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Morphological characterization of *Colletotrichum gloeosporioides* causing leaf twister blight disease in *Allium cepa* (onion)

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Abstract

Onion twister blight disease is a major rainy season disease in major onion cultivating areas of Madurai and Virudhunagar districts. The disease showed characteristic symptoms of twisting, curling, chlorosis of leaves, conidial mass in the neck region, and slender bulbs. The pathogen was isolated and its morphological characteristics were studied. It showed greyish white to grey mycelium which was hyaline and spectate. The pathogen produced conidia which was hyaline, cylindrical in shape, and spore size ranged between 15-20x4-6µm. The artificial inoculation test proves its pathogenicity which satisfied Koch's postulates and the morphological characteristics of various isolates of *C. Gloeosporioides* were confirmed.

Keywords: Twister blight, Pathogenicity, morphological characters

Introduction

The onion (*Allium cepa* L.) is a bulbous, biennial herb known as the "Queen of the Kitchen." It is a major vegetable crop cultivated in India, with a total area of 14.05 lakh hectares and a yield of 224.27 lakh tonnes with the productivity of 17.18 Tonnes per hectare (NHRDF 2016-2017, Nashik). Onion is a prominent vegetable crop farmed in the Tamil Nadu districts of Perambalur, Dindigul, Madurai, Virudhunagar, Trichy, Namakkal and Erode. Numerous fungal and bacterial diseases severely limit onion yield. In recent days, onion crop has been infected with twister blight disease, which has resulted in yield losses ranging from 50% to 100% in Tamilnadu's key onion-growing districts. Onion twister blight caused by *Colletotrichum Gloeosporioides* is a rainy season disease. The symptoms of the disease are twisting, curling and chlorosis of leaves, elongated necks and sunken lesions on leaf sheaths. The current study was conducted to determine the incidence and severity of the disease in the major onion-growing areas of Madurai and Virudhunagar and to confirm its etiology by morphological characters and proving its pathogenicity.

Materials and methods

Disease incidence and severity

During 2021–2022, a survey was conducted in the predominant onion-growing areas of Madurai and Virudhunagar districts to determine the incidence and severity of the onion twister blight disease. A roving survey was conducted in the villages of Narasingam, Kalvelipatti, Allathur, Alanganallur, and Mullaiperiyar river bank of Madurai district and in Rayarpatty, Kallanai, Arasakulam, Virudhunagar, and Kambakudi of Virudhunagar district. The disease incidence (Vidhyasekaran, 2004) ^[10] was calculated by using the formula

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

Where PDI is Percent Disease Incidence

Isolation of pathogen

The diseased samples were collected from the infected area, which were brought to the lab and the pathogen was isolated *in vitro* by tissue isolation method. The infected leaves were selected and cut into small bits of about 0.5 to 1 cm using a sterilized scalpel or blade. The leaf bits were surface sterilized with 0.1% sodium hypochlorite for 2 minutes. These cut leaf bits were

The disinfected leaf bits were transferred to the Petri plates, which contained 15 ml of Potato Dextrose Agar medium (PDA) under aseptic conditions. The plates were incubated at 27°C for 5 to 10 days. The plates were observed frequently for fungal growth. To obtain pure fungus colonies, mycelium was purified based on the colour of the colony on PDA.

Cultural and Morphological characteristics

The fungus was distinguished by morphological and microscopic characteristics. Colour, shape, hyphae (estate or acetate) and spores were noticed and documented. The colony diameter of fungal isolates is used to calculate growth rate. Spore shapes and sizes were observed and measured using a light microscope (400X magnification).

Pathogenicity test

To test for pathogenicity, Co (on) 5 onion variety was used. Six onion bulbs of the Co (on) 5 variety were planted in each pot. At 35–40 DAS, the onion plants were sprayed with the spore suspension at a rate of 2.5×10^6 spore/ml and the pots sprayed with sterile distilled water served as control (Gyempeh, N., 2015; Ebenebe AC., 1980) [3, 2]. The data was analyzed through CRD analysis.

Result and Discussion

Symptoms: Twisting, curling, and chlorosis of the leaves, and also the presence of acervuli on the neck region of onion plants and slender bulbs are the characteristic symptoms recognized as onion twister blight (Ebenebe AC 1980, Sikirou R *et al.*, 2011) [2, 8], Fig.1.



Fig 1: Symptoms of onion twister blight

Survey and assessment

A roving survey was executed in major onion cultivating areas of Madurai and Virudhunagar districts *viz.*, Narasingam, Kalvelipatti, Allathur, Alanganallur, and Mullaiperiyar river bank of Madurai district and in Rayarpatty, Kallanai, Arasakulam, Virudhunagar, and Kambakudi of Virudhunagar district and specimens were collected from the infected plants exhibiting leaf twisting symptoms. In the areas surveyed, the

prevalence of infection varied between 23.3 and 76.67 per cent. The study indicated that Rayarpatty village in Virudhunagar district had the highest disease incidence of twister blight with 76.67 per cent, followed by Arasakulam village which registered 73.33 per cent, and the lowest incidence was observed in Allathur village of Madurai district (23.33%), (Fig 2).

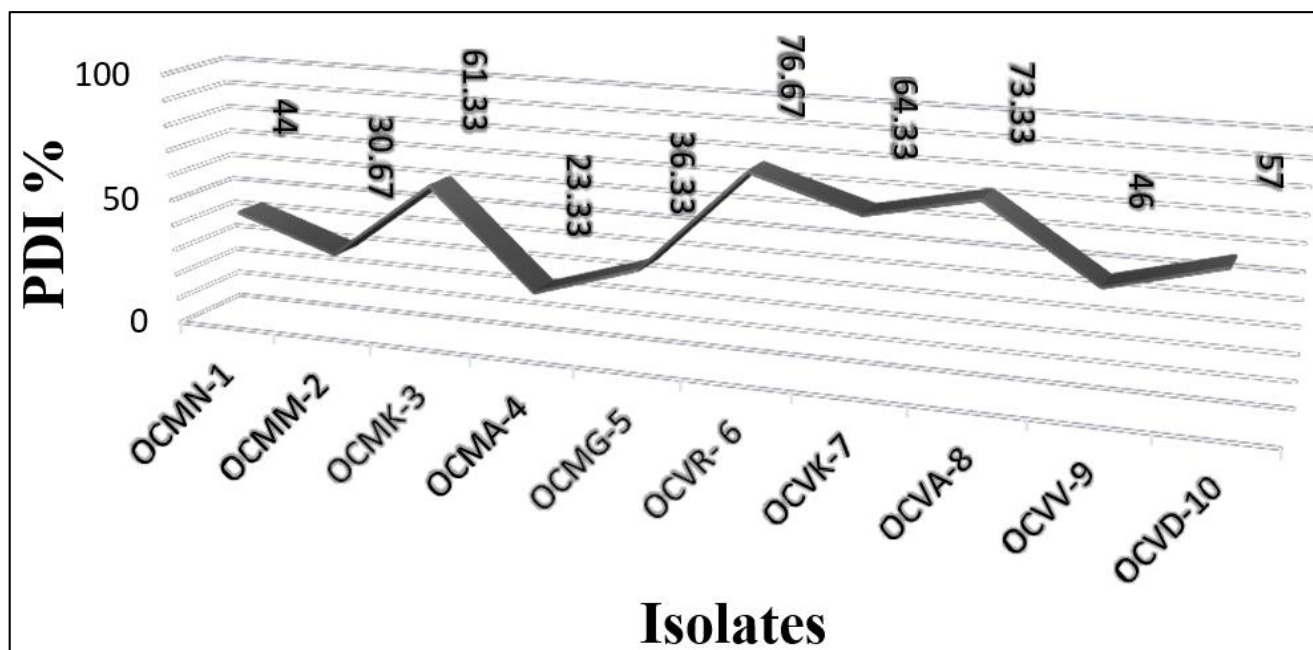


Fig 2: Disease incidence and severity of Onion twister blight disease in Madurai and Virudhunagar district

Isolation of pathogen

The pathogen was isolated from the infected plants, which were collected from 10 different locations in Madurai and Virudhunagar district. The colony showed greyish white cottony growth on PDA medium and it was subculture in order to maintain pure culture of the isolates.

Cultural and Morphological characters

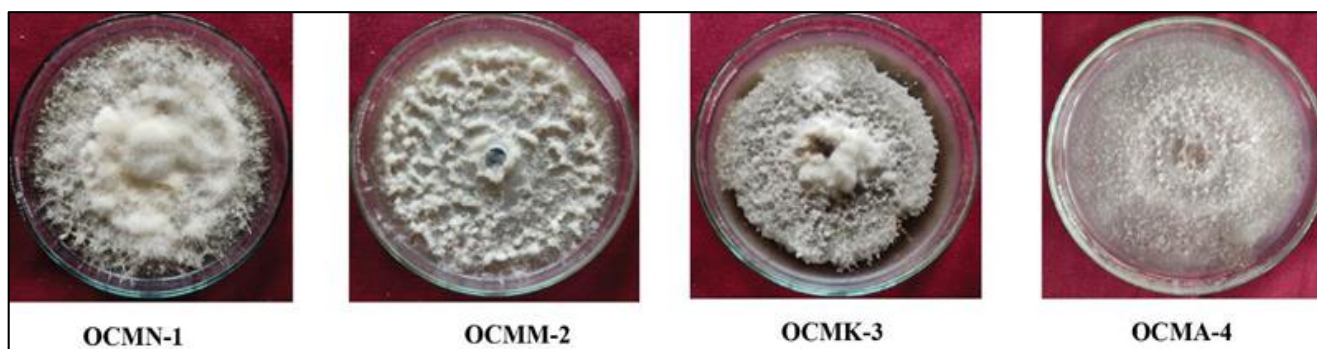
The mycelium produced by the ten isolates varied from whitish grey to dark grey on the upper side and orange to dark black on the lower side of the plate similar with the description given by (Rangkuti *et al.*, 2017; Vengadaramana A, D De Costa, 2014) [6, 9]. The fungus produced hyaline, septate mycelium and hyaline, cylindrical conidia with

rounded ends when examined under a microscope (Sikirou R *et al.*, 2011; Sattar and Malik 1939) [8, 7]. The isolates OCVR-6, OCVK-7 and OCVA-8 showed greyish white, thick and cottony mycelium with faster growth in PDA. The characters of other isolates were described in the Table 1(Fig 3). The size of conidia of *Colletotrichum Gloeosporioides*, which was isolated from dragon fruit was 6–10 x 2-2.5 µm. (Masyahit *et al.*, 2009) [5]. The length of conidia of *Colletotrichum Gloeosporioides* ranged from 10.5-17.8 µm and width 3.22 to 6.9µm which was isolated from mango anthracnose (Amsalu Abera *et al.*, 2016). Likewise the isolate OCVR-6 had conidial size of 19.2x5.5 µm and spore size of other isolates ranged between 15-20x4-6 µm (Fig 4).

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

S. No	Isolates	Place	Mycelium growth pattern	Colony colour	Growth rate	Sporulation
1	OCMN-1	Narasingam	Mycelium is whitish grey with fluffy centre	Whitish grey	Moderate	++
2	OCMM-2	Mullaiperiyar river bank	Sparse and thin mycelium	Creamy white	Slow	+
3	OCMK-3	Kalvelipatti	Thick and aggregated at centre	Whitish grey	Fast	+++
4	OCMA-4	Allathur	Sparse and thin mycelium	Grey	Slow	+
5	OCMG-5	Allanganallur	Mycelium is whitish grey with fluffy centre	Whitish grey	Moderate	++
6	OCVR- 6	Rayarpathy	Thick and cottony mycelium	Greyish white	Very fast	++++
7	OCVK-7	Kallanai	Thick and cottony mycelium	Greyish white	Fast	+++
8	OCVA-8	Arasakulam	Thick and cottony mycelium	Greyish white	Very fast	++++
9	OCVV-9	Virudhunagar	Thick and aggregated at centre	Whitish grey	Fast	+++
10	OCVD-10	Kambikudi	Mycelium is grey with fluffy centre	Grey	Moderate	++

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



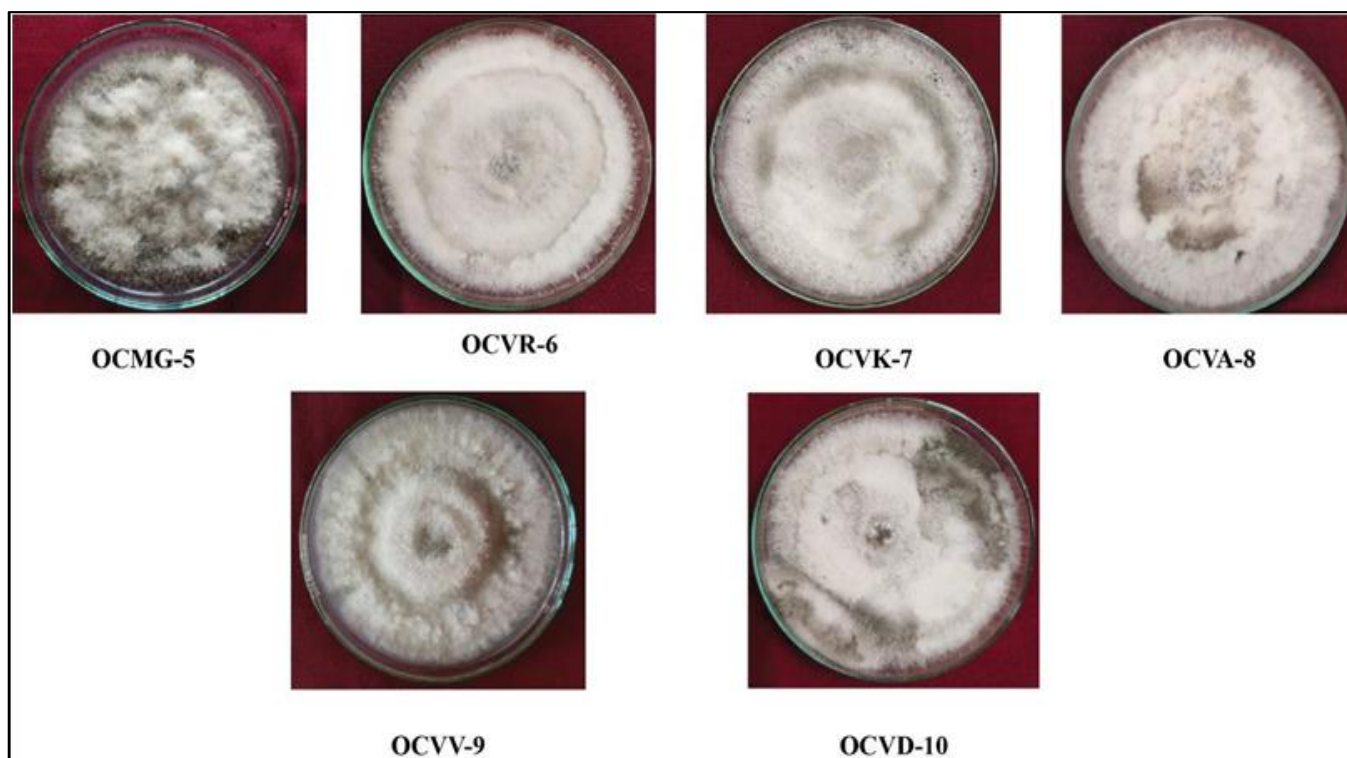


Fig 3: Isolates of *Colletotrichum Gloeosporioides* from onion growing areas of Madurai and Virudhunagar district



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

Pathogenicity test

Ten isolates were tested for pathogenicity under pot culture. All the isolates proved their pathogenicity by producing similar symptoms observed in the field. Among the ten isolates, OCVR-6 was the most virulent with pathogenicity of

94.44 percent, followed by OCVA-8 with 81.11 percent, and the least incidence was observed in OCMN-1 with 17.77%. The pathogen was re-isolated and its morphological character was observed (Fig 5 & 6).

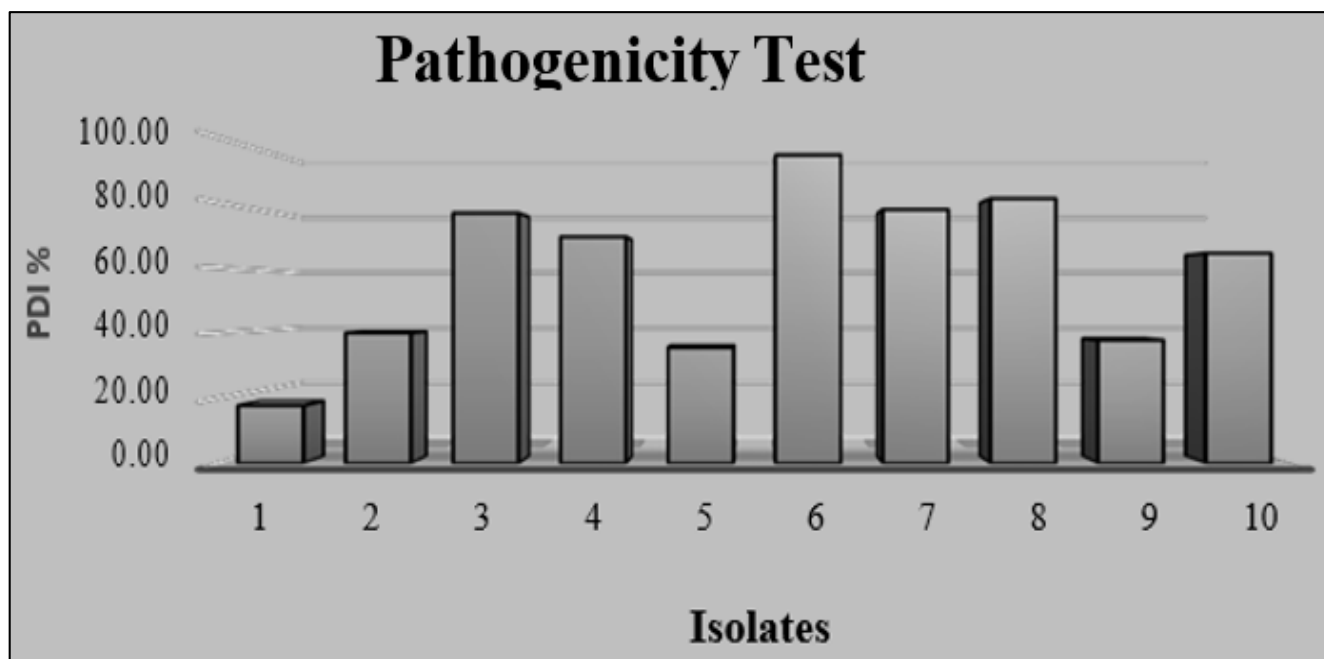


Fig 5: Pathogenicity test 1-OCMN-1, 2-OCMM-2, 3-OCMK-3, 4-OCMA-4, 5-OCMG-5, 6-OCVR-6, 7-OCVK-7, 8-OCVA-8, 9-OCVV-9, 10-OCVD-10



Fig 6: Pathogenicity test under glass house condition

Conclusion

The present study revealed that the leaf twister blight disease caused by *Colletotrichum Gloeosporioides*, was confirmed by its morphological characteristics. The pathogen produced hyaline, estate mycelium and cylindrical conidia with the size ranges from 15x4-20x6 μm .

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