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Survey, isolation and morphological variation of different isolates of anthurium anthracnose disease incited by *Colletotrichum gloeosporioides*

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Abstract
Anthurium is tropical ornamental plant and it is one of the most valued cut flowers which have a great export potential. Anthracnose or spadix rot disease leads to massive flower loss to anthurium growing farmers. Survey was conducted for anthurium anthracnose disease during 2017-2018 at anthurium growing areas of Tamil Nadu. Maximum disease incidence was observed in Thandikudi (56.66 PDI) and Pandrimalai (54.43 PDI) village of Dindugal district. The fungus *C. gloeosporioides* was isolated and the morphological variations were studied among the isolates. The result revealed that all the isolates of *C. gloeosporioides* were circular and wavy pattern and the topography were observed as raised fluffy growth. The I piiboyfgCg1 of Thandikudi isolates recorded 89.12 mm radial mycelial growth with excellent sporulation and least mycelial growth on ICg6 (64.15mm) with poor sporulation. The length of the conidia ranged from10.28-16.28 µm. Highest length of conidia was observed in ICg1 isolates (16.28 µm) followed by ICg10 (15.39 µm). Width of the conidia ranged from 5.77-3.31 µm. Isolate ICg1 recorded the highest width of conidium (5.77 µm) and was followed by ICg10 (5.23 µm).

Keywords: Anthracnose, *Colletotrichum gloeosporioides*, pathogenicity, sporulation

Introduction
Anthurium is tropical ornamental plant and it is one the most valued cut flowers which has a great export potential. Anthurium is a native of Central and South America and the genus Anthurium, with over 700 species. The name anthurium is derived from the Greek word 'anthos' means flower and 'aura' means tail, referring to the spadix. The popularity of growing anthurium as cut flowers has risen tremendously in the past few years and it has now become an important export-oriented crop. Successful production of anthurium requires the management of numerous pests and diseases. The major diseases are bacterial blight, anthracnose, bacterial wilt, root rot, and black nose.

Among the fungal diseases, anthracnose or spadix rot caused by *Colletotrichum gloeosporioides* leads to massive losses in terms of quality and quantity. Anthracnose caused severe rotting symptom in anthurium resulting in 100 per cent death of plants in Alleppy district of Kerala (Santhakumari *et al.,* 2001) [7]. Severity of anthracnose of anthurium ranged from 21.67 to 54.89 per cent in Tamil Nadu (Nandinidevi, 2008) [5].

Materials and methods
Survey and collection of disease samples
Disease survey was conducted during 2017-2018 to assess the severity of anthracnose disease in different anthurium growing areas of Tamil Nadu, India. The disease intensity was assessed on a 0-9 scale (TNAU, 1980) [10]. Disease severity was scored using the 0-9 scale, where: 0 = no disease, 1 = less than 1% infection, 3 = 1 - 10% infection, 5 = 11 - 25% infection, 7 = 26 - 50% infection and 9 = more than 50% infection. The Per cent Disease Index (PDI) was calculated by McKinney (1923) [4] formula *viz.*

$$\text{PDI} = \frac{\text{Sum of individual ratings}}{\text{Total number of leaves assessed}} \times \frac{100}{\text{maximum disease grade}}$$

Isolation of the fungus
Leaves of anthurium showing typical symptoms of brown coloured lesion were collected separately from anthurium growing areas of Tamil Nadu. The infected leaf was first washed with tap water to remove dust and other contaminants. The periphery of the lesions were cut into small bits and surface sterilized with 10 per cent sodium hypochlorite for 5-10 min.

In order to remove the residue of the chemical, the tissue bits were washed with three changes of sterile distilled water. The surface sterilized bits were placed on potato dextrose agar (PDA) medium in sterilized Petri dishes. These plates were incubated at room temperature (28±2 ºC) for seven days. After incubation the cultures were purified by hyphal tip method (Sinclair and Dhingra, 1985) [8] and the fungal cultures were maintained separately in agar slants/Pertiplates.

Pathogenicity

Pathogenicity was confirmedby Koch's Postulates testat farmer's field, Thaandikudi, Dindugal district. Anthurium plants of healthy temptation variety were maintained in 75% shade net. Inoculum of each isolated fungal culture was prepared and the spore suspension was prepared to 5×10^6 conidia/ml. The replications were maintained and the plants sprayed with sterile distilled water served as control.

Morphological variations among the isolates

Cultures of *C. gloeosporioides* isolated from infected anthurium plants collected from different locations in Tamil Nadu were used. Nine mm mycelial disc of the pathogen from the individual cultures was placed on the centre of sterilized PDA in sterilized Petri dishes separately and they were incubated at laboratory conditions (28±2 ºC). Observations on radial mycelial growth, colony characters and conidial characters were recorded.

Results and Discussions

Survey on the occurrence of anthracnose disease of anthurium

A detailed survey was conducted during 2017-2018 in various places of Tamil Nadu. The occurrence of anthracnose disease on anthurium cultivars was recorded and the result was presented in Table 1.

Among the ten villages surveyed, Thandikudi village (Dindugal Dt.) recorded maximum disease occurrence of 56.66 per cent disease index (PDI). This was followed by Pandrimalai of Dindugal District (54.43 per cent), Parasalai (52.21 per cent) and Pechipparai (48.88 per cent) of Kanyakumari district. Wellington of Ooty district showed 41.10 per cent disease index and it was followed by Thuckkalay (38.88 per cent) of Kanyakumari district, TNAU campus (36.66 per cent) of Coimbatore district. The minimum anthracnose disease incidence was observed in Yercaud (31.10) of Salem district, Moolachanvilai (31.10 per cent) of Kanyakumari district and Nagalur (17.77 per cent) of Salem district.

Table 1: Survey on the incidence of anthurium anthracnose disease on different anthurium growing areas of Tamil Nadu

S. No.	Village name	District	Isolate name	Per cent Disease Index (PDI)*
1.	Thandikudi	Dindugal	ICg1	56.66^{a} (57.35)
2.	Pechipparai	Kanyakumari	ICg2	48.88^{d} (48.68)
3.	Thuckkalay	Kanyakumari	ICg3	38.88^{f} (39.22)
4.	Parasalai	Kanyakumari	ICg4	52.21^{c} (51.78)
5.	Moolachanvilai	Kanyakumari	ICg5	31.10^{h} (31.40)
6.	Yercaud	Salem	ICg6	31.10^{i} (20.11)
7.	Nagalur	Salem	ICg7	17.77^{j} (17.57)
8.	TNAU	Coimbatore	ICg8	36.66^{g} (36.63)
9.	Pandrimalai	Dindugal	ICg9	54.43^{b} (53.57)
10.	Wellington	Ooty	ICg10	41.10^{e} (41.50)

*Mean of three replications

Values in the parentheses are arcsine transformed values

Means in a column followed by same superscript are not significantly different by Duncan's Multiple Range Test at P 0.05

Symptomatology

In the majority of the cases, the disease was observed on old leaves and the lesions were mainly formed on the tips or at the edges of the leaves. At first, a small and brown discoloration appeared on the surface of the affected leaves and the size of the lesion gradually increased. The affected leaves showed a large circular lesion or a lesion with irregular shape exhibiting a slow discoloration from brown to greyish-brown or light gray. Small, black and spherical acervuli were abundantly formed on the lesion.

Pathogenicity

Pathogenicity was performed by spraying leaves of healthy potted *A. andreanum* plants with a conidal suspension of $5x10^6$ conidia per ml. Control plants were sprayed with sterile water. Plants were covered with polythene bags and kept on the green house at 30±2 ºC. After 2 weeks typical symptoms were produced on the inoculated leaves. Symptoms on the leaf appeared as small brown discolouration on the surface of leaf and size of the lesion gradually increased.

Variation among the isolates

Morphological variations among the ten isolates of *C. gloeosporioides* isolated from anthurium diseased leaves collected from different locations of Tamil Nadu were studied and the result was presented in Table 2.

Table 2: Morphological variation of different isolates of anthurium anthracnose caused by *Colletotrichum gloeosporioides* on PDA

S. No.	Isolate	Colour of mycelial mat	Growth pattern	Topography	Radial mycelia growth (mm) *	Days to cover the plate (days)*	Sporulation
1.	ICg1	Greyish white	Circular	Raised fluffy growth	89.12^{a}	10	+++
2.	ICg2	Whitish black	Circular	Raised fluffy growth	78.73^{d}	11	++
3.	ICg3	Black	Circular	Raised fluffy growth	72.16^{e}	9	+
4.	ICg4	Whitish black	Wavy	Mycelium flat growth	84.12^{b}	10	+++
5.	ICg5	Greyish black	Circular	Raised fluffy growth	83.31^{b}	9	++
6.	ICg6	Blackish grey	Circular	Mycelium flat growth	64.15^{g}	10	+
7.	ICg7	Cottony white	Circular	Raised fluffy growth	68.57^{f}	9	+

8.	ICg8	Greyish black	Circular	Mycelium flat growth	81.13[b]	11	+++
9.	ICg9	Brownish black	Wavy	Mycelium flat growth	87.62[a]	10	+++
10.	ICg10	Brownish black	Wavy	Mycelium flat growth	76.00[d]	9	++

(-) no sporulation, (+) poor sporulation, (++) moderate sporulation, (+++) good sporulation, (++++) excellent sporulation;
*Mean of three replications
Means in a column followed by same superscript are not significantly different by Duncan's Multiple Range Test at P 0.05

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

S. No.	Isolates	Colour	Shape of conidia	Length of conidia (µm)*	Width of conidia (µm)*	Perithecium	Setae
1.	ICg1	Hyaline	Cylindrical	16.28[a]	5.77[a]	Present	Present
2.	ICg2	Hyaline	Cylindrical	14.18[c]	4.62[e]	Absent	Present
3.	ICg3	Hyaline	Cylindrical	11.34[g]	4.50[e]	Absent	Present
4.	ICg4	Hyaline	Cylindrical	12.31[f]	3.31[h]	Present	Present
5.	ICg5	Hyaline	Cylindrical	10.28[h]	4.08[g]	Present	Present
6.	ICg6	Hyaline	Cylindrical	13.21[e]	4.80[d]	Absent	Absent
7.	ICg7	Hyaline	Cylindrical	12.18[f]	5.05[c]	Present	Present
8.	ICg8	Hyaline	Cylindrical	13.72[d]	4.32[f]	Absent	Present
9.	ICg9	Hyaline	Cylindrical	12.75[f]	4.31[f]	Present	Absent
10.	ICg10	Hyaline	Cylindrical	15.39[b]	5.23[b]	Present	Present

*Mean of three replications
Means in a column followed by same superscript are not significantly different by Duncan's Multiple Range Test at P 0.05

The morphological characteristics of different isolates of C. gloeosporioides on PDA were studied. The result revealed that all the isolates of *C. gloeosporioides* were circular and wavy pattern and the topography were observed as raised fluffy growth. The ICg1 of Thandikudi isolates recorded 89.12 mm radial mycelial growth with highest sporulation at 10 days. The ICg6 of Yercaud isolate showed least mycelial growth (64.15mm) with poor sporulation

Different isolates of *C. gloeosporioides* were studied on PDA medium and the result revealed that all *C. gloeosporioides* produced hyaline cylindrical conidia and perithecium contains setae. Significant variations were observed with respect to conidial dimensions among the isolates. The length of the conidia ranged from10.28-16.28µm. Highest length of conidia was observed in ICg1 isolates (16.28 µm) followed by ICg10 (15.39 µm) and ICg2 (14.18 µm). The minimum conidial length were recorded in isolate ICg3 (11.34 µm) and ICg5 (10.28 µm). Width of the conidia ranged from 3.31– 5.77 µm. Isolate ICg1 recorded the highest width of conidium (5.77 µm) and was followed by Icg10 (5.23 µm), ICg7 (5.05 µm). The least size of conidial width were recorded in isolate ICg5 (4.08 µm) and ICg4 (3.31 µm). (Table 3).

On an average, the conidia measured 14.7x7.1µm with a centrally placed oil globule. These characters agreed with the original descriptions given by Lemme and Sonoda (1978) [3] and Sutton (1980) [9]. Das Gupta (1986) [2] also reported the variation in the spore size (17.36-21.8 µm x 2.66-2.88 µm) among the isolates of *C. capsici* causing anthracnose of betelvine. The average size of the spores however, did not vary among the isolates. Chakrabarty *et al*. (1988) [1] reported that in *C. lindemuthianum* also the average size of the spores did not vary much among the isolates. Nandinidevi (2008) [5] reported that the conidiophores were hyaline and septate bearing ovoid to cylindrical conidia which were one celled with one or two oil globules, measuring 22.5 x 10 µm. Quimo and Quimo (1975) [6] found differences in the degree of virulence of eleven *C. gloeosporioides* isolates of mango and it was further reported that the conidial size was 12.0-17.0 x 3.5-6.0 µm. *C. gloeosporioides* isolates obtained from apple, peach, pecan and other hosts varied greatly in their growth, virulence and conidial size.

From this study, it is concluded that the maximum occurrence of anthurium anthracnose was reported up to 56.66 per cent disease index (PDI) with conidial size of 16.28 x 5.77 µm in Thandikudi village, Dindugal District of Tamil Nadu.

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