International Journal of Applied and Pure Science and Agriculture
ISSN Online - 2394-5532 Impact Factor: 3.762 by SJIF ISSN Print - 2394-823X

Studies on morphological characteristics of *Colletotrichum* sp the causal organism of snake gourd anthracnose

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Abstract

Snake gourd is one of the most important cucurbitaceous crops in India. During surveys conducted at Kalliyoor panchayath, (2015-2016), five isolates of the pathogen Colletotrichum sp were obtained. Disease incidence and disease severity of each surveyed locations were observed. Snake gourd field of Instructional Farm, Vellayani recorded a maximum Disease incidence (90.00 per cent) and Percentage disease index (44.22 per cent). The least disease incidence (70.00 per cent) and percentage disease index (21.89 per cent) was recorded for snake gourd fields in Palapoor. Mophological studies the five isolates C1,C2,C3 and C5 were larger in size and cylindrical shape with obtuse ends while isolate C4 was smaller in size and ellipsoidal in shape. The isolates C1, C2 C3 and C5 were identified as C coffeanum and the isolate C4 as C musae.

Key words: Colletotrichum sp, Conidia, Acervuli, Disease incidence, Percentage disease index.

I. INTRODUCTION

Anthracnose is a widely prevalent disease where ever snake gourd is cultivated. It causes infection of both foliage and fruits of the crop. . Field losses caused by *Colletotrichum* species have been reported to be more than 60% in the United States [1]. On leaves lesions are pale brown to reddish, and centers may crack and fall out [2]. However there are very few reports on the occurrence of disease in field as well as the nature of the pathogen. Hence the morphological characters and cultural characters of the pathogen isolated from anthracnose affected snake gourd were studied. Different synthetic and natural media were screened to identify the most suitable medium growth of the pathogen.

II. MATERIALS AND METHODS

A. Isolation of pathogen from diseased leaves of snake gourd plants showing symptoms of anthracnose caused by *Colletotrichum*sp

Leaf samples showing typical symptoms of anthracnose were collected from five different locations in and around College of Agriculture Vellayani, Thiruvananthapuram district for further isolation of the pathogen *Colletotrichum* sp. In order to collect leaf samples, surveys were conducted during which, the disease intensity and severity of anthracnose disease affecting the crop of the surveyed locations were also studied.

Table 1. Different surveyed locations in Kalliyoor panchayath of Thiruvananthapuram.

Designated isolate	Locations
C1	Instructional Farm, College of Agriculture- Vellayani
C2	Department of Olericulture- Vellayani
C3	Kalliyoor
C4	Kakkamoola
C5	Palapoor

B. Intensity and Severity of anthracnose plants in different locations.

Disease intensity / Disease incidence (DI) is theproportion or percentage of infected plant units (diseased leaves/ stalk/ tillers/ seedlings) in the field. Disease incidence of snake gourd plants in each of the selected plants was assessed during each survey by observing the infection in a random sample of fifty plants.

The Disease incidence (DI) was recorded according to **[3; 4]** as follows:-

$$\text{Disease Incidence} = \frac{\text{Number of infected plants}}{\text{Total number of plants observed}} \times 100$$

Percentage Disease Index, (PDI) /severity denotes the percentage of relevant host tissue (or) organ covered by symptom (or) lesion damaged by the disease and it depends on number and size of lesion present on infected part.

Disease index was calculated based on the score chart proposed by [5] Table1,Figure-1 as follows;

Table 2. Score chart used for assessing anthracnose of snake gourd.

Disease scale	Per cent leaf area affected
0	0%
1	1-10 %
3	11-15 %
5	16-25%
7	26-50
9	More than 51 %

Disease index (severity) was calculated according to disease index formula [6]

$$\text{Disease Severity /Index} = \frac{\text{Sum of all ratings}}{\text{Total number of leaves examined}} \times \frac{100}{\text{maximum disease grade}}$$

C. Isolation of pathogen and pathogenicity studies

The pathogen was isolated from the leaf samples collected from different locations on potato dextrose agar (PDA) medium. Leaves exhibiting characteristic symptoms of anthracnose were cut into small pieces of 1.0–1.5 cm, surface sterilized with 0.1% mercuric chloride for 1 min and washed in sterile distilled water thrice and blot dried or sterilized filter paper. The sterilized leaf bits were transferred to Petri plates containing PDA medium which was amended with streptomycin sulphate. The plates were incubated at 28 ± 2^{o}C for seven days and observed for the fungal growth [7]. Fungal colonies exhibiting typical characters of the anthracnose leaf spot pathogen, that were consistently obtained during the isolation from the disease specimens of anthracnose leaf spot, were transferred to PDA slants and stored at room temperature (28 ± 2^{o}C) for conducting subsequent studies. The isolated samples were designated from C1-C5.

D. Morphological Characters

The pathogenic isolates grown on PDA were examined under 40 X magnification for observing the morphological characters like color, width of hyphae which were recorded. The culture was also observed for presence of acervuli and spores which if present were examined for recording the size and shape of acervuli, number of setae per acervuli and size and shape of spores.

The isolates obtained from surveyed locations were tentatively identified based on morphological and cultural characters observed in the above study. The identity of the isolates were further confirmed by the morphological characterization undertaken at NFCCI, Pune.

III. RESULTS

B. Intensity and Severity of anthracnose plants in different locations.

Five fungal isolates with similar cultural characters were consistently obtained from leaf samples collected from the various surveyed locations, as indicated in Table-1.Isolate C1 recorded maximum disease incidence (90.00%) and percentage disease index (44.22 %) which was followed by

isolate C2. Least disease incidence (70.00%) and percentage disease index (21.89) was recorded in isolate C5.

Table 3. Disease Incidence and Disease severity on infected leaves of snake gourd field in different locations of Kalliyoor panchayat, Thiruvananthapuram.

No	Locations	Disease incidence (%)	Percentage Disease Index /severity (%)
L1	Snake gourd field of IF-Vellayani	90.00	44.22
L2	Snake gourd field of Department of Olericulture	84.00	40.66
L3	Kalliyoor	80.00	37.33
L4	Kakkamoola	82.00	30.22
L5	Palapoor	70.00	21.89

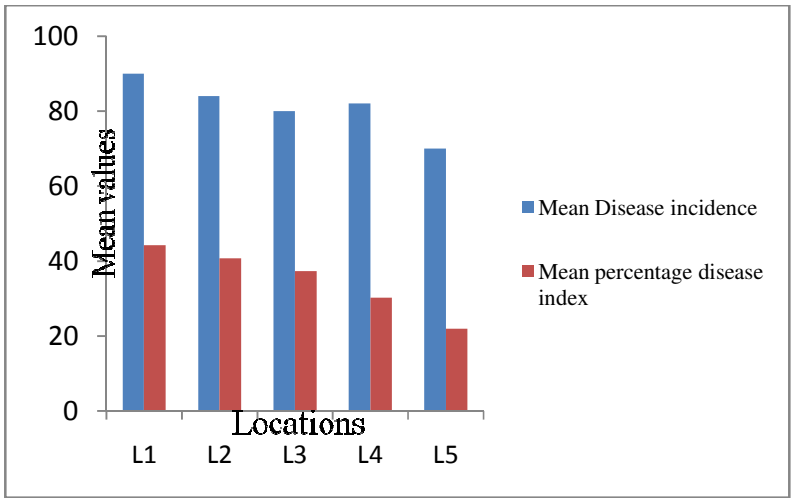


Figure 1. Incidence and severity of anthracnose of snake gourd in different locations.

C. Isolation of pathogen and pathogenicity studies

Studies on pathogenicity revealed that isolate C1 recorded higher lesion size of (10.06± 0.152) which was followed by isolate C2 (7.63±0.057). Lowest lesion size of (1.53 ± 0.115) was recorded in isolate C4.

D. Morphological characters

Hyphae of all four isolates (C1,C2,C3 and C5) were hyaline and septate with a width ranging from 2.22- 3.86µm.The isolate C1 had maximum hyphal width of 0.0238 µm and minimum hyphal width was recorded in the isolate C4 (1.92µm).

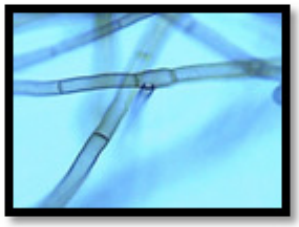
Conidia produced in all the isolates were hyaline and cylindrical with obtuse to slightly round ends. The average conidial size of four isolates (C1, C2, C3 and C5) was 11- 15 µm in length and 4-5 µm in breadth. Maximum average size (13.14 x 4.82 µm) was recorded for conidia produced by isolate C1 which was followed by C2 (12.28x 4.73), C3 (11.40x 4.52) and C5 (11.30x 4.48) µm. The least conidial size was observed in isolate C4 (10.52 x 4,40) µm. (Table-6, Plate-13-17)

Acervuli bearing setae were produced in aged culture of each of the five isolates. The acervuli were varied from ovoid, slightly irregular to irregular in shape, brown in colour and there diameter ranged from 85.52- 123.25 µm Setae were few in number, brown , straight to slightly curved , swollen at the base and narrowing towards the apex.

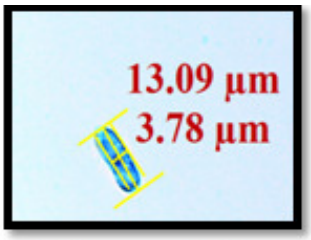
Table 3. Microscopic observations of different isolates of Colletotrichum sp obtained from surveyed locations

Isolate	Shape of conidia	Conidial dimension (Length x Breadth) (µm)*	Colour of conidia	Hyphal dimension Breadth) (µm)*	Diameter of acervulus (µm) **	No of setae
C1	Cylindrical with obtuse to slightly round ends.	13.14x 4.82	Hyaline	3.86	123.25[a]	24.00 [a]
C2	Cylindrical with obtuse to slightly round ends.	12.28 x 4.73	Hyaline	3.50	112.56 [b]	22.00 [b]
C3	Cylindrical with obtuse to slightly round ends.	11.40x 4.52	Hyaline	2.65	98.13 [d]	17.00 [d]
C4	Ellipsoidal	10.52 x 4.40	Hyaline	1.92	82.52 [e]	6.00 [e]
C5	Cylindrical with obtuse to slightly round ends.	11.30 x 4. 48	Hyaline	2.22	105.67 [c]	19.00 [c]
CD (0.05)					0.675	0.930

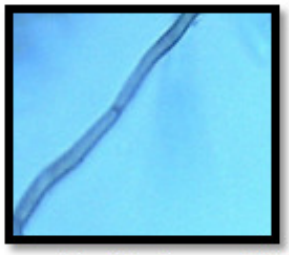
*Mean of ten replications
** Mean of fifteen replication



A) Hyphae of C1

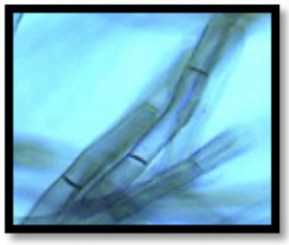


B) Spore of C1

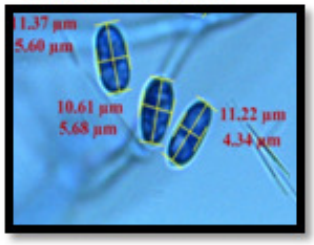


A) Hyphae of C2

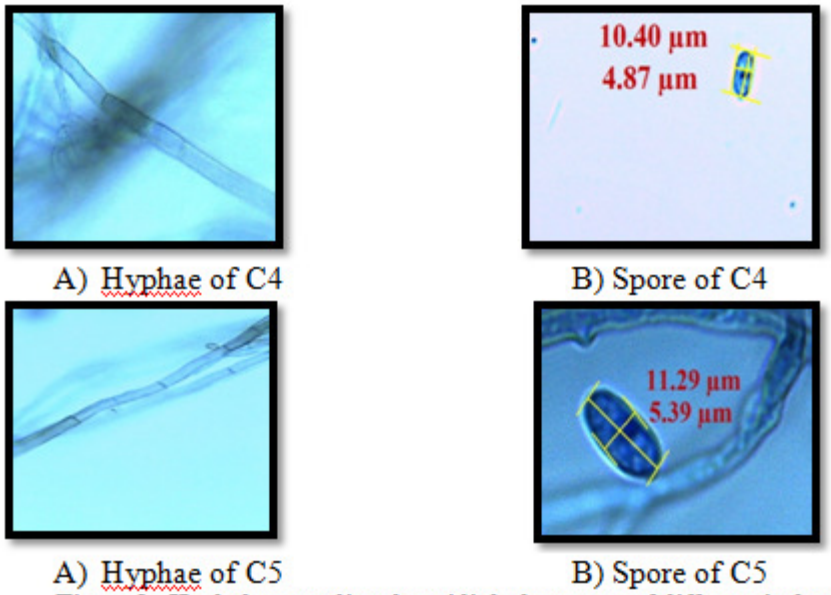
C) Spore of C2



A) Hyphae of C3



B) Spore of C3



A) Hyphae of C4 B) Spore of C4

A) Hyphae of C5 B) Spore of C5

Figure 2. Hyphal, acervuli and conidial characters of different isolates

IV.DISCUSSION

Surveys were conducted in snake gourd fields of five different locations of KaliyoorPanchayat viz., IF, COA Vellayani, Dept. of Olericulture, COA Vellayani, Kakamoola, Palapoor and Kaliyoor, in order to determine the prevalence of anthracnose of snake gourd in the fields and also to make a comparative assessment of the disease in the surveyed locations by finding out the Disease Incidence (DI) and Percentage Disease Index (PDI)/ Disease severity of the plants observed during the period of survey. Accordingly the (DI) and (PDI) observed in the fields ranged from 70 to 90 per cent and 21.89-44.22 per cent and the highest disease parameters were recorded in the fields of Instructional Farm, Vellayani followed closely by those of Dept. of Olericulture, College of Agriculture Vellayani . (DI) and (PDI) were lowest in the snake gourd fields of Palapoor. There was large scale and continuous cultivation of the crop in the fields of Instructional Farm, College of Agriculture Vellayani and Dept. of Olericulture, College of Agriculture Vellayani whereas the acreage under the crop was comparatively (10 cents) in Palapoor. Besides, there were other cucurbitaceous crops like cucumber, pumpkin and bittergourd surrounding the fields of Instructional Farm, College of Agriculture Vellayani and Dept. of Olericulture, College of Agriculture Vellayani while in Palapoor the snakegourd field was located in an isolated area with no other crops cultivated nearby.

Morpho-taxonomic criteria such as conidial shape and size, morphology and size of acervuli, setae morphology, temperature response on potato dextrose agar medium (PDA) and host specificity, as well as molecular identification techniques, are currently in use for identification of *Colletotrichum* spp. [8; 9].

Results of morphological studies indicated that all isolates had almost similar hyphal characters, which were hyaline and septate. However there was slight variations observed in hyphal width with regard to isolate C4(1.92µm) which was narrow compared to remaining isolates which had a hyphal width of (2.22- 3.86 µm).Isolate C4 varied from the other four isolates in size and shape of conidia. The conidia of isolate C4 was ellipsoidal in shape and had a size of 10.52 x 4.40 µm, where as in all other isolates the size ranged from 12.28-13.14 µm x 4.73-4.82 µm and they all were cylindrical in shape with obtuse ends. Conidia of all the five isolates were single celled. The acervuli of isolate C4 was 82.52 µm in diameter and produced an average of six number of setae, but diameter of C1,C2,C3 and C5 isolates ranged from 98.13- 123.25µm and had an average number of 19 – 24 setae. Thus the isolate C4 was morphologically smaller compared to other four isolates with variations in conidial characters. All the five isolates were tentatively identified as belonging to *Colletotrichum* sp based on morphological characters *viz*., size and shape of conidia, hyphal characters, diameter of

acervuli and number of setae. [10] observed the size of conidia of *Colletotrichum*sp varied from 11-16 x 4-6 µm and 13.8 x 4.8 µm, The identities of all the five isolates were later confirmed by conducting morphological studies at Agarkhar Research Institute , Pune. Accordingly isolate C1,C2 C3 and C5 were identified as *C.coffeanum* with an accession number NFFCI/2015-8/AKC/2293-03/SKS/DKMand C4 was identified *C. musae* which was recorded with an accession number NFFCI/2015-8/AKC/2293-07/SKS/DKM. In many of earlier reports , the conidial size of *C. coffeanum* was ranged from 10- 19 x 4-5.5µm [11;12; 13 and 14]. In morphological description of *C coffeanum* isolated from coffee plants, [15] indicated that the conidial size of the pathogen ranged from 10- 20 x 4-5µm and they produced dark brown acervuli bearing lower number of setae.

Similar observations of present study [16] reported that conidia of *C.musae* were aseptate, hyaline, mostly ellipsoidal, ranging from 10-18 µm and 4-9 µm. [17] recorded that the pathogen *C.musae* produced coloured acervuli in case of anthracnose in banana.

The isolates C1,C2,C3 and C5 were particularly identified as*C. gloeosporioides* due to the characteristic shape of their cylindrical conidia (Sutton,1980).*C.offeanum* which caused infection specifically on coffee plants were renamed as *Ckahawae* by [18] . At the same time, [19] showed that *C. kahawae* is very close to *C. gloeosporioides* on the basis of rDNA sequences. Affinity of these two species (*C. kahawae* and*C. gloeosporioides*) was based on a single base difference in rDNA sequences data used to discriminate related taxa and there fore, due to limited number of informative sites identified the underlying difference is very small. However the studies conducted later by [20], recorded that that *C.kahwae* is closely related to *C. gloeosporioides* based on rDNA-ITS sequence analysis. The above studies confirmed to the results of the previous investigation in which four isolates (C1,C2,C3 and C5) were identified initially as *C. gloeosporioides* and later confirmed as *C.coffeanum*.

The isolate C4 from Kalliyoor was identified as *C.musae.* Von Arx [12] included *C.musae* under *C. gloeosporioides* as specific to Musa, while Sutton [8; 13] accepted this as distinct species which was supported by recent molecular work (L. Cai, pers.comm).

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