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Unravelling the morphological features of *Colletotrichum gloeosporioides*: The causative agent of leaf twister blight disease in onion (*Allium cepa* L.)

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Abstract
Onion twister blight disease is a major constraint in onion cultivation during the rainy season. The present study was carried out with the aim of studying the morphological characters of onion twister blight pathogen. Survey was conducted in major onion cultivating areas of Perambalur, Dindigul, Madurai and Virudhunagar districts, Tamil Nadu and percent disease incidence ranged from 34.44 and 78.88%. The infected plants of onion showed various symptoms, including chlorosis, leaf twisting, curling, the presence of acervuli in the neck region, and the development of slender bulbs. The pathogen isolated from infected leaves of onion exhibited a greyish white to grey mycelium, hyaline and septate. The pathogen produced cylindrical, hyaline conidia with a size ranging from 15 to 20µm in length and 4 to 7 µm in width. Isolated pathogens proved its virulence in symptom development and satisfied Koch's postulates during pathogenicity test. This provided conclusive evidence of the pathogen's role in causing the twister blight disease.

Keywords: *Colletotrichum gloeosporioides*, percent disease incidence, pathogenicity, onion twister blight

Introduction
The onion scientifically named *Allium cepa* L. which holds the label "Queen of the Kitchen". India holds the esteemed position of being the second-largest producer of onion globallywith a production of 22.07 million metric tons and a productivity of 16.78 tons per hectare (Kumawat & Raheman, 2022) [5]. It is a bulbous, biennial herb, prominent vegetable crop and cultivated in various districts of Tamil Nadu, *viz*., Perambalur, Dindigul, Madurai, Virudhunagar, Trichy, Namakkal and Erode. Onion yield is significantly hampered by numerous fungal and bacterial diseases. Among biotic stresses, twister blight disease caused by *Colletotrichum gloeosporioides* has been identified as a major threat to onion cultivation in Tamil Nadu. It was first reported from Zaria, north Nigeria, in 1969 (Ebenebe, 1980) [2]. In India, it was first reported from the Lonand, Satara district, Maharashtra during 1981. Then, it was further spread to other states of India and it may cause 50 to 100% yield losses in the onion cultivation areas of Tamil Nadu. The typical disease symptoms are chlorosis, leaf curling, twisting, elongated necks, sunken lesions on leaf sheaths and finally plant death. Knowing the distribution pattern and characterization of pathogen are very much important for the development of management strategies. Hence, the present study was to assess the disease occurrence and intensity in the major onion-growing regions of Madurai, Virudhunagar, Perambalur and Dindigul and to characterize the pathogen morphologically.

Materials and Methods
Disease incidence
Aroving survey was conducted in the commercial cultivation areas of onionat various districts of Tamil Nadu, *viz*., Madurai (Kesampatti, Kalvelipatti, Panangudi villages), Virudhunagar (Parankudi village), Perambalur (Veppanthattai, Esanai, Kaikalathur villages) and Dindigul (Toppampatti, Oddanchatram, Ethachankottai villages) during 2022–2023, to assess the incidence and severity of the onion twister blight disease. The percent disease incidence (PDI) was calculated by using the following formula (Vidhyasekaran, 2004) [11].

$$\text{PDI (\%)} = \frac{\text{No.of plants infected}}{\text{Total number of plants observed}} \times 100$$

Isolation of pathogen

Leaf samples of onion showing twisting and curling collected from the major cultivation areas of the onion crop, labeled properly and brought to the laboratory for isolation of the pathogen using the tissue isolation method under *in vitro* condition. The infected leaves sliced into small pieces using a sterilized scalpel, and the leaf bits were surface sterilized with 0.1% sodium hypochlorite for 2 minutes followed by sterile water wash to remove the excess of sodium hypochlorite. The surface sterilized leaf bits were transferred to the Petri plates containing Potato Dextrose Agar medium (PDA) under aseptic conditions. The plates were incubated at a temperature of 27 °C for 7 to 10 days and the plates were regularly monitored for any signs of fungal growth. Then the cultures were purified and stored for further use under refrigerated condition.

Cultural and Morphological characterization

The morphological features such as color, shape, type of hyphae were observed and recorded to categorize the fungal pathogen. In addition to that, shape and size of the spores were also recorded using a light microscope at a 40X magnification. The growth rate of fungal isolates was determined by measuring the colony diameter.

Proving Koch's postulates

To prove the pathogenicity of the pathogen, the onion variety Co (on) 5was utilized in the experiment. Each pot sown with six onion bulbs of the Co (on) 5 variety. The spore suspension ($2.5x10^6$ spores/ml) of the pathogen was sprayed to the onion plant at 35–40 days after sowing (DAS) to induce the disease pressure, while onion plants sprayed with sterile distilled water served as the control. The onion plants were observed for symptom development and recorded the disease incidence and nature of symptoms developed. The data obtained from the experiment was statistically analyzed (Gyempeh, 2015 and Ebenebe, 1980) [3, 2].

Result and Discussion

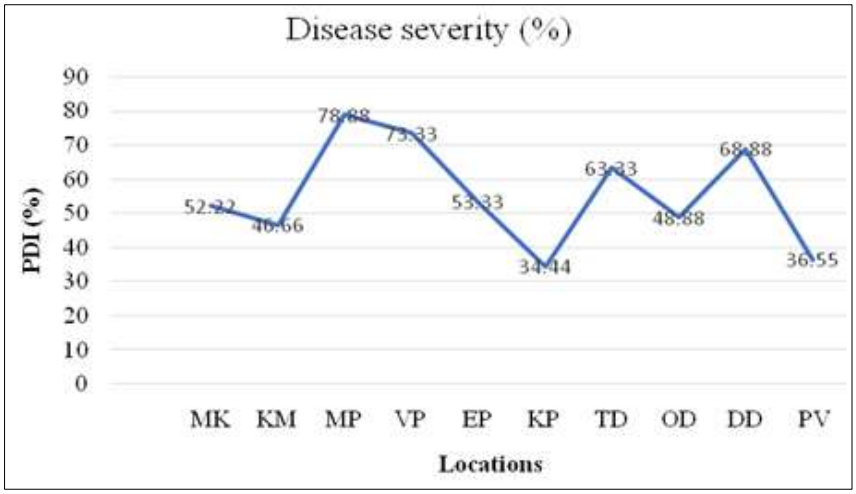
Symptoms

Onion twister blight disease was identified based on symptoms such as chlorosis, leaf twisting, curling, the presence of acervuli on the neck region of onion plants and the formation of slender bulbs (Ebenebe AC., 1980, Sikirou *et al*., 2011) [2, 9], as illustrated in Figure 1. Similar observation was reported by Dutta *et al*, (2022) [1], where the symptom exhibited the pale-yellowwater-soaked oval depressed lesion on leaf blade and laterally it turns to black color lesion.

Fig 1: Onion plants showing symptoms of twister blight disease

Survey and assessment

Aroving survey was conducted in the commercial cultivation areas of onionat various districts of Tamil Nadu, *viz*., Madurai (Kesampatti, Kalvelipatti, Panangudi villages), Virudhunagar (Parankudi village), Perambalur (Veppanthattai, Esanai, Kaikalathur villages) and Dindigul (Toppampatti, Oddanchatram, Ethachankottai villages) during 2022–2023. The twister blight disease incidence ranged from 34.44 and 78.88 per cent. The per cent incidence of twister blight disease was high inPanangudi village, Madurai (78.88 %), whereas low incidence was observed in Kaikalathur village, Perambalur (34.44%) (Fig 2).



MK-Madurai Kesampatti; KM-Kalvelipatti Madurai; MP-Madurai Panangudi; VP-Veppanthattai Perambalur; EP- Esanai Perambalur; KP- Kaikalathur; TD-Toppampatti; OD-Ottanchatram Dindigul; DD- Ethachankottai; PV -Parankudi Virudhunagar

Fig 2: Percent disease incidence of Onion twister blight disease in various districts of Tamil Nadu (Madurai, Perambalur, Dindigul and Virudhunagar)

Isolation of pathogen

The pathogen was isolated from the infected onion plants, which were collected from various villages located in different districts, Madurai, Perambalur, Dindigul, Virudhunagar. The isolated fungal colony exhibited a greyish-white cottony appearance on PDA medium, and preserved all the isolates as a pure culture.

Cultural and Morphological characterization

The mycelium arisen from ten isolates showed a range of colors, from whitish-grey to dark grey on the upper side of the plate and orange to dark black on the lower side. This observation aligns with the descriptions provided by (Rangkuti *et al*., 2017; Vengadaramana A, D De Costa, 2014) [6, 10]. The fungus exhibited hyaline, septate mycelium, and hyaline, cylindrical conidia with rounded ends under microscope, which is consistent with findings from previous studies (Sikirou *et al*., 2011; Sattar and Malik 1939) [9, 7] (Table 1 and Fig. 3 and 4).

Table 1: Cultural characteristic of *C. gloeosporioides* causing twister blight disease in onion

S. No	Isolates	Place	Mycelium growth pattern	Colony colour	Growth rate	Sporulation*
1	CKM1	Kesampatti	Mycelium is whitish grey with fluffy centre	Whitish grey	Moderate	++
2	CMK2	Kalvelipatti	Mycelium is grey with fluffy centre	Whitish red	Fast	+++
3	CMP3	Panangudi	Mycelium is whitish grey with fluffy centre	Creamy white	Very fast	++++
4	CVP4	Veppanthattai	Thick and cottony mycelium	Greyish white	Slow	+
5	CEP5	Esanai	Sparse and thin mycelium	Grey	Moderate	+++
6	CKP6	Kaikalathur	Thick and aggregated at centre	Greyish white	Slow	++
7	CTD7	Toppampatti	Thick and cottony mycelium	Grey	Fast	+++
8	COD8	Oddanchatram	Thick and cottony mycelium	Grey	Very fast	++++
9	CDD9	Ethachankottai	Thick and aggregated at centre	Creamy white	Fast	++
10	CPV10	Parankudi	Sparse and thin mycelium	Creamy white	Very fast	++++

*++++ -very fast sporulation, +++- Fast sporulation, ++- moderate sporulation, + - slow sporulation

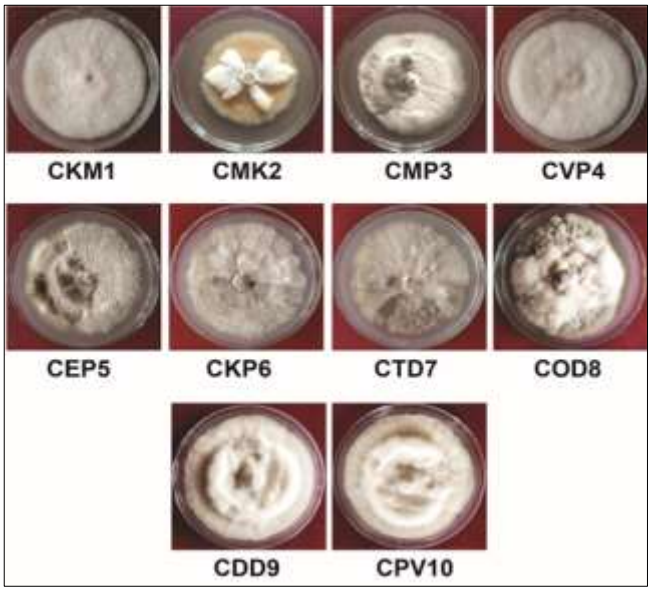


Fig 3: Isolates of *Colletotrichum gloeosporioides* from different onion growing areas of Madurai, Perambalur, Dindigul and Virudhunagar district

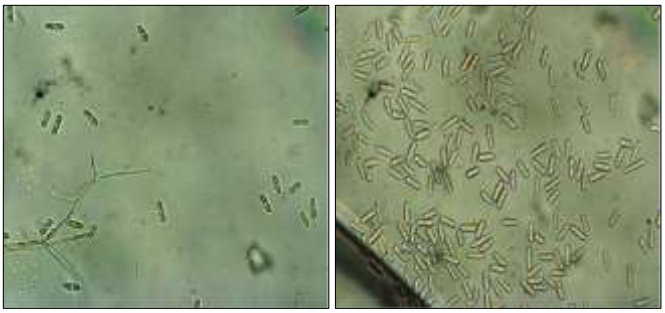


Fig 4: Microscopic image of *C. gloeosporioides* A- Mycelium (Hyaline and estate) B-Conidia (cylindrical with rounded ends)

Pathogenicity test

Ten isolates were subjected to pathogenicity testing under pot culture conditions and observed for the symptom's development, which was identical to those observed in the field. Out of the ten isolates tested, CMP-3 exhibited the highest virulence with a pathogenicity rate of 78.89%, followed by COD-8 (77.88%), and the least incidence was recorded in CKM-1(16.66%). Then, the pathogen isolates were re-isolated, and observed its morphological characteristics (Figures 5 and 6).

a. Healthy Plant b. Infected Plant

Fig 5: Pathogenicity studies

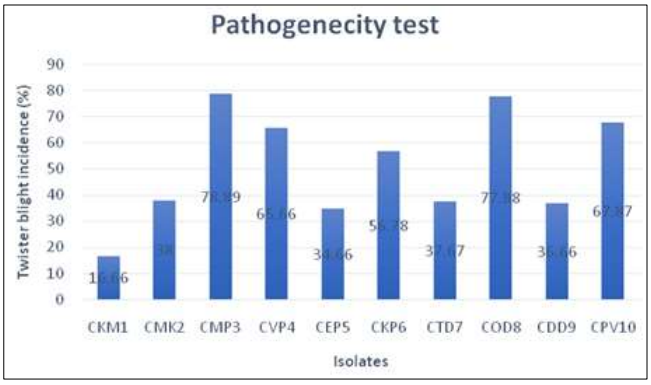


Fig 6: Percent disease incidence while proving Koch's postulates under pot culture condition

Conclusion

The current investigation indicated that the leaf twister blight disease, incited by *Colletotrichum gloeosporioides*. The percent disease incidence ranged from 34.44 and 78.88% in commercial growing areas of onion. The pathogen displayed hyaline, elongated mycelium, and cylindrical conidia.

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References

1. Dutta Ram, Jayalakshmi K, Sharath M. Nadig, Dalasanuru Chandregowda Manjunathagowda, Vishal S. Gurav, and Major Singh. Anthracnose of onion (*Allium cepa* L.): A twister disease. Pathogens. 2022;11(8):884.
2. Ebenebe AC. Onion twister disease caused by *Glomerella cingulate* in northern Nigeria. No. Research; c1980.
3. Gyempeh N, Offei SK, Cornelius EW, Honger JO. Importance of the onion leaf twister disease in Ghana and the effect of *Trichoderma asperellium* on the mycelia growth and sporulation of the causal agent. Ghana Journal of Science. 2015;55:51-65.
4. Khanzada, Muhammad Ali, Arsheema Ansari, Muhammad Aslam Rajput, Sultan Maitlo, Abdul Qayoom Rajput et al. Effect of different abiotic factors on the growth and sporulation of *Colletotrichum gloeosporioides* causing anthracnose of mango. Plant Protection. 2018;2(1):23-30.
5. Kumawat L, Raheman H. Mechanization in onionharvesting and its performance: A review and aconceptual design of onion harvester from Indian perspective. Journal of The Institution of Engineers (India): Series A; c2022, p. 1-10.
6. Rangkuti, Eryna Elfasari, Suryo Wiyono, Widodo. Identifikasi *Colletotrichum* spp. Asal Tanaman Pepaya. Jurnal Fitopatologi Indonesia. 2017;13(5):175-175.
7. Sattar A, Malik SA. Some studies on anthracnose of mango caused by *Glomerella cingulata* (Stonem.) Spauld Sch. *Colletotrichum gloeosporioides*; c1939. p. 511-521.
8. Sattar A, Malik SA. Some studies on anthracnose ofmango caused by *Glomerella cingulata* (Stonem.) Spauld Sch. *Colletotrichum gloeosporioides*; c1939. p. 511-521.
9. Sikirou R, Beed F, Hotègni J, Winter S, Assogba-Komlan Reeder FR, et al. First report of anthracnose caused by *Colletotrichum gloeosporioides* on onion (*Allium cepa*) in Benin. New Dis Rep. 2011;23(7):583-584.
10. Vengadaramana A, de Costa D. Morphological and pathogenic variations of the causal organisms of leaf twister disease of red onion (*Allium cepa* L.) In Jaffna district of Sri Lanka. Tropical Agricultural Research. 2014;25(3):412-431.
11. Vidhyasekaran, Perumal. Concise encyclopedia of plant pathology. CRC Press; c2004.