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RESEARCH PAPER

Morphological and pathogenic variability of *Colletotrichum gloeosporioides* inciting anthracnose in betel vine (*Piper betle* L.)

A. NISHA AND G. HEERA

Department of Plant Pathology
Kerala Agricultural University, College of Agriculture, Vellayani - 695 522, India
E-mails: nishapinky1993.na@gmail.com, heerag@kau.in

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Abstract: A survey conducted to assess betel vine anthracnose caused by *colletotrichum* sp in three southern districts of Kerala revealed that disease incidence varied from 20 to 80 per cent and severity from 5.70 to 20.00 per cent. Five different isolates (C1, C2, C3, C4 and C5) of *Colletotrichum* sp. obtained from the survey showed wide cultural and morphological variability in PDA medium. The nature of mycelial growth was either fluffy or sparse and colony colour showed a multitude of variation from white, off white, orange to pinkish among the different isolates. The mycelia were hyaline, branched and septate. The mean septal width ranged from 2.65 µm - 3.45 µm and distance from 8.50 µm - 25.56 µm were recorded among the *C. gloeosporioides* isolates. The conidia and appressorial dimensions varied from 9.6 - 12.2 µm x 3.7 - 4.3 µm and 8.37 - 10.08 x 5.02 - 6.28 µm in length and breadth respectively. The isolates had a significant pathogenic variability with respect to the symptom development, lesion size and rate of lesion development on artificial inoculation. Among the five isolates of *C.gloeosporioides*, C2 was the most virulent producing maximum lesion size (14.75 mm) with in two days after inoculation when compared to the other isolates.

Keywords: Anthracnose, Betel vine, Conidia, Isolates

Introduction

Anthracnose or leaf spot disease caused by *Colletotrichum* sp. is a devastating disease widely noticed in betel vine growing areas. The incidence was first reported by Hector (1925) from West Bengal. The foliage infection and stem damage of 20 - 80 per cent and 10 - 60 per cent respectively were noticed in severe cases (Singh and Shankar, 1971; Dasgupta and Sen, 1999). The anthracnose in betelvine has produced a yield loss of 10 - 60 per cent (Singh and Joshi, 1971; Maiti and Sen, 1982). The morphological variability among the *C. gloeosporioides* has been reported by several researchers (Jagtap *et al.,* 2015; Chavan *et al.,* 2016; Udayakumar *et al.,* 2019). Betel vine isolates of *C. gloeosporioides* showing cultural, morphological and pathogenic has been reported by Naik and Hiremath (1986) and Poornima, (2007). The present study focused on the assessment of the disease incidence and severity of anthracnose of betel vine through survey, symptomatological variations and morphological, cultural and pathogenic variability among the incitant from Southern districts of Kerala.

Material and methods

Survey for the collection of diseased samples: A survey was conducted during December 2016 - April 2017 in the Southern districts of Kerala *viz.,* Thiruvananthapuram (Kalliyoor, Vellayani and Kattakada), Kollam (Kareepra) and Alappuzha (Cherthala) for betelvine anthracnose incidence and severity. Ten plants were selected randomly from each field and five leaves from each plant were graded for determining the percentage disease index (PDI) according to 0-9 score chart (Sankar, 2002).

Score chart adpted was 0 - No infection, 1 - Lesions covering upto 10 per cent leaf area, 3 - Lesions covering 11- 25 per cent leaf area, 5 - Lesions covering 26- 50 per cent leaf area, 7 - Lesions covering 51-75 per cent leaf area and 9 - Lesions covering > 75 per cent leaf area

Percentage disease index was calculated using the formula of Mayee and Datar (1986),

$$PDI = \frac{\text{Sum of the grades of each leaf}}{\text{Number of leaves assessed}} \times \frac{100}{\text{Maximum grade used}}$$

Disease incidence was calculated using the formula of Singh (2002).

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

Symptoms of anthracnose on the foliage and stem were observed and studied. The symptom variation in different surveyed areas were also studied.

Isolation of the pathogen : The diseased leaves were cut into small bits containing diseased portion along with healthy portion after initial washings with running water. The bits were surface sterilized using 0.1% mercuric chloride for 1 minute followed by three washings in sterile distilled water. The excess moisture was removed by placing the surface sterilised bits over a sterile filter paper. The surface sterilised bits were transferred on to Petri dishes containing potato dextrose agar

(PDA) medium under aseptic conditions. The Petri dishes were incubated at room temperature (27 ± 3p C) for 24 - 48 hr. The fungal growth observed on the Petri dishes was transferred to PDA slant (Aneja, 2003). The fungal isolates were purified by single spore isolation technique (Dhingra and Sinclair, 1985) and proved its pathogenicity using Koch postulates.

Cultural and morphological variability of *Colletotrichum gloeosporioides* from betel vine : The cultural characteristics of the five isolates were studied on PDA medium. A mycelial disc of 5 mm from five day old culture was placed at the centre of the Petri dish. The Petri dishes were incubated at room temperature (27 ± 3p C). Five replications were maintained for each treatment. Observations were made on the radial growth, rate of growth of each isolates, growth pattern, colony colour and number of days taken to complete full growth in Petri dish. The morphological characters of the various isolates *viz.,* the mycelial characters, sporulation, size and shape of conidia and appresoria were studied with seven day old *C.gloeosporioides* isolates by microscopic observation under Leica DM 750 at 400X magnification.

Pathogenic variability studies: The pathogenic variability of *C. gloeosporioides* isolates were studied by virulence rating. Healthy leaves of betel vine (var. *Pannikarpooram)* collected from farmer's field were used for virulence study. The leaves were initially washed with tap water and surface sterilized by with 70 per cent ethanol using a cotton swab. The leaves were gently wounded by pin pricks lower surface and inoculum was placed at the pricked area . The mycelial bit of 5 mm from five day old culture of *C. gloeosporioides* grown on PDA was used for inoculating the leaves The inoculated leaves were incubated in moist chamber for symptom development. Observations were made at on the lesion size, time for symptom appearance and rate of lesion development.

Results and discussion

Survey and symptomological studies of betel vine anthracnose

The survey was conducted from December to April 2018. The maximum temp of the surveyed areas ranged between 31-34 ^{o}C while the minimum temp range was between 20 .7 to 27 ^{o}C. In the surveyed locations, the disease incidence ranged from 20 to 80 per cent and severity from 5.70 to 20.00. The highest DS (20.00) and DI (80.00) was recorded from Cherthala (Table 1). Kalliyoor and Vellayani region of Thiruvananthapuram districts had a disease incidence of 20 per cent and disease index of 16.42 and 17.86, respectively. The least disease incidence was noticed in Kareepra (4 per cent). The warm humid condition of the Cherthala area favoured the disease development and more disease severity. Similar observations were recorded by Haralpatil (2006) in a survey conducted in different parts of Maharashtra to assess the anthracnose of betel vine . The disease severity of 11.0 to 20.0 was noticed. Ahmed *et al.* (2014) also reported anthracnose incidence of 20 to 80 per cent in betel vine growing areas during December - July.

Symptom variations were noticed in betel vine plants in the surveyed areas. Initially symptoms appeared as circular necrotic spots surrounded by a yellow halo, mostly towards the tip of the leaf lamina. Later, these spots coalesced leading to extensive leaf blight under high humid conditions. Defoliation was associated with leaf blight in severe cases. LSevere cases of leaf infection led to petiole and stem infection ultimately leading to girdling and drying up of the entire vine (Plate 1). The symptoms observed in the surveyed areas were necrotic spots with a yellow halo (Kaliyoor, Vellayani and Cherthala) and leaf blight along the margin (Kattakada and Kareepra) (Plate 2). Maiti and Sen, (1979) and Chandra and Sagar, (2004), observed incidence of anthracnose symptom aas irregular spots with a yellow halo and black centre on the foliage. Stem lesions were also observed leading to girdling and death of entire vines. (Chattopadhyay and Maiti, 1990; Ahmed *et al.,* 2014).

Variability studies of the anthracnose pathogen

Cultural variability: Five different isolates of *Colletotrichum* sp. (C1 , C2, C3 ,C4 and C5) were obtained from the surveyed areas. These five isolates of *C. gloeosporioides* exhibited wide variation in its cultural characters (Table 2) (Plate 3). The isolates had either fluffy or sparse mycelial growth with a regular margin. The isolate C2 had a sparse growth while all other isolates exhibited fluffy growth . The colony of the C1 isolate appeared dark grey in the front view and and rear view. The isolate C2 appeared as dark greyish on front view while on rear view as orangish with concentric zonation. The colony growth of the isolate C3 and C4 appeared as whitish in the front and rear view. C5 had off white growth in front view and pinkish colouration on rear view. Naik and Hiremath (1986) identified *C. gloeosporioides* isolates from betel vine which produced smooth greyish black colonies in PDA medium. Sankar (2002) observed *C. gloeosporioides* from black pepper with a colony colour ranging from white, light to dark grey. Jagtap *et al.* (2015) also observed raised fluffy colony growth of *C. gloeosporioides* from betel vine with intermixed black and white colour in PDA. The isolate C2 had fast growth with a growth

Table 1. Epidemiological factors in relation to disease incidence and severity of betel vine anthracnose in the surveyed areas

Sl. No.	Locations	Max. temp. (^{0} C)	Mini. temp.(^{0} C)	Relative humidity	Disease incidence (%)	Per cent disease index*
1.	Kalliyoor	31.0	26.0	82.0	20	17.86
2.	Vellayani	31.9	20.7	94.4	20	16.42
3.	Kattakada	32.7	20.9	91.7	70	13.57
4.	Kareepra	34.0	26.0	84.0	4	5.70
5.	Cherthala	33.0	27.0	84.0	80	20.00

*Mean of 50 leaves per location

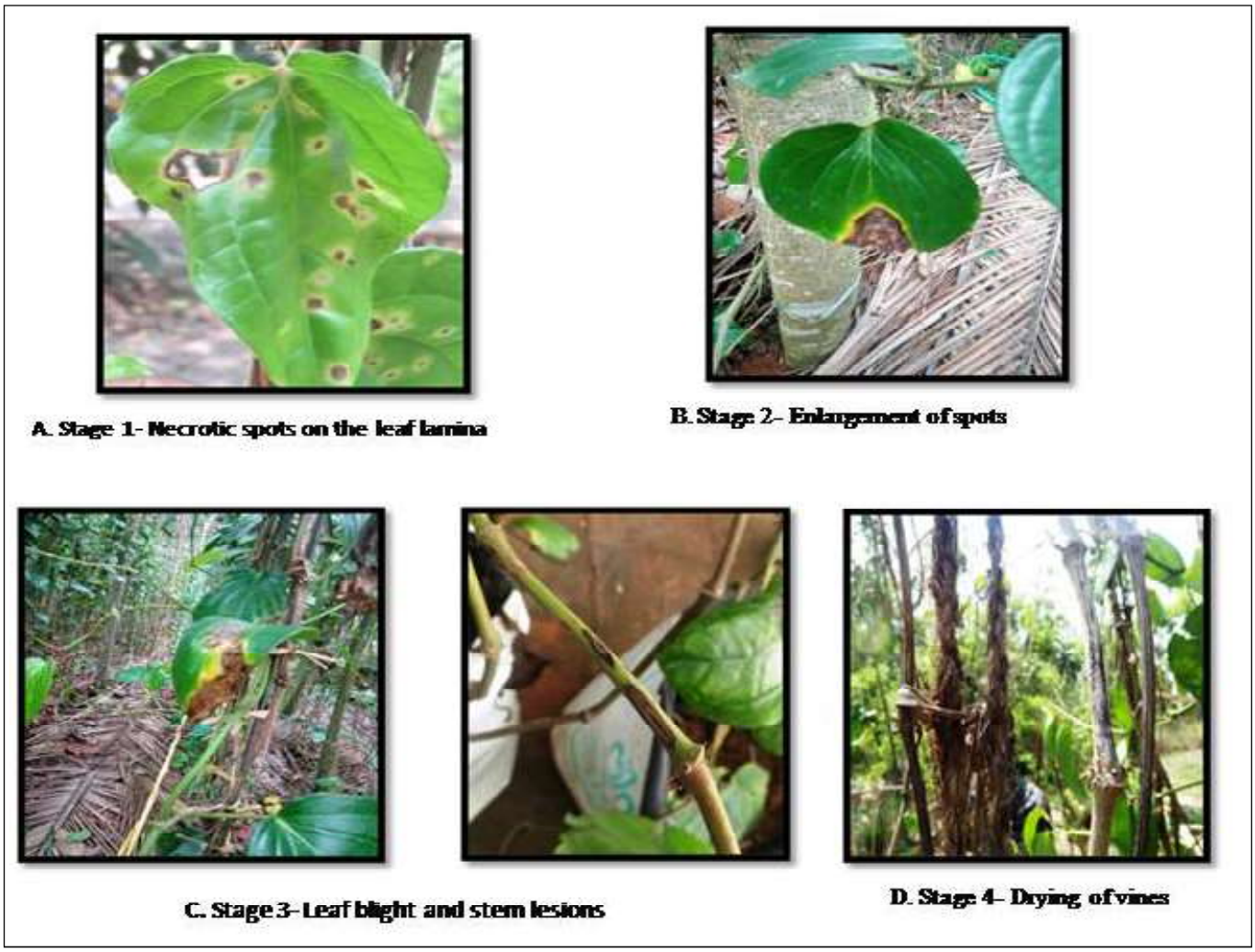


Plate. 1. (A-D). Stages in symptom development of anthracnose of betel vine

Table 2. Cultural and morphological characters of different isolates of *Colletotrichum* sp.

Isolates	Cultural characters					Morphological characters					
	Growth in petri dish (cm) after 7 days *	Rate of growth (cm/day)*	Colony characters			Characteristics of the mycelium				Conidia Size and shape (length x breadth) (µm)**	Appressoria Size (length x breadth) (µm)**
			Growth pattern	Appearance	Margin	DTCP	Width (µm**)	Septal distance (µm**)	Nature		
C1	8.1	1.15	Fluffy	White to dark grey	Regular	8	2.65	25.56	Septate Hyaline	11.4 x 4.7 Cylindrical	8.73 x 5.02
C2	9.0	1.28	Sparse	Dark grey to Orangish	Regular	7	2.98	19.15	Septate Hyaline	11.3 x 3.9 Dumbbell	10.08 x 5.23
C3	8.5	1.21	Fluffy	White	Regular	7	3.32	13.86	Septate Hyaline	11.2 x 3.7 Oblong	9.57 x 6.28
C4	7.3	1.04	Fluffy	White	Regular	9	3.45	9.80	Septate Hyaline	12.2 x 4.3 Oblong	9.28 x 5.71
C5	8.6	1.22	Fluffy	Off white to Orangish	Regular	8	3.02	8.50	Septate Hyaline	9.6 x 3.8 Cylindrical	8.37 x 5.11
C.D. (0.05)	0.269	0.037			0.224						
S.Em.±	0.178	0.031			0.148						

*Mean of four replications

**Mean of ten replications (Observations were made from seven day old culture)

DTCP: Days taken to cover 9 cm in petri dish

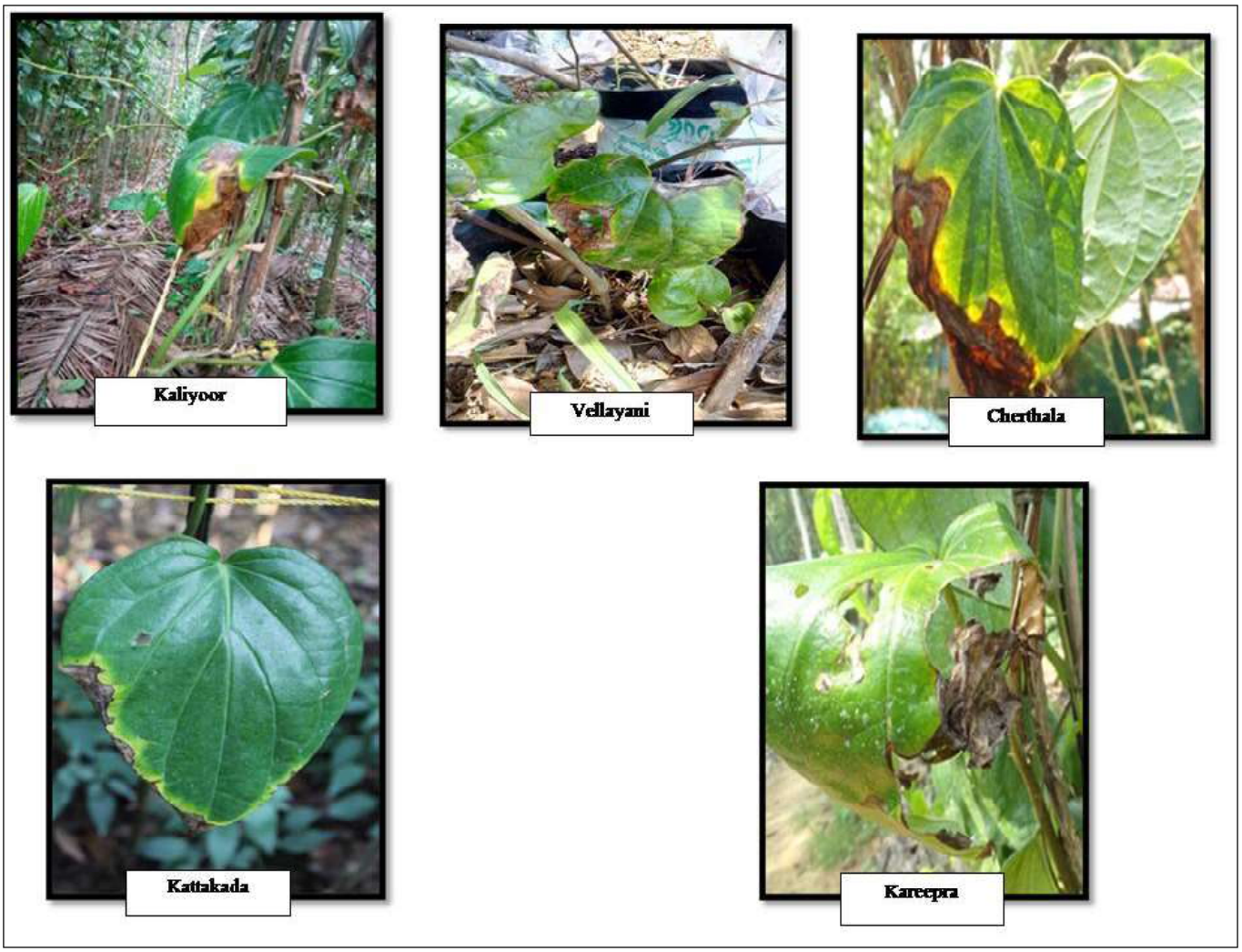
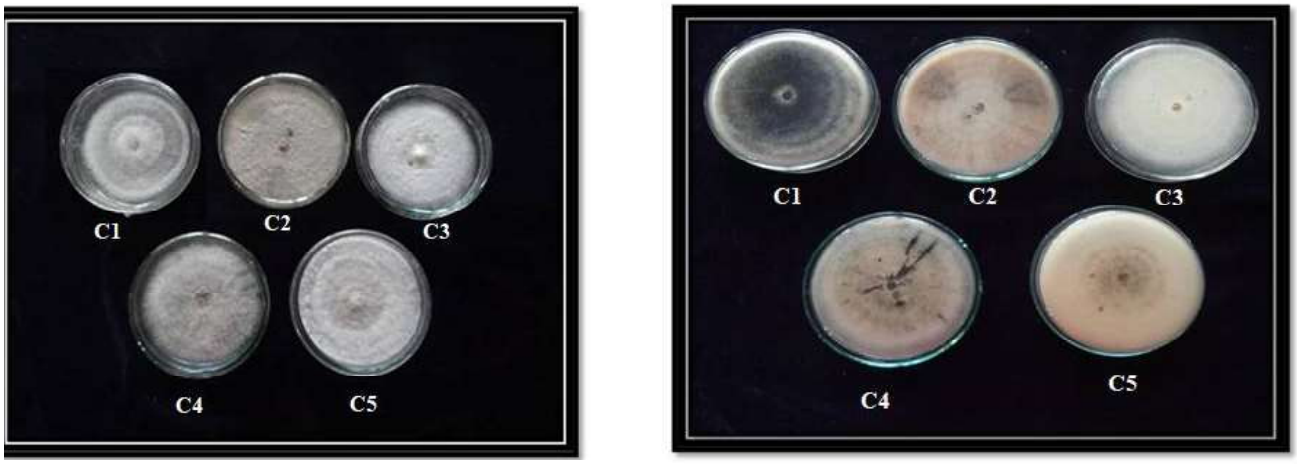


Plate. 2. Symptoms observed at the surveyed areas



Front view Rear View

C1- Isolate from Kalliyoor, C2- Isolate from Vellayani, C3- Isolate from Cherthala,
C4- Isolate from Kattakada, C5- Isolate from Kareepra

Plate. 3. (A-B). Mycelial growth of isolates of *C. gloeosporioides* causing anthracnose of betel vine on PDA medium (7th day)

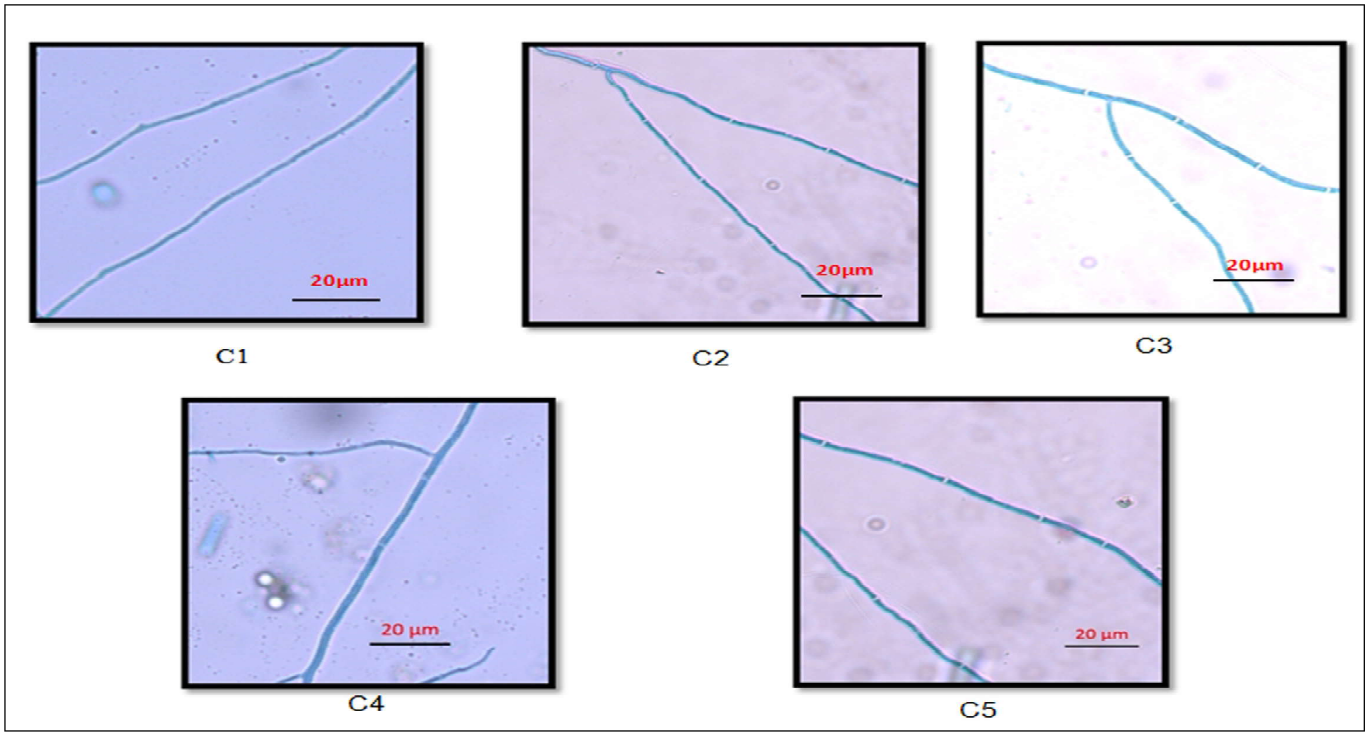


Plate. 4. (C1-C5). Mycelia characteristics of isolates of *C. gloeosporioides* causing anthracnose of betel vine (400X)

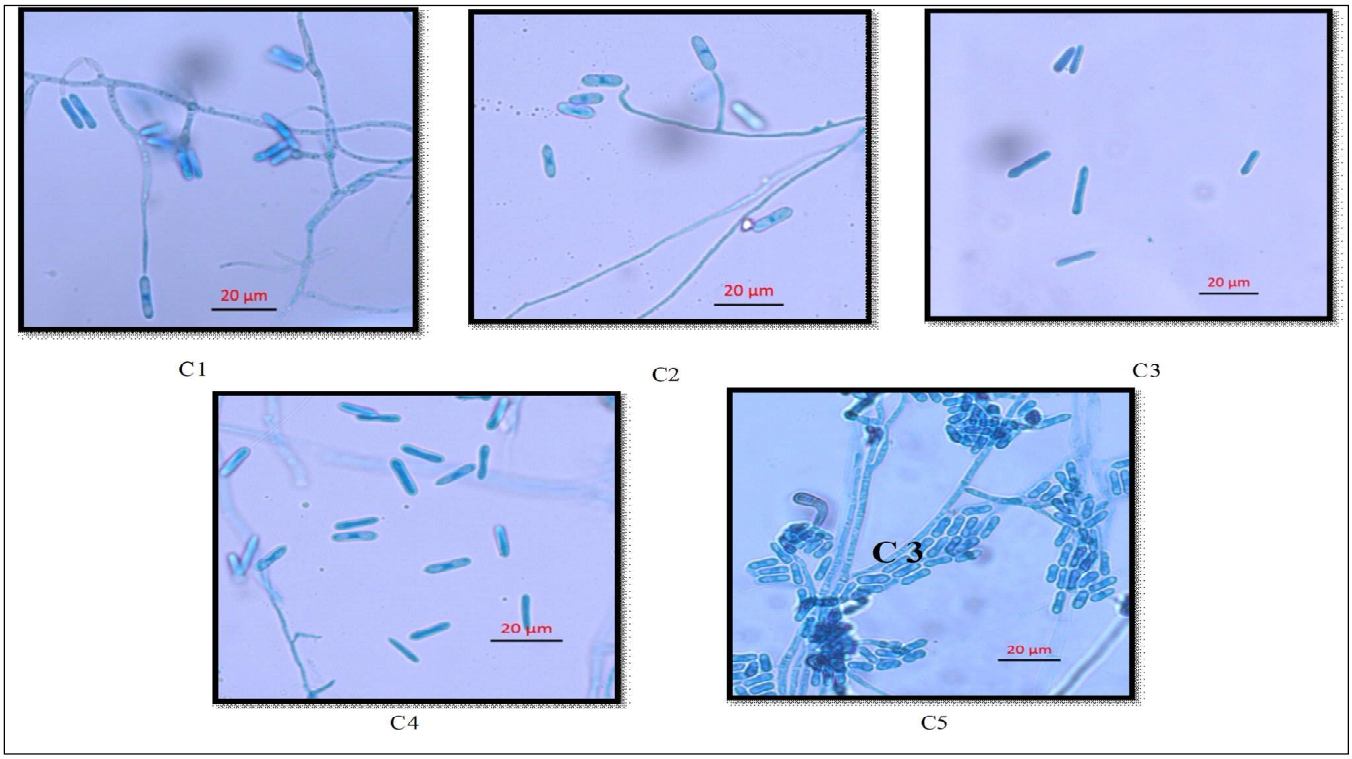


Plate. 5. (C1-C5). Conidia of isolates of *C. gloeosporioides* causing anthracnose of betel vine (400X)

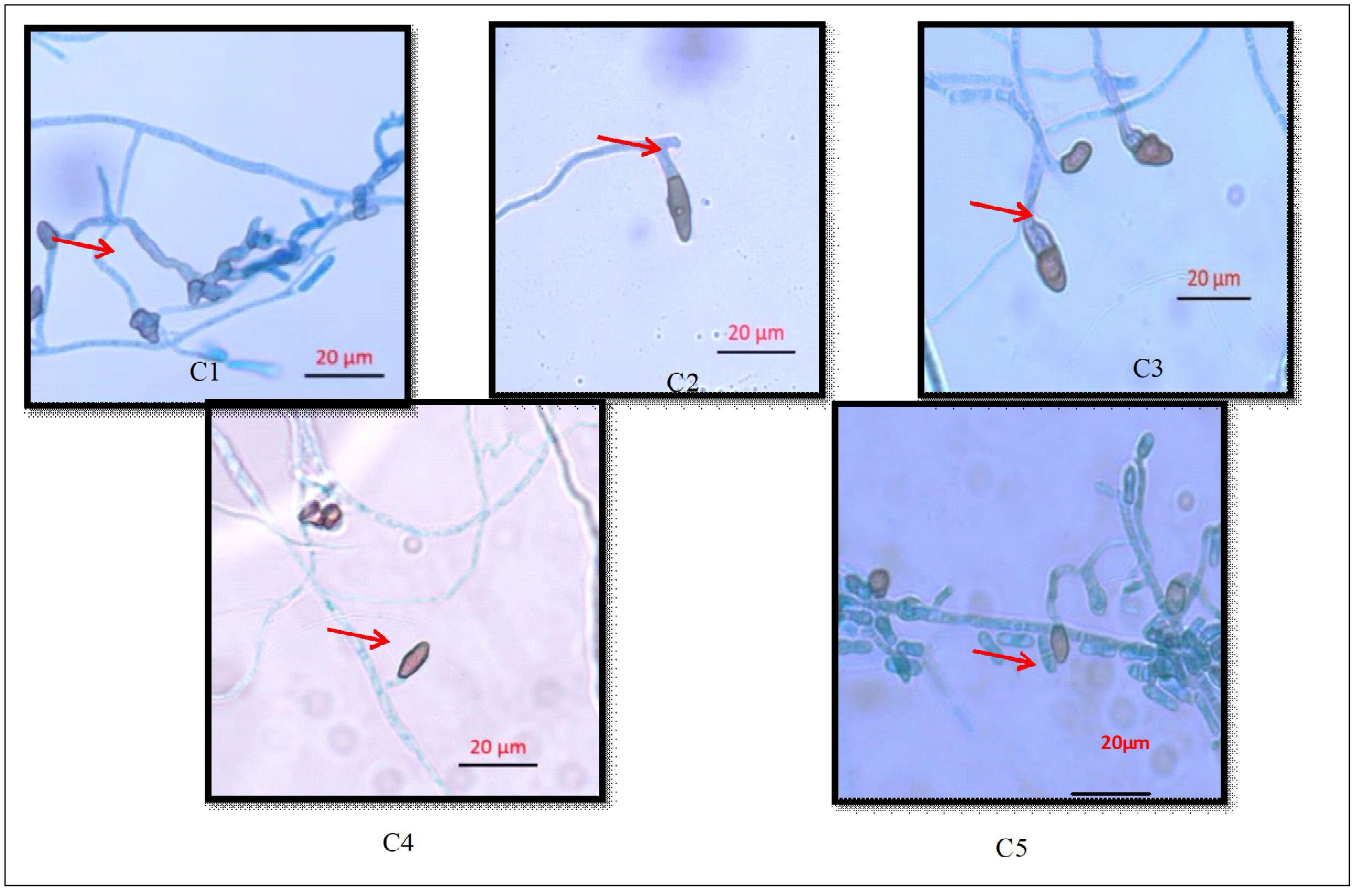


Plate. 6. (C1-C5). Appressoria of collected isolates of *C. gloeosporioides* (400X)

rate of 1.28 cm day^{-1} and took seven days to complete full growth in Petri dish. The isolate C4 with minimum growth rate of 11.04 cm day^{-1} took 9 days for growth completion in Petri dish. The isolate C3, C1 and C5 took 7, 8 and 8 days respectively for completing growth in petri dish. Similar results were obtained by Sreeja (2014) where an average growth rate of *C. gloeosporioides* from cowpea ranged from 0.80 to 1.38 cm day^{-1} and took 6 - 10 days for completion of growth.

Table 3. Lesion size, rate of lesion development and days for symptom expression (virulence rating) of *C. gloeosporioides* causing anthracnose in betel vine

Isolates	Mean lesion size at 5 DAI (mm²) *	Time taken for lesion development (days)	Rate of lesion development (mm day $^{-1}$)
C1	2.50^{b}	5	0.5
C2	14.75^{a}	2	2.95
C3	3.52^{b}	4	0.70
C4	3.00^{b}	4	0.60
C5	5.07^{b}	4	1.01
C.D. (0.05)	6.95		
S.Em.±	4.615		

*Mean of four replications

Values followed by similar superscripts are not significantly different at 5% level

DAI- Days after inoculation

Morphological studies revealed that mycelium of the *C. gloeosporioides* isolates were hyaline, branched and septate. These five isolates showed variation in its morphological characters. The mycelial width varied from 2.65 µm to 3.45 µm among isolates (Table 3) (Plate 4). The maximum mycelial width of 3.45 µm was recorded for the isolate C4 and the minimum width of 2.65 µm for the isolate C1. The mycelial width of 3.32 µm was recorded for the isolate C3 followed by C5 and C2 with 3.02 µm and 2.98 µm respectively. The septal distance was also varied between 9.80 to 25.56 µm among the various isolates. The maximum septal distance was recorded for isolate C1 (25.56 µm). This was followed by C2, C3 and C4 with 19.15 µm, 13.86 µm and 9.80 µm respectively. The minimum septal distance was recorded for the isolate C5 (8.50 µm). Gautam (2014) reported that mycelium of *C. gloeosporioides* from different crops were hyaline, septate and branched which was in accordance with the present study. Mammooty (2003) reported isolates of *C. gloeosporioides* from black pepper with a mycelial width of 1.25 - 4 µm. Aswani *et al.* (2016) reported mycelial width of *C. gloeosporioides* from snake gourd was in the range of 2.22 - 3.8 µm.

The conidia of *C.gloeosporioides* were borne on elongated acervular conidiomata. The conidial shape varied from cylindrical, oblong to dumbbell with an oil globule at centre. The size varied from 9.6 µm to 12.2 µm in length, 3.8 µm to 4.7 µm in breadth among the collected isolates of Colletotrichum

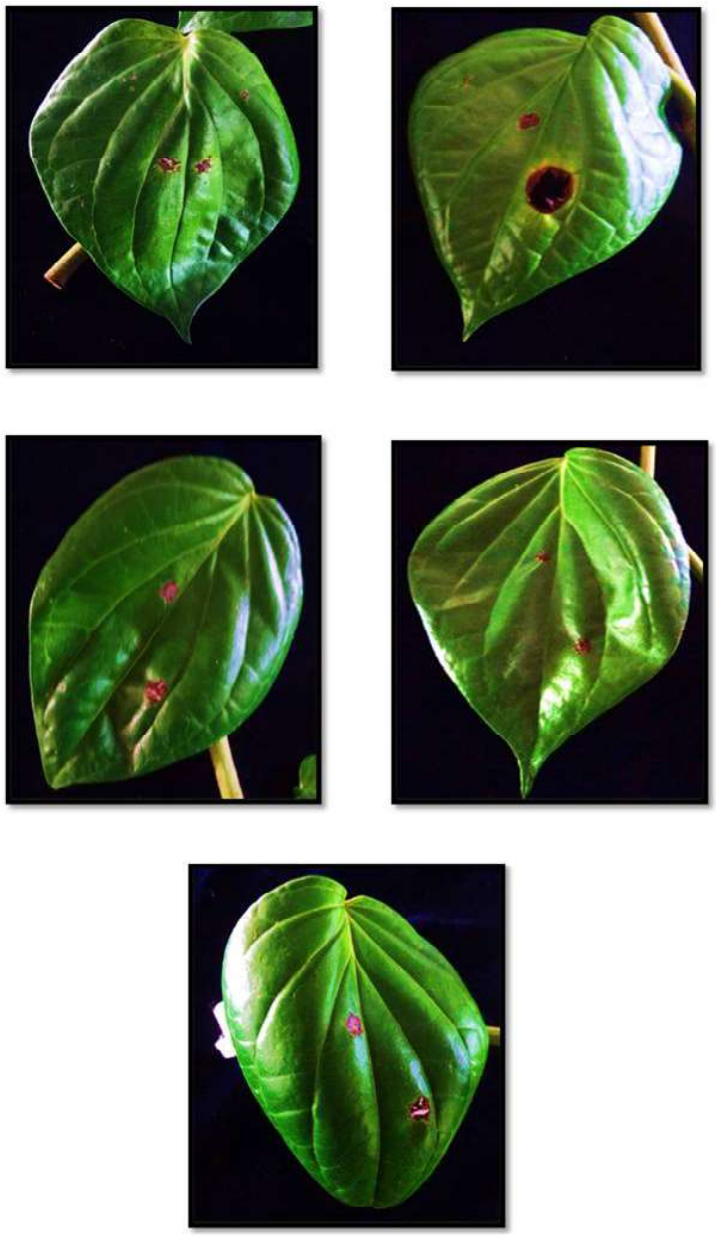


Plate 7 (C1-C5). Lesion development of *C. gloeosporioides* causing anthracnose of betel vine on excised betel vine leaves (5 DAI)

(Table 2) (Plate 5). The isolate C1 had a conidial size of 11.4 µm x 4.7µm. The isolate C3 had oblong shaped conidia with the size of 11.2 µm in length and 3.7 µm in width. The isolate C4 and C2 had oblong and dumbbell shaped conidia with a dimension of 12.2 µm x 4.3 µm and 11.3 µm x 3.9 µm respectively. The isolate C5 had cylindrical shaped conidia with a dimension of 9.6 µm x 3.8 µm. Sreeja (2014) and Aswani *et al.* (2014) reported size of conidia of *C. gloeosporioides* from cowpea and snakegord as 8.6 - 11.3 µm x 3.5 - 4.3 µm and 11 – 15 µm x 4 - 5 µm respectively which was in consonance with the study. *C. gloeosporioides* having hyaline , aseptate, oblong to slightly dumbbell shaped conidia with an average size 15.74 × 5.43 µm were also observed from betel vine. (Jagtap *et al.,* 2015).

The appressorial size also varied among isolates between 8.73 - 10.08 µm and 5.02 - 6.28 µm length and breadth respectively (Table 2)(Plate 6). The maximum appressorial length was recorded for the isolate C2 and breadth for the isolate C3. The isolate C1 had an appressorial size of 8.73 µm x 5.02 µm, while the isolate C2 had 10.08 µm x 5.23 µm sized appressoria. Similarly, the isolate C3 had an appressorial size of 9.57 µm x 6.28 µm while for the isolates C4 and C5 the size ranged about 9.27 µm x 5.71 µm and 8.37 µm x 5.11 µm respectively. Appressorial dimensions of 5.10 - 8.30 x 4.24 - 6.0 µm in length and width were recorded for *C. gloeosporioides* isolated from citrus (Agostini *et al.* 1992). Parashar (2013) observed *C. gloeosporioides* from anthracnose infected chilli produced appressoria with a similar dimension of 11.06 - 12.95 µm x 10.08 - 12.10 µm.

The pathogenic variability among isolates were assessed by virulence rating, based on lesion size, rate of lesion development and time taken for symptom expression. The days taken for symptom appearance on artificial inoculation of five isolates ranged between 2-5. The isolate C2 expressed the symptom within two days after inoculation (DAI) and produced 14.75 mm sized lesion on 5th day of inoculation (Table 4) (Plate 7). Similarly the isolates C5, C3, C4, C1 produced 5.07 mm, 3.52 mm, 3.00 mm and 2.50 mm sized lesions at 5 DAI. The rate of lesion development varied from 0.5 mm day^{-1} to 2.95 mm day^{-1}. The highest rate of lesion development and minimum days for symptom initiation was recorded for the isolate C2, while the isolate C1 was the least virulent with respect to the minimum lesion size and maximum time for symptom development. Based on the lesion size produced, rate of lesion development and days for symptom expression, C2 was identified as the most virulent among the five isolates of *C. gloeosporioides*. Pathogenic variability of *C. gloeosporioides* in black pepper was assessed by leaf inoculation method (Sankar, 2002). The study revealed that isolates of *C. gloeosporioides* from black pepper produced lesion size between 0.9 to 11.6 mm on 8 DAI. The isolate which produced larger lesion size (C6) was identified as most virulent as in confirmation with the present study. Ahmed *et al.* (2014) studied pathogenicity of *C.gloeosporioides* on betel vine stems by virulence rating on the basis of lesion size developed on artificial inoculation.

Conclusion

Anthracnose of betel vine caused by *collectotrichum* sp. is a devasting disease of this mastigatory crop. The five isolates of c. *gloeosporioides* from surveyed areas showed wide variation in their morphological culture and pathogenic characters. The virulent isolate (c6) induced symptoms with in two days after inoculation and produce maximum lesion size.

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