Journal of Spices and Aromatic Crops
Vol. 29 (1) : 48-58 (2020)
doi : 10.25081/josac.2020.v29.i1.6334

Indian Society for Spices

Occurrence of teleomorphic phase of *Colletotrichum gloeosporioides sensu lato*, the incitant of black pepper anthracnose

C N Biju[1,*], R Praveena[1], M F Peeran[2], A M Sujatha[2] & S J Ankegowda[2]

[1]*ICAR-Indian Institute of Spices Research, Kozhikode-673 012, Kerala.*
[2]*ICAR-Indian Institute of Spices Research Regional Station*
Appangala, Madikeri-571 201, Karnataka.
**E-mail: Biju.CN@icar.gov.in*

Received 19 March 2020; Revised 21 May 2020; Accepted 01 June 2020

Abstract

Anthracnose caused by *Colletotrichum gloeosporioides sensu lato*, the ascomycetous pathogen is a major constraint in black pepper cultivation. In the present study, surveys carried out in black pepper cultivating regions of Karnataka, India revealed the prevalence of anthracnose disease manifested as diverse array of foliar symptoms. An atypical foliar symptom was also noticed in the black pepper nurseries, characterized by grayish necrotic lesions with brown-blackish margins and randomly distributed blackish structures of pin-head size in the lesion area manifested particularly on the older leaves. The pin-head structures produced orangish exudation embedded with asci, ascospores and perithecia, when incubated under high humid conditions. Typical anthracnose symptoms were developed on susceptible host in pathogenicity studies and subsequent isolation yielded two distinct colonies designated as black and orange. The perithecia were induced artificially under *in vitro* conditions, which retained fertility and infectivity more than three months. Alternation of generation was observed when the perithecia were cultured on potato dextrose medium which resulted in the formation of acervuli with abundant conidiation. The results of present investigation shed light into the occurrence and potential role of perithecial (teleomorphic) phase in the survival of *C. gloeosporioides s. l*. infecting black pepper.

Keywords: anthracnose, *Colletotrichum gloeosporioides sensu lato, Piper nigrum*, perithecia

Introduction

Black pepper (*Piper nigrum* L.) is one among the highly valued spices originated in the evergreen forests of Western Ghats, India. Black pepper is commercially cultivated in Brazil, India, Indonesia, Malaysia, Sri Lanka and Vietnam for its dried mature fruits referred as berries. In India, cultivation of black pepper is chiefly confined to Karnataka, Kerala and Tamil Nadu (Ravindran 2000). Black pepper is highly vulnerable to several diseases like foot rot, slow decline and stunt disease of which, anthracnose also known as "*fungal pollu*" is an economically important disease prevalent in black pepper cultivating regions. *Colletotrichum*

gloeosporioides sensu lato (Penz.) Penz and Sacc., incitant of the disease infects all the aerial plant parts including stem, foliage, spikes and berries (Sarma *et al*. 1988; Anandaraj & Sarma 1995; Anandaraj 2000; Biju *et al*. 2013). Earlier studies revealed that, different species of *Colletotrichum* which survive in soil, crop debris and collateral hosts serve as potential sources of inoculum and could initiate disease under favourable environmental conditions (Yoshida & Shirata 1999; Sankar & Kumari 2002; Amusa *et al*. 2005). It is also reported that, related species of *C. gloeosporioides* perpetuate as persistent survival structures in crop residues and collateral hosts, which serve as primary sources of inoculum for the succeeding season. *Colletotrichum*, the hemi-biotrophic ascomycetous fungus employs conidia, borne on acervuli as a mode of rapid dissemination mechanism in a broad spectrum of economically important crop species. Production and dissemination of infective conidial propagules that are ephemeral in nature are generally favoured by conducive environmental conditions. However, in annual crops during unfavourable environmental conditions, the pathogen tend to produce hardy, more persistent perennating structures like microsclerotia (asexual morph) (Biju *et al*. 2017) or perithecia (sexual morph) as a survival mechanism to over-winter or over-summer which subsequently gets activated and acts as primary sources of inocula for the succeeding cropping season. It is reported that different species of *Colletotrichum* over-winter in their teleomorphic form (*Glomerella*) in a wide range of crop species and possibly serve as primary foci of infection under field conditions (Kim *et al*. 2002; Padilla *et al*. 2002). Intra-specific analysis of *Colletotrichum* populations revealed the existence of strains as obligate homothallic or heterothallic and also occurrence of strains exhibiting unbalanced heterothallism due to gene mutations in the self-recognition (homothallic) pathway (De Silva *et al*. 2017). Species-specific primer *CgInt*, coupled with ITS 4 are used for precise identification of *C. gloeosporioides* (Freeman *et al*. 2000a; Maymon *et al*. 2006). Though information on the occurrence of teleomorphic phase are reported in several economically important horticultural crops, pertinent information on the occurrence of perithecial (teleomorphic phase) of *C. gloeosporioides sensu lato* infecting black pepper is lacking. Hence, the present study was formulated with the objective to explore the possibility of occurrence of teleomorphic stage in the field populations of *C. gloeosporioides s. l*. infecting black pepper.

Materials and methods

Survey and collection of samples

The black pepper nurseries and plantations representing high altitudinal, high rainfall zone of Appangala (12°26'N Latitude and 75°45'E Longitude 920 m above MSL) and adjacent locations in Kodagu District, Karnataka, India were surveyed to explore the possible occurrence of oversummering structures of the pathogen *in planta*. During the period of study, temperature profile of the surveyed regions ranged from 11° to 34°C with an average annual rainfall of 2800 mm, which generally registered the peak during July to August. During the surveys, symptomatic leaves exhibiting characteristic anthracnose symptoms, infected spikes and runner shoots with necrotic lesions were collected from nurseries and plantations. The samples exhibiting characteristic symptoms (typical) as well as atypical were collected in polythene bags, sealed and subsequently brought to the laboratory for further processing.

Isolation and phenotypic characterization

For isolation, peripheral healthy zones along with the necrotic portions were incised from advancing margin of the lesions, dissected into bits (1 × 1 cm), surface sterilized with 70% ethanol for 30 sec followed by treating with 1% sodium hypochlorite for 2 min and washed with sterile distilled water consecutively three times. Subsequently, the bits were transferred aseptically to potato dextrose agar (PDA) and incubated at 25±2°C with 12:12 h photoperiod (alternating with light and dark conditions).

The hyphal initials emerging out of the bits were transferred aseptically to PDA in Petri dishes and maintained at 25±2°C under continuous illumination. Subsequently, the single spore cultures were derived from each isolate and maintained at 4°C for further studies. For analyzing phenotypic features, mycelial plugs of size 5 mm were punched out aseptically from the periphery of 7 days old cultures of each isolate, transferred to PDA (in triplicates) and incubated at 25±2°C. For examining conidial morphology, the conidia harvested from the colony maintained in the culture plates were mounted in water, stained with lactophenol cotton blue and dimensions were recorded at 100 X magnification. Similarly, the ascus, ascospores and perithecia were examined under the microscope (LEICA DM 5000B) and photomicrographs were documented.

Pathogenicity

To prove Koch's postulates *in planta*, the plants of susceptible black pepper variety (Panniyur 1) at 4 leaf stage were established under controlled conditions in polyhouse and the succulent second fully opened leaves were used for inoculation. Prior to inoculation, the leaves were subjected to surface sterilization with 0.5% sodium hypochlorite for 2 min, washed with sterile distilled water and blot dried.

Black pepper leaves expressing atypical symptoms were incubated at 25±2°C, 90±5% relative humidity with 12:12 h photoperiod to induce sporulation. The leaves with exudates were made into bits (1 cm × 1 cm) and placed over the leaf of Panniyur 1 with the exudate facing the adaxial surface, covered with a thin mat of moistened cotton and the entire plant was covered with polythene bags to maintain adequate humidity. The cotton bits were assured with sufficient moisture by misting distilled water with hand-driven atomizer at 12 h interval till development of conspicuous symptoms, if any.

For inoculation with conidial/ascospore suspension, the conidia/ascospores were collected by adding 10 mL of sterile distilled water into the Petri dish and swirled gently to dislodge conidia/ascospores. The concentration of conidia/ascospores was adjusted to 3×10^6 mL^{-1} using haemocytometer and subsequently used as standard inoculum density for pathogenicity test. Fifty µL of the suspension was spotted on the leaf surface, followed by placing moistened cotton at the portal of inoculation. The humidity and other parameters to facilitate infection were adopted as described above. The control plants received sterile distilled water without conidia/ascospores. The inoculated plants were monitored regularly for the development of symptoms, if any.

In vitro induction of teleomorphic stage

The experiment was designed based on mating-test model, using sterilized toothpicks, dried leaves and twigs of black pepper as well as twigs of silky oak (*Grevillea robusta*), a common standard used to trail black pepper which were placed between the confronting inoculum sources (infected young and dried leaves of black pepper as well as pathogen culture) which served as inert platforms for perithecial induction. The formation of perithecia in the combinations was assessed based on 0–7 scale adopted from Guerber & Correll (2001).

Scale for assessing formation of perithecia

Scale	Inference
0	No structures formed
1	Minute sterile structures
2	Sterile perithecia with beaks, no asci
3	Sterile perithecia with asci, no ascospores
4	Asci with few ascospores
5	Fertile perithecia with many ascospores, few asci with 8 ascospores
6	Fertile perithecia with abundant ascospores, many asci with 8 ascospores
7	Perithecia exuding ascospores from asci

Molecular characterization

The total DNA was extracted from mycelial mat of the cultures representing teleomorphic stage and *C. gloeosporioides s. l*. CP1 adopting cetyl trimethyl ammonium bromide (CTAB) protocol described by Knapp & Chandlee (1996). The mycelial mat was harvested by filtration using sterile filter paper and subsequently stored at -70°C until used for DNA extraction. 1 g of frozen mycelium was made into fine powder in liquid nitrogen and incubated in 5 mL 2% CTAB extraction buffer [consisted of 10 mM trisbase (pH 8.0), 20 mM EDTA (pH 8.0), 1.4 M NaCl, CTAB (2%), mercaptoethanol (0.1%) and PVP (0.2%)] at 65°C for 1 h to which equal volume of phenol-chloroform-isoamylalcohol (25:24:1) mixture was added. The two phases were mixed by vortexing, followed by centrifugation at 12,000 rpm for 5 min. The DNA was precipitated by transferring the supernatant to a clean tube, mixing with equal volume of ice cold isopropanol and incubating at 25°C. Further, by centrifugation the precipitate was collected, washed with ethanol (70%) and the final pellet was re-suspended in TE buffer. The concentration of the DNA was estimated using spectrophotometer (Genway Genova, UK) at 260 nm. For PCR amplification, ITS 4 primer coupled with specific primer (*CgInt*) (5′-GGCCTCCCGCCTCCGGGCGG-3′) were used which was performed in a total reaction volume of 20 μL in Eppendorf master cycle gradient thermal cycler. The PCR programme was as follows: initial denaturation for five min at 95°C, 30 cycles of 30 sec at 95°C, 30 sec at 48°C and 90 sec at 72°C and a final extension of 10 min at 72°C (Maymon *et al*. 2006). The PCR products were resolved in 1.2% agarose gel and documented using an Alpha Imager (Alpha Innotech Corporation, CA, USA). The amplified products were eluted from the gel and sequenced. The phylogenetic analysis was carried out by Neighbor-Joining method using MEGA X (Kumar *et al*. 2018) along with the corresponding sequences of other species and related genera retrieved from NCBI nucleotide database.

Results and discussion

Survey, disease symptoms and collection of samples

During the surveys in plantations, the disease symptoms were noticed as minute dark brown spots surrounded by yellow halo on the newly emerged leaves. Whereas, on the older leaves, randomly distributed circular or discrete spots with or without yellow halo were noticed. In few locations, the spots were characterized with gray center or with a shot-hole. Leaf blight symptoms at distal end of the leaves were also observed (Fig. 1). In nurseries, the most common symptoms observed were small pin-head size dark brown necrotic spots more or less evenly distributed throughout the entire leaf surface (Fig. 2a). These spots later developed yellow halo surrounding the necrotic region. Besides, an unusual symptom characterized by grayish necrotic lesions with dark margin surrounded with yellow halo (Fig. 2b) and randomly distributed blackish structures of pin-head sized on the lesion area (Fig. 2c) on the matured old leaves was noticed in one of the nurseries. A total of 12 symptomatic samples from black pepper plantations and 6 samples (5 exhibiting characteristic anthracnose symptoms and one atypical symptom) from the nurseries were collected.

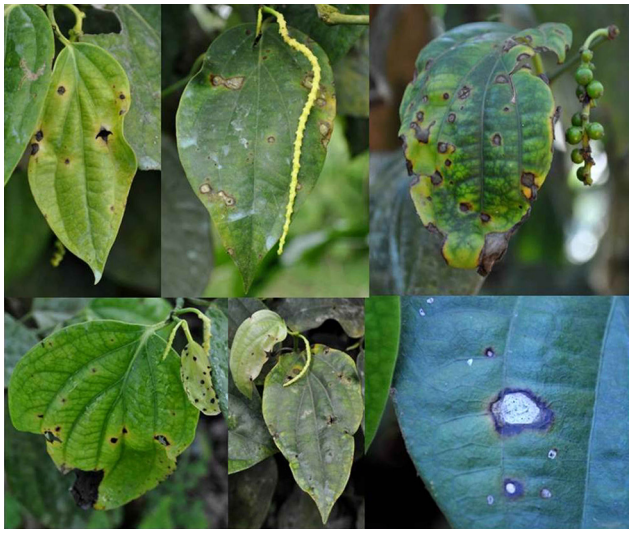

Fig. 1. Variation in symptoms of black pepper anthracnose under field conditions

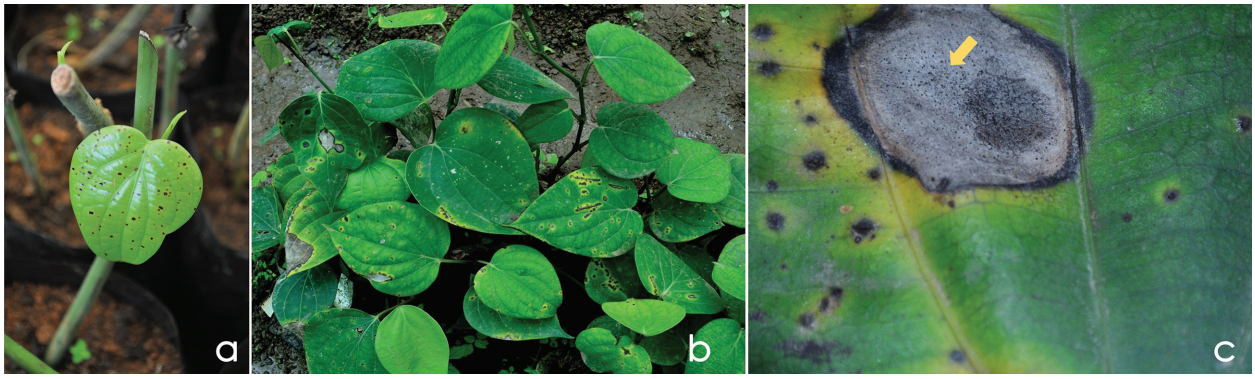

Fig. 2. Symptoms of anthracnose under nursery conditions (a) typical symptoms (b) atypical symptoms (c) blackish structures of pin-head size on lesion area

Isolation, phenotypic characterization and pathogenicity

The isolation carried out from the samples collected from nurseries and plantations yielded morphologically distinct colonies. The colonies were subcultured and identified as *Colletotrichum gloeosporioides sensu lato* based on colony, conidial as well as appressorial characters. In general, the colonies were gray or white with puffy growth with an average growth rate of 8.5 mm/day (8 days after inoculation), the conidia were cylindrical/elliptical and measured 13.4 × 6.7 µm (average) and characterized by single to two lobed appressoria with dimension of 14.3 × 7.1 µm (average). The leaf bits derived from plants exhibiting typical and atypical symptoms when exposed to high humidity produced copious exudation of two different kinds; dull creamish (Fig. 3a) and orange coloured (Fig. 3b), respectively. The dull creamish exudate on microscopical examination revealed the presence of characteristic conidia of *C. gloeosporioides s. l.* embedded in a gelatinous matrix and the orange coloured exudation revealed the presence of asci, ascospores and perithecia embedded in the exudate. The perithecia were predominantly globose and measured 172 to 298 µm (Fig. 4a), while the dimensions of intact asci were in the range of 93 to 115 µm (Fig. 4b). The size of the ascospores (Fig. 4c) varied between 14 to 26 µm. In the present investigation, atypical symptoms (necrotic spots) observed on the older leaves of black pepper plants maintained under nursery conditions revealed the

Fig. 3. Dull creamish (a) orange (b) exudations developed on leaves with typical and atypical anthracnose symptoms, respectively

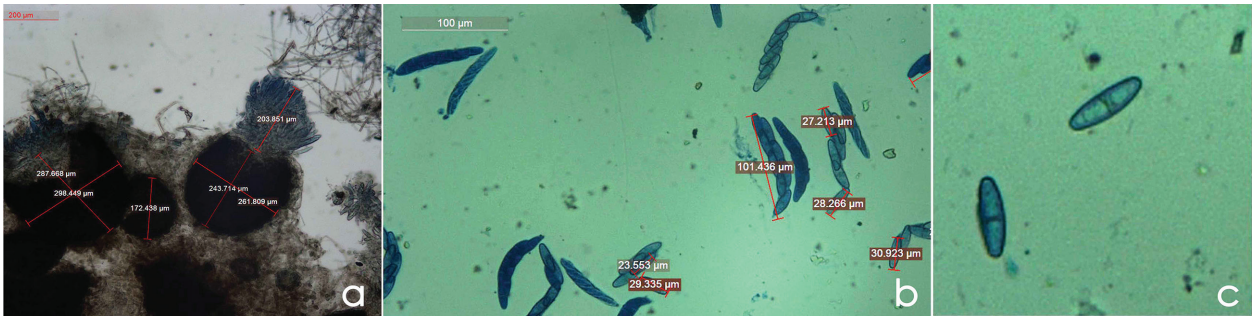

Fig. 4. Teleomorphic phase (perithecia) of *Colletotrichum gloeosporioides s. l.* (a) perithecia (b) intact asci (c) ascospores

presence of teleomorphic phase of *C. gloeosporioides* (*Glomerella cingulata*) associated with the moribund tissues. Talgø *et al*. (2007) reported the formation of both conidial (*C. acutatum*) and the teleomorphic (*G. acutata*) stages on naturally infected blueberry fruits. The perithecial phase was also readily formed on blueberries and strawberries artificially inoculated with high bush blueberry isolate as well as on artificial media (both on potato dextrose agar and strawberry leaf agar). The perithecia were dark brown measuring 210–390 × 115–190 µm, asci, 52.5–95.0 × 7.5–12.5 µm and ascospores, 10.0–16.3 × 4.3–5.5 µm. The ascospores were slightly curved and were pointed at one end and rounded at the other end or pointed at both ends or rounded at both ends. It was also observed that, under high humid conditions, orangish ascospore mass oozed out of perithecia which was drier compared to the conidial spore masses. Kim *et al*. (2002) observed the formation of teleomorphic stage of *C. gloeosporioides* infecting *Gardenia* under laboratory conditions. *C. gloeosporioides* developed the perithecial stage (*Glomerella cingulata*) on the medium after 4 weeks of incubation period. The perithecia were dark brown and globose and measured 113–225 µm. The perithecial, ascal and ascospore dimensions recorded in the present study were in conformation with earlier reports. The perithecia formed on the older leaves could act as primary source of inoculum which might trigger initiation and rapid spread of the disease especially under nursery conditions, if adequate management measures are not adopted.

While assessing the disease causing potential of the exudates, the leaf bits with dull creamish and orange coloured exudates inoculated on the black pepper variety Panniyur 1, resulted in the development of characteristic anthracnose symptoms. Subsequent isolation from the lesions inoculated with dull creamish exudates yielded typical *C. gloeosporioides s. l.* colony. However, isolation from the lesion induced by orange exudate yielded two distinct colonies designated as black and orange. The black colony was characterized with slight puffy growth, formation of creamish exudate at the center with abundant conidiation and recorded a growth rate of 11 mm/day (Fig. 5a). While, the orange colony with a growth rate of 7.8 mm/day, appeared puffy with randomly distributed black hard structures, produced asci and ascospores embedded in the orange exudation (Fig. 5b). For further confirmation, conidia and ascospores derived from the test cultures were inoculated both singly and in combination on black pepper leaves (variety Panniyur 1). In single inoculations, the symptoms developed 3 days post inoculation as small pin head sized yellow spots. On 6th day after inoculation, size of the spots increased from 0.1 to 0.2 cm and later turned brownish with yellow halo. Inoculation with conidia and ascospores in combination resulted in the formation of necrotic lesions with yellow halo, which measured 0.9 cm. Padilla *et al*. (2002) hypothesized that, strawberry fruit rot epidemics caused by *C. acutatum* and crown rot under field conditions were due to *Glomerella cingulata*, the teleomorph of *C. gloeosporioides*. Further, they

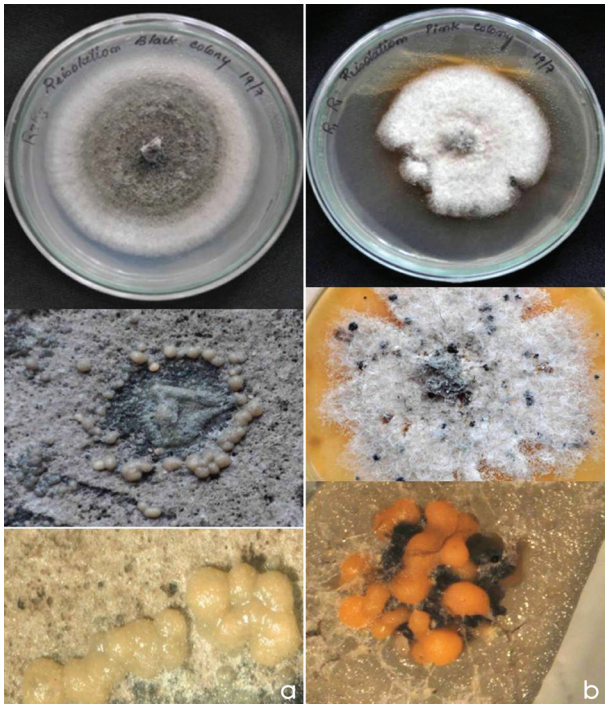

Fig. 5. Colony morphology and exudations (a) blackish culture (b) orange culture

suggested that, genetic recombination would have resulted in low linkage disequilibrium and high degree of diversity in the population of *C. gloeosporioides*. Latham & Williams (1983) reported the role of *G. cingulata*, the perfect stage of *C. gloeosporioides* in causing bitter rot of apple. Shane & Sutton (1981) noticed the occurrence of perfect stage of *C. gloeosporioides; G. cingulata* in the orchards of North Carolina. They further hypothesized that, ascospores that are forcibly discharged from the perithecia aid in the dissemination of the pathogen. The study of Sutton & Shane (1983) confirmed that isolates of *G. cingulata* (perithecial isolates) have the potentiality to contribute significantly to apple bitter rot epiphytotics in the orchards. The perithecial strains spread in the orchards from overwintering sites primarily through rain liberated, wind-blown ascospores.

Molecular characterization

PCR amplification with *CgInt* and ITS 4 species-specific primers generated the expected 450 bp amplicon as reported earlier by Chowdappa *et al*. (2012). The band was subsequently eluted, sequenced and BLASTN analysis confirmed identity of the pathogen (*C. gloeosporioides s. l.)* and its teleomorphic phase (*G. cingulata*). The sequence was submitted to NCBI carrying accession number KY236318. Phylogenetic analysis showed a clear separation of *G. acutata* clade indicating that the isolate CPI belongs to *C. gloeosporioides s. l.* (Fig. 6). Nucleic acid-based tools such as PCR employing species-specific primers are used in delineating different species of *Colletotrichum* hitherto distinguished using morphological protocols (Lewis Ivey *et al*. 2004; Freeman *et al*. 2000b; Sawant *et al.* 2012).

In vitro induction of teleomorphic stage

Under *in vitro* conditions, perithecial production was observed in all the combinations of inert substrates and different inoculum sources. However, the formation of ascospores was noticed only in the combination of dried black pepper twig + infected young or dried black pepper leaves (Fig. 7). The copious exudation embedded with ascospores produced from fertile perithecia was evident in the combination; black pepper twig + infected young leaf even three months after incubation (Fig. 8) indicating, fertile nature and longevity of the perithecia (Table 1). The twigs with ascospore exudates, partly or wholly, when assessed for infectivity on the susceptible black pepper variety, Panniyur 1, resulted in the production of characteristic anthracnose symptoms 4-6 days after inoculation. Subsequent re-isolation from the lesions yielded the conidial phase of the pathogen. Alternation of generation was also observed as a shift from perithecial (sexual phase) to conidial (asexual phase), when aggregates of perithecia were inoculated on potato dextrose agar medium.

The alternation of generation suggests the adaptive nature of the pathogen to adverse environmental conditions. Unfavourable weather conditions and depletion of nutrients in the media would have resulted in the shift from the ephemeral phase (conidia) to more

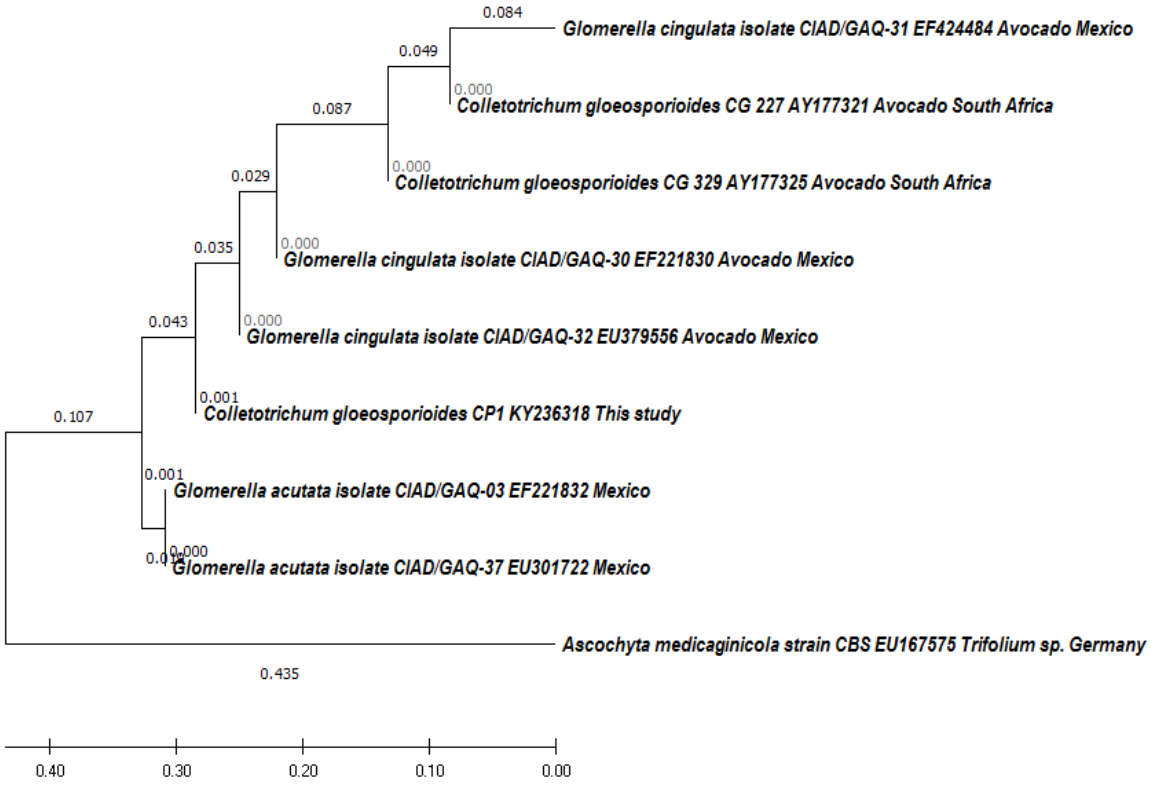

Fig. 6. Phylogenetic tree drawn by Neighbor-Joining method using *CgInt* and ITS 4 sequence of *C. gloeosporioides s. l.* black pepper isolate (CPI) with related species from the GenBank

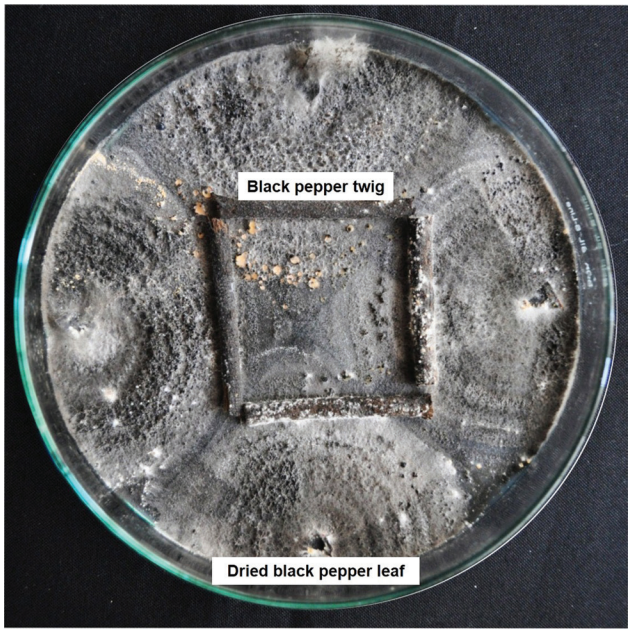

Fig. 7. *In vitro* induction of teleomorphic phase

persistent quiescent structures (perithecia). Several ascomycetous pathogens are reported to readily produce teleomorphic stage under *in vitro* conditions. Nevertheless, formation of teleomorphic phase of *Colletotrichum* species

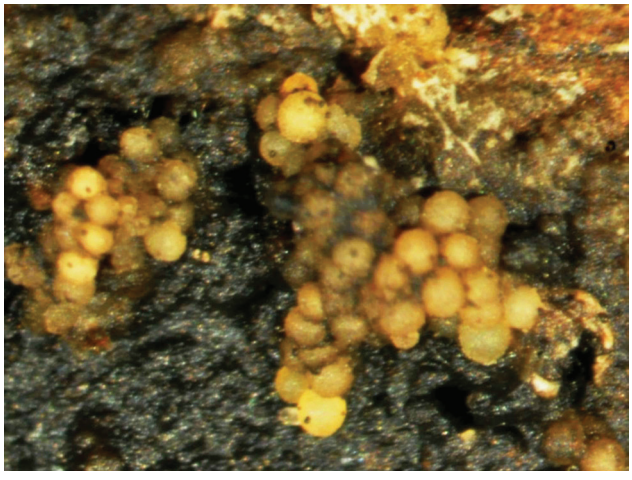

Fig. 8. Orange perithecial exudates formed on dried black pepper twig

Table 1. *In vitro* induction of perithecia of *Colletotrichum gloeosporioides s. l.* on inert substrates with different sources of inoculum

Inoculum source	Dried black pepper twig				Dried black pepper leaves				Dried unsplit twig of silky oak				Dried split twig of silky oak			
	P	C	ASP	ACER	P	C	ASP	ACER	P	C	ASP	ACER	P	C	ASP	ACER
Pathogen culture	+	+	-	-	+	+	-	-	+	+	-	-	+	+	-	-
*Infected young black pepper leaf	+	+	+	-	+	+	-	-	+	+	-	-	+	-	-	-
Infected dried black pepper leaf	+	+	+	-	+	-	-	-	+	+	-	-	+	-	-	-

P=Perithecia; C=Conidia; ASP=Ascospores; ACER=Acervulus

*=Orange exudation noticed even after 3 months

cultured in laboratory are reported to be rare. Induction of perfect stage under *in vitro* conditions would help in analyzing longevity, retention of fertility and infectivity of the propagules, thus deciphering the disease cycle comprehensively. Politis (1975) artificially induced perithecia of *C. graminicola* isolates infecting maize on sterilized maize leaves under *in vitro* conditions. These isolates were found to be homothallic, since the cultures derived from mono-conidia produced perithecia. The present investigation demonstrated that, perithecia of *C. gloeosporioides s. l.* could be induced artificially under *in vitro* conditions, which retained fertility and infectivity for more than a period of three months. It is hypothesized from the present study that, the field populations of *C. gloeosporioides s. l.* might comprise of heterothallic forms which have the potentiality to interbreed. Since conidia are considered as the primary source of inocula in black pepper anthracnose, infectivity of ascospores under *in vitro* conditions indicates that, the propagules produced by the oversummering quiescent teleomorph may also act as primary inoculum which could initiate disease under favourable environmental conditions. The existence of perithecial phase of *C. gloeosporioides s. l*. infecting black pepper under natural conditions indicates the possible occurrence of outcrossing heterothallic population in nature.

Acknowledgments

The authors thank The Director, ICAR-Indian Institute of Spices Research, Kozhikode, Kerala, India and Indian Council of Agricultural Research, New Delhi for providing financial support through the Outreach Programme on Diagnosis and Management of Leaf Spot Diseases in Field and Horticultural Crops.

References

Amusa N A, Adegbite A A & Oladapo M O 2005 Investigations into the role of weeds, soil and plant debris in the epidemiology of foliar fungal diseases of yam in Western Nigeria. Int. J. Bot. 1: 111–115.

Anandaraj M & Sarma Y R 1995 Diseases of black pepper (*Piper nigrum* L.) and their management. J. Spices Arom. Crops. 4: 17–23.

Anandaraj M 2000 Diseases of black pepper. In: Ravindran P N (Ed.), Black pepper (*Piper nigrum*) (pp. 239–266). Harwood Academic Publishers.

Barakat R M, Johnson D A & Grove G G 1995 Factors affecting conidial exudation and survival and ascospore germination of *Leucostoma cincta*. Plant Dis. 79: 1245–1248.

Biju C N, Praveena R, Ankegowda S J, Darshana C N & Jashmi K C 2013 Epidemiological studies on black pepper anthracnose caused by *Colletotrichum gloeosporioides*. Indian J. Agri. Sci. 83: 1199–1204.

Biju C N, Praveena R, Mohammed F P, Darshana C N, Jashmi K C & Ankegowda S J 2017 Significance of microsclerotia in the epidemiology of black pepper anthracnose and an approach for disease management in nurseries. J. Phytopathol. 165: 342–353.

Chowdappa P, Chabanahalli S C, Bharghavi R, Sandhya H & Rajendra P P 2012 Morphological and molecular characterization of *Colletotrichum gloeosporioides* (Penz) Sac. isolates causing anthracnose of orchids in India. J. Biotechnol. Bioinform. Bioengg. 2: 567–572.

De Silva D D, Crous, P W, Ades, P K, Hyde, K D & Taylor P W 2017 Life styles of *Colletotrichum* species and implications for plant biosecurity. Fungal Biol. Rev. 31: 155–168.

Eastburn D M & Gubler W D 1990 Strawberry anthracnose: Detection and survival of *Colletotrichum acutatum* in soil. Plant Dis. 74: 161–163.

Forster H & Adaskaveg J E 2000 Early brown rot infections in sweet cherry fruit are detected by *Monilinia*-specific DNA primers. Phytopathol. 90: 171–178.

Freeman S, Minz D, Jurkevitch E, Maymon M & Shabi E 2000a Molecular analyses of *Colletotrichum* species from almond and other fruits. Phytopathol. 90: 608–614.

Freeman S, Shabi E & Katan T 2000b Characterization of *Colletotrichum acutatum* causing anthracnose of anemone (*Anemone coronaria* L.). Appl. Environ. Microbiol. 66: 5267–5272.

Guerber J C & Correll J C 2001 Characterization of *Glomerella acutata*, the teleomorph of *Colletotrichum acutatum*. Mycologia. 93: 216–229.

Kim G H, Lee J G, Hur J S & Koh Y J 2002 Anthracnose of gardenia (*Gardenia jasminoides* var. *grandiflora* Makino) caused by *Glomerella cingulata*. Plant Pathol. J. 18: 228–230.

Knapp J E & Chandlee J M 1996 RNA/DNA mini-prep from a single sample of orchid tissue. Biotechniques. 21: 54–56.

Kumar S, Stecher G, Li M, Knyaz C & Tamura K 2018 MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. Mol. Biol. Evol. 3: 1547–1549.

Latham A J & Williams J C 1983 Cultural characteristics and pathogenicity of *Glomerella cingulata* isolates from apples in Alabama. Plant Dis. 67: 1065–1068.

Lewis Ivey M, Nava-Diaz C & Miller S 2004 Identification and management of *Colletotrichum acutatum* on immature bell peppers. Plant Dis. 88: 1198–1204.

Maymon M, Zveibil A, Pivonia S, Minz D & Freeman S 2006 Identification and characterization of benomyl resistant and sensitive populations of *Colletotrichum gloeosporioides* from statice (*Limonium* spp.). Phytopathol. 96: 542–548.

Norman D J & Strandberg J O 1997 Survival of *Colletotrichum acutatum* in soil and plant debris of leather leaf fern. Plant Dis. 81: 1177–1180.

Padilla A R U, Mackenzie S J, Bowen B W & Legard D E 2002 Etiology and population genetics of *Colletotrichum* species causing crown and fruit rot of strawberry. Phytopathol. 92: 1245–1252.

Politis D J 1975 The identity and perfect state of *Colletotrichum graminicola*. Mycologia. 67: 56–62.

Ravindran P N 2000 Introduction. In: Ravindran P N (Ed.) Black pepper (*Piper nigrum*) (pp. 01–22). Harwood Academic Publishers.

Sankar A & Kumari S P 2002 Survival of *Colletotrichum gloeosporioides*, the causal organism of anthracnose disease of black pepper. J. Spices Arom. Crops. 11: 129–131.

Sawant I S, Narkar S P, Shetty D S, Upadhyay A & Sawant S D 2012 Emergence of *Colletotrichum gloeosporioides sensu lato* as the dominant pathogen of anthracnose disease of grapes in India as evidenced by cultural, morphological and molecular data. Aust. Plant Pathol. 41: 493–504.

Shane W W & Sutton T B 1981 The *Glomerella cingulata* perfect stage and apple bitter rot. Plant Dis. 65: 135–137.

Sutton T B & Shane W W 1983 Epidemiology of the perfect stage of *Glomerella cingulata* on apples. Phytopathol. 73: 1179–1183.

Talgø V, Aamot H U, Strømeng G M, Klemsdal S S & Stensvand A 2007 *Glomerella acutata* on highbush blueberry (*Vaccinium corymbosum* L.) in Norway. Plant Health Progress doi:10.1094/PHP-2007-0509-01-RS.

Yoshida S & Shirata A 1999 Survival of *Colletotrichum dematium* in soil and infected mulberry leaves. Plant Dis. 83: 465–468.