

## Morphological variability, mycelial compatibility and fungicidal sensitivity of *Colletotrichum gloeosporioides* causing leaf spot of ginger (*Zingiber officinale* Rosc.)

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### Abstract

*Colletotrichum gloeosporioides* isolates causing leaf spot of ginger were collected from diverse agro-ecological regions of Kodagu, Hassan, Shimoga and Chikmagalur districts of Karnataka (KAG 1-6) and Idukki and Wayanad districts of Kerala (KLG 1-3). Diversity analysis of the isolates revealed existence of considerable variability in the field populations of the leaf spot pathogen. Colour of the colony varied from white to dull grey and considerable variation was observed in the growth rate of the isolates. The conidial shapes varied with regions and were cylindrical with tapering ends, cylindrical, elliptical or dumbbell, whereas, most of the isolates belonged to cylindrical with tapering ends category. The appressoria were either unlobed, single lobed or multilobed. The size of conidia and appressoria ranged from  $12.2-18.3 \times 6.1-6.9 \mu\text{M}$  and  $9.0-14.8 \times 6.2-11.0 \mu\text{M}$ , respectively. Mycelial compatibility studies among the nine isolates revealed that, the isolate, KLG 3, originating from Kerala was most compatible with other isolates, while KLG 1 and KAG 6 exhibited a highly incompatible reaction. Microscopic observations to study the process involved in compatible reaction showed the aggregation of hyphae at region of contact, fusion of hyphae (formation of 'H' – like structure) and subsequent generation and growth of new hyphae from the point of contact. Among the fungicides tested at recommended dosages, propiconazole was found to be promising followed by carbendazim + mancozeb. The present study indicated that leaf spot caused by *C. gloeosporioides* is emerging as a major foliar disease in the ginger growing tracts.

**Keywords:** *Colletotrichum gloeosporioides*, diversity, fungicidal sensitivity, ginger, leaf spot, mycelial compatibility

### Introduction

Ginger (*Zingiber officinale* Rosc.) is one among the most valued and widely cultivated spice crops in the world. The major limitation to the production of ginger is its susceptibility to a wide range of pests and diseases. Crop losses

due to various diseases incited by a myriad of pathogenic microbes belonging to diverse groups represented by fungi, bacteria and viruses are a major concern, as it inflicts damage to a considerable extent in most of the ginger growing regions. Among the diseases affecting aerial parts especially the foliage, leaf spot

incited by *Colletotrichum gloeosporioides* is a major cause of concern in many of the ginger growing tracts (Dohroo 1997). *Colletotrichum* leaf spot was first reported by Sundararaman (1922) from the Godavari district of Andhra Pradesh and the species *C. zingiberi* was identified by Butler & Bisby (1931). Similarly, *Colletotrichum* leaf spot incited by species, *C. gloeosporioides* and *C. capsici* has been reported from South-East Asian countries (Xizhen *et al.* 2005). The disease affects the foliage, leading to extensive damage of the effective photosynthetically active surface area, coupled with a concomitant reduction in the ability of the developing rhizome to store food reserves, thus adversely affecting the intrinsic qualitative parameters of the produce.

Earlier research attempts carried out in India and elsewhere addressed areas related to identification of the causal agent and management of the disease through chemical strategies. However, limited progress was made in understanding and analyzing pathogen diversity, compatibility among the isolates belonging to diverse agro-climatic regions and sensitivity of the pathogen towards currently used fungicides. In light of the above, the present investigation was formulated to investigate the diversity existing in the field populations of leaf spot pathogen, mycelial compatibility among the isolates and fungicidal sensitivity of the isolates originating from different geographical regions.

## Materials and methods

### Survey, isolation, identification and maintenance of isolates

Surveys were undertaken in the major ginger growing tracts *viz.*, Karnataka and Kerala during 2009–10. The samples exhibiting characteristic leaf spot symptoms were collected from various geographical locations representing different altitude and rainfall regions. The infected tissues were incised from advanced margin of the lesions, cut into small pieces and disinfested with 3% sodium hypochlorite solution followed by rinsing with sterile distilled water three times. The bits were transferred aseptically to potato dextrose agar (PDA) medium supplemented with

streptomycin sulphate (100 ppm) to prevent bacterial contamination, incubated at room temperature (25°C) and observed periodically for 7 days. The growing edges of hyphal initials emerging from the leaf tissues were transferred aseptically to growth medium in Petridishes. The cultures were identified by comparing the colony as well as conidial characters with published literature. Pure cultures of the pathogen were transferred to PDA slants supplemented with streptomycin sulphate (100 ppm) and maintained at 4°C, for subsequent studies.

### Morphological characterization

Observations on macro-morphological characteristics like colony morphology, growth rate and colour (top and reverse) were recorded by culturing the isolates on PDA medium. For morphological characterization, 5 mm mycelial plugs were aseptically punched out from actively growing area near the edges of 7 day old cultures of each isolate using a sterile cork borer. Each plug was subsequently transferred to the growth medium (PDA) and maintained in triplicates at temperature range of 25–27°C for 7 days. The mean radial mycelial growth (mm per day) of each isolate was recorded daily and after 7 days, colony size and colour were recorded.

Further, dimensions and shape of microscopic structures like conidia and appressoria were also recorded. For examination of conidial morphology, the isolates were cultured on PDA at 25°C for 7 days for enhancing conidial production. The conidia harvested from the culture plates of each isolate were mounted in water, stained with lactophenol cotton blue and the size was measured at 10X magnification. The production of conidial appressoria was induced by mixing the conidia in 2% sucrose solution. The conidial suspension was smeared on a glass slide and incubated in a moisture chamber at room temperature overnight. On the succeeding day, the slides were observed for the production of appressoria formed at the tip of germ tube from conidia and size as well as number of lobes was recorded for each isolate.

### Pathogenicity

The cultures of each isolate were grown on PDA for seven days at 25°C with 12 h photoperiod (alternating light and dark conditions) to enhance conidial production (Hong *et al.* 2008; Than *et al.* 2008). The conidia were then harvested by adding 10 mL of sterilized distilled water onto the Petridish, which was subjected to gentle swirling in order to dislodge conidia from the mycelial tuft. The concentration of conidia was adjusted to  $10^6$  conidia mL<sup>-1</sup> using a haemocytometer and subsequently used as the standard density of inoculum for pathogenicity test. Pathogenicity of the isolates was tested on the ginger variety Rio-de-Janerio under glass house conditions. The rhizomes were sown separately in pots maintained in glass house to prevent infection from external sources and inoculation was effected on young succulent leaves, at 4–6 leaf stage. Healthy leaves were surface sterilized with sterile distilled water and then injured softly using sterilized needles (pin - pricking method). Further, 5 µL of conidial suspension was placed on the wound and the inoculated plants (in quadruplicates) were enclosed in a moistened polythene hood to maintain sufficient humidity. The plants were observed regularly for the manifestation and development of symptoms.

### Determination of mycelial compatibility

Each isolate was paired with itself and with other isolates in all possible combinations on PDA medium. Each pairings were replicated thrice, incubated at 25°C and observed for 21 days. After incubation period, the reaction between each isolate pair was evaluated. The reactions were recorded as incompatible, when an apparent line of demarcation; a barrage zone or a mycelial free zone was observed between the confronting paired isolates. The pairings were scored compatible, when the two confronting isolates merged to form one confluent colony, with no distinct interaction zone.

### Examination of hyphal interaction/ anastomosis

The hyphal interaction studies were carried out on sterilized glass slides. The glass slides were

flame sterilized and placed at the center of a 90 mm petridish, which served as a moist chamber. 5 mm diameter mycelial discs derived from the edges of the colony from seven day old cultures were placed 5 cm apart on either side of the glass slide. Another flame-sterilized slide was carefully placed over the mycelial discs, so that the discs were sandwiched between the two glass slides and incubated at 25°C. The Petridishes were examined daily for hyphal interaction at 4.5X magnification. The upper glass slide was removed carefully, inverted and stained with cotton blue, especially at the region of contact and a cover slip was placed over it. Microscopic examinations were carried out using fresh direct mounts after staining with lactophenol cotton blue. The slides were observed for different hyphal interactions at 10X and 40X magnifications and were photographed.

### Evaluation for fungicidal sensitivity

Sensitivity of the isolates was tested against contact, systemic and combination fungicides *viz.*, carbendazim + mancozeb, hexaconazole, propiconazole, carbendazim, mancozeb, benomyl, triadimefon, metalaxyl + mancozeb, Bordeaux mixture and chlorothalonil at recommended dosages under *in vitro* conditions by employing poisoned food technique. Fungicidal suspensions were prepared by dissolving requisite quantities of each fungicide in warm PDA. About 20 mL of the medium was poured into Petridishes and medium without fungicide served as control. Mycelial discs (5 mm diameter) from the advanced margin of five day old culture of the isolates were placed at the center of each petridishes and each treatment was replicated thrice. The plates were incubated at 25°C and observations on radial growth of the colony were recorded seven days after the incubation period (Zentmeyer 1955; Kumar *et al.* 2007).

The percent inhibition of the colony growth was calculated using the formula:

$$C - T / C \times 100$$

Where, C=Growth of culture in control plate;  
T=Growth of culture in fungicide treated plate

### Data analysis

The *in vitro* bioassay experiments were laid out in completely randomized design (CRD) and the data recorded in percent were transformed to arc sine transformation. The transformed data were statistically analyzed using the software package AGRES version 7.01 @ 1994 Pascal Intl Software Solutions.

### Results and discussion

#### Survey, isolation, identification and maintenance of isolates

The surveys were carried out during the months of March to October. It was noticed that occurrence and severity of the disease were higher during June to September, which coincided with the active growth phase of the crop, marked with the emergence of new succulent tillers and young leaves. In the surveyed areas, *C. gloeosporioides* leaf spot was found to be widespread (Fig. 1) compared to other major foliar diseases like *Phyllosticta* leaf spot. A total of nine isolates of *Colletotrichum* were collected from Karnataka (KAG 1-6) and Kerala (KLG 1-3) (Table 1).

During the surveys, two distinct types of symptoms were noticed in the Suntikoppa region, Kodagu, Karnataka. The first was characterized with a discrete, unevenly distributed circular to oval, generally non-coalescing brown necrotic spots bordered with a bright yellow halo and was designated as leaf spot (induced by the isolate KAG 6) (Fig. 2). On contrary, the second could be distinguished



Fig. 1. Field view of ginger crop severely affected with leaf spot diseases

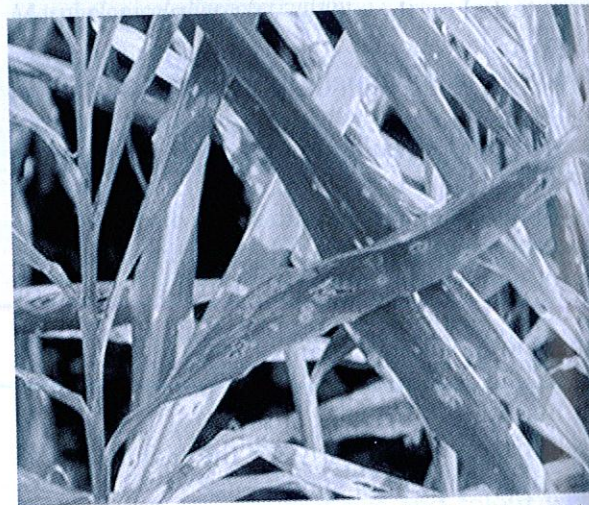


Fig. 2. Brown necrotic spots with a bright yellow halo

by brown coalescing necrotic lesions, especially manifested along the margins of the leaves causing extensive leaf blight (induced by the isolate KAG 5) (Fig. 3). Xizhen *et al.* (2005)

Table 1. Origin of isolates representing different ginger growing regions of South India

Isolate	Origin	District	State
KLG 1	Mananthavady	Wayanad	Kerala
KLG 2	Ambalavayal	Wayanad	Kerala
KLG 3	Idukki	Idukki	Kerala
KAG 1	Virajpet	Kodagu	Karnataka
KAG 2	Hanbal	Chikmagalur	Karnataka
KAG 3	Sakleshpur	Hassan	Karnataka
KAG 4	Shimoga	Shimoga	Karnataka
KAG 5	Suntikoppa	Kodagu	Karnataka
KAG 6	Suntikoppa	Kodagu	Karnataka

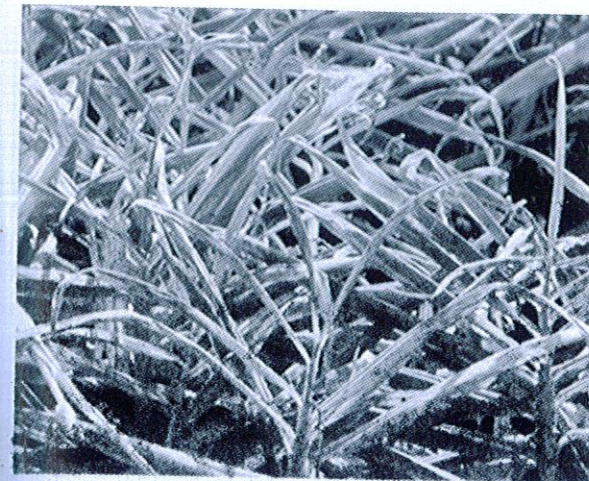


Fig. 3. Brown coalescing necrotic lesions along the margins of leaves

described the symptomatology of *Colletotrichum* leaf spot in South-East Asia as, inward spreading brown spots, which later turns ellipsoidal/ spindle shaped speckles with halo. The affected leaves eventually turn brown and results in dry rot. While, according to Sundararaman (1922), *Colletotrichum* leaf spot in India was characterized with small round to oval, light yellow spots on leaves and leaf sheaths, which gradually increase in size and coalesce to form large discoloured areas. The infected areas often dry up at the center, forming shot holes. However, the symptoms observed in the present investigation were in agreement with the earlier reports, except to that of the symptoms induced by the isolate KAG 6.

#### Morphological characterization

*C. gloeosporioides* exhibits extensive cultural and morphological diversity and have overlapping host ranges, often being associated with at least

470 different genera, which has made the identification process extremely difficult (Hyde *et al.* 2009). Traditional identification and characterization of *Colletotrichum* species has relied predominantly on differences in morphological features like, colony colour, size and shape of conidia and appressoria, optimal temperature for growth, growth rate, presence/absence of setae and existence of the teleomorphic phase, *Glomerella* (Smith & Black 1990; Gunnell & Gubler 1992; Photita *et al.* 2005). Diversity analysis of the isolates collected from varied agro-ecological zones encompassing Kodagu, Hassan, Shimoga and Chikmagalur districts of Karnataka and Idukki and Wayanad districts of Kerala revealed the existence of considerable variability among field populations of the pathogen (Table 2). All the nine isolates of *Colletotrichum* sp. were isolated from lesions of the infected leaf samples and subsequently identified as *C. gloeosporioides* based on macro and micro-morphological characters. Among the Karnataka isolates, KAG 1, KAG 4, KAG 5 and KAG 6, produced grey coloured colony with grey, black or orange colours at the bottom (Fig. 4). Whereas, the isolates representing Kerala *i.e.*, KLG 1-3 had white to whitish-grey colonies with cream or black coloured bottom. The colonies of the isolates from different locations were puffy in appearance and the margins were circular. Prihastuti *et al.* (2009) described the colony characters of *C. gloeosporioides* infecting coffee in Northern Thailand, as grey, becoming dark grey to black, with black circular zones in reverse, which attained 83 mm diameter after incubating at 28°C for seven days. Two different kinds of conidial ooze were noticed in the cultures of the isolates *viz.*, orange coloured,

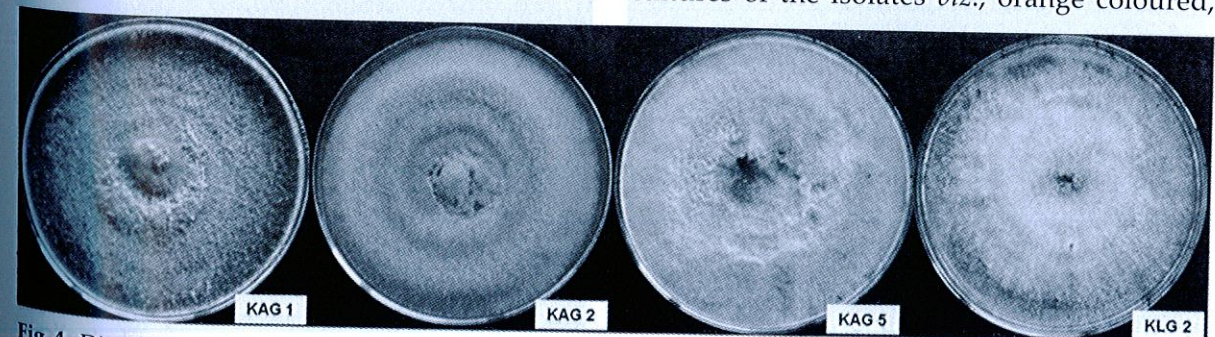


Fig. 4. Diversity in colony characters of *C. gloeosporioides* isolates

**Table 2.** Radial growth, colony, conidial and appressorial characteristics of *C. gloeosporioides* isolates

Isolate code	Diameter of the colony (mm)	Colony characteristics		Conidia		Appressoria	
		Top	Reverse	Shape	Dimension ( $\mu\text{m}$ )	No. of lobes	Dimension ( $\mu\text{m}$ )
KLG 1	8.5	Whitish grey	Black	CT	15.4 × 6.9	SL	9.0 × 6.2
KLG 2	8.5	White	Black	E	12.2 × 6.1	SL	10.8 × 6.5
KLG 3	7.7	White	Cream	CT	18.3 × 6.1	1-2 L	14.2 × 11.0
KAG 1	7.5	Grey	Black	C	14.4 × 6.8	SL	11.3 × 6.7
KAG 2	9.0	Greyish white	Black	D	16.3 × 6.7	UL	13.9 × 6.8
KAG 3	7.7	Greyish white	Black	C	13.6 × 6.8	UL	13.8 × 6.5
KAG 4	7.3	Grey	Orange	CT	13.9 × 6.8	UL	14.8 × 7.4
KAG 5	8.8	Grey	Black	CT	14.6 × 6.7	UL	14.1 × 6.5
KAG 6	9.0	Grey	Grey	E	14.1 × 6.8	UL	13.8 × 7.4

E=Elliptical; D=Dumbbell; C=Cylindrical; CT=Cylindrical with tapering ends; L=Lobed; SL=Single Lobe; UL=Unlobed

which was mostly concentrated at center of the colony (Fig. 5) and lemonish - yellow, which was distributed unevenly on the colony surface (Fig. 6).

Conidial morpho-metrics also revealed considerable variation among the isolates. The conidial shapes varied with regions and were cylindrical with tapering ends, cylindrical, elliptical or dumbbell (Fig. 7). Conidial morphology of most of the isolates was cylindrical with tapering ends and the dimensions were in the range of 12.2-18.3 (L) ×

6.1-6.9 (B)  $\mu\text{m}$ . The conidial size of the fungus was described as 12.0-22.0 × 4.0-6.0  $\mu\text{m}$  and 9.0-24.0 × 3.0-6.0  $\mu\text{m}$  by Cai *et al.* (2009) and Mordue (1971), respectively. Photita *et al.* (2005) analyzed morphological diversity among three groups of phylogenetically distinct *C. gloeosporioides* isolated from *Musa acuminata*, *Euphorium thymifolia*, *Alpinia malaccensis* (a member of *Zingiberaceae*), *Draceana sanderiana* and *Mangifera indica* and found that the conidial dimensions were in the range of 12-24 × 3-10  $\mu\text{m}$ . The conidial shape of the grape dry rot

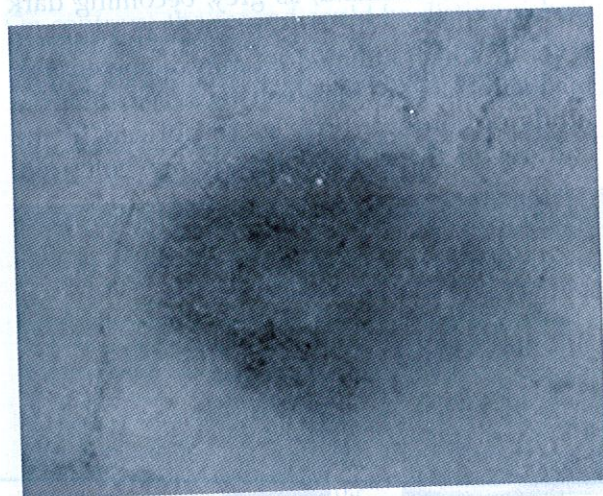


Fig. 5. Conidial ooze with orange colour

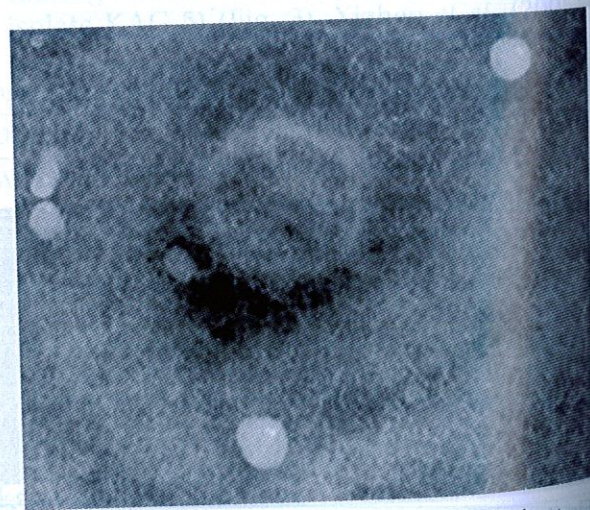


Fig. 6. Conidial ooze with lemonish-yellow colour

Fig. 7. Conidial morphology of *C. gloeosporioides* isolates

pathogen, *C. gloeosporioides*, was found to be cylindrical and dimensions were reported to be in the range of 9.5-29.8 × 3.6-9.1  $\mu\text{m}$  (Hong *et al.* 2008). The conidia of *C. gloeosporioides*, associated with coffee berries in Northern Thailand were cylindrical with obtuse end and slightly tapered, occasionally slightly rounded ends to oblong and having dimensions of 8-11 × 3-4.5  $\mu\text{m}$  (Prihastuti *et al.* 2009). It is evident from the observations of present investigations that, the ginger isolates of *C. gloeosporioides* have more similarity with respect to conidial size as described by Cai *et al.* (2009).

Appressorial shape and size are important characters employed for identifying different species of *Colletotrichum* (Crouch *et al.* 2009) and conidial appressoria are easier to characterize compared with mycelial appressoria as suggested by Cai *et al.* (2009). Both shape and dimension of appressoria have been used as criteria for taxonomically delineating the genus *Colletotrichum*. In the present study, appressoria of the isolates were variable with respect to the number of lobes (unlobed to 1 or 2 lobes) (Fig. 8) and the size varied between 9.0-14.8 × 6.2-11.0  $\mu\text{m}$ . The appressoria of *C. gloeosporioides*, the incitant of grape dry rot had the dimensions of 6.7-16.9 × 4.2-11.2  $\mu\text{m}$  (Hong *et al.* 2008). The appressoria produced by *C. gloeosporioides*, implicated as a pathogen of coffee berry disease,

is characterized by circular to slightly irregular, pale to medium brown, with a size of 4.5-10 × 4-7.5  $\mu\text{m}$  (Prihastuti *et al.* 2009).

Earlier reports from India indicated that, the causal agent of ginger leaf spot as *C. zingiberi* (Butler & Bisby 1931). Further, Xizhen *et al.* (2005) described *C. gloeosporioides* and *C. capsici* as the incitants of ginger leaf spot in South-East Asian countries. However, in the present study, identity of the *Colletotrichum* isolates that were found to be consistently associated with leaf spot, representing different agro-ecological regions was identified as *C. gloeosporioides*, by employing morphological parameters and pathogenicity tests.

#### Pathogenicity

Inoculation of *C. gloeosporioides* isolates on the ginger variety Rio-de-Janerio under glass house conditions showed that, five isolates (KAG 1, KAG 3, KAG 6, KLG 2 and KLG 3) induced brownish necrotic lesion with a distinct yellow halo, while brown coloured lesions were observed with other isolates (KLG 1, KAG 2, KAG 4 and KAG 5). The size of the lesions ranged from 2-5 cm and four isolates *viz.*, KLG 2, KLG 3, KAG 3 and KAG 6 developed symptoms five days after inoculation.

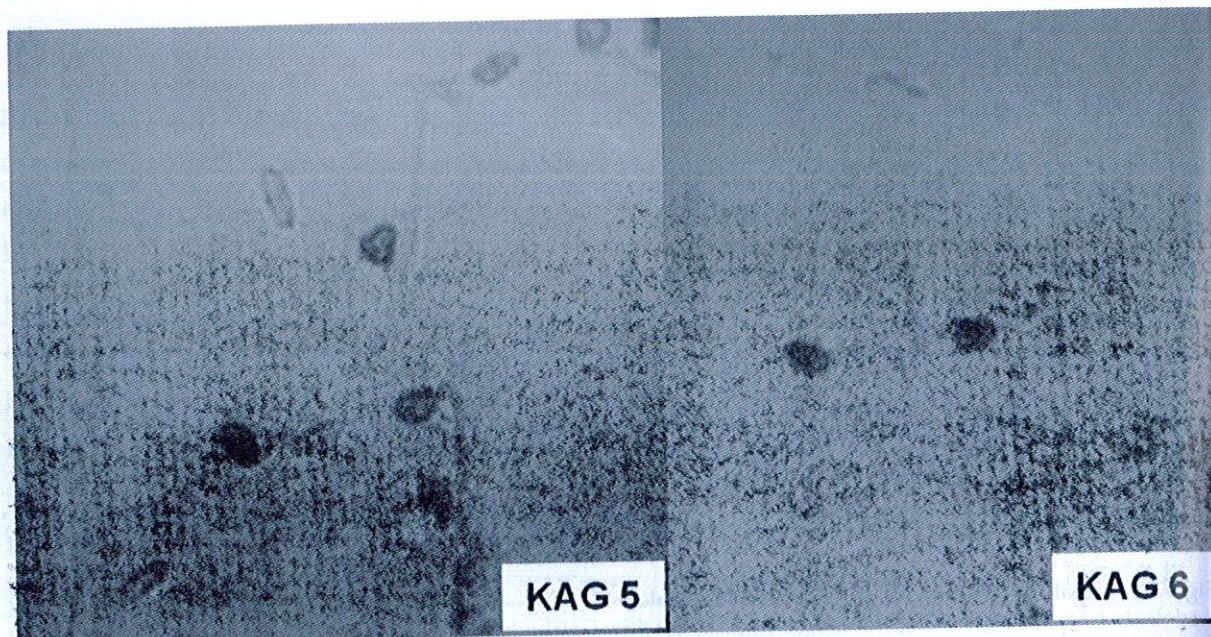


Fig. 8. Appressorial morphology of *C. gloeosporioides* isolates

#### Mycelial compatibility and hyphal interaction

The ability of cells to fuse is governed by complex genetic systems in fungi especially in Ascomycetes. Mycelial/vegetative incompatibility has been studied extensively in a number of plant pathogenic fungi (Leslie 1993). Strong correlations between origin of isolates, host specificity as well as cultivar specificity (pathotypes/races) with vegetative compatibility have been extensively investigated and proven in several patho-systems, exemplified with asexual- sexual morphs of Ascomycetous fungi such as *Fusarium*, *Colletotrichum* and *Sclerotinia* (Anagnostaki 1987; Correl *et al.* 1988; Ahn *et al.* 2003). In filamentous fungi, mycelial compatibility/ incompatibility is considered as a self/ non-self - recognition system controlled by multiple alleles. The mycelial compatible isolates are considered as clones of a single parental strain and possess a particular set of VCG alleles. While, isolates that are incompatible are assumed not to be clones and might have developed pathogenic capabilities independently (Leslie 1993). Reproduction in many *Colletotrichum* populations associated with a broad spectrum of crops is chiefly or exclusively mediated through vegetative means, the only mode of exchanging genetic material

between two genetically distinct individuals, facilitated by anastomosis and heterokaryosis (Wharton & Dieguez 2004). Mycelial compatibility is a mechanism by which genetic diversity is generated and considered as a valuable tool to measure population diversity among the field populations in different species of *Colletotrichum* (Cai *et al.* 2009). The compatible isolates may potentially share a common gene pool and are generally isolated from other isolates by the mechanism of incompatibility (Irani *et al.* 2011). Mycelial compatibility test is a phenotypic, macroscopic assay to determine self or non-self recognition system controlled by multiple loci that are common in fungi (Li *et al.* 2008).

Mycelial compatibility studies with the nine test isolates revealed that the isolate KLG 3 originating from Idukki, Kerala was most compatible, resulting in the formation of a confluent colony (Figs. 9a & 9b) with all other isolates tested, followed by KAG 2 and KAG 5. While KLG 1 and KAG 6 exhibited a highly incompatible reaction with other isolates, as indicated by the formation of a barrage zone between the confronting isolates (Figs. 10a & 10b) (Table 3). In the present study, mycelial compatibility groups representing different

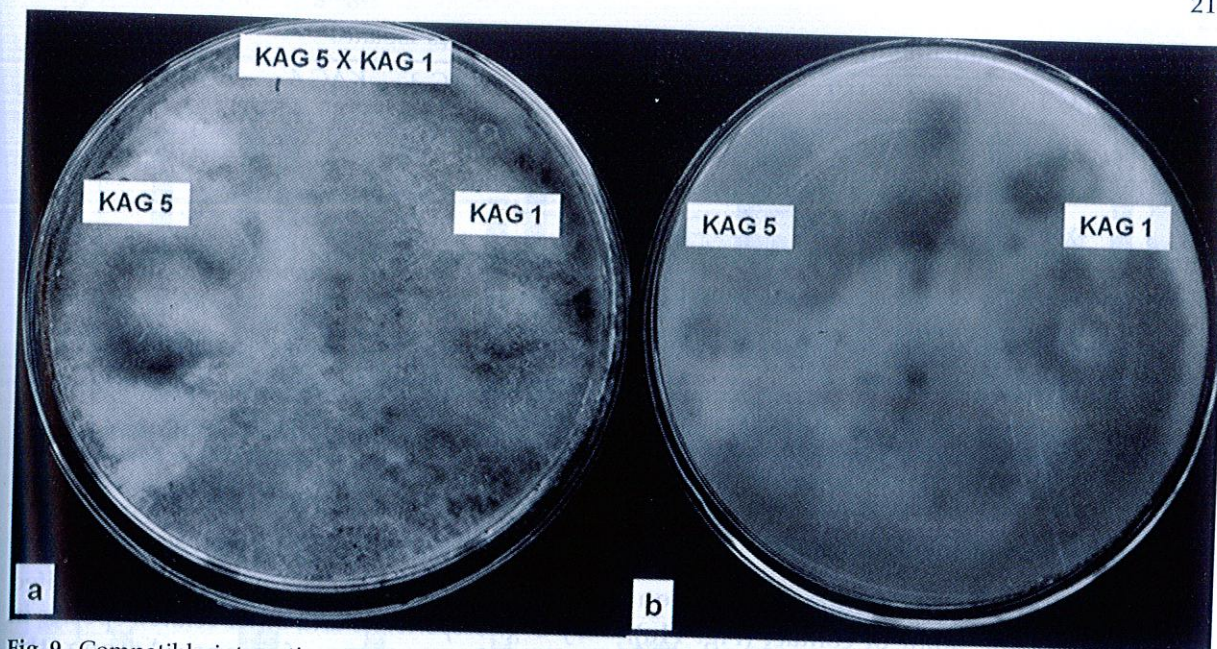


Fig. 9. Compatible interaction among *C. gloeosporioides* isolates (a) Top (b) Reverse

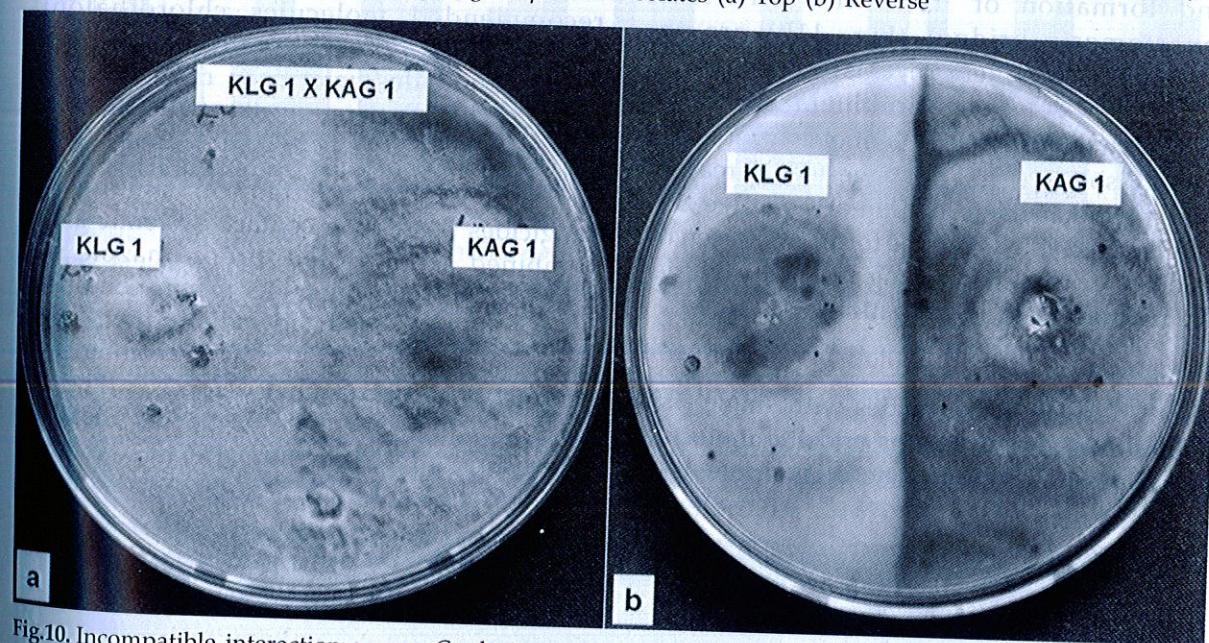


Fig. 10. Incompatible interaction among *C. gloeosporioides* isolates (a) Top (b) Reverse

clades of isolates representing the regions surveyed could not be elucidated. This was primarily due to the out-crossing of KLG 3, KAG 2 and KAG 5 with other isolates, which was trans-regional and non-dependent on location specific origin of the isolates. It is inferred that these isolates perhaps would have acted as "bridge" isolate, which was capable of carrying out anastomoses with other isolates

originating from different agro-ecological and diverse climatic regions.

Microscopical examinations on mycelial compatibility or cross-mating studies provide much insight into the sequential events associated with hyphal anastomoses and the consequential developments leading to the formation of heterokaryons. Hyphal homing (trophic movement of hyphae of confronting

**Table 3.** Mycelial compatibility interaction among *C. gloeosporioides* isolates

	KLG 1	KLG 2	KLG 3	KAG 1	KAG 2	KAG 3	KAG 4	KAG 5	KAG 6
KLG 1	C	X	X	X	C	X	C	X	X
KLG 2	X	C	C	X	C	C	C	C	X
KLG 3	X	C	C	C	C	C	C	C	C
KAG 1	X	X	C	C	C	X	C	C	C
KAG 2	C	C	C	C	C	C	X	C	X
KAG 3	X	C	C	X	C	C	X	C	X
KAG 4	C	C	C	C	X	X	C	X	X
KAG 5	X	C	C	C	C	C	X	C	C
KAG 6	X	X	C	C	X	X	X	C	C

C=Compatible interaction; X=Incompatible interaction

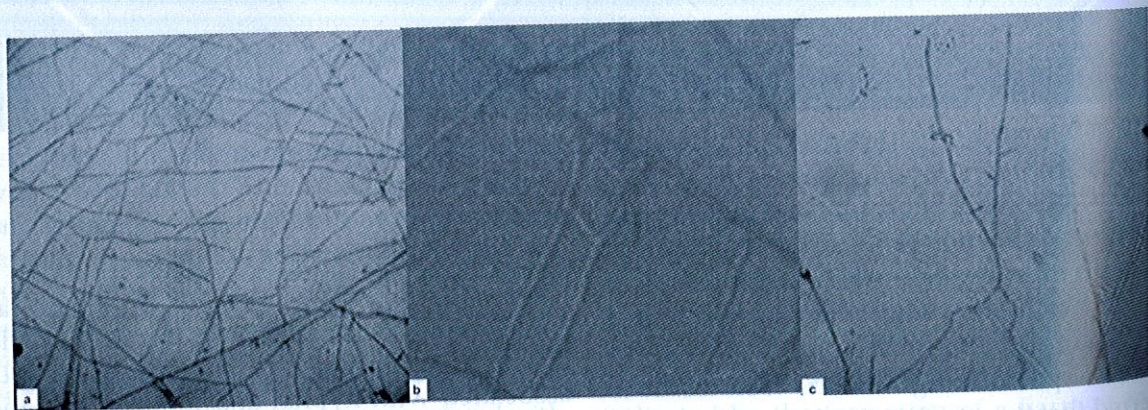
isolates towards each other), subsequent aggregation at the point of contact (Fig. 11a), and formation of 'H' - like structures (anastomosis bridge) (Fig. 11b) are the commonly encountered events in a compatible interaction. Subsequent proliferation of new hyphal strands from the point of contact of two distinct isolates (Fig. 11c) suggested the reaction as compatible. The formation of a barrage zone (demarcated as a dark zone formed between the colonies of two confronting isolates) signified the reaction to be incompatible.

#### Fungicidal sensitivity

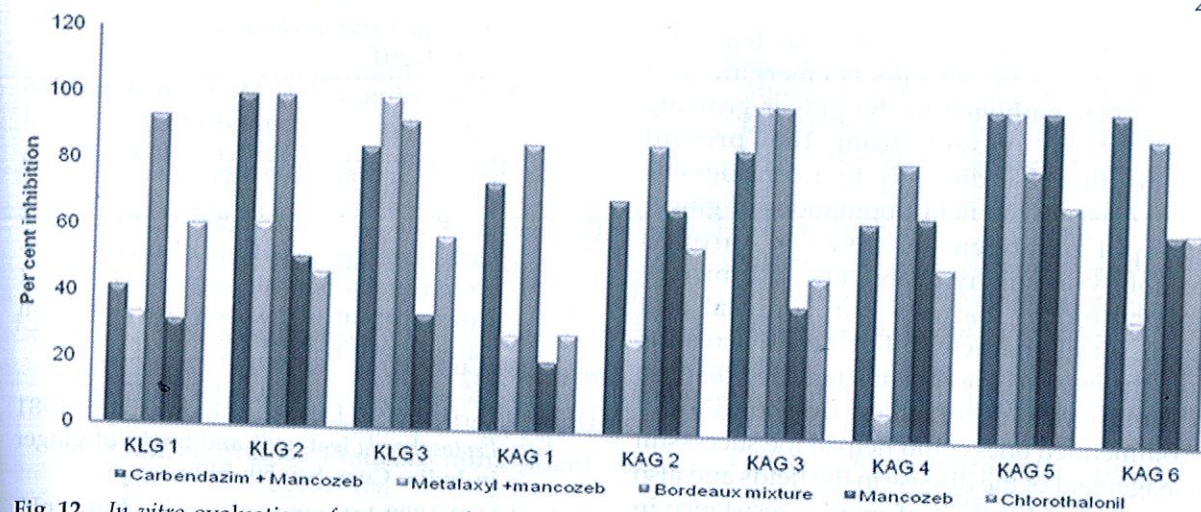
A broad range of fungicides with single - site or multiple site of actions and the molecules that possess systemic activity have been

extensively used to manage the diseases incited by *C. gloeosporioides* and other related species in a broad spectrum of crops. Among the recommended molecules chlorothalonil, benzimidazoles, triazoles, carbamates, copper based fungicides have been reported to be effective under *in vitro* as well as field conditions (Dillard 1988; La Mondia 2001; Kumar 2007).

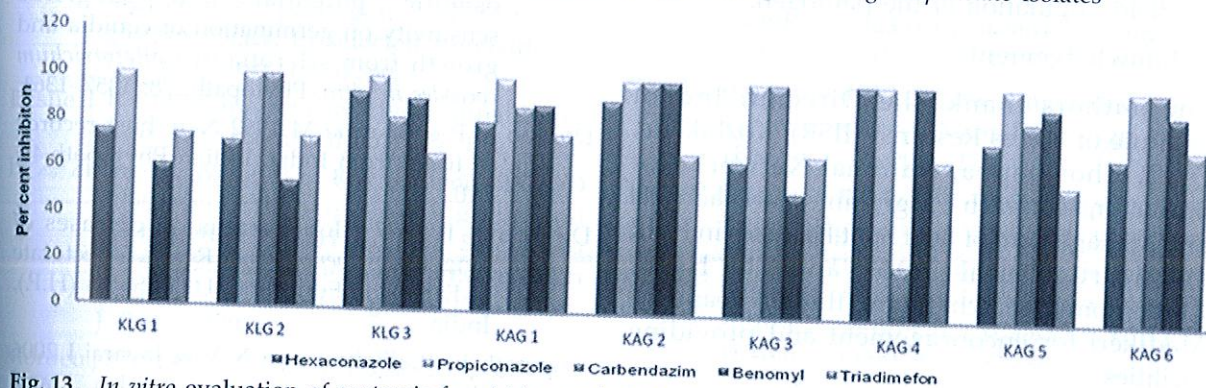
In the present investigation, among the fungicides possessing contact, systemic and a combined action tested, propiconazole was found to be promising under *in vitro* conditions as it completely inhibited radial growth of all the ginger isolates followed by the combination product of carbendazim + mancozeb (Figs. 12 & 13). Earlier, Iyer (1987) reported that two sprays of Bordeaux mixture (1%) at an interval



**Fig. 11.** Microscopic events in compatible interaction between *C. gloeosporioides* isolates (a) Aggregation of hyphae at the point of contact (b) Formation of 'H' - like structure (c) Emergence and growth of new hyphae



**Fig. 12.** *In vitro* evaluation of contact and combination fungicides against *C. gloeosporioides* isolates



**Fig. 13.** *In vitro* evaluation of systemic fungicides against *C. gloeosporioides* isolates

of six weeks effectively managed the disease under field conditions.

Dillard (1988) observed addition of copper hydroxide to either chlorothalonil or captafol significantly reduced radial colony growth originating from the sclerotial structures of *C. coccodes*. Diniconazole, flusilazol, benomyl, propiconazole, nuarimol, fenarimol, bitertanol, PCNB, mancozeb, maneb, myclobutanil, thiram, DCNA, iprodione, triforine, chlorothalonil and triadimefon were found effective against *C. fragariae*, under *in vitro* conditions. However, in general the systemic fungicides (particularly the ergosterol biosynthesis inhibitors) reduced growth of *C. fragariae* much more than the conventional fungicides tested (Smith & Black 1993). Triazole fungicides *viz.*, propiconazole and difenoconazole were found effective in managing the chilli anthracnose under

greenhouse and field conditions (Gopinath *et al.* 2006).

*In vitro* studies on sensitivity of *C. lindemuthianum*, the bean anthracnose pathogen revealed that, 16 isolates showed a high sensitivity to trifloxystrobin, while 19 isolates showed high sensitivity to propiconazole and to trifloxystrobin + propiconazole. The results revealed seven isolates with low sensitivity to carbendazim and thiophanate-methyl thus suggesting the existence of cross-resistance in the field isolates. The *in vitro* sensitivity of five isolates of *C. lindemuthianum* to benomyl, carbendazim, thiophanate-methyl and chlorothalonil observed a low sensitivity of isolates to fungicides of the group of benzimidazoles (benomyl, carbendazim and thiophanate-methyl) although those isolates were found to be sensitive to chlorothalonil (Sartori & Maringoni 2008).

The present study indicated that leaf spot caused by *C. gloeosporioides* is emerging as a major foliar pathogen in the ginger growing areas. It is evident from the present investigation that diversity to a considerable extent exists in the field populations of ginger leaf spot pathogen, as revealed through variability in colony characters and micro-morphological features like conidial and appressorial characters. Further, the results from the *in vitro* experiment suggested that use of fungicides other than the commonly recommended ones could help in the successful management of the disease in the fields and also prevents development of cross-resistance in the field population of the pathogen.

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