materials and Methods

Collection of samples

Samples were collected in many districts throughout Tamil Nadu, including Perambalur, Namakkal, Trichy, Dindugal, and Coimbatore. Infected onion leaf samples were collected in a polythene bag from the examined field. During survey onion twister are serious diseases of raining season. Yield losses are estimated at 50-100% in several fields. Onion twister disease is characterized by curling, twisting, and chlorosis of the onion leaves, abnormal elongation of the necks, and formation of slender bulbs. Some diseased plants

rot before harvest while others decay rapidly when stored. Acervuli of a *Colletotrichum* were founded on the necks and leaf blades of diseased plants. Inoculation test satisfied Koch's postulates and demonstrated for the first time that this fungus is the causal agent of onion twister disease. It is possible to explain that onion twister and leaf anthracnose are two phases of the same disease. Because there was no survey data for twister disease, a scale was created and used to score the disease, similar to that used for purple blotch (Bhangale and Joi, 1985).

Table 1

Grade	Twisting (%)	Sign or symptoms.
0	No disease	There are no signs or symptoms of illness.
1	Up to 10%	Curling and chlorosis are two symptoms of leaf chlorosis.
2	11-20%	Leaves and neck elongation that is abnormal
3	21-40%	Acervuli clusters in concentric rings, as well as shallow, depressed necrotic patches and root galling, are visible on the leaf sheath.
4	41-60%	Dieback signs include a tall neck, narrow bulbs, and leaves.
5	>61%	Severe dieback, rotting bulbs, and a poorly established root system with discoloured roots

The following formula was used to determine the Percent Disease Index (PDI) (Wheeler, 1969).

$$\text{Per cent disease index} = \frac{\text{Sum of individual ratings}}{\text{Total number of plants observed} \times \text{Maximum disease grade}} \times 100$$

Computation of area under disease progress curve (AUDPC) on survey

To determine the measure of disease development throughout the period, Area under disease progress curve (AUDPC) (Reynolds and Neher, 1997) ^[13] was calculated using the formula given by Campbell and Madden (1990) ^[4].

$$A_k = \sum_{i=1}^{N_i-1} \frac{(y_i + y_{i+1})}{2} (t_{i+1} - t_i)$$

Y_i = the severity of twister blight disease on the given date.

n = number of dates on which the disease was scored

T_i = date on which the disease was scored

Isolation of the pathogen

The pathogen was isolated from the sick onion leaves. The diseased along with healthy portions of the leaves were chopped into small pieces and surface sterilised for 20-30 seconds with a 1 percent sodium hypochlorite solution. The cut leaf parts were then rinsed three times in sterile distilled water to eliminate any traces of sodium hypochlorite. The fragments are then dried after being placed on sterilised tissue paper. Then, in a laminar air flow chamber, these fragments were placed into Petri plates containing 15 ml of Potato Dextrose Agar medium (PDA). At 25±2°C, the plates were incubated for 7-10 days. The plates were checked for fungal development on a regular basis. The pure fungal culture was obtained from tissue isolation method. Confirmation of the pathogenicity was done by following Koch's Postulates.

Single spore isolation

10 ml of sterilized water agar was poured into Petri plates and allowed to solidify. Spore suspension was prepared using sterile distilled water from ten days old culture. 1 ml of spore suspension was poured to Petri plates uniformly, again 2% agar was poured aseptically and allowed to solidify. Then the plates were observed under compound microscope to view the pathogenic conidia. Single isolated conidium was then

marked under the microscopic field with ink on the surface of the Petri plate. Then the marked areas were cut and transferred aseptically onto PDA slants with the help of cork borer and incubated at 28±10°C.

Pathogenicity study

Onion seedlings (30 days old) was planted in the earthen pots which contain mixture of sandy loam soil and cowdung (4:1) in glasshouse. The plants were sprayed with distilled water and covered using polythene bags for 24 hr. The spore (conidia) suspension of *Colletotrichum goleosporiodes* was prepared from 10 days old fungal culture using sterile distilled water at the concentration of 10⁸ spores/ml. Then, onion bulbs were punctured using sterile toothpick. After that pathogenic spore suspension was sprayed onto the injured leaves of 30 days old plants. The inoculated plants were covered with polythene bags and incubated for 3-4 days to maintain relative humidity and also for easy penetration of the conidia into the leaves. The polythene bags were removed after 5 days and in oculated plants were kept under glasshouse. The plants were observed regularly for symptom expression. After the appearance of disease symptoms, re-isolation was made from the diseased tissues of artificially inoculated plants. The isolate obtained was compared with the original culture for confirmation of fungus.

Statistical analysis

Data entry and processing were carried out in MS Excel-10. Randomized Block Design was used for analysis. Analysis was done at 5% level of significance (Gomez and Gomez, 1984).

Results and Discussion

Assessing the virulence of the pathogen

These symptoms produced by the pathogen were found to be in agreement with Ebenebe (1980) ^[2]. For proving pathogenicity on onion host, the pathogen (*C. goleosporiodes*) was artificially inoculated on the onion bulbs and leaves by creating artificial injuries to facilitate the pathogen to enter and establish the symptoms. Inoculation tests of *Colletotrichum* state of *G. cingulata* satisfied Koch's

postulates and thus demonstrated causal agent of onion twister disease (Kanlong *et al.*, 1988) [17]. After 96 hours after inoculation, typical onion anthracnose symptoms with salmon coloured mucilaginous spore matrix were observed on the infected leaf surface (Panday *et al.*, 2012) [12].

The symptoms started to appear on inoculated onion leaves on 4th day after inoculation. The symptoms appears as a small, water soaked lesions. The typical symptoms were noticed after 30 days inoculation and leaves turns to be twisted.

In the present study, disease symptoms given above were agreed with the typical symptoms described by earlier workers (Suresh patil *et al.*, 2018) [17]. Chethana (2000) proved pathogenicity on onion plants by spraying spore suspension on the plant surface. The pathogen was re-isolated from artificially infected leaves and compared with the original culture. Hence, finally confirmed that as *C. golesporiodes* the causal agent of the twister blight disease of onion.

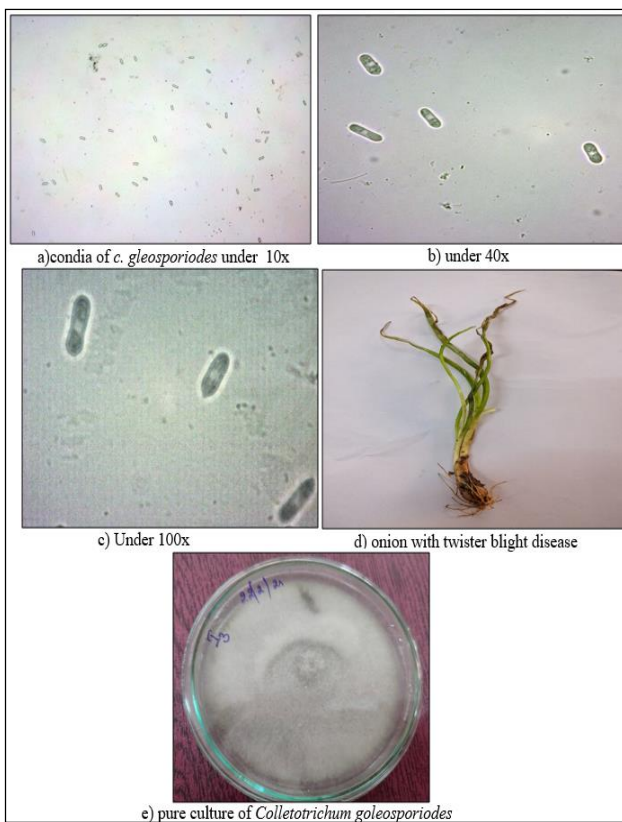


Fig 1

Survey for the occurrence twister blight of onion

The survey for distribution, symptomatology, and severity of onion twister disease was recorded and the data was presented in the Table 1. The survey results showed that disease incidence in different regions and severity ranges from 31.30% to 52.23% percent disease index (PDI) in different districts of Tamil Nadu. The highest twister blight severity of 52.23% PDI was observed at Chettikulam village in perambalur district and with highest AUDPC value 1908.52, least severity 31.30% PDI was noticed in Pavitram village of Trichy district and lowest AUDPC value is 1117.35.

The AUDPC readings were taken at intervals of 45-60 DAS, 60-75 DAS, and 75-90 DAS. The five districts were separated into four divisions based on the AUDPC values.

Table 2

AUDPC Values	Division	Disease Progress status	Districts
≥1200	1	Low	Dindugal
1300 – 1400	2	Moderate	Coimbatore, Trichy
1400– 1600	3	Severe	Namakkal
≥1600	4	More Severe	Perambalur

As twister blight disease is spread by air and seed, variations in the magnitude of damage may be related to epidemiological factors such as air currents, moisture levels in the air, temperature conditions, the amount of agricultural inputs employed, and the variety of plant selected for production. Table 2 showed the disease severity of twister blight incidence of different isolates in pot culture during the Rabi (September-November) season. The type of onion variety Co -5. The greatest PDI was observed from Perambalur district, Chettikulam village isolate 51.3%, followed by Rasipuram 48.9% PDI from Nammakal, and the lowest PDI was recorded from coimbatore district, Pollachi village isolate 26.9% and Chettikulam with highest AUDPC value 1269 and Pollachi with lowest AUDPC value 809.25. This is concluded by the results of sudhasha *et.al* 2020 [16].

Table 3 shows the disease severity of twister blight incidence of different isolates in pot culture during the Kharif (Feb-May) season. The type of onion variety Co -5. The greatest PDI was observed from Perambalur district, Chettikulam village isolate 25.8%, followed by odichattiram 23.3% PDI from Nammakal, and the lowest PDI was recorded from comibatore district, Pollachi village isolate 15.9%, Chettikulam with highest AUDPC value 801.75 and Pollachi with lowest AUDPC value 578.25. This is concluded by the results of sudhasha *et.al* 2020 [16]. During the Rabi season the disease severity is recorded high in the perambalur district, chettikulam village with PDI of 51.3% and in kharif season with PDI of 25.8% whereas the lowest PDI is recorded from the Coimbatore district of Pollachi village with 26.9% in rabi season and with the PDI Of 15.9 % is recorded in the kharif season. These variations are due to the weather parameters prevailing during the seasons. This is concluded by the results of Suresh patil *et.al* 2018 [17]. Table 4 shows the morphological differences between *colletotrichum* isolates. It was identified as the pathogen, which had white, grey, pink colony colour with hyaline oblong cylindrical conidia, and an oil globule in the centre. These also revealed size diversity, with conidia sizes ranging from (9.52 x 3.85 µm to 23.20 x 5.15 µm). The smallest conidial size is 9.52 x 3.85 µm in isolate from V.P kovil village, and the largest conidial size is 23.20 x 5.15 µm in isolate from tiruppur village.

Conclusion

The outcomes of this study found that the soil cum seed carried twister blight disease is prevalent in the places studied, have varied in their cultural and morphological attributes which is very important factor in the pathogen genetic stability. In addition, the study found that the weather factos played a significant role in the disease severity and their spread. The first step in creating disease management techniques is to have a better understanding of the morphological differences and virulence of isolates of the pathogen, as well as their climatic preferences. By combining with epidemiological studies, the farmers community can be forecasted in advance for betterment in management practices.

Table 3: Surveillance of twister blight disease of onion incited by *Colletotrichum gloeosporioides* Penz. in major onion growing regions of Tamil Nadu

S. no.	Districts	Location	Percent disease Index (PDI) at harvest	AUDPC VALUES			
				45-60 Das	60-75 Das	75-90 Das	Total AUDPC
1	Perambalur	Nattarmangalam	50.35	281.4	450.82	640.875	1373.1
		Chettikulam	52.23	498.37	655.2	754.95	1908.52
		Mavalingai	49.85	436.2	548.92	657.9	1643.02
		Siruvayalur	48.98	463.35	582.45	677.4	1723.2
		Padalur	51.64	498.22	621.15	716.1	1835.47
		Mean	50.61	435.51	571.71	689.44	1696.66
2	Trichy	Pavitram	31.30	305.85	372	439.5	1117.35
		Jadamangalam	37.68	267.45	449.85	542.7	1260
		Thuraiyur	48.54	337.95	523.8	660.6	1522.35
		Sengattypatti	45.75	422.1	598.125	661.5	1681.72
		Uppiliyapuram	38.05	358.42	462.6	537.75	1358.77
		Mean	40.26	338.35	481.27	568.41	1388.03
3	Namakkal	Erumapatti	45.38	431.32	539.325	636.45	1607.1
		Rasipuram	41.09	410.77	509.475	584.4	1504.65
		Puduchatram	46.71	458.62	597.075	659.4	1715.1
		Vennandur	33.93	294.67	389.85	475.95	1160.47
		V.P kovil	40.04	295.57	486.075	575.85	1357.5
		Mean	41.43	378.05	396.49	586.41	1468.96
4	Dindugal	Ottanchathiram	35.54	385.72	480.45	508.35	1374.525
		Nudupatti	36.43	284.17	449.55	524.325	1258.05
		Kurumbapatti	38.18	367.35	458.62	530.77	1356.75
		Vakkampatti	31.95	359.32	442.87	469.5	1271.7
		Adhilakshampuram	36.47	248.62	381.75	495	1125.375
		Mean	35.71	329.03	442.64	505.58	1266.47
5	Coimbatore	Tiruppur	41.64	442.87	544.275	613.2	1600.35
		Palladam	37.69	307.95	459.9	542.325	1310.175
		Udumalliaipettai	36.31	297.37	417	505.125	1219.5
		Ningara	34.80	266.62	394.575	483.225	1144.425
		Pollachi	39.57	413.25	512.1	569.85	1495.2
		Mean	38.00	345.61	465.57	542.74	1353.92

PDI: Percent Disease Index; AUDPC: Area UNDER Disease Progress Curve

Table 4: Estimation of disease severity of twister blight incidence of different isolates in Pot culture (Rabi season)

Isolate	Isolate number	PDI at harvest (%)	AUDPC values			
			45-55 Das	65-75 Das	85-110 Das	Total AUDPC
Padalur	C-1	36.7	171.75	311.25	471	954
Chettikulam	C-2	51.3	249	393.75	626.25	1269
V.P kovil	C-3	44.5	216.75	307.5	495	1019.25
Rasipuram	C-4	37.9	256.5	392.25	504.75	1153.5
Odichattiram	C-5	42.1	198.75	336.75	520.5	1056
Nadupatti	C-6	35.4	216.75	311.25	437.25	965.25
Thuraiyur	C-7	29.7	270	344.75	412.5	1026.75
Sengattypatti	C-8	36.4	264	381	495.75	1140.75
Tiruppur	C-9	32.7	272.25	340.5	458.25	1071
Pollachi	C-10	26.9	184.5	267	357.75	809.25
		C.D.	3.534			
		SE(m)	1.18			
		SE(d)	1.669			
		C.V.	5.471			

Table 5: Estimation of disease severity of twister blight incidence of different isolates in Pot culture (Kharif season)

Isolate	Isolate number	PDI at harvest (%)	AUDPC values			
			45-55 Das	65-75 Das	85-110 Das	Total AUDPC
Padalur	C-1	21.4	125.25	184.5	276	585.75
Chettikulam	C-2	25.8	200.25	255.75	345.75	801.75
V.P kovil	C-3	20.1	152.25	228	297	677.25
Rasipuram	C-4	22.9	200.25	243.75	300	744.00
Odichattiram	C-5	23.3	153.75	196.5	284.25	634.50
Nadupatti	C-6	18.6	172.5	204	248.25	624.75

Thuraiyur	C-7	20.6	245.25	271.5	296.25	712.50
Sengattypatti	C-8	21.1	222	258.75	300.75	781.50
Tiruppur	C-9	17.7	186.75	224.25	249.75	660.75
Pollachi	C-10	15.9	155.25	192.75	230.25	578.25
		C.D.	1.355			
		SE(m)	0.453			
		SE(d)	0.64			
		C.V.	3.78			

Annexure: Rabi season which starts with September, the last month of the rainy (monsoon) season, in Chidambaram, with average temperature fluctuating between 24.3°C– 33.4°C (75.74°F-92.12°F), 255mm-412mm of precipitation and the average length of the day was 12.2h-11.9h (Online resource- Weather Atlas). Whereas, the Kharif season starts with Feb-

May is a hot summer month in Chidambaram, with average temperature fluctuating between 33.7°C-36.4°C (93°F-97.5°F), 23-47mm of precipitation the average length of the day in Feb-May is 12.2- 12.6h (Online resource- Weather Atlas).

Table 6: Morphological variation among the isolates of *C. gloeosporioides*

S.No	Isolate	Isolate number	Colony colour	Colony morphology	Colony in diameter(mm)	Conidial size (um)
1.	Padalur	C-1	creamy white	Fluffy, No sectoring	85	16.24 x 4.62
2.	Chettikulam	C-2	Dull black	Fluffy, sectoring	90	19.65 x 5.49
3.	v.p kovil	C-3	Greyish	Fluffy, No sectoring	90	9.52 x 3.85
4.	Rasipuram	C-4	Creamy white	Flat, No sectoring	87	15.18 x 4.30
5.	Odichattiram	C-5	Greyish	Fluffy, sectoring	90	15.46 x 5.83
6.	Nadupatti	C-6	Greyish	Fluffy, sectoring	88	10.54 x 4.76
7.	Thuraiyur	C-7	Off white	Light fluffy, No sectoring	90	25.30 x 6.48
8.	Sengattypatti	C-8	Off white	Fluffy, sectoring	90	12.79 x 4.01
9.	Tiruppur	C-9	Greyish	Fluffy, No sectoring	90	23.20 x 5.15
10.	Pollachi	C-10	Greyish	Fluffy, sectoring	90	17.62 x 4.33

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