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Survey, isolation and pathogenicity of twister blight disease of onion caused by *Colletotrichum gloeosporioides* in Tamil Nadu

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Abstract
Onion (*Allium cepa* var. *aggregatum*) is a prominent bulbous vegetable crop grown worldwide for its pungency and flavour. Onion production is affected by various fungal and bacterial diseases. Among the fungal diseases affects onion, the twister blight of onion (*Colletotrichum gloeosporioides*) causes yield loss of 20 to 30 per cent. A survey was conducted to assess the incidence and severity of the twister blight disease in six onion growing districts of Tamil Nadu. The highest twister blight disease incidence (48.57 PDI) was recorded in Kurippangulam village of Tirunelveli district. The lowest disease incidence (28.00 PDI) was recorded in Sankarankovil of Tenkasi district. During the survey ten isolates (IS 1 to IS 10) of *Colletotrichum gloeosporioides* were isolated from the diseased samples. Among these isolates, Kurippangulam isolate (IS 1) was found to be the most virulent.

Keywords: Onion, *Allium cepa* var. *aggregatum*, twister blight, *Colletotrichum gloeosporioides*

1. Introduction
The onion (*Allium cepa* var. *aggregatum*) belongs to the family Alliaceae, is a widely grown vegetable crop in India and is used for salad, spice and medicine. It is an important commercial vegetable crop (Sinnadurai, 1970) [14]. India occupies second place next to China in onion production (26.74 million tonnes) and productivity of 18.64 MT/ha from an area (14.34 lakh hectares). In India, Maharashtra alone contributes 40.94 per cent of the total area under onion cultivation. Madhya Pradesh is the second-largest in terms of production (16.36%), followed by Karnataka (8.71%) and Gujarat (5.45%) (Anonymous, 2020) [1]. Among the foliar diseases affecting onion, twister blight disease of onion is one of the most devastating disease. This disease was initially discovered in 1969 near Zaria Nigeria and the causal organism is identified as *Colletotrichum gloeosporioides* by Ebenebe (1980) [3]. Kuruppu (1999) [8] reported the twister blight disease on shallot onions (*Allium cepa* var. *ascalonicum*) which caused the yield loss of 20 to 30 per cent. The extent of loss varies with the time of infection and stage of crop growth (Hegde *et al*., 2012) [5]. In the recent years, onion twister blight disease has extended in most of the onion growing districts of Tamil Nadu. Therefore, the present study has been focused on survey and assessment of disease incidence in major onion growing areas of Tamil Nadu.

2. Materials and Methods
2.1 Survey and collection of infected samples
A Survey was conducted to assess the incidence and severity of twister blight in five onion growing districts of Tamil Nadu. The twister blight infected samples were collected from the surveyed area (Fig 1) and kept in labelled polythene bag. In each surveyed area, observation on disease severity was recorded based on 0 to 5 grade (Bhangale and Joi, 1985) [2] as detailed below.

In each surveyed area, observation on disease severity was recorded based on 0 to 5 grade detailed below

Grade	Description
0	No Symptom.
1	Upto 10% Curling and chlorosis of leaves.
2	11 to 20% Abnormal elongation of leaves and neck.
3	21 to 40% Leaf-sheath showing cluster of acervuli concentric rings.
4	41 to 60% Elongated neck, slender bulbs leaves show dieback symptoms.
5	>60% Severe dieback, rotten bulbs, root system underdeveloped with discoloured roots.

Per cent Disease Index (PDI) of twister blight disease was calculated by using the formula proposed by (Mckinney, 1923) [10].

$$\text{Per cent disease index (PDI)} = \frac{\text{Sum of all individual disease ratings X 100}}{\text{Total No. of plants observed X Maximum disease grade}}$$

2.2 Isolation and purification of the pathogen

Isolation of the pathogen was done on the PDA medium from twister blight infected leaves of the onion plants by using tissue segmentation method (Rangaswami, 1958) [12]. The infected leaves were washed in sterile distilled water and cut into small pieces with the help of a sterilized blade. The washed pieces were surface sterilized with mercuric chloride (0.1%) for 30 seconds. After surface sterilization the tissues were washed three times with sterile water to remove the traces of mercuric chloride and blot dried in sterile blotter paper. The diseased leaf bits were then transferred aseptically to the sterilized PDA medium and incubated at 28 ± 2°C for seven days. After seven days of incubation, the mycelial growth of each isolate was observed separately. The actively growing hyphal tips of fungus were transferred to the PDA slant under aseptic conditions and the pure culture was maintained at 25 °C.

2.3 Morphological characterization of *Colletotrichum gloeosporioides*

The culture was identified based on the morphological characters *viz.*, colony colour, mycelial growth, shape of the conidia and presence of oil globules with the use of compound microscope.

2.4 Pathogenicity test

Pathogenicity test was conducted for all the ten isolates of *Colletotrichum gloeosporioides* under pot culture condition by pin prick method. After 30 days of inoculation, the severity of twister blight incidence was assessed based on 0-5 grade proposed by Bhangale and Joi, (1985) [2] and re-isolation of the pathogenic isolates were also made.

2.5 Statistical analysis

The experimental results were analysed using WASP Stat, which is now being applied to compare the treatment means using DMRT. Whenever the percentage values reached crucial difference levels with a five percent level of significance, they were transformed into an arc sine.

3. Results and Discussion

3.1 Survey and occurrence of onion twister blight disease

A survey report revealed that the twister blight disease incidence in surveyed areas was ranged between 28.00 to 48.57 PDI (Table 1; Fig 2). Among all the surveyed areas, the maximum disease incidence was found in Kurippangulam village (48.57 PDI) of Tirunelveli district followed by Vaalikandapuram (48.00 PDI) of Perambalur district, the least disease incidence of 28.00 PDI was recorded from Sankarankovil of Tenkasi district. The maximum disease severity of 55.00 per cent and 24.05 percent in Uttar Kannada district in 2011 and 2012 respectively were recorded by Patil *et al*., (2016) [11]. The disease severity range of 0 to 77.65 per cent was recorded by Manthesha *et al*., (2022) [9] in six districts of Kalyan-Karnataka region.

3.2 Isolation and identification of the pathogen

Ten isolates of *Colletotrichum gloeosporioides* were isolated from the infected samples. The isolates were named as IS 1, IS 2, IS 3, IS 4, IS 5, IS 6, IS 7, IS 8, IS 9 and IS 10. Pure culture of the pathogenic isolates were maintained (Fig 3). Morphological and cultural characters of *Colletotrichum gloeosporioides* was studied in PDA medium. Among the ten isolates, Kurippangulam isolate (IS 1) produced greyish white cottony mycelium and covered the entire plate within ten days (Table 2). Sankarankovil isolate (IS 1) and Vaalikandapuram isolate (IS 5) showed moderate fluffy mycelial growth. Alandha isolate (IS 3) and Manjanayaganpatty isolate (IS 9) produced brownish white mycelial growth. Singhathakurichy isolate (IS 4) showed white colour fluffy mycelium, whereas Sridevimangalam isolate (IS 6) and Satharapatty isolate (IS 10) showed circular cottony mycelium with grey white. Ottanchathiram isolate (IS 7) and Veeralapaty isolate (IS 8) showed white colour cottony mycelium. The maximum radial growth of 90.00 mm was observed in IS 1 isolate and found to be more virulent which has taken 10 days to completely cover the plates. All the isolates were produced hyaline conidia. The shape of the conidia is cylindical (IS1, IS 3, IS 4, IS 5), elliptical (IS 2, IS 7), oblong shape (IS 8), fusiform shape (IS 10). The length of the conidia ranged from 9.05 to 17.25 µm. The maximum conidial length was recorded in IS 1 isolate (17.25 µm) followed by IS 7 isolate (14.30 µm). The minimum conidial length of 9.05 µm IS 6 isolate. Width of the conidia was ranged from 3.36 to 5.49 µm. The maximum width of conidia was observed in IS 1 isolate (5.49 µm) followed by 4.73 µm (IS 5 isolate). The minimum conidial width was noticed in IS 6 isolate (Table 3; Fig 4). Similarly, Sikirou *et al*., (2011) [13] observed white to grey mycelia, which later transformed into dark brown colour. Acervuli were profuse, dark brown to black in appearance, globose to irregular in shape and glabrous. Conidia were hyaline, unicellular, aseptate and oval to cylindrical with rounded ends and were 10 to 20 µm long and 3 to 5 µm wide whereas they also generated greyish-white colonies with dark grey to black colony colour on back side of the Petri plate, which is a feature of *C. gloeosporioides* colonies (Than *et al.*, 2008) [15].

3.3 Assessing the virulence of the pathogen

Pathogenicity was done through pot culture experiment for all the ten isolates of *C. gloeosporioides*. Among the ten isolates, Kurippangulam isolate (IS 1) recorded the highest disease severity (Grade 5). The infected plant showed curling, twisting, chlorosis, abnormal elongation of the neck and finally death of plants were observed on thirty days after inoculation. Hence, IS 1 isolate of *C. gloeosporioides* was identified as the most virulent isolate and used for further studies (Table 4; Fig 5). Hill (2008) [7] confirmed the pathogenicity of onion twister disease by spray inoculation technique on onion seedlings. Gyempeh *et al.*, (2015) [4] conducted the pathogenicity test by using foliar and soil inoculation method.

Table 1: Survey and incidence of twister blight disease of onion in various places of Tamil Nadu

S. No.	Location	District	Isolate Name	Geographic coordinates	Variety	Percent disease Index (PDI)*
1	Kurippangulam	Tirunelveli	IS 1	8.90^0 N 77.50^0 E	Local	48.57^a
2	Sankarankovil	Thenkasi	IS 2	9.17^0 N 77.51^0 E	Local	28.00^g
3	Alandha	Thoothukudi	IS 3	8.78^0 N 77.86^0 E	Local	43.80^b
4	Singhathakurichy		IS 4	11.10^0 N 78.82^0 E	Local	40.74^d
5	Vaalikandapuram	Perambalur	IS 5	11.31^0N 78.92^0 E	Local	48.00^a
6	Sridevimangalam		IS 6	10.48^0 N 77.68^0 E	Local	42.20^c
7	Ottanchathiram	Dindugal	IS 7	10.48^0N 77.66^0 E	Local	28.80^g
8	Veeralapaty		IS 8	10.49^0 N 77.67^0 E	Local	33.50^f
9	Manjanayaganpatty		IS 9	10.47^0 N 77.63^0 E	Local	23.40^h
10	Satharapatty		IS 10	10.47^0 N 77.68^0 E	Local	35.80^e
SE(d)= 0.584						
CD (P=0.05) = 1.126						
CV= 2.083						

*Mean of four replications.
The treatment means are compared using Duncan Multiple Range Test (DMRT).
In a column, means followed by a common letter (s) are not significantly different (P=0.05).

Table 2: Morphological characters of *C. gloeosporioides*

S. No	Isolates	Cultural characteristics	Colony diameter (mm)*	Days to cover full plate
1	IS 1	Greyish white cottony mycelium	90.00^a	10
2	IS 2	Moderate fluffy mycelium	85.58^b	11
3	IS 3	Brownish white mycelial growth	75.00^f	13
4	IS 4	White colour fluffy mycelium	78.00^e	14
5	IS 5	Moderate fluffy mycelium	68.00^h	12
6	IS 6	Circular cottony mycelium with greyish white	80.00^d	14
7	IS 7	White colour cottony mycelium	69.25^h	13
8	IS 8	White colour cottony mycelium	72.55^g	11
9	IS 9	Brownish white mycelial growth	82.12^c	12
10	IS 10	Circular cottony mycelium with greyish white	66.00^i	14
		CD (P =0.05)	2.154	
		CV	1.937	

*Mean of four replication.
The treatment means are compared using Duncan Multiple Range Test (DMRT).
In a column, means followed by a common letter (s) are not significantly different (P=0.05).

Table 3: Conidial variation among the isolates of *Colletotrichum gloeosporioides*

S. No	Isolate	Colour of conidia	Shape of conidia	Length of conidia (µm)*	Width of conidia (µm)*
1.	IS 1	Hyaline	Cylindrical and straight	17.25^a	5.498^a
2.	IS 2	Hyaline	Elliptical	13.395^c	4.25^d
3.	IS 3	Hyaline	Cylindrical and straight	11.27^e	4.523^c
4.	IS 4	Hyaline	Cylindrical and	10.15^g	3.503^g
5.	IS 5	Hyaline	Cylindrical	12.103^d	4.73^b
6.	IS 6	Hyaline	Oval to cylindrical with rounded ends	9.05^h	3.36^h
7.	IS 7	Hyaline	Ellipsoidal	14.3^b	4.25^d
8.	IS 8	Hyaline	Oblong	10.5^f	3.75^f
9.	IS 9	Hyaline	Cylindrical and rounded ends	11.25^e	4.023^e
10.	IS 10	Hyaline	Fusiform	9.253^h	3.803^f

CD (P =0.05)	0.275	0.115
CV	1.596	1.898

*Mean of four replication.
The treatment means are compared using Duncan Multiple Range Test (DMRT).
In a column, means followed by a common letter (s) are not significantly different (P=0.05).

Table 4: Severity level of various isolates of *Colletotrichum gloeosporioides*

S. No.	Isolate	Grade	Description
1	IS 1	5	Severe dieback, rotten bulbs, root system under developed with discolored roots.
2	IS 2	4	Elongated neck, slender bulbs leaves show dieback symptoms
3	IS 3	3	Leaf-sheath showing cluster of acervuli concentric rings along with shallow, sunken necrotic spots.
4	IS 4	1	Curling and chlorosis of leaves
5	IS 5	2	Abnormal elongation of leaves and neck
6	IS 6	3	Leaf-sheath showing cluster of acervuli concentric rings along with shallow, sunken necrotic spots a
7	IS 7	4	Elongated neck, slender bulbs leaves show dieback symptoms
8	IS 8	1	Curling and chlorosis of leaves
9	IS 9	1	Curling and chlorosis of leaves
10	IS 10	2	Abnormal elongation of leaves and neck

Fig 1: Symptoms and sign of the twister blight disease

Onion twister blight disease incidence in Tirunelveli district Onion twister blight disease incidence in Tenkasi district

Fig 2: Survey and incidence of onion twister blight disease

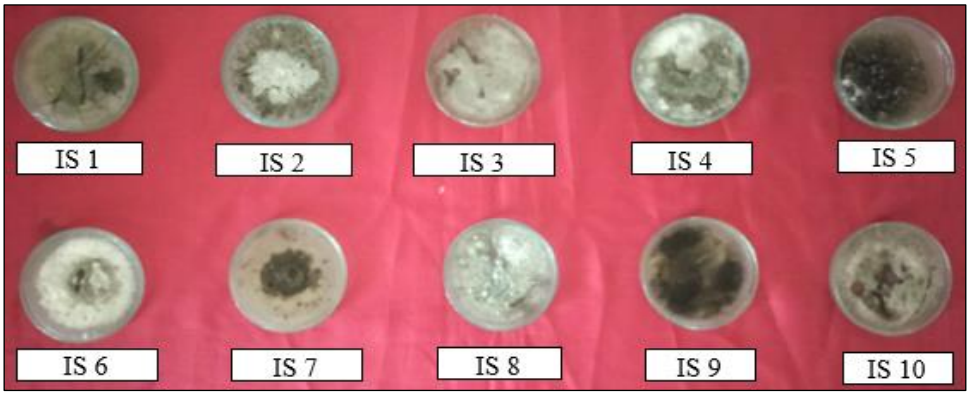

Fig 3: Various isolates of *Colletotrichum gloeosporioides*

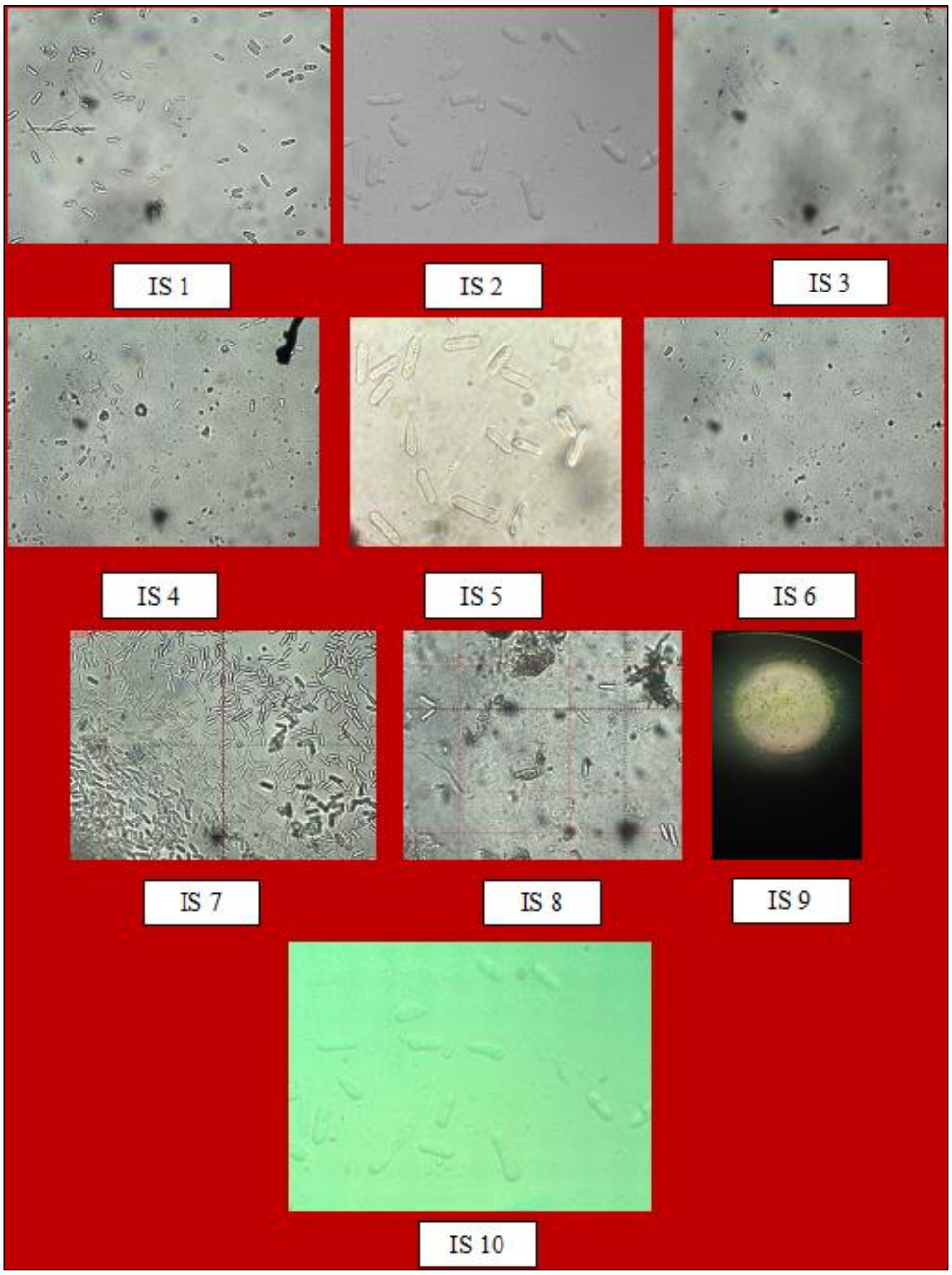

Fig 4: Conidial variations of *Colletotrichum gloeosporioidess*

Healthy plant Diseased Plants

Fig 5: Pathogenicity Test

4. Conclusion

The disease incidence of onion twister blight was the highest (48.57 PDI) in Kurippangulam village of Tirunelveli district. The least disease incidence (28.00 PDI) was noticed in Sankarankovil of Tenkasi district. The pathogenic isolates (IS 1) obtained from Kurippangulam village of Tirunelveli district was found to be the most virulent.

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