Effect of nutritional and environmental conditions on growth of *Colletotrichum gloeosporioides* causing anthracnose of anthurium

ABSTRACT

Five isolates of *Colletotrichum gloeosporioides* causing anthracnose disease of anthurium were collected from five different anthurium growing areas of Tamil Nadu and Kerala and their pathogenicity was established. Effect of different varying pH level, light intensity, media and carbon and nitrogen sources were tested against the growth of *C. gloeosporioides* under *in vitro* conditions. The results of experiment experiment results indicated that the growth of *C. gloeosporioides* was maximum in pH range of 6.0-6.5. The exposure of the fungus to alternate cycles of 12 hour 12-hour light and 12 hour 12-hour darkness resulted in the maximum mycelial growth of *C. gloeosporioides* compared to continuous light and darkness. Among the different media tested, potato dextrose agar medium increased the growth of mycelium followed by oatmeal agar. The maximum growth of *C. gloeosporioides* was observed in mannitol followed by starch and maltose as carbon source. Among the nitrogen sources, potassium nitrate was found to promote more mycelial growth in *C. gloeosporioides*.

Keywords: *Colletotrichum gloeosporioides*, mycelial growth, media

INTRODUCTION

Anthurium or spadix rot incited by *C. gloeosporioides* is the most serious disease widely distributed in all anthurium raising regions of the the world and is a major constraint on the expansion of export trade of anthurium. The crop is being debilitated by an array of opportunistic pathogens. Among all *C. gloeosporioides* which causes anthracnose is a major limiting factor in

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Comment [R2]: Please reference this as it is a claim. There is a 2019 paper by Rex et al looking at anthurium losses in TN

production leading to heavy losses in terms of quality and quantity (Naseema *et al.*, 1997). Understanding of the role of environmental conditions and its effect on infection and survival of the pathogen is necessary to develop cultural disease management practices. T~~Therefore t~~he objectives of this study included isolation, purification and identification of pathogenic fungus causing anthracnose disease of anthurium and ~~determine~~ determiningnutritional requirement and optimal conditions for ~~the~~ mycelial growth.

Comment [R3]: I would suggest a newer reference point here as this is quite dated.

MATERIALS AND METHODS

Collection of samples

Survey was conducted during 2010-2011 at five locations in Tamil Nadu and two places at Kerala to assess the intensity of anthracnose in anthurium. Disease severity was recorded in 0-9 scale (Anonymous, 1980) and per cent disease index (PDI) was calculated. Infected samples of leaves were collected from these areas.

Comment [R4]: Perhaps this paragraph could be worded better for understanding by non-native English speakers

Isolation and identification of pathogens

The pathogens causing anthracnose in anthurium were isolated from the collected samples by tissue segment method and they were purified by single spore isolation and maintained on potato dextrose agar (PDA). The causal organisms were identified based on spore morphology.

Comment [R5]: A reference here would be ideal such as:
Hallmann, J., Berg, G., & Schulz, B. (2006). Isolation procedures for endophytic microorganisms. In *Microbial root endophytes* (pp. 299-319). Berlin, Heidelberg: Springer Berlin Heidelberg.

Pathogenicity test

a. **Preparation of spore suspension**

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The pathogenicity of purified cultures of *C. gloeosporioides* was confirmed by Koch's Postulates. Sporulating cultures of *C. gloeosporioides*were inoculated on PDA and incubated at room temperature (28 ± 2°C) to attain full growth. After incubation, the dishes were flooded with 10 ml of distilled water and the spores were collected using a small brush. The spore suspension was filtered through a six-layer-sterile cheese cloth to remove mycelial debris. Using a haemocytometer spore concentration of *C. gloeosporioides*was adjusted to 5×10^5 spores ml^{-1}respectively with distilled water.

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b. Inoculation

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Single leaf inoculation technique was followed and the spore suspension was sprayed using a syringe until run-off on to the leaves of anthurium raised in glasshouse. Such single leaves were covered with polythene bags and symptom expression was observed regularly. Proper controls were also maintained.

Effect of pH on the growth of *C. gloeosporioides*

Sterilized PDA medium was distributed in 250 ml Erlenmeyer flasks @100 ml per flask and the pHof the medium was adjusted to p^H levels *viz*., 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0 and 8.5 with 0.1N HCl or 0.1N NaOH and autoclaved at 1.4 kg cm^{-2} for 20 min. Fifteen ml of the medium from each p^H level was poured onto sterilized Petri dishes and allowed to solidify. The pathogen was inoculated as mentioned in 3.7. and the mycelial growth was measured after 9 days of inoculation of *C. gloeosporioides*.

Effect of light intensity on the growth of *C. gloeosporioides*

The effect of light on the growth of the pathogens was studied by exposing them on PDA to alternate cycles of 12 h light, 12 h dark, continuous light and continuous darkness in an environment chamber maintained at 30°C. ~~Mycelial~~ A mycelialdisc of 9-mm was used to inoculate Petri plates. Three replications were maintained for each treatment. Inoculated plates were kept in environmental chamber and light intensity was adjusted to the required level. The mycelial growth was recorded 10 days after inoculation.

Standardization of culture media for the rapid growth of *C. gloeosporioides*

Cultures of *C. gloeosporioides*were grown on PDA, oatmeal agar, carrot dextrose agar, beetroot dextrose agar, host leaf extract agar, Czapek's Dox agar, Richard's agar, starch agar and Martin's rose bengal agar to standardize the one which supported rapid growth of the pathogen. Sterilized warm medium was poured @15 ml in sterile Petri dishes and the medium was allowed to solidify. The pathogen was inoculated at the centre of the plate by placing a 9-day-old ~~(*C. gloeosporioides*),~~ 9-mm culture disc of the fungus. The plates were incubated at room temperature (28±2ºC) and three replications were maintained for each treatment. The radial growth was measured after 9 days of inoculation of *C. gloeosporioides*.

Growth of *C. gloeosporioides* on different liquid media

Liquid broths *viz.*, potato dextrose, oatmeal, carrot dextrose, beetroot dextrose, host leaf extract, Czapek's Dox, Richard's, starch and Martin's rose bengal were prepared. From the prepared medium 100 ml was distributed in 250 ml Erlenmeyer flasks and autoclaved at 1.4 kg cm^{-2} for 20 min and cooled. The flasks were separately inoculated with 9-day-old (*C. gloeosporioides*), 9-mm culture disc of the pathogen. After the incubation period, the mycelial

mat was filtered through pre weighed Whatman No.1 filter paper, dried in hot air oven at 100º C until constant weight was obtained.

Effect of carbon, nitrogen sources on the growth of *C. gloeosporioides*

Richard's agar medium as well as broth was substituted with different carbon sources *viz.*, starch, mannitol, fructose, glucose, carboxy methyl cellulose (CMC), sucrose, maltose and nitrogen sources such as ammonium nitrate, ammonium molybdate, ammonium oxalate, peptone, potassium nitrate, sodium nitrate, urea and sterilized. The medium without carbon and nitrogen source served as control. The colony diameter was measured after 9 days of inoculation of *C. gloeosporioides*.

RESULTS AND DISCUSSION

Occurrence of anthracnose

A survey was conducted during 2010-2011 to assess the severity of anthracnose in different anthurium growing areas of Tamil Nadu and Kerala. The severity of anthracnose as per cent disease index (PDI) ranged from 12.69 to 44.44 (Table 1). The causal organism isolated was identified as *Colletotrichum gloeosporioides*(Penz) Sacc. based on colony characters and spore morphology.

Pathogenicity test

The pathogenicity of *C. gloeosporioides*was confirmed by artificial inoculation. The results indicated that the isolated pathogens reproduced the typical anthracnose symptoms in

anthurium. The pathogens were reisolated from infected tissues and Koch's postulates were fulfilled.

Hydrogen ion concentration and the growth of *C. gloeosporioides*

In our investigation,the ideal pH observed for culturing *C. gloeosporioides* was 7.0 with a mean mycelial growth of 8.90 cm followed by pH 6.5 (8.60 cm) and 6.0 (8.45 cm). The growth was very slow at pH 4.0 with a mean colony diameter of 3.90 cm (Fig 1). The observation is in agreement with the results of Nandinidevi (2008) and Manjunath (2009) who also had observed the maximum growth of the fungus at pH 7.0 followed by pH 6.0 which was preferred for sporulation. Gina (1999) identified pH 6.0 as optimum for the growth of *C. gloeosporioides*.

Light intensity and the growth of *C. gloeosporioides*

Diurnal light when compared to continuous light and darkness was conducive for the growth of *C. gloeosporioides*. Exposure of the *C. gloeosporioides*to alternate cycles of 12 h light and 12 h darkness for 10 days yielded maximum mycelial growth and dry weight of the pathogen (8.80 cm and 448 mg). The mycelial growth and dry weight of the fungus when exposed to continuous light was 7.45 cm and 410 mg respectively. Continuous darkness recorded 6.50 cm growth and 338 mg dry mycelial weight respectively (Fig 2). Similarly Sudhakar (2005), Ashoka (2005), Narendra kumar (2006) and Tasiwaland Benagi(2009) observed that exposure of *C. gloeosporioides* to alternate cycles of 12 h light and 12 h darkness yielded maximum biomass and spores.

Culture media for the growth of *C. gloeosporioides*

Every living being requires food for its growth and reproduction and fungi are not an exception to it (Kiryu, 1939). Fungi secure food and energy from the substrate upon which they live in nature. In order to culture fungus in the laboratory, it is necessary to furnish essential elements and compounds in the medium for their growth and other life processes. All media are not equally good for all fungi, nor ~~there can a universal substrates or artificial media upon which all fungi grow well~~is there a universal substrate for fungi culturing. ~~So~~Therefore, different media including both synthetic and non-synthetic media were tried for *C. gloeosporioides*in the present investigation.

Among the nine different media tested, PDA significantly supported the maximum growth of *C. gloeosporioides*with a mean mycelial growth of 9.00 cm which was on par with oatmeal agar wherein the fungal growth was 8.90 cm (Table 2). Lowest mycelial growth was recorded in Martin's rose Bengal agar (4.60 cm). Nine different liquid media were used for culturing *C. gloeosporioides*(Fig 3). Among them oatmeal broth significantly was the best by recording maximum dry mycelial weight of the fungus (394 mg). These results are in conformity with the findings of Naseema *et al.* (1997) who recorded the maximum growth of *C. gloeosporioides* of anthuriumon PDA. Similarly, Anand and Bhaskaran (2009) also observed that the growth of *C. gloeosporioides*from chillies was more on PDA. Tasiwal and Benagi (2009) recorded the maximum mycelial weight of the fungus in Richard's broth.Among the seven media tested for the growth of *C. gloeosporioides*,hostleaf extractrecorded the maximum mean colony diameter followed by PDA and corn meal agar (Nandinidevi, 2008). Manjunath *et al.* (2011) observed that host leaf extract agar followed by PDA supported the maximum growth of *C. gloeosporioides*that causes anthracnose of noni.

Carbon sources and the growth of *C. gloeosporioides*

Carbon occupies a unique position among the essential elements required by the living organisms. Carbon utilization is speculated to be dependent upon enzyme system. The utilization of various carbon compounds may depend either on the activity of the fungus to utilize certain simple forms or on its power to convert the complex carbon compounds into simple forms which may be utilized. As a component of both structural and functional constituents carbon comprises about 50% of the total dry mycelia weight in fungi (Bilgrami and Verma, 1978) Monosaccharides are the better sources for the growth and sporulation of the pathogen. The fungus *C. gloeosporioides*varied in its ability to utilize different carbon sources. *C. gloeosporioides*grew well in all the carbon sources tested (Table 3). Mannitol ranked first in promoting the mycelial growth of the pathogen with a mean growth of 8.90 cm. It was followed by starch (8.80 cm), maltose (8.70 cm) and fructose (8.50 cm) that were at par with mannitol. Our results are in agreement with the observations of Reddy (2000), Manjunatha and Rawal (2002) and Sangeetha and Rawal (2008). Chaturvedi (1966) also noticed that starch and fructose induced more growth and sporulation in *C. gloeosporioides*than other carbon sources. Sucrose was found to be a better carbon source followed by glucose and dextrose for the growth of betel vine anthracnose pathogen C. *gloeosporioides*(Naik *et al.,* 1988).Hegde *et al*. (1990) observed dextrose and sucrose as good carbon sources for *C. gloeosporioides*isolated from arecanut.Enhanced mycelial growth and sporulation of bean anthracnose pathogen *C. gloeosporioides*was recorded in starch added medium while poor sporulation was recorded in galactose and lactose (Deshmukh *et al*., 2012).

Nitrogen sources and the growth of *C. gloeosporioides*

The fungi for their structural and functional processes use nitrogen, like carbon. Nitrogen is very important element in the protein synthesis. But all the sources of nitrogen are not equally good for the growth of all fungi (Lilly and Barnett, 1951). Purkayastha and Sengupta (1975) found that peptone, casaminoacid and potassium nitrate were favourable for both mycelial growth and sporulation of *C. gloeosporioides*the incitant of jute anthracnose. *C. gloeosporioides*of mango utilized potassium nitrate more efficiently while ammonium nitrate was used less effectively for its growth and sporulation (Ekbote, 1994). In the present investigation, p~~P~~otassium nitrate was found to promote more mycelial growth in *C. gloeosporioides* with a mean colony diameter of 8.70 cm andleast growth was observed in urea (4.20 cm). Saxena (2002) reported potassium nitrate as the best source for growth and sporulation of *C. gloeosporioides*isolated from ~~betelvine~~betel vine and pomegranate while Deshmukh et al., (2012) noted maximal growth and sporulation within similar media for anthracnose caused in bean. In contrast, Sangeetha and Rawal (2012) had observed ammonium nitrate to support good growth and sporulation of the fungus from mango.~~Deshmukh *et al.* (2012) observed that maximum growth and sporulation of *C. gloeosporioides* causing anthracnose of bean was recorded in potassium nitrate.~~

The results of present study are summarized that the *Colletotrichum gloeosporioides*(Penz) Sacc. was confirmed as the causal agents of anthracnose of anthurium based on colony characters, spore morphology and pathogenicity. *C. gloeosporioides* were found to grow well in pH ranging from 6.0 to 7.0. Exposure of *C. gloeosporioides* to alternate cycles of 12 h light and 12 h darkness yielded maximum mycelial growth and mycelial dry weight of the pathogens. Among the culture media tested, Potato dextrose agar and oat meal broth were found

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to promote maximum growth and dry weight of *C. gloeosporioides.* mannitol as carbon sources and potassium nitrate as sources of nitrogen were found to be equally effective for the growth of *C. gloeosporioides.*

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Table 1. Occurrence of anthracnose/spadix rot in anthurium growing areas

Sl. No.	Isolate code	Location	Variety	Disease severity (PDI)	Symptoms
1	CI_1	Shade net house, TNAU	Lady jane	44.44	Dark brown margin with grey brown centre on leaves
2	CI_2	Wyanad	Temptation	25.39	Spadix rot
3	CI_3	Munnar	Sweet	15.87	Small to irregular brown spots

			orange		on leaves
4	CI_4	Yercaud	Temptation	20.63	Spadix rot
5	CI_5	Thadiyankudisai	Lady jane	12.69	Dark brown margin with grey brown centre on leaves

PDI: Percent disease index

Table 2. Growth of *C. gloeosporioides* on different solid media

Sl. No.	Media	Mycelial growth (cm)
		C. gloeosporioides
		(9 DAI)
1	Potato dextrose agar	9.00
2	Oatmeal agar	8.90
3	Carrot dextrose agar	5.20
4	Beetroot dextrose agar	5.30
5	Host leaf extract agar	7.20
6	Czapek's Dox agar	6.10
7	Richard's agar	7.80
8	Starch agar	6.80
9	Martins rose Bengal agar	4.60
CD (0.05)		1.05

DAI - days after incubation

Table 3. Effect of carbon sources on the growth of *C. gloeosporioides*

Sl.	Carbon source	Mycelial growth (cm)

No.		*C. gloeosporioides* (9 DAI)
1	Starch	8.80
2	Mannitol	8.90
3	Fructose	8.50
4	Glucose	8.30
5	Carboxy methyl cellulose	8.10
6	Sucrose	8.00
7	Maltose	8.70
8	Control	1.20
CD (0.05)		0.59

DAI - days after incubation

Table 4. Effect of nitrogen sources on the growth of *C. gloeosporioides*

Sl. No.	Nitrogen source	Mycelial growth (cm) *C. gloeosporioides* (9 DAI)
1	Ammonium nitrate	6.60
2	Ammonium sulphate	8.10
3	Ammonium oxalate	4.50
4	Peptone	6.40

5	Potassium nitrate	8.70
6	Sodium nitrate	8.20
7	Urea	4.20
8	Control	1.40
CD (0.05)		0.49

DAI - days after incubation

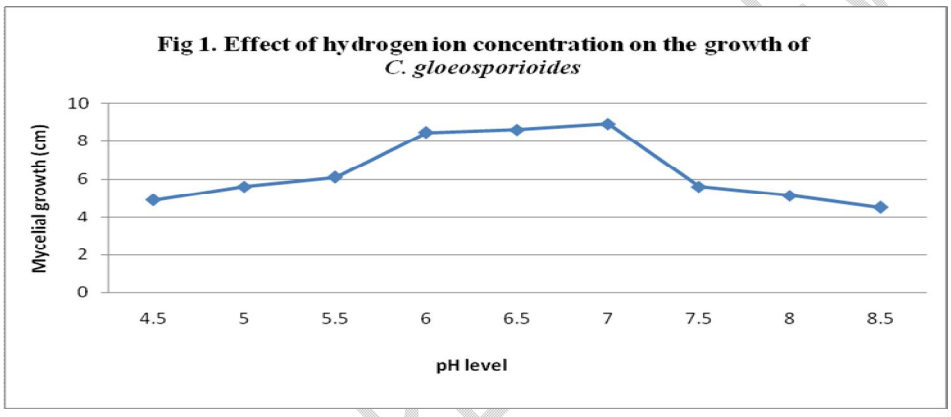

Fig 1. Effect of hydrogen ion concentration on the growth of *C. gloeosporioides*

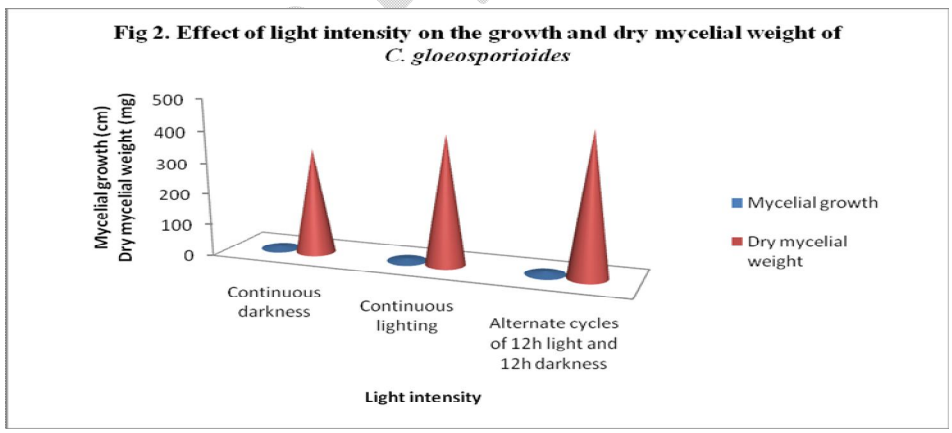

Fig 2. Effect of light intensity on the growth and dry mycelial weight of *C. gloeosporioides*