



Isolation and characterization of *Colletotrichum gloeosporioides* causing twister blight disease in Onion

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Abstract

Onion (*Allium cepa* L.) is a bulbous herb which belongs to the Alliaceae family, commonly referred to as the "Queen of the Kitchen." It is a biennial crop that constitutes one of the most important vegetables cultivated in India, having the highest export value. The onion productivity was limited by numerous bacterial and fungal diseases viz., bacterial soft rot, twister blight, stemphylium blight, downy mildew and purple blotch. Among them twister blight disease causes severe yield loss. A roving survey was executed in Chengalpattu and Kanchipuram districts and assessed the prevalence of twister blight disease in onion. According to the survey, Ullavur village in Kanchipuram district had the lowest frequency of twister blight (38.34%), whereas Thirukzalkundram village in Chengalpattu district had the highest incidence at 47.53 percent. Based on the morphological features, four isolates of the pathogen have been identified as belonging to *Colletotrichum gloeosporioides*. Among the four isolates that conducted pathogenicity experiments, COCM-2 showed the highest level of disease incident (76.65%), followed by COCT-1 (37.77%), while COKU-4 (17.77%) was the lowest the incident.

Keywords: Onion, Bacterial, Fungal, *Colletotrichum gloeosporioides*

Introduction

Onion (*Allium cepa* L.) is a bulbous herb which belongs to the Alliaceae family, commonly referred to as the "Queen of the Kitchen" (Gohi *et al.*, 2016). It is a biennial crop that constitutes one of the most important vegetables cultivated in India, having the highest export value. It is a temperate crop which is grown in various climate zones throughout the world where lower temperatures (12.8–23°C) are required for vegetative growth and 20 to 25°C are required later for proper bulb development. The pungency of onion is due to the presence of allyl-propyl-disulphide. The yellow colour of onion is due to the presence of pigment quercetin and red colour is due to anthocyanin pigment (Nagpal *et al.*, 2022) [10]. It has medical benefits like lowering disease risk of cardiovascular disease, diabetes and atherosclerosis. It possess antimicrobial, anti-inflammatory and antioxidant properties (Wilson and Demmig-Adams., 2007) [16]. The bulb is useful as diuretic and heart stimulant (Ahmad *et al.*, 2016) [1].

In India, onion is grown in an area around 12.20 lakhs ha with production of 22.82 million tonnes during the year 2018-2019 (Ministry of Agriculture and Farmers welfare). The major onion producing states are Andhra Pradesh, Maharashtra, Tamil Nadu, Punjab and Bihar. In Tamilnadu onion is grown in the districts of Dindigul, Madurai, Perambalur, Trichy, Virudhunagar, Erode and Namakkal. The onion productivity is limited by numerous bacterial and fungal diseases viz., bacterial soft rot, twister blight, stemphylium blight, downy mildew and purple blotch which causes severe yield loss. Farmers in 2009 reported an elongated neck, depressed lesions on leaf sheaths, curling, twisting, and chlorosis of leaves as symptoms of a leaf twisting disease. During 2009, in Kwahu South (Amartey,

Amanfrom, Adawoso) and Fanteakwa (Papra, mantam, Diedeso, Mpaemu) districts of the Eastern region, reported disease incidence of twister blight (Awuah *et al.*, 2009) [2]. In recent times, critical onion-growing areas in Tamil Nadu suffered yield losses ranging from 50% to 100% due to the onion crop suffering twister blight disease. According to Awuah *et al.* (2009) [2] and Ebenebe. (1980) [4], the disease appears as twisting, curling, chlorosis of the leaves, elongated necks, and sunken lesions on the leaf sheath. Gyempeh *et al.* (2015) [6] reported on the onion Twister blight disease caused by *Colletotrichum gloeosporioides*. The pathogen penetrate through cuticle by producing melanised appressoria, then develop hyphae and infection vesicle in the host. At later stage it develops secondary hyphae which spread to kill the host cell. Hence the pathogen is known as hemibiotropic (Munch *et al.*, 2008). The pathogen survives in soil, infected plant debris and alternate host. High humidity, continuous raining and temperature of 23-30°C favour this disease. The spores from the soil and infected plant debris are dispersed by splash mechanism to the lower leaf surface, then by wash off mechanism it reaches the neck region of the bulb. Later on the spores deposits in the neck region which later spread through air, insect and rain which causes infection in the healthy plants (Peregrine, 1970) [13].

Materials and methods

1. Collection of sample

A survey was conducted in the onion-growing regions of Chengalpattu and Kanchipuram district in 2022–2023 to determine the incidence and severity of the onion Twister blight disease. In Kolathur, Ulavur, Thirukkazhukundram and Madhurandhagam villages, a roving survey was carried out.

$$PDI (\%) = \frac{\text{No of plants infected}}{\text{Total no of plants observed}} * 100$$

Where PDI is Per cent Disease Incidence

The disease incidence was calculated by using the formula.

2. Symptomatology:

Twisting, curling, and chlorosis of the leaves, elongated necks, and sunken lesions on the leaf sheath were the most common symptoms of the disease (Dutta *et al.*, 2022) ^[3].

3. Isolation of the pathogen

The typical twisting symptoms on onion leaves have been collected in the field and stored in refrigerator. Using the tissue segment methodology, the pathogen *C. gloeosporioides* was isolated from the infected onion leaves. To eliminate soil particles, the collected samples were rinsed with running tap water. Using a sterile scapel or blade, the diseased leaves were chopped into small pieces ranging between 0.5 and 1 centimeters. For two minutes, the leaf pieces were surface sterilized using 1% sodium hypochlorite. After that, the leaf bits were dried on sterile tissue paper and thoroughly cleaned three times using sterile distilled water to get rid of any excess sodium hypochlorite. Under aseptic conditions, sterilized leaf pieces were transferred to Petri plates containing 10 mL of Potato Dextrose Agar medium (PDA). For five to ten days, the plates were incubated at 25±2°C. The plates were frequently examined for the growth of fungi. The mycelial bits were placed in sterile Petri dishes containing PDA media. Subsequently, it was purified by the single hyphal tip technique and transferred to potato dextrose agar (PDA) slants, where pure cultures of the pathogen were maintained for future research at 4 °C (Vengadaraman *et al.*, 2015).

4. Morphological characteristics of *Colletotrichum sp.*

A nine mm culture disc of the pathogen taken from the 5 to 10 days old culture was placed at the centre of Petri plate containing sterilized PDA medium separately for each isolates. Three replications were maintained for each isolate and incubated at 25 ±2 °C for five to ten days. The cultural characters like mycelial growth, colony type, colony colour, morphology of mycelia were noted for each isolates of *Colletotrichum sp.*

Result and discussion

1. Disease incidence

In the major onion-growing districts of Chengalpattu and Kanchipuram, especially Thirukzalkundram, Maduranthakam, Kolathur, and Villavur, a roving study was conducted. Specimens were taken from infected plants showing symptoms of leaf twisting. The estimated range of infection prevalence in the examined locations was 23.67 to 47.53 percent. According to the study, the highest disease incidence of twister blight was found in Madhuranthakam village in Chengalpattu district (47.53 percent), followed by Kolathur village (38.34 percent), and the lowest incidence was found in Ullavur village in Kanchipuram district (23.67). (Table1 & Plate 1)

2. Symptomatology

Infected plants exhibited twisting, curling of leaves and showed chlorosis of leaves, elongated neck region and twisted leaves. In the neck region acervuli were seen. The disease infected plants produced slender bulbs (Plate 2).

3. Isolation of the pathogen

The pathogen was isolated from the affected plants, which were collected from four different areas around the district of Kanchipuram and Chengalpattu. The isolated culture categorized as COCT 1, COCM 2, COKK 3, and COKU 4 is derived from four different locations. On PDA medium, the colony showed cottony, greyish white growth, and it was subcultured to maintain pure culture of the isolates (Plate 3).

4. Morphological characteristics of *Colletotrichum sp.*

The mycelium produced by the four 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 and de Costa., (2014) ^[15]. The isolates COCT 1 and COKV-4 showed greyish white, thick and cottony mycelium with faster growth in PDA medium. The characters of other isolates were described in the Table 1(Plate 3).

Discussion

In chengalpattu and kanchipuram districts, which are important onion-growing regions, a roving survey was conducted, and samples were taken from infected plants showing symptoms of leaf twisting. The estimated range of infection prevalence in the evaluated locations was 23.67 to 56.0 percent. According to the study, the highest disease incidence of twister blight was recorded in Maduranthakam village in Chengalpattu district (47.53 percent), followed by Kolathur village (38.34 percent), and the lowest disease incidence was found in Ullavur village in Kanchipuram district (23.63%). Patil *et al.* (2016) ^[11] recorded 24.05 and 29.37 per cent disease incidence in Uttar Kannada district during 2012-2013. In Koppal, Bidar and Ballari districts of Karnataka, the disease incidence was recorded as 52.34, 44.20 and 18.09 per cent respectively (Manthesha *et al.*, 2022) ^[8].

Infected plants exhibits twisting, curling of leaves, chlorosis of leaves, elongated neck region and twisted leaves. In the neck region acervuli were seen and emergence of pale oval sunken lesions on leaf sheath was also observed. The disease infected plants produced slender bulbs. Similar symptoms were observed by Gyempeh *et al.*, (2015) ^[6]; Ebenebe, (1980) ^[4]; Sikirou, *et al.*, (2011) ^[14]. Patil *et al.* (2018) ^[12] also revealed the symptoms of root discoloration, underdeveloped root system and root knot in the infected plants.

The pathogen *Colletotrichum gloeosporioides* from the infected tissues was isolated from the sample collected in the surveyed area by tissue segmentation method. In order to maintain the pure culture of the isolates, the colony was sub cultured after it exhibited cottony, greyish-white growth on PDA media. For isolation of the pathogen, the disease samples that exhibited typical fungal symptoms on leaf, neck and root were employed. By using conventional tissue isolation techniques, the pathogen was isolated (Patil *et al.*, 2018) ^[12]. Kefialew and Ayalew (2008) ^[7] isolated *C. gloeosporioides* from the infected tissues of mango leaf and fruit by tissue transplanting techniques.

Table 1: Disease incidence of Twister blight in onion growing areas Chengalpattu and Kanchipuram districts

| S. No | Location | District | Isolates | Geographical location | Twister blight incidence |
|-------|-----------------|--------------|----------|-----------------------|--------------------------|
| 1 | Thirukalkundram | Chengalpattu | COCT 1 | 12.60°N 80.65°E | 38.34 |
| 2 | Maduranthakam | Chengalpattu | COCM 2 | 12.50°N 79.88°E | 47.53 |
| 3 | Kolathur | Kanchipuram | COKK 3 | 13.12°N 80.21°E | 35.12 |
| 4 | Ullavur | Kanchipuram | COKU 4 | 13.45°N 80.24°E | 23.67 |

Table 2: Morphological characters of *Colletotrichum gloeosporioides* causing twister blight

| S. No | Isolates | Mycelium growth | Colony Color | Growth rate | Sporulation |
|-------|----------|---------------------|---------------|-------------|-------------|
| 1 | COCT 1 | Fluffy | Whitish grey | Moderate | +++ |
| 2 | COCM 2 | Spase thin mycelium | Creamy white | Slow | ++++ |
| 3 | COKK 3 | Spase thin mycelium | Creamy grey | Very fast | ++ |
| 4 | COKU 4 | Thick and cottony | Greyish white | Fast | + |

+ slow, ++ moderate, +++ fast, ++++very fast



Plate 1: Collection of twister blight disease infected samples from major onion growing areas of Chengalpattu and Kanchipuram district



Plate 2: Symptoms of Twister blight disease in onion caused by *Colletotrichum gloeosporioides*

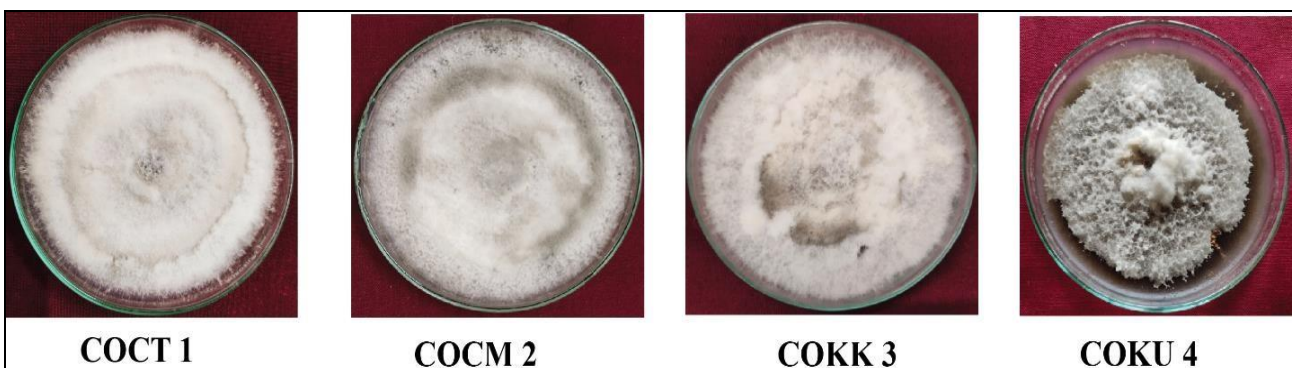


Plate 3: Morphological characteristics of *Colletotrichum gloeosporioides*

Conclusion

According to the current study, the leaf twister blight disease caused by *Colletotrichum gloeosporides* was confirmed by its morphological characteristics. The pathogen produces whitish-grey to creamy-white colonies with fluffy, thick, cottony mycelial growth.

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