

**STRAIN VARIATION IN *COLLETOTRICHUM*
GLOEOSPORIOIDES (PENZ.) PENZ. & SACC.**

By

K. J. ALICE

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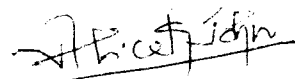
to

My Parents, Husband and Children

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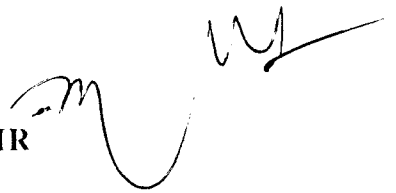
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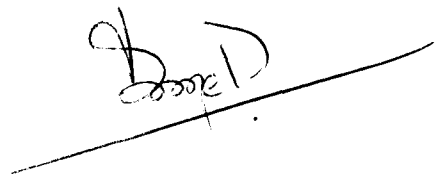
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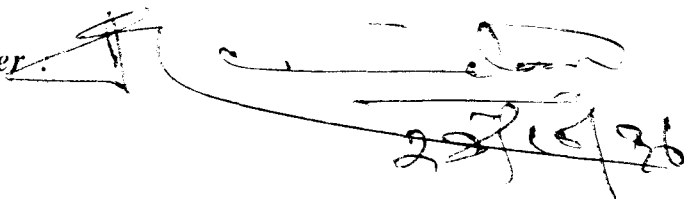
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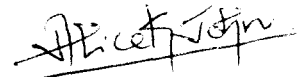
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A handwritten signature in black ink, appearing to read 'K. J. Alice', written over a horizontal line.

K. J. ALICE

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INTRODUCTION

INTRODUCTION

Colletotrichum gloeosporioides (Penz.) Penz. and Sacc. is observed as a major pathogen infecting a number of plants including crop plants, ornamentals and weeds. The pathogen causes diseases of economic importance.

The isolates of *C. gloeosporioides* infecting different hosts varied greatly in pathogenicity, symptom expression, cultural and morphological characters. Variability in colony colour and fruiting bodies was observed by Andes and Keitt (1950), Strobel (1960) and Singh *et al.* (1966). Different isolates of the pathogen showed great variability within the species consisting of different strains as recognised by Shear and Wood (1913) Von Arx (1957), Scharpf (1964), Souch and Corka (1979), Tan (1979) and Karunakaran (1981).

The objective of the present investigation was to study the variability of *C. gloeosporioides* infecting important vegetables and ornamental plants in different locations of Thiruvananthapuram and Thrissur districts of Kerala state. Attempts were therefore made to study the following aspects.

- i. Isolation of the pathogen
- ii. Testing pathogenicity of isolates

- iii. Cultural characters of the isolates viz. growth and sporulation, protein content and enzyme activity
- iv. Influence of environmental conditions on growth and sporulation
- v. Host range and cross infectivity of the isolates
- vi. Production of toxins by the isolates
- vii. Biological grouping of the sixty nine isolates obtained based on cultural characters, growth and sporulation, protein content, enzyme activity, toxin production and symptom expression.

The present investigation may help to identify the different strains of the pathogen infecting important vegetable and ornamental plants. The information gathered from the present study will therefore be useful for breeding resistant varieties and to identify collateral hosts with the ultimate aim of managing the disease.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Colletotrichum gloeosporioides (Penz.) Penz. and Sacc. is the most ubiquitous of all *Colletotrichum* species being universal in occurrence on a wide range of hosts, causing disease symptoms commonly known as anthracnose.

Von Arx (1957) named the species as *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. occurring frequently on anona, apple, avacado, pear, cocoa, capsicum, cherry, citrus (lemon, orange, grape fruit) cotton, mango, papaya, rubber, tea, tomato, yams and many other cultivated and wild plants (Von Arx, 1957; Mordue, 1971). This fungal pathogen was reported on more than 85 species of host plants from India (Bilgrami *et al.* 1979; Edward *et al.* 1972).

Prasad and Singh (1960) found this pathogen as the causal agent for anthracnose of *Dioscorea alata*. Prasad and Varma (1966a) reported this species on *Saraca indica* and Wilson *et al.* (1979) on clove. Nair *et al.* (1978) observed this on nutmeg leaf. Verma (1979) described three species of *Colletotrichum* pathogenic on chillies. Leaf spot of *Dioscorea alata* was caused by *C. gloeosporioides* (Abraham *et al.* 1980).

Karunakaran and Nair (1980a; 1980b; 1980c) reported a new leaf spot and die back disease of *Cinnamomum zeylanicum*, twig blight and flower shedding disease of clove trees and anthracnose of papaya caused by *C. gloeosporioides*. Karunakaran *et al.* (1980) found that the clove pathogen *C. gloeosporioides* survived on the weed *Clerodendron*. Nair *et al.* (1980) recorded leaf blight of *Dioscorea esculenta* caused by *C. gloeosporioides*. Leaf spot disease of zinnia caused by *Colletotrichum* was reported by Srivastava and Gupta (1980).

Catharanthus roseus, *Clitoria ternatea*, *Cosmos bipinnatus*, *Gardenia jasminoides*, *Murraya exotica* and *Quisqualis indica* were found to be hosts of *C. gloeosporioides* (Santhakumari and Nair, 1981). Abraham *et al.* (1982) reported a new leaf spot disease of jungle jack (*Artocarpus hirsuta*) caused by *C. gloeosporioides*. Raju *et al.* (1982) recorded *Heliconia psittacorum* and *Thunbergia grandiflora* as new hosts of *C. gloeosporioides*.

Adikaran *et al.* (1983) observed that *Glomerella cingulata* infected *Capsicum annuum* fruit. *Colletotrichum* caused wide spread leaf spot disease of *Xanthosoma sagittifolium* (Teri and Shaona, 1985). A severe leaf blight was observed by Iqbal Singh and Chohan (1986) on potted plants and commercial plantations of Jasmine due to *Glomerella cingulata*. Occurrence of *C. crassipes* on clove, *Clerodendron infortunatum*, *Croton tiglium* and *Erythrina indica* was reported by Chandra Mohanan and Kaveriappa (1986).

A severe outbreak of anthracnose caused by *C. gloeosporioides* on *Neoregelia carolinae* was observed by Kim (1987). Host range of

three strains of *C. gloeosporioides* from tropical pastures was reported by Vinijs Sanun *et al.* (1987). Fruit rot of lemon caused by *C. gloeosporioides* was studied by Jagdish Babu and Reddy (1988).

Zakaria and Ahamad (1988) illustrated symptoms and disease development of *C. gloeosporioides* on ornamental trees in Malaysia. A severe leaf blight of *Bauhinia malabarica* in a forest nursery in Karnataka caused by *C. gloeosporioides* was reported by Bhat *et al.* (1989). Hegde *et al.* (1989) reported *C. gloeosporioides* on arecanut. Leaf spots on *Dracaena deremensis* and *Cordyline terminalis* were observed by Kagiwata (1989) and leaf spots on *Dieffenbachia amoena* were observed by Mirabolfathy (1989) to be caused by *Colletotrichum*.

Naseema *et al.* (1989) reported *C. gloeosporioides* as the causal organism for leaf spot of *Philodendron melanochrysum*. Red pepper, *Capsicum annum*, was found to be susceptible to *C. gloeosporioides* (Park *et al.* 1989). Stem blight of *Kalanchoe pinnata* caused by *G. cingulata* and *C. dematium* was recorded in Maharashtra state by Rao *et al.* (1989). *C. gloeosporioides* was reported as causal agent of anthracnose of arecanut, banana, betelvine, cardamom, cocoa, coconut, coffee and sapota (Hegde *et al.* 1990).

Circular or irregularly shaped lesions were produced on leaves of *Anthurium scherzerianum* and *A. andraeanum* by *C. gloeosporioides* in Japan (Kagiwata, 1990). *C. gloeosporioides* was reported to cause leaf blight of *Costus malortieanus* and *C. sanguineus* by Naseema and Wilson (1990). Ogle *et al.* (1990) reported infection of *Stylosanthus* by

C. gloeosporioides. *Ipomoea quamodit*, *Pteris longifolia*, *Smilax macrophylla* and *Tephrosia candida* were recorded as hosts of *C. gloeosporioides* from Assam by Ali and Saikia (1991). Anthracnose caused by *C. gloeosporioides* on 24 species of ornamental plants was reported by Kim *et al.* (1991).

In a survey, all 150 cultivars of roses were affected by die back yielding isolates of *C. gloeosporioides* (Pratibha Shukla and Choudhury, 1991) Boruah *et al.* (1992) reported leaf blight of *Dioscorea composita* by *G. cingulata* in N.E. India. Gunnel and Gubler (1992) reported *Colletotrichum* species as pathogenic to strawberry. *Curcuma zedoaria* was reported as a host of *C. gloeosporioides* from Jabalpur (Bhawani Singh, 1993).

Blackening confined to the cuticle and/or outer epidermal cell walls of mature leaves of yams was found to be associated with *C. gloeosporioides* (Winch *et al.* 1993). *G. cingulata*, was reported as the causal agent of anthracnose of *Dieffenbachina picta* by Gally *et al.* (1994).

2.1 Symptomatology / Pathogenicity

Marks *et al.* (1975) found that the penetration pegs successfully penetrated the epidermal walls of young leaves within 24 to 48 h after inoculation. Eldon Brown (1975) found that the spore germinated on the surface of citrus fruit and formed appressoria which in turn produced infectious hyphae that remained latent.

Munjal and Gupta (1965) observed that the cells of the diseased tissue lost their shape and later the hyphae collected underneath the epidermis from which conidiophores were produced.

Tebeest *et al.* (1978) found that the spores germinated and produced appressoria in four to five h and penetrated the host epidermis after 48 hours. The mycelium grew within the cortex, cambium, xylem and pithway tissues and death of seedlings was caused by collapse of infested stem tissue.

According to Bruggen and Vander Marate (1987) infection through undamaged epidermis was limited to epidermal cells. The cells turned brown within a day and their walls were lignified seven days after penetration. A brown margin developed on the edge of the spreading mycelium. The lesions could spread to the entire stem, more often in detached stem pieces than in the field.

Boruah *et al.* (1992), described the leaf blight of *Dioscorea composita*. The disease symptoms first appeared as light-brown small circular spots on the upper surface of leaves and progressively spread resulting in defoliation and death of the vines.

Bhawani Singh (1993) reported that on *Curcuma zedoaria*, the disease appeared soon after the rains as numerous dull pinhead spots scattered all over the lamina. Quite often the spots coalesced to form large lesions. The leaves were attacked on both the surfaces. The lower leaves were affected first and the disease then progressed upwards on the plants. Severely infected leaves turned yellow and shed prematurely.

2.2 Cultural characters of isolates

The genus *Colletotrichum* is known for its variability in cultural and morphological characters. These pathogens cause damage to roots, stems, leaves, flowers and fruits, but are often highly specific to individual tissues. Many are also specific to particular plant species or cultivars. Physiologic specialization is a commonly occurring phenomenon in *Colletotrichum*.

Different isolates of *C. gloeosporioides* show great variations in their morphological characters like shape and size of acervuli, setae, conidiophores and conidia. Acervuli may be globose to saucer shaped in culture media (Prasad and Singh, 1960) on *Dioscorea alata* or disc shaped (Varadarajan, 1964) on *Rauwolfia serpentina* or oval to circular (Singh and Katiyar, 1969 and Narendra *et al.* 1974) on *Alstonia scholaris* and on groundnut respectively. The size of the acervuli varied from 71 μ m (Roy, 1965) on *Inga dulcis* to 400 μ m (Rawla and Rawla, 1970) on citrus.

A few isolates did not produce setae while others produced setae abundantly in culture. No setae were found associated with acervuli on dwarf mistletoe (Parmeter *et al.* 1959) on *Dioscorea alata* (Prasad and Singh, 1960) on avocado (Agnihotrudu and Madappa, 1966) on loquat (Lele and Asha Ram, 1969) and on cassava (Vyas and Joshi, 1976). However, setae of various sizes, shapes and septations were observed by Prasad (1962) on leaf spot of *Nephelium litchi*, Varadarajan (1964) on *Rauwolfia serpentina*, Munjal and Gupta (1965) on anthracnose of celosia,

Prasad and Verma (1966 a&b) on *Saraca indica* and on *Sweitenia macrophyllata* and by Rawla and Rawla (1970) on citrus. The size of the setae was found varying from 22.8 μm (Narendra *et al.* 1974) on groundnut to 181.5 μm (Prasad and Verma, 1966a) on *Saraca indica*.

The conidiophores may be phial-shaped as reported by Munjal and Gupta (1965) on *Celosia* and by Moses and Govinda Rao (1969) on coriander or simple cylindrical as reported by Agnihotrudu and Madappa (1966) in avocado. These may be simple as reported by Prasad and Verma (1966b) on *Sweitenia macrophyllata* or closely packed as reported by Varadarajan (1964) on *Rauwolfia serpentina*. The length of conidiophores varied from 8.10 μm as recorded by Munjal and Gupta (1965) on *Celosia* to 24.60 μm as observed by Prasad (1962) on *Nephelium litchi*.

The conidia may be oblong or cylindrical with rounded ends as observed by Prasad and Singh (1960) on *Dioscorea alata* and Prasad (1962) on *Nephelium litchi*, Singh and Katiyar (1969) on *Alstonia scholaris* or reniform as observed by Swarup and Mathur (1964) on *Euphorbia lactea* or broadly oval to oblong with rounded ends as observed by Vyas and Joshi (1976) on cassava. The length of conidia varied from seven μm (Lele and Asha Ram, 1969) on loquat to 23.28 μm (Prasad and Verma, 1966b), on *Sweitenia macrophyllata*.

The perithecia were spherical as reported by Srivastava and Bilgrami (1963) on *Bougainvillea glabra* or globose to subglobose as observed by Prasad and Verma (1966a) on *Saraca indica* and by Rawla and Rawla (1970) on citrus. The size of the perithecia varied from 80 μm

(Rawla and Rawla, 1970) to 156 μ m (Ramsey *et al.* 1951) in association with anthracnose of peaches.

In majority of cases, the asci were clavate to cylindrical (Rawla and Rawla, 1970 and Jack Tayler, 1971). The size of the asci varied from 35 μ m (Rawla and Rawla, 1970) on citrus to 68 μ m (Jack Tayler, 1971) in association with fruit rot of apple.

The shape of ascospores may be cylindrical or slightly curved. The size of ascospores varied from 5.7 to 18.6 μ m (Ramsey *et al.* 1951) in association with anthracnose of peaches.

The morphology of perithecial isolates of *G. cingulata* obtained from different hosts was compared by Stipes and Winstead (1964). The fungus causing anthracnose disease of *Dioscorea alata* was recognised as a new form 'alatae' under *C. gloeosporioides* (Singh *et al.* 1966).

Mordue (1971) reviewed and enumerated the morphological characters of *C. gloeosporioides* (*Glomerella cingulata*). Based on growth rate, colour, sporulation, acervuli, perithecia, setae and conidia, isolates were separated by Hindorf (1973) in *C. coffeanum*, *C. acutatum* and *C. gloeosporioides*.

Variations in the pathogenicity of certain isolates of *C. gloeosporioides* associated with stalk end rot and their effect on quality of the fruits of sweet orange were studied by Cheema *et al.* (1976).

Five isolates of *C. falcatum* showed cultural and morphological variations (Khirtat *et al.* 1980). Evaluation of 12 isolates of *C. capsici* did not give positive correlation between virulence and acervuli production or sporulation (Dasgupta, 1981). Nine isolates of *Colletotrichum* spp. were obtained by Verma (1982) from anthracnose affected chillies showing various symptoms.

Kim *et al.* (1986) classified 48 monoconidial isolates of *C. gloeosporioides* from diseased *Capsicum* fruits into G or R Strains based on pathogenicity, morphology and cultural characteristics. Bhombe *et al.* (1988) found that isolates of *C. gloeosporioides* from *Vigna mungo*, citrus, groundnut, *Artabotrys odoratissima* and mango were comparable with each other.

Physiological specialisation in *C. lindemuthianum* was studied by Chakrabarty *et al.* (1988). Cox and Irwin (1988) suggested that only dimensions of conidia from conidiomata should be used in taxonomic work.

Dale *et al.* (1988) reported that *C. gloeosporioides* isolates causing different anthracnose diseases on *Stylosanthes* in Australia, carried distinct double stranded RNA's. Gubler and Gunnel (1990) concluded that setae for the three species *C. gloeosporioides*, *C. acutatum* and *C. fragariae* differed morphologically and the shape of the conidia was more important in distinguishing the species than the conidial size.

Smith and Black (1990) found that all the isolates of *C. fragariae* induced disease symptoms when wound inoculated into strawberry leaves

and fruits where as all the *C. acutatum* isolates caused fruit rot but none caused leaf lesions. Thind and Jhooty (1990) observed marked variation among the isolates of *Colletotrichum capsici* and *C. gloeosporioides*. Based on lesion type and morphological characters, more than 150 isolates were grouped into eight categories. A significant difference existed in the pathogenic behaviour of different types of isolates and concluded that different strains of these fungi were prevalent in Punjab.

Agostini and Timmer (1992) developed a semi-selective medium to isolate slow growing orange strain of *C. gloeosporioides* from citrus and to differentiate it from the fast growing grey saprophytic strain. Three strains of *C. gloeosporioides* from citrus in Florida were described by Agostini *et al.* (1992). Two genetically distinct populations of *C. gloeosporioides* from citrus were reported by Liyanage *et al.* (1992).

Various morphological and genetic characteristics were compared among six isolates of *Colletotrichum* from maize and six from sorghum (Vaillancourt and Hanau, 1992). The slow growing SGO strain of *C. gloeosporioides* caused post-blossom fruit drop disease of citrus, whereas the fast-growing grey FGG strain was primarily saprophytic (Agostini and Timmer, 1994).

2.3 Effect of environment

The environmental conditions like temperature and relative humidity affect the pathogen, host and host-pathogen system. *Colletotrichum gloeosporioides* grows and sporulates well from 22 to 28°C. Higher

temperature, sunlight and diffused light favoured formation of acervuli (Singh and Prasad, 1967). The action of UV and blue light on *Glomerella cingulata* (sexual avirulent strain) and *C. musae* (sexual virulent strain) was described by Grand Pernet (1972). UV produced perithecia only in *G. cingulata*. It induced modifications in morphology and segregation in the asci, but not biochemical mutations. Both UV and blue light increased the production of perithecia in *G. cingulata*.

Singh and Shankar (1972) studied the effect of physical factors and nutrition on growth and perithecial development of *G. cingulata*. The maximum incidence of anthracnose of mungbean was recorded between 26° and 30° C and RH 90-100 per cent during morning and 80-93 per cent during noon was the most favourable (Thakur, 1988).

Effect of temperature, wetness, duration and inoculum density on infection and lesion development of *C. cocodes* on tomato fruit was studied by Dillard (1989). Kagiwata (1989) found pH 8.5 and temperature 25°C as optimum conditions for growth of *C. gloeosporioides*.

Bruggen *et al.* (1990) studied temperature-induced alteration in the expression of susceptibility by cassava to *C. gloeosporioides*. At 25°C, the cv. TMS. 30211 was less susceptible than the cv. TMS. 30337. At 35°C as compared at 25°C, the lesion diameter was reduced in both the cultivars. At 15°C, the lesions in both the cvs. extended more rapidly than at the other temperatures, even though 15°C as compared to 25°C caused a 50 per cent reduction of mycelial growth rate on oat meal agar.

The infection of *C. gloeosporioides* on piper betle was found by Naik *et al.* (1990) to be the highest in August (disease index 44%) followed by September (43%) and July (32%) while the lowest was in April (2%). The disease development had been favoured by the continuous rainfall between June and September. The low disease index during early summer was associated with low RH and high temperature.

Sushil Sharma (1990) studied the effect of temperature and humidity on the development of bitter rot of pear and found that conditions favouring infection caused by *C. gloeosporioides* on inoculated fruits included a temperature of 25°C and RH 95 per cent. No infection occurred at 10, 35 or 40°C when combined with 20 per cent RH.

Studies by Thakur (1990) revealed that in mungbean anthracnose the maximum lesion size was recorded with 100 per cent RH in two species of *Colletotrichum*. The temperature ranging from 22 to 27°C was found to be optimum for the maximum disease development.

Light intensity had slight effect on development of anthracnose as compared to RH and temperature. However the maximum development of disease (lesion diameter 6.9mm²) was recorded under conditions of continuous light (72 h) and alternate light and darkness (12 h each) in *C. lindemuthianum* and *C. dematium* respectively.

The optimum temperature for infection of rooted cuttings of grapes by *C. gloeosporioides* was 25 to 30°C and a wetness period of at least eight h was required for infection at this temperature (Yun and Park, 1990). Considerable differences were observed in the rate of development of

C. gloeosporioides by Green and Simons (1991) at eight field sites due to variations in environmental conditions.

Lai *et al.* (1993) recorded that growth and development of *C. gloeosporioides* occurred at 10-38°C; the germination required more than 96 per cent RH and the mycelial growth was the best at pH 4.2 to 6.1. Li and Matt (1993) found that the optimum and the maximum temperature for mycelial growth of *Colletotrichum* were 28 and 34°C respectively and mycelium was killed beyond 55°C. The fungus grew on PDA over the pH range 4 to 9, while the optimum being 6.7. Spore germination required high RH and none occurred at RH less than 79.3 per cent.

The role of temperature, humidity and host age on sporulation and spore germination of two species of *Colletotrichum* from mungbean was reported by Thakur and Khare (1993). The maximum sporulation of *C. lindemuthianum* and *C. dematium* was at 25°C. RH between 90-100 per cent was optimum for the maximum sporulation of both the species. The maximum number of conidia of *C. lindemuthianum* (300.78/mm²) was seen in the leaves of 40 day old plants. But *C. dematium* had the maximum sporulation (224.25/mm²) in the leaves of 30 day old plants. In both the cases, with increase in age of plant, there was decrease in sporulation per mm² area.

Abha Mishra and Om Gupta (1994) reported that the maximum radial growth, sporulation and spore germination of *C. dematium* occurred at 27°C as compared to 20, 25 and 30°C. But the same counts were the highest of all at 100 per cent RH and at room temperature (28 ± 2°C) as

compared to incubation at other temperatures alone and at room temperature with 80, 85 and 90 per cent RH. The maximum increase in lesion size was obtained with continuous light at room temperature as compared to alternate light and dark and total dark followed by room temperature with 100 per cent RH and 25°C temperature alone. Increased number of spores of *Colletotrichum dematium* was trapped at drizzling rains, 28.11°C temperature and 81.78 per cent RH.

2.4 Toxin and enzyme production

Infection and establishment of a pathogen involve a series of biochemical changes which generally determine the severity of the disease.

Wolf and Flowers (1957) reported the production of toxin by *C. nicotianae* inciting anthracnose of tobacco. They demonstrated the toxic effect on the leaves and petioles of tobacco. Goodman (1959 and 1960) enumerated the production, physiological activity and chemical nature of 'Colletotin' a toxin produced by *C. fuscum*. The toxin production appeared on the 11th day of incubation and its effect on tomato foliage became more intense by 17th and 18th days. Toxins produced in axenic culture of *C. fuscum*, produced an intense foliar spotting of tomato (Sally Lewis and Goodman 1962).

Production of toxin by *C. gloeosporioides* causing citrus die back was reported by Sharma and Sharma (1969). Production of toxin *in vitro* took place in Richard's solution after 22 days of growth. Narain and Das (1970) reported the production of toxin by *C. capsici* on chillies.

Janardhanan and Husain (1970) found that the toxic metabolite produced by *C. gloeosporioides* causing blight of jasmine, induced rapid wilting and necrosis of jasmine cuttings and symptoms similar to those caused by the fungus. Nair and Ramakrishnan (1973) reported that the toxin produced by *C. capsici* causing leaf spot of turmeric, induced visible alterations in the inoculated area of turmeric leaves within four h of inoculation.

Nair and Ramakrishnan (1974) reported alteration of cell permeability with very low concentration of the toxin, leading to leakage of water soluble constituents. Sahani *et al.* (1974) reported the toxic metabolites of *C. capsici* to be non specific.

Santhakumari (1980) studied the production of toxic metabolites by *C. gloeosporioides*. Fries', Richard's and host leaf extract broths were found to be good in supporting toxin production. Bioassay using vegetable seeds gave positive results and toxic activity could be expressed quantitatively. Necrotic effect on host leaves was also observed when the toxic principle was inoculated on the detached host leaves. The culture filtrates retained their toxic effect even after autoclaving showing their thermostability.

Karunakaran (1981) found that all the three isolates of *C. gloeosporioides* obtained from clove, nutmeg and cinnamon produced toxic metabolites in different media. Richard's medium was found to be the best suited for the production of the maximum quantity of the toxins. No growth of organism was observed on the clove and cinnamon leaf extract dextrose media, possibly because of the fungicidal action of clove oil and cinnamon oil present.

Partially purified toxins showed the same host specificity on their respective host leaves and produced more or less identical symptoms. Production of toxins in the media started on the 10th day of growth and reached the maximum on the 20th day after which it declined slowly, showing that the organism was capable of excreting toxins during its active growth stage.

In vitro toxin production by six isolates of *C. capsici* revealed that Fries' medium induced toxin production and symptoms similar to that observed in the field (Dasgupta, 1986). Kumar and Mahmood (1986) assayed culture filtrates of five isolates of *C. dematium* and observed marked inhibition in rate of seed germination. Toxin produced by *C. gloeosporioides* killed all the cells from callus of the rubber clones. (Suwarto *et al.* 1988).

Naik *et al.* (1989) found that Richard's medium was the best for supporting toxin production by *C. gloeosporioides*.

A substance extracted from leaves of *Dioscorea alata* infected by *C. gloeosporioides*, produced necrotic lesions on yam leaves similar to those caused by the pathogen (Amusa *et al.* 1993).

Effect of nutrients on the production of toxic metabolites by *Sarocladium oryzae* was studied by Alagarsamy (1989).

The role of cell wall degrading enzymes of pathogen and terminal oxidases of host is well known. Reddy and Reddy (1987) found that activities of endogluconase, pectin esterase, exo-PMG, exo-PL and exo-

PAL increased in *Coccinea indica* fruits infected by *C. gloeosporioides*. These effects were greater in a wild than in a cultivated variety. *C. gloeosporioides* produced pectolytic proteolytic and cellulolytic enzymes in culture media incorporated with water soluble components from unripe and ripe mango fruit (Kanakaratne and Adikaram, 1990).

Bonde *et al.* (1991) successfully distinguished strawberry pathogens *C. acutatum*, *C. fragariae* and *C. gloeosporioides* by comparing isoenzymes for 12 enzymes and 14 putative isoenzyme loci.

Fungal infection in apple caused a marked decline in phenol oxidase and the maximum activity in peroxidase (Kaul and Munjal, 1980).

Jagdish Chandra and Tyagi (1993) reported peroxidase activity associated with leaf blight of mung bean (*Vigna radiata*).

MATERIALS AND METHODS

MATERIALS AND METHODS

3.1 Collection of diseased specimens

Specimens suspected to be infected by *Colletotrichum gloeosporioides* of the following seven vegetables and 150 ornamentals were collected from Thiruvananthapuram and Thrissur districts of Kerala and used for the studies

Vegetables

1. *Amaranthus gangeticus* Linn
2. *Amorphophallus campanulatus* Blume
3. *Capsicum annuum* Linn
4. *Coccinea indica* Wight & Arn.
5. *Colocasia antiquorum* Schott
6. *Dioscorea alata* Linn.
7. *Solanum melongena* Linn.

Ornamental plants

1. *Acalypha hispida* Linn.
2. *Aglaonema commutatum* Schott
3. *Aglaonema costatum* Veitch.
4. *Aglaonema pseudobraceatum* Schott
5. *Allamanda cathartica* Linn.

6. *Alocasia amazonica* Neck.
7. *Alpinia purpurata* Roxb.
8. *Anthurium andraeanum* (Lind) Schott
9. *Anthurium grande* (Lind) Schott
10. *Anthurium imperiale* Lind.
11. *Antigonon leptopus* Endl.
12. *Arachnis magieoi* Linn.
13. *Asplenium nidus* Linn.
14. *Aster amellus* Linn.
15. *Bauhinia purpurea* Linn.
16. *Begonia diadema* Linn.
17. *Begonia epipsila* Linn.
18. *Begonia grandis* Linn.
19. *Begonia metallica* Linn.
20. *Begonia 'rex'* Putz.
21. *Begonia sachsen* Linn.
22. *Begonia semperflorens* Link & Otto.
23. *Bougainvillea spectabilis* Willd.
24. *Caladium 'candidum'* Vent.
25. *Calathea acuminata* G.F.W. Mey.
26. *Calathea insignis* G.F.W. Mey.
27. *Calathea kegeliana* G.F.W. Mey.
28. *Calathea leuconeura* G.F.W. Mey.
29. *Calathea ornata* G.F.W. Mey.
30. *Calathea picturata* V. 'Argentea' G.F.W. Mey.
31. *Calathea picturata* V. 'Vandenheckei' G.F.W. Mey.
32. *Calathea splendida* 'hort'. G.F.W. Mey.
33. *Calathea undulata* G.F.W. Mey.
34. *Calathea zebra* G.F.W. Mey.

35. *Canna indica* Linn.
36. *Celosia argentea* 'Plumosa' Linn.
37. *Chlorophytum comosum* Ker-Gawl.
38. *Chrysothemis pulchella* Linn.
39. *Cissus discolor* Linn.
40. *Clerodendrum thomsonae* Linn.
41. *Codiaeum variegatum* Blume
42. *Cordyline terminalis* Adans.
43. *Costus malortieanus* Linn.
44. *Costus sanguineus* Linn.
45. *Dieffenbachia amoena* Schott
46. *Dieffenbachia barraquiniana* Schott
47. *Dieffenbachia maculata* Schott
48. *Dieffenbachia* 'Exotica alba' Schott
49. *Dieffenbachia* 'Rud Roehrs'.
50. *Dracaena deremensis* Linn.
51. *Dracaena fragrans* 'Victoriae' Linn.
52. *Dracaena godseffiana* Linn.
53. *Dracaena goldieana* Linn.
54. *Dracaena hookeriana* Linn.
55. *Dracaena marginata* Linn.
56. *Dracaena reflexa* Linn.
57. *Dracaena sanderiana* Linn.
58. *Duranta plumieri* Linn.
59. *Episcia cupreata* Linn.
60. *Epiphyllum oxypetalum* Linn.
61. *Euphorbia milliprostrata* CH - DES Moulins
62. *Eurycles amboinensis* Linn.
63. *Ficus elastica* Roxb.

64. *Ficus benjamina* 'Variegata' Linn.
65. *Gardenia jasminoides* Ellis.
66. *Gardenia spatulifolia* Ellis.
67. *Gerbera jamesonii* L. Bolus.
68. *Graptophyllum pictum* Nees.
69. *Hamelia patens* Jacq.
70. *Hedychium coronarium* Koenig
71. *Heliconia metallica* L.
72. *Heliconia psittacorum* L.
73. *Hibiscus mutabilis* Linn.
74. *Hibiscus rosa - sinensis* Linn.
75. *Hippeastrum reticulatum* Herb.
76. *Hippeastrum* sp.
77. *Homalomena lindenii* Schott
78. *Homalomena miamiensis* Schott
79. *Homalomena rubescens* Schott
80. *Homalomena wallisii* Schott
81. *Hydrangea hortensia* Sm.Dc.
82. *Hymenocallis americana* Roem.
83. *Hymenocallis lillioasafoetida* Linn.
84. *Hymenocallis littoralis* (Jacq) Salish.
85. *Impatiens balsamina* Linn.
86. *Iris tectorum* Max.
87. *Ixora arborea* Roxb
88. *Ixora coccinea* Linn.
89. *Ixora singaporensis* Linn.
90. *Jasminum multiflorum* (Burmf) Andr.
91. *Jasminum odoratissimum* Linn.
92. *Jasminum sambac* (Linn) Ait.

93. *Jasminum simplicifolium* Linn.
94. *Jatropha panduraefolia* Andr
95. *Jatropha podagrica* Hook.
96. *Kalanchoe blossfeldiana* Adans
97. *Kalanchoe gastonis* Bonnierii Adans.
98. *Kalanchoe laciniata* Adans
99. *Kopsia fruticosa* A.Dc.
100. *Lagerstroemia indica* Lyth.
101. *Lantana camara* Linn
102. *Maranta arundinacea* 'Variegata' Linn
103. *Murraya exotica* Linn.
104. *Murraya koenigii* Linn
105. *Mussaenda erythrophylla* Schum & Thonn.
106. *Neomarica gracilis* Linn.
107. *Neoregelia* sp.
108. *Nephrolepis exaltata* Schott
109. *Ochna squarrosa* Linn.
110. *Ophiopogon intermedius* D. Don.
111. *Pandanus sanderi* 'Roehrsianus'.
112. *Pedilanthus tithimaloides nanus* Poiti.
113. *Pedilanthus tithimaloides Variegatus* Poiti.
114. *Pentas lanceolata* Linn.
115. *Peperomia scandens* Ruiz & Pav.
116. *Philodendron elongatum* Kunth & Bouche.
117. *Philodendron goldiana* Kunth & Bouche
118. *Philodendron haustatum* Kunth & Bouches.
119. *Philodendron scandens* Kunth & Bouche
120. *Pilea cadierei* Lindl.
121. *Pilea nummulariifolia* Lindl.

122. *Pilea* sp.
123. *Pleomele reflexa* Linn.
124. *Pleomele reflexa Variegata* Linn.
125. *Plumeria acutifolia* Poir.
126. *Polianthes tuberosa* Linn.
127. *Polyscias balfouriana* Bialek.
128. *Polyscias balfouriana pennockii* Forst & Forst. F
129. *Polyscias guilfoylei 'Victoriae'* Forst & Forst. F.
130. *Polyscias paniculata 'Variegata'* Forst & Forst. F.
131. *Pothos scandens* Linn.
132. *Pritchardia* sp
133. *Pseuderanthemum atropurpureum* L.
134. *Quisqualis Indica* Linn.
135. *Rheo discolor* L.
136. *Sansevieria aethiopica* Thumb.
137. *Sansevieria 'Hahnii'*.
138. *Sansevieria trifasciata* Prain.
139. *Spathiphyllum commutatum* Linn.
140. *Spathoglottis plicata* Linn.
141. *Stenotaphrum secundatum Variegatum* Trin.
142. *Syngonium podophyllum* Linn.
143. *Tagetes erecta* Linn.
144. *Tecomaria capensis* (Thumb) Spach.
145. *Thunbergia erecta* Retz.
146. *Tithonia speciosa* Desf. ex Juss.
147. *Vinca rosea* Linn.
148. *Xanthosoma lindenii* Schott
149. *Xanthosoma roseum variegatum* Schott
150. *Zinnia elegans* Linn.

3.1.1 Isolation, purification and maintenance of pure cultures of the pathogen

The pathogens were isolated by standard mycological techniques. The fungal growths obtained were examined under the microscope and isolates yielding *C. gloeosporioides* were transferred to PDA slants. These were further purified by single spore isolation to get pure cultures of *C. gloeosporioides*. Such cultures were maintained on PDA slants by periodical subculturing.

Cultures from PDA slants were transferred to petri plates and mycelial discs of five mm diameter were cut with a sterile cork borer from the periphery of five -day-old cultures and these were used for the studies.

3.2 Symptomatology and Pathogenicity

3.2.1 Symptomatology

The symptoms produced in the naturally infected plants in the field were studied.

3.2.2 Pathogenicity of isolates

The isolates of *C. gloeosporioides* isolated from the diseased plants were used in this study. Disease-free plants were raised in pots kept in green house. Inoculations were conducted by placing seven-day-old culture bits of the respective isolate on the pinpricked part on the upper side of the healthy leaves. The culture bits were covered with moist cotton wool. The inoculated leaves were covered with polythene bags containing moist cotton to ensure high humidity. After incubation for 48 h, the polythene bags were removed. The pathogen was reisolated from the artificially produced lesions and compared with the original isolates, after purification by single spore isolation technique.

In all the experiments, three plants were inoculated for each crop. The experiment was repeated two times and the following observations were recorded upto a period of one month in all the cases.

1. Symptom expression in the inoculated plants
2. Incubation period for the initiation of symptom in the inoculated plants

3.3 Cultural characters of isolates

3.3.1 Rate of growth

Fifteen ml of sterilized medium were transferred to petri plates and the mycelial discs were placed in the centre of the plates. The colony diameter was measured on PDA on fourth, sixth and eighth days and rates of growth were calculated using the formula:

Rate of growth =

$$\frac{\text{Sum of colony diameters measured at different intervals}}{\text{Number of days grown (8) } \times \text{ Number of measurements}}$$

3.3.1.1 Growth in liquid media

The isolates were grown in liquid media, viz. potato dextrose broth and Richard's medium in 250 ml conical flasks containing 50 ml media. After ten days, the weight of the mycelium was determined on oven dry basis.

3.3.2 Colony characters

Eight-day-old colonies of the isolates maintained on PDA were examined to study the characters such as colony colour, production of aerial mycelium and texture of the colony. The microscopic characters such as presence/absence of acervuli, shape and size of conidia, size of acervuli, setae, perithecia, asci and ascospores, whenever present, were recorded. The cultures which did not produce perithecia on the 10th day were incubated under room temperature for 30 days to induce the production of perithecia (Chandra Mohanan, *et al.* 1989).

3.3.3 Degree of sporulation

To assess the degree of sporulation five mm diameter mycelial discs, three each from the periphery, middle and centre of the colony were cut with a sterile cork borer. The discs were transferred to a test tube containing five ml sterile distilled water and centrifuged for 15 min under 1500-2000 rpm. From this 0.2ml suspension was transferred to a slide and three such slides was prepared for each replication. The number of conidia per microscopic field was recorded. The average counts of conidia from 15 microscopic fields (five from each slide) were considered as the degree of sporulation for each isolate.

3.3.4 Relationship between rate of growth and sporulation

To determine the relationship between rate of growth and sporulation, the isolates were grouped into five classes based on the degree of sporulation as detailed below :

| Sporulation class | Degree of sporulation (average no. of conidia/ microscopic field (6.3 x 40)) |
|-------------------|--|
| I Poor | 0-1 |
| II Average | 2 -5 |
| III Good | 6-15 |
| IV Very good | 16-25 |
| V Abundant | above 25 |

3.3.5 Size of conidia

The length (L) and breadth (B) of 150 conidia, 50 from each replication were measured for each isolate using micrometers and L/B ratios were calculated.

3.3.6 Protein estimation

The soluble proteins of *C. gloeosporioides* were determined (Mahadevan and Sridhar, 1986).

The fungus, *C. gloeosporioides* was grown in 30ml of potato-dextrose broth for five and eight days, respectively. The cultures were filtered through Whatman No. I filter paper using a Buchner funnel under suction. The mycelial mat was washed repeatedly with cold distilled water and homogenized in a pre-chilled mortar placed on an ice bath with pestle using cold Tris-HCl buffer. The homogenate was centrifuged at 10,000g for 30 minutes and the supernatant was used for protein estimation. The following reagents were prepared :

1. Albumin standard 0.20 μ g i.e. 200 μ g in 1 ml.
2. 0.2% sodium carbonate in 0.1N. NaOH (Solution A)
3. 0.5% copper sulphate in 1% sodium citrate (Solution B)
4. 0.2 N sodium hydroxide
5. Phenol reagent 10ml : 20ml of water

Fifty ml of solution A were mixed with one ml of solution B to get reagent C.

Pipetted out 0.5ml of protein extract and 0.5ml of 0.2 N. NaOH and five ml of reagent C. The mixture was shaken and kept for 10 minutes. Then 0.5ml of phenol reagent was added and the optical density (OD) was read at 500nm or 650nm after 30 seconds in a calorimeter with green filter.

A standard curve was prepared using albumin as standard

Protein values for the cultures on the fifth and eighth day were determined from the standard curve drawn and expressed as μ g per l.

3.3.7 Enzyme activity

Enzyme activity was estimated calorimetrically after oxidation with catechol, (Mahadevan and Sridhar, 1986).

3.3.7.1 Polyphenol oxidase

The enzyme extract (0.25ml), citrate phosphate buffer at pH 7.0 (0.75ml) and 0.5% catechol (3ml) were allowed to react and the optical

density (OD) was read at 400nm using blue filter. Popyphenol oxidase activity was calculated using the formula

$$\text{PPO} = \frac{\text{OD} \times \text{time factor} \times 2.7}{\text{protein value} \times 1000} \quad \text{and}$$

expressed as units /10² protein/min

3.3.7.2 Peroxidase

Pipetted out two ml of pyrogallol 40µm molar to 1.5ml citrate phosphate buffer at pH 7 and 0.5ml of 3 per cent hydrogen peroxide along with one ml of enzyme extract. The reaction mixture was incubated for five min at 37°C and then stopped by adding five ml of five normal sulphuric acid. The coloured purpurogallol developed was extracted twice with five ml each of solvent ether. The ether was then evaporated at room temperature. The residue was dissolved in five ml ethanol and OD was read at 430nm using blue filter.

3.4 Studies on the effect of environment

The effect of environmental conditions on the growth and sporulation of the isolates was studied in detail. For this, the host plants were grouped into four categories/types based on the disease symptoms produced on the host. The disease symptoms used for this grouping were:

1. Leaf spot with yellow halo G₁
2. Leaf spot without yellow halo G₂
3. Leaf blight with yellow halo G₃
4. Leaf blight without yellow halo G₄

Two isolates were selected from each group, viz. G₁ isolates from *Amorphophallus campanulatus* (I₂) and *Dioscorea alata* (I₄), G₂ from *Hydrangea hortensia* (I₆) and *Ixora singaporensis* (I₇), G₃ from *Homalomena miamiensis* (I₅) and *Philodendron goldieana* (I₈) and G₄ from *Anthurium andraeanum* (I₃) and *Aglaonema commutatum* (I₁). The effect of temperature, relative humidity and light on the growth of these eight isolates was studied as detailed hereunder :

3.4.1 Effect of temperature

Culture plates were prepared in sterile Petri plates pouring 15ml PDA and the discs were placed as described in 3.3.1 and incubated at 15, 20, 27, 33 and 38°C in incubators for eight days. Three replications were maintained for each isolate. The observations on colony diameter were taken on the fourth, sixth and eighth days. The colony diameter was measured in two directions at right angles to each other and the average of two such measurements was recorded for each replication. The data were analysed statistically (factorial CRD). The rate of growth of each isolate was calculated using the formula given under 3.3.1.

3.4.2 Effect of relative humidity

The culture plates were incubated at room temperature in desiccators maintained at 75.6, 82.9, 92.9 and 100 per cent R.H. using different concentrations of sulphuric acid in water, for eight days. Three replications were maintained for each isolate. Observations on radial

growth were taken as in temperature studies and the data were analysed in the same manner as described in 3.4.1. The rate of growth was also calculated by using the same formula. The degree of sporulation of the isolates under different R.H was calculated as given under 3.3.3.

3.4.3 Effect of light

The culture plates were incubated for different periods of light / darkness, as given below, for eight days.

1. Continuous light (60 Watts electric bulb) for 24 h at a distance of 50cm from the source (125lux)
2. Continuous darkness for 24 h
3. Light for twelve h followed by darkness for twelve h

Three replications were maintained for each isolate. The observations on colony diameter were taken and analysed as already described. The rate of growth also was calculated. The degree of sporulation of isolates under different light treatments was calculated as given under 3.3.3.

3.5 Growth and sporulation of selected isolates

To determine the influence of various media on growth and sporulation and to study the variation among the selected isolates of G₁, G₂, G₃ and G₄ groups, three types of solid and liquid media were used. The source material was prepared as described in 3.1.1.

3.5.1 Growth on solid media

The variability in growth rate was studied on PDA, oatmeal agar and malt extract agar media prepared as per the standard procedure. The pH of the media were adjusted to 5.5. Fifteen ml of the sterilized medium was transferred to 90mm diameter Petri plates. The culture discs of five mm diameter from each isolate were placed separately at the centre of each plate and incubated at room temperature. The colony diameter was measured at fourth, sixth and eighth day. The rate of growth was calculated as explained earlier, under 3.3.1.

3.5.2 Growth in liquid media

Three liquid media viz., Coon's medium, Basal medium and Richard's medium were prepared. The composition of the media are given in Appendix I. Each isolate was inoculated separately in 15ml of the liquid medium taken in 100ml conical flasks (two sets) and incubated at room temperature. After 10 days of incubation, the mycelial mats from one set of flasks were harvested on previously dried and weighed Whatman No. 1 filter paper, washed in distilled water and dried at 60°C for 72 hours. Thereafter these were allowed to cool in a desiccator and the dry weight was determined. To determine the degree of sporulation, the mycelial mat from each of the second set of flasks was transferred to Petri plates. The mycelial discs were cut from the mycelial mat and the spore suspension was prepared as explained earlier, under 3.3.3.

3.5.3 Sporulation of selected isolates

Sporulation of the selected eight isolates was studied, under different relative humidity, light periods and in different liquid media.

3.5.3.1 Sporulation under different relative humidity

The culture discs of five mm size of the selected isolates were inoculated as described in 3.5.2.

The conical flasks were kept in desiccators maintained at 75.6, 82.9, 92.9 and 100 per cent R.H, as described in 3.4.2. Three replications were maintained.

After ten days of growth spore suspension was made and degree of sporulation was assessed as in 3.3.3. The data were statistically analysed.

3.5.3.2. Sporulation under different light periods

The culture discs of five mm size of the selected isolates were inoculated as described in 3.5.2.

The conical flasks were exposed to different periods of light viz. continuous light, continuous darkness for 24 h and alternate light and darkness for twelve h each, as described under 3.4.3. Three replications were maintained.

After ten days, the degree of sporulation was assessed and the data were analysed statistically.

3.5.3.3 Sporulation in different liquid media

The culture discs of five mm size of the selected isolates were inoculated as described in 3.5.2 and degree of sporulation was assessed after ten days of growth in different liquid media viz. Coon's medium, basal medium and Richard's medium. Three replications were maintained. The data were statistically analysed.

3.6 Host range studies

Eight isolates selected from G₁, G₂, G₃ and G₄ groups were used for these studies. Twenty four plants belonging to vegetables, fruit crops, plantation crops and ornamental plants were used. These were :

1. *Amaranthus gangeticus* Linn.
2. *Anthurium grande* Lind.
3. *Artocarpus integrifolia* Linn. f.
4. *Bauhinia purpurea* Linn.
5. *Canna indica* Linn.
6. *Capsicum annuum* Linn.
7. *Carica papaya* Linn.
8. *Cinnamomum zeylanicum* Breyn.
9. *Citrus aurantifolia* (Christm.) Swingle
10. *Colocasia antiquorum* Schott
11. *Eugenia caryophyllata* Thumb.
12. *Hibiscus rosa-senensis* Linn.
13. *Jasminum sambac* (Linn.) Ait.
14. *Lycopersicon esculentum* Mill.
15. *Mangifera indica* Linn.
16. *Mirabilis jalapa* Linn.

17. *Mussaenda erythrophylla* Schum & Thonn
18. *Myristica fragrans* Houtt.
19. *Piper nigrum* Linn.
20. *Psidium guajava* Linn.
21. *Sauropus androgynous* Merrill.
22. *Solanum melongena* Linn.
23. *Talinum triangulare* Willd
24. *Theobroma cacao* Linn.

All the isolates were inoculated on the above hosts and observations on the symptom expression and the extent of damage were recorded.

3.6.1 Cross inoculation studies

Cross inoculation studies were conducted with the selected eight isolates of *C. gloeosporioides* obtained from

- Aglaonema commutatum* Schott
- Amorphophallus campanulatus* Blume
- Anthurium andraeanum* (Lind) Schott
- Dioscorea alata* Linn
- Homalomena miamiensis* Schott
- Hydrangea hortensia* DC
- Ixora singaporensis* Linn
- Philodendron goldieana* Kunth and Bouche

Inoculation studies were conducted as per the methods described in 3.2.1. The per cent infection obtained in each case was calculated. The variations in incubation periods and symptom expressions were also recorded.

3.7 Studies on toxin production

The selected eight isolates of *C. gloeosporioides* obtained from the leaf spot of *Amorphophallus campanulatus*, *Dioscorea alata*, *Hydrangea hortensia* and *Ixora singaporensis* and leaf blight of *Aglaonema commutatum*, *Anthurium andraeanum*, *Homalomena miamiensis* and *Philodendron goldieana* were used for the following studies.

3.7.1 Effect of different media and incubation periods on toxin production

The following five liquid media were tried to assess their comparative merits in the production of toxic metabolites of the eight isolates under study.

1. Potato dextrose broth
2. Fries' medium
3. Czapek's medium
4. Richard's medium
5. Coon's medium

The compositions of the above media are given in Appendix II.

Each medium was prepared and dispensed at the rate of 30ml per 250ml conical flask and sterilized by autoclaving at 1.05 kg/cm² for 15 minutes. The media were inoculated with the mycelial discs of five mm diameter of the respective isolates obtained from the actively growing periphery of five-day-old cultures on PDA medium. For each treatment three replications were maintained. The inoculated flasks were incubated at room temperature (27°C).

For potato dextrose medium, three incubation periods viz. 15, 20 and 25 days were tried. For the other four media only one incubation period, viz. 20 days, was tried.

After the desired incubation period, the cultures were filtered through Whatman No.1 filter paper and the culture filtrate was designated as crude exotoxin. This exotoxin was used for studying its effect on inhibition of germination of two vegetable seeds viz. cowpea (*Vigna unguiculata*) and amaranthus (*Amaranthus gangeticus*) and seeds of three ornamental plants viz. balsam (*Impatiens balsamina*), periwinkle (*Catharanthus roseus*) and caesalpinia (*Caesalpinia pulcherrima*).

Inhibition of germination of seeds was studied by the method described by Vidhyasekaran *et al.*, (1970). Seeds of amaranthus, cowpea, balsam, periwinkle and caesalpinia were surface sterilized with 1:1000 mercuric chloride and washed in three changes of sterile water. These were then spread on Whatman No. 1 filter paper placed in sterile 90mm petridishes at the rate of five seeds per plate. Three replications were maintained. An aliquot of five ml of the test solution were poured into the plates over the filter paper and the dishes were incubated at room temperature. Controls were run simultaneously with sterile water. The seeds germinated on the third day in each of the treatments was counted and the per cent germination over control was calculated.

3.7.2 Partial purification of toxin

The mycelium was homogenised with five volumes of water and centrifuged at 2000 rpm, for 15 min and the pellets discarded. The

supernatant solution was again centrifuged for 15 minutes. The supernatant after second centrifugation was designated as 'Endotoxin' (crude).

The supernatant was reduced to 1/10th of its original volume under reduced pressure, combined with equal volume of methanol; stirred well and stored at 5°C overnight. The methanol solution was clarified by filtration and removed by evaporation under vacuum. The left out aqueous solution was adjusted to pH 3.5 with dilute hydrochloric acid and shaken well with an equal volume of ether in a separating funnel. The ether phase was separated and the remaining solution was again mixed with ether and extracted. The ether phase was combined together and mixed with an equal volume of five per cent sodium bicarbonate solution, shaken well and the aqueous phase was discarded. The ether solution was evaporated to dryness under reduced pressure. This left an ashy colored precipitate. This residue was again dissolved in ether and dried.

3.7.2.1. Effect of various media on toxin production

All the endotoxins thus extracted from 20-day-old eight isolates of *C. gloeosporioides* were assayed by bioassay technique

The toxic activity was measured by the intensity of symptoms produced on two broad leaved ornamental plants viz. *Philodendron goldieana* and *Aglaonema commutatum*. To detect the toxic activity, purified toxins were dissolved in sterile water and applied on the attached and detached leaves. Drops of 0.05ml of the test solution were applied on

one half of the leaf and slightly pricked under the drop. Other half of the same leaf served as control. Drops of purified toxin solutions of all the eight isolates, obtained from different media were assayed.

The inoculated leaves were incubated at room temperature and observations were recorded. Development of necrotic spots on the leaves was measured after 72 h and comparison made.

3.7.2.2 Effect of incubation periods on toxin production

Endotoxins produced by the selected eight isolates after incubation for different periods, viz. 15, 20 and 25 days, were partially purified, as described in 3.7.2. These were assayed by bioassay technique as described in 3.7.2.1. on *P. goldiciana* and *A. commutatum*. The necrotic spots developed were measured after 72 h and comparison was made, to find out the optimum period of incubation of the isolates for the maximum toxin production.

3.8 Biological grouping of different isolates

Biological grouping of different isolates was done, to study the strain variation of the pathogen in the state. The purified cultures were subjected to the cultural, morphological and physiological tests required for classification (adensonian classification) employing the key of Bradbury, (1970).

4

Researcher's name: _____

RESULTS

RESULTS

4.1 Collection of diseased specimens

Diseased specimens of vegetable plants and ornamental plants were collected from selected localities of Thiruvananthapuram and Thrissur districts of Kerala to isolate the pathogen, *Colletotrichum gloeosporioides*. Isolations from the diseased materials of seven vegetable plants and 150 ornamental plants were done. Out of the total 157 plants screened, the following 69 plants consistently yielded *Colletotrichum gloeosporioides*.

1. *Acalypha hispida* Linn.
2. *Aglaonema commutatum* Schott
3. *Allamanda cathartica* Linn.
4. *Alpinia purpurata* Roxb.
5. *Amorphophallus campanulatus* Blume
6. *Anthurium andraeanum* (Lind).
7. *Anthurium grande* (Lind).
8. *Antigonon leptopus* Endl.
9. *Asplenium nidus* Linn.
10. *Bauhinia purpurea* Linn.
11. *Begonia* 'rex' Putz.
12. *Begonia sachsen* Linn.
13. *Begonia semperflorens* Link. & Otto.

14. *Bougainvillea spectabilis* Willd.
15. *Calathea acuminata* G.F.W.Mey.
16. *Calathea insignis* G.F.W.Mey.
17. *Calathea kegeliana* G.F.W.Mey.
18. *Calathea leuconeura* G.F.W.Mey.
19. *Calathea ornata* G.F.W.Mey.
20. *Calathea picturata* G.F.W.Mey.
21. *Calathea* "splendida" 'hort' G.F.W.Mey.
22. *Calathea zebrina* G.F.W.Mey.
23. *Canna indica* Linn.
24. *Chrysothemis pulchella* Linn.
25. *Colocasia antiquorum* Schott
26. *Costus sanguineus* Linn
27. *Costus malortieanus* Linn.
28. *Dieffenbachia* 'Exotica alba'
29. *Dieffenbachia maculata* 'Rud Roehrs' Schott
30. *Dioscorea alata* Linn.
31. *Dracaena deremensis* Linn.
32. *Dracaena fragrans* 'Victoriae' Linn.
33. *Dracaena godseffiana* Linn.
34. *Dracaena marginata* Linn.
35. *Dracaena reflexa* Linn.
36. *Dracaena sanderiana* Linn.
37. *Duranta plumieri* Linn.
38. *Eurycles amboinensis* Linn.
39. *Gerbera jamesonii* L.Bolus
40. *Hamelia patens* Jacq.

41. *Hedychium coronarium* Koenig
42. *Heliconia metallica* Linn.
43. *Hibiscus rosa-sinensis* Linn.
44. *Hippeastrum reticulatum* Herb.
45. *Hippeastrum* sp.
46. *Homalomena miamiensis* Schott
47. *Hydrangea hortensia* DC.
48. *Hymenocallis lillioasafoetida* Salish.
49. *Iris tectorum* Max.
50. *Ixora singaporensis* Linn.
51. *Kopsia fruticosa* A.DC.
52. *Lantana camara* Linn.
53. *Maranta arundinacea* 'Variegata' Linn.
54. *Mussaenda erythrophylla* Schum and Thonn.
55. *Neomarica gracilis* L.
56. *Ochna squarrosa* Linn.
57. *Philodendron goldieana* Kunth & Bouche
58. *Pleomele reflexa* L.
59. *Plumeria acutifolia* Poir.
60. *Polyscias paniculata* 'Variegata' Forst & Forst. f.
61. *Pothos scandens* Linn.
62. *Quisqualis indica* Linn.
63. *Sansevieria* 'Hahnii'
64. *Sansevieria trifasciata* Prain
65. *Spathiphyllum commutatum* L.
66. *Syngonium podophyllum* L.
67. *Tagetes erecta* Linn.

68. *Tecomaria capensis* (Thumb) Spach.

69. *Thunbergia erecta* Retz.

Out of the above 69 plants, *Amorphophallus campanulatus*, *Colocasia antiquorum* and *Dioscorea alata* are vegetables and the remaining 66 are ornamental plants.

4.1.1 Isolation, purification and maintenance of pure cultures of the pathogen

Isolates of *C. gloeosporioides* obtained from each of the above plants were purified and maintained separately for further studies.

4.2. Symptomatology and pathogenicity

4.2.1. Symptomatology

Different types of symptoms were produced by *C. gloeosporioides* on the selected vegetables and ornamental plants under field conditions and these are presented in table 1 and plates 1 to 28.

Under natural conditions there were variations in symptom expression on different hosts. During the course of the survey, four major types of symptoms could be distinguished, as detailed below:

- a. Leaf spots with yellow halo (Plate 1 to 4)
- b. Leaf spots without yellow halo (Plate 5 to 10)
- c. Leaf blight with yellow halo (Plate 18 to 23)
- d. Leaf blight without yellow halo (Plate 24 to 28)

Table 1. Symptomatology

| Sl No. | Name of Host Plant | Spot | Blight | Colour | Size | Presence of halo | Shot hole | Shredding | Acervuli | Others |
|--------|--|------|-------------------------------|------------------------|-----------------------------|------------------|-----------|-----------|----------|---|
| 1 | <i>Acalypha hispida</i> Linn | + | — | Brown | Pinhead to 2mm | — | — | — | — | Spots turn dark brown as grew older. number of spots leaf 5-7 |
| 2 | <i>Aglaonema commutatum</i> Schott | + | Later blighting | Brown | 5-10 mm | — | — | — | + | Spots enlarged, coalesced and caused blighting mainly at tip of leaves |
| 3 | <i>Allamanda cathartica</i> Linn | + | — | Black | Pinhead like | — | — | — | + | Circular spots and scattered |
| 4 | <i>Alpinia purpurata</i> Roxb | + | Later blighting | Light brown | 3-5 mm | — | — | — | — | Oval spots, spots enlarged, coalesced and formed large blighted patches |
| 5 | <i>Amorophallus campanulatus</i> Blume | + | Blighting in later stages | Brown with yellow halo | 2-5 mm | + | + | — | — | Spots coalesced and caused blighting with yellow halo |
| 6 | <i>Anthurium andreaeanum</i> (Lind) Schott | + | + | Brown | More than half of leaf area | — | — | — | + | Large blighted areas which covered more than half of leaf area |
| 7 | <i>Anthurium grande</i> (Lind) | + | Blighting as disease advanced | Greyish white | 2-5 mm | + | — | + | — | Spots enlarged to form blighted patches yellow halo observed |
| 8 | <i>Antigonon leptopus</i> Endl | + | Later blighting | Reddish brown | 5-10 mm | — | — | + | + | Spots irregular in shape |
| 9 | <i>Asplenium nidus</i> Linn | + | — | Black | Pinhead to 2mm | — | + | — | + | Centre of spots turned grey with dark margin as the spots grew old |

Contd

Table 1. (Contd...)

| Sl No. | Name of Host Plant | Spot | Blight | Colour | Size | Presence of halo | Shot hole | Shredding | Acervuli | Others |
|--------|--|------|-----------------|------------------------|---------|------------------|-----------|-----------|----------|---|
| 10 | <i>Bauhinia purpurea</i> Linn. | + | - | Light brown | 4-8 mm | - | - | — | + | Symptoms ranged from spots to blighted areas |
| 11 | <i>Begonia 'rex'</i> Linn | - | - | Brown | 2-5 mm | - | - | - | - | Spots were water soaked and irregular |
| 12 | <i>Begonia sachsen</i> Linn. | + | Later blighting | Dark brown | 2-5 mm | - | - | + | - | Water soaked sunken spot which enlarged and caused blighting |
| 13 | <i>Begonia semperflorens</i> Link. & Otto. | - | — | Dark brown | — | — | - | + | — | Water soaked sunken spots without yellow halo |
| 14 | <i>Bougainvillea spectabilis</i> Willd. | - | - | Grey white | 1-3 mm | - | - | — | + | Marginal blighting |
| 15 | <i>Calathea acuminata</i> G.F.W.Mey. | - | Tip blighting | Black | 2-5 mm | — | — | — | - | Irregular spots enlarged and formed blighted areas towards leaf tip |
| 16 | <i>Calathea insignis</i> G.F.W.Mey. | - | Later blighting | Dark brown | 2-5 mm | - | - | — | - | Spots coalesced and formed blighted patches with yellow halo |
| 17 | <i>Calathea kegeliana</i> G.F.W.Mey. | - | - | Grey white | 2-5 mm | — | - | - | — | Spots coalesced, caused blighting towards leaf tip and margins. Affected tissues withered |
| 18 | <i>Calathea leuconeura</i> G.F.W.Mey. | - | Tip blighting | Brown with yellow halo | 2-10 mm | - | - | — | - | Blighting of leaf tip and margins with yellow halo |
| 19 | <i>Calathea ornata</i> G.F.W.Mey. | + | + | Grey white | 2-4 mm | + | — | - | - | Blighted areas at tip and margins of leaf, affected tissues withered away |

Table 1. (Contd...)

| Sl. No. | Name of Host Plant | Spot | Blight | Colour | Size | Presence of halo | Shot hole | Shred- ding | Acerv- uli | Others |
|---------|---|------|-----------------|------------------------------|-----------------------------|------------------|-----------|-------------|------------|--|
| 20. | <i>Calathea picturata</i> G.F.W.Mey. | + | — | Grey white with brown margin | 2-4 mm | — | — | — | - | Spots coalesced and formed bigger spots |
| 21. | <i>Calathea splendida</i> 'hort' G.F.W.Mey. | + | — | Dark brown | 2-5 mm | — | — | — | — | Irregular spots |
| 22. | <i>Calathea zebrina</i> G.F.W.Mey. | + | — | Pale yellow | 1-3 mm | + | — | — | — | Water soaked spots with yellow halo |
| 23. | <i>Canna indica</i> Linn | + | + | Brown | 2-5 mm | + | — | + | — | Symptoms ranged from small spots to irregular blighted areas towards margin. Yellow halos observed |
| 24. | <i>Chrysothemis pulchella</i> Linn | + | Later blighting | Light brown | 3-5 mm | — | — | — | — | Water soaked spots which coalesced and caused drying of leaves |
| 25. | <i>Colocasia antiquorum</i> Schott | + | Later blighting | Dark brown | 3-5 mm | + | + | — | + | Spots enlarged and were irregular in shape. Yellow halo and shot hole symptoms observed |
| 26. | <i>Costus sanguineus</i> Linn. | + | + | Grey white | Pinhead to 10mm | + | + | — | — | Blighting symptoms with yellow halo observed |
| 27. | <i>Costus malorticanus</i> Linn. | + | + | Grey white | Pinhead to 10mm | — | + | — | — | Marginal blighting as the disease advanced |
| 28. | <i>Dieffenbachia</i> 'Exotica alba' | + | + | Light brown | More than half of leaf area | — | — | — | — | Water soaked areas coalesced and caused severe blighting of entire leaf lamina. Affected portions turned thin and papery |
| 29. | <i>Dieffenbachia maculata</i> 'Rud. Roehrs' Schott. | — | + | Dark | Half of leaf area | — | — | + | — | Blighting covers more than half of leaf area, blighting more towards leaf tip |

Contd.

Table 1. (Contd...)

| Sl No | Name of Host Plant | Spot | Blight | Colour | Size | Presence of halo | Shot hole | Shredding | Acervuli | Others |
|-------|--|------|--------|-----------------------------------|-----------------------|------------------|-----------|-----------|----------|---|
| 30 | <i>Dioscorea alata</i> Linn | + | - | Brown with yellow halo | 3-4 mm | - | — | — | + | Leaves withered and caused blighting and defoliation. Yellow halo observed |
| 31 | <i>Dracaena deremensis</i> Linn. | + | - | Reddish brown with grey centre | 2-4 mm | - | — | + | + | Leaves showed circular spots with yellow halo |
| 32 | <i>Dracaena fragrans victoriae</i> Linn. | — | + | Grey | Half of leaf area | - | - | + | + | Large blighted areas with concentric zonations towards margins |
| 33 | <i>Dracaena godseffiana</i> Linn. | + | - | Reddish spots | 2-3 mm turn blighting | - | - | + | + | Spots coalesced, turned to blighting covering half of leaf area towards tip of leaves. Yellow halo observed |
| 34 | <i>Dracaena marginata</i> Linn. | - | - | Greyish spots with reddish margin | 1-2 mm | - | - | + | - | Spots were more towards margin of leaf |
| 35 | <i>Dracaena reflexa</i> Linn. | + | — | Grey with reddish border | Minute | - | — | - | - | Anthracnose symptoms developed in young leaves |
| 36 | <i>Dracaena sandersoniana</i> Linn. | - | - | Grey white | Large areas | - | - | + | + | Large patches with concentric zonation |
| 37 | <i>Duranta plumieri</i> Linn | + | - | Grey with black border | 2-4 mm | - | + | + | + | Spots enlarged and formed necrotic spots and shot hole symptoms |

Table 1. (Contd...)

| Sl No. | Name of Host Plant | Spot | Blight | Colour | Size | Presence of halo | Shot hole | Shredding | Acervuli | Others |
|--------|--------------------------------------|------|-----------------|----------------------------------|-------------------------|------------------|-----------|-----------|----------|---|
| 38 | <i>Eurycles amboinensis</i> Linn. | - | Later blighting | Golden yellow | 5-10 mm | - | - | - | - | Spots coalesced to form blighting. Yellow halo observed |
| 39 | <i>Gerbera jamesonii</i> L. Bolus | - | + | Brown | 2-5 mm | --- | --- | --- | --- | Severe blighting in the advanced stages |
| 40 | <i>Hamelia patens</i> Jacq. | - | --- | Grey | 1-2 mm | --- | + | --- | --- | Spots enlarge and produce shot hole symptoms |
| 41 | <i>Hedychium coronarium</i> Koenig | - | + | Yellowish brown | --- | + | - | --- | --- | Severe blighting covering almost the whole leaf area. Yellow halo |
| 42 | <i>Heliconia metallica</i> Linn. | - | + | Reddish brown | 2-3 mm | - | - | --- | --- | Spots coalesced and caused severe blighting. |
| 43 | <i>Iibiscus rosa-sinensis</i> Linn. | - | + | Dirty white with brown margin | 3-5 mm | --- | + | --- | - | Spots enlarged coalesced and turned to blighting |
| 44 | <i>Hippeastrum reticulatum</i> Herb. | - | --- | Reddish brown | 1-2 mm | --- | --- | --- | - | Spots enlarged later |
| 45 | <i>Hippeastrum</i> sp. | - | --- | Reddish spots | 2-3 mm | --- | + | --- | + | Irregular spots without halo |
| 46 | <i>Homalomena miamiensis</i> Schott | - | - | Dark brown with yellowish margin | Cover half of leaf area | + | --- | + | - | Initial symptoms appeared as marginal blighting. Yellow halo observed |

Contd.

Table 1. (Contd...)

| Sl. No. | Name of Host Plant | Spot | Blight | Colour | Size | Presence of halo | Shot hole | Shredding | Acervuli | Others |
|---------|--|------|-----------------|--------------------------------------|-----------------------------|------------------|-----------|-----------|----------|--|
| 47. | <i>Hydrangea hortensia</i> DC. | - | + | Dark purple | 2-5 mm | — | + | — | + | Severe blighting as the disease advanced |
| 48. | <i>Hymenocallis lillioasafoetida</i> Salisb. | + | tip blighting | Grey brown to brownish | 1-2 mm | + | — | — | — | Tip burning and blighting of leaves commonly observed |
| 49. | <i>Iris tectorum</i> Max | + | Brown blighting | Grey spots with brown border | 2-5 mm | — | — | - | + | Concentric zonations in the blighted area observed |
| 50. | <i>Ixora singaporensis</i> Linn. | + | — | White to cream colour | 1-3 mm | — | — | — | — | Spots are irregular in shape and turned brownish |
| 51. | <i>Kopsia fruticosa</i> A.DC. | + | + | Grey spots with reddish brown margin | 2-5 mm | — | + | — | + | Marginal and tip blighting |
| 52. | <i>Lantana camara</i> Linn. | - | — | Chlorotic turning brown | 2-3 mm | — | — | — | — | Usually angular in shape |
| 53. | <i>Maranta arundinacea</i> 'Variegata' Linn. | — | + | Light brown | More than half of leaf area | — | — | + | — | Margins of leaf became blighted — Infected region turn thin and papery |

Table 1. (Contd...)

| Sl. No. | Name of Host Plant | Spot | Blight | Colour | Size | Presence of halo | Shot hole | Shredding | Acervuli | Others |
|---------|---|------|--------|---------------------------------------|-----------------|------------------|-----------|-----------|----------|---|
| 54. | <i>Mussaenda erythrophylla</i> Schum and Thonn. | + | + | Brown with yellow margin | 3 to several mm | — | + | — | — | Marginal blighting |
| 55. | <i>Neomarica gracilis</i> Linn. | + | + | Chlorotic spots | 1-3 mm | + | — | — | — | Spots coalesced and turned to blighting |
| 56. | <i>Ocimum squarrosum</i> Linn. | + | + | Brown | 2-5 mm | — | — | — | + | Blighting of leaf margins at advanced cases |
| 57. | <i>Philodendron goldiana</i> Kunth & Bouche | + | + | Dark brown water soaked | 5-10 mm | + | — | — | — | Severe blighting covering whole leaf area. Yellow halo observed |
| 58. | <i>Pleomele reflexa</i> Linn. | + | — | Grey white with reddish brown boarder | 2-5 mm | — | + | — | + | Spots enlarged and shot hole symptoms in advanced stages observed |
| 59. | <i>Plumeria acutifolia</i> Poir | + | — | Black | Pin head sized | — | — | — | — | Innumerable minute spots |
| 60. | <i>Polyscias paniculata</i> 'Variegata' Forst & Forst. f. | + | — | Reddish brown | 1-2 mm | — | + | — | + | Centre spots turned grey as the spots matured shot hole symptoms observed |
| 61. | <i>Pothos scandens</i> Linn. | — | + | Black | Large areas | — | — | — | — | Blighting extended to half of leaf area |
| 62. | <i>Quisqualis indica</i> Linn. | + | — | Purplish spots with grey centre | 3-5 mm | — | + | — | — | Necrosis developed in the centre of spots and shot hole symptoms observed |

Table 1. (Contd...)

| Sl No. | Name of Host Plant | Spot | Blight | Colour | Size | Presence of halo | Shot hole | Shredding | Acervuli | Others |
|--------|--|------|--------|-----------------------------|----------------|------------------|-----------|-----------|----------|--|
| 63. | <i>Sanseveria 'Hahnii'</i> | - | + | Light brown sunken | 3-5 mm | --- | --- | - | + | Spots were usually at the tips and margins of leaf and were sunken |
| 64. | <i>Sanseveria trifasciata</i> Prain. | + | --- | Brown sunken | 2-5 mm | --- | --- | --- | + | Spots towards margin |
| 65. | <i>Spathiphyllum commutatum</i> L. | + | + | Grey with dark brown margin | 3-10 mm | --- | --- | + | + | Blighting of whole leaf in advanced stages of infection observed |
| 66. | <i>Syngonium podophyllum</i> L. | + | + | Brown | 5mm to 3cm | - | --- | --- | + | Blighting of leaf lamina in advanced stages observed |
| 67. | <i>Tagetes erecta</i> Linn. | - | --- | Black spots | Pin head sized | --- | --- | --- | --- | Numerous spots in leaf lamina |
| 68. | <i>Tecomaria capensis</i> (Thumb) Spach. | + | --- | Light brown | 2-5 mm | --- | --- | --- | --- | Brown irregular spots observed |
| 69. | <i>Thunbergia erecta</i> Retz. | + | --- | Light brown | 3-5 mm | --- | + | --- | --- | Irregular spots |

+ Present - Absent



Plate 1

Leaf spot with yellow halo in *Amorphophallus campanulatus*



Plate 2

Leaf spot with yellow halo in *Dioscorea alata*

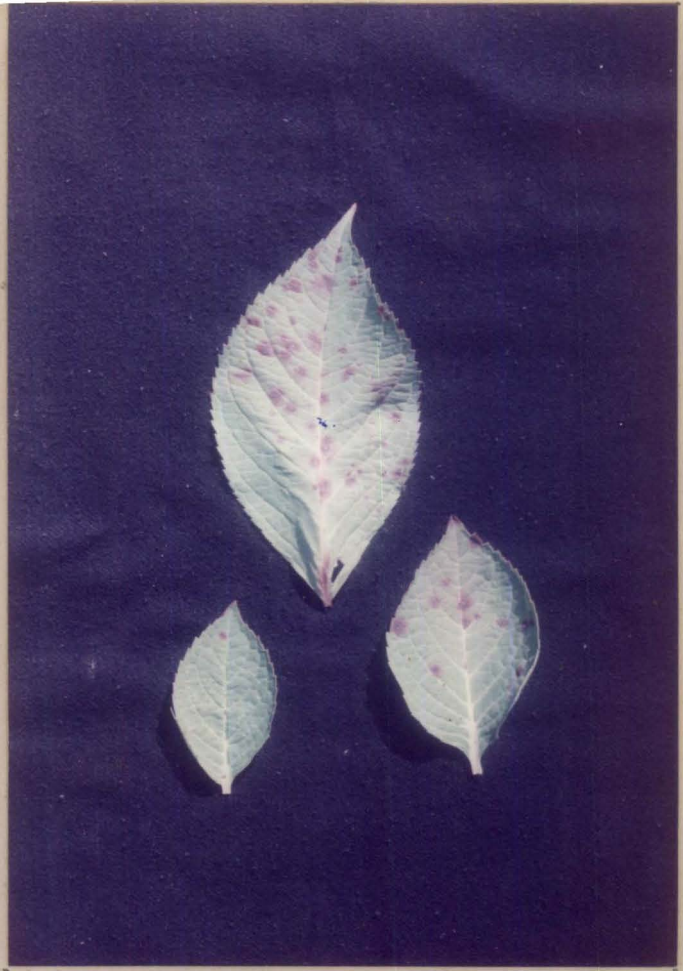


Plate 5

Leaf spot without yellow halo in *Hydrangea hortensia*



Plate 6

Leaf spot without yellow halo in *Begonia 'rex'*



Plate 7

Leaf spot without yellow halo in *Gerbera jamesonii*



Plate 8

Leaf spot without yellow halo in *Polycias paniculata* "Variegata"



Plate 9
Leaf spot without yellow halo in
Ixora singaporensis



Plate 10
Leaf spot without yellow halo in
Hippeastrum sp.



Plate 11

Leaf spot later turning to blighting in *Syngonium podophyllum*



Plate 12

Leaf spot later turning to blighting in *Sansevieria 'Hahnii'*



Plate 13

Leaf spot later turning to blighting in *Dracaena sandariana*



Plate 14

Leaf spot later turning to blighting in *Kopsia fruticosa*



Plate 15
Leaf spot with shot-hole symptom in *Calathea kegeliana*



Plate 16
Leaf spot with shot-hole symptom in *Begonia semperflorens*



Plate 17

Leaf spot with shot-hole symptom in *Hibiscus rosa-sinensis*

Plate 18
Leaf blight with yellow halo in
Homalomena miamiensis



Plate 19
Leaf blight with yellow halo in
Canna indica



Plate 20

Leaf blight with yellow halo in *Dracaena godseffiana*



Plate 21

Leaf blight with yellow halo in *Hedychium coronarium*



Plate 22

Leaf blight with yellow halo in *Philodendron goldieana*



Plate 23



Plate 24

Leaf blight without yellow halo in *Dieffenbachia maculata* "Red roehrs"



Plate 25

Leaf blight without yellow halo in *Maranta arundinacea* "Variegata"



Plate 26

Leaf blight without yellow halo in *Dieffenbachia* "Exotica alba"



Plate 27

Leaf blight without yellow halo in
Anthurium andraeanum



Plate 28

Leaf blight without yellow halo in
Dracaena fragrans victoriae

The isolates 5, 7, 16, 18, 22, 25, 30, 31, 38, and 55 produced leaf spot symptoms which were surrounded by yellow halo. The spots were circular to irregular in shape and were usually scattered (Plate 1 to 4).

The isolates 1, 2, 3, 4, 6, 8, 9, 10, 11, 12, 13, 14, 15, 17, 20, 21, 24, 27, 28, 34, 35, 36, 37, 39, 40, 42, 43, 44, 45, 47, 49, 50, 51, 52, 54, 56, 58, 59, 60, 62, 63, 64, 65, 66, 67, 68 and 69 produced necrotic spots on the leaf lamina without yellow halo (Plate 5 to 10). In both the above cases, the size of spots varied considerably from minute dots to 10 mm in diameter.

The isolates 17, 36, 51, 63, 65 and 66, initially produced leaf spots which later coalesced and formed blighting (Plate 11 to 14).

It was also observed that the isolates 5, 9, 10, 11, 13, 14, 17, 25, 26, 27, 37, 38, 40, 43, 45, 51, 54, 58, 60, 62 and 69 produced 'shot-hole' symptoms as a result of shedding of central portion of the necrotic region. However, a portion of the necrotic tissue remained intact and was sharply demarcated from the rest of the healthy tissues (Plate 15 to 17).

The isolates 16, 18, 19, 23, 26, 30, 33, 38, 41, 46, 48 and 57 produced blighting symptoms of variable sizes and shapes on the leaf lamina which were surrounded by yellow halo (Plate 18 to 22).

The isolates 6, 28, 29, 32, 39, 53, 54, 61, 65 and 66 produced blighting symptoms without yellow-halo. It was observed that the isolates

6, 28, 29, 53, 61 and 66 produced bigger patches covering a major portion of the leaf surface (Plate 23 to 27).

In severely infected plants drying of the leaves and defoliation were observed. The isolates 1, 10, 12, 30, 40, 43, 59, 62 and 69 produced defoliation symptoms on their respective host plants.

The isolates 7, 11, 12, 13, 17, 23, 29, 31, 32, 33, 34, 36, 38, 46, 53 and 65 produced leaf shredding symptoms in advanced stages of infection (Plate 15).

The isolates 32, 36 and 49 produced concentric zonations on the necrotic blotches. These zonated spots were sharply demarcated from the green healthy tissues by a well defined dark brown banding (Plate 27). It was also observed that these zonations were prominent under alternate wet and dry conditions.

The isolates 2, 3, 6, 9, 10, 14, 20, 25, 30, 31, 32, 33, 36, 37, 38, 43, 45, 46, 47, 49, 51, 56, 57, 58, 60, 62, 63, 64, 65 and 66 produced black acervuli of the organism on the upper surface of the central region of the blighted area.

4.2.2 Pathogenicity of isolates

Pathogenicity of 69 isolates were studied separately as described in 3.2.1.

Successful infection by artificial inoculations were obtained in all the 69 isolates. Pathogenicity was confirmed in all these cases.

The observations on the symptoms produced on the inoculated plants and incubation period are presented in Table 2.

Table 2. Pathogenicity

| Sl. No. | Name of Host Plant | Symptoms observed | Incubation period |
|---------|---|---|-------------------|
| 1. | <i>Acalypha hispida</i> Linn. | Brown water soaked lesions of 3 x 3 cm followed by defoliation | 4 days |
| 2. | <i>Aglaonema commutatum</i> Schott. | Water soaked spots of .5 x .5cm which turned brown and blighted | 5 days |
| 3. | <i>Allamanda cathartica</i> Linn. | Water soaked spots which enlarged in size to 1.5 x 1cm on 6th day | 4 days |
| 4. | <i>Alpinia purpurata</i> Roxb. | Water soaked brown lesions of 2 x .5 cm formed on 6th day | 5 days |
| 5. | <i>Amorphophallus campanulatus</i> Blume. | Necrotic areas with yellow halo | 5 days |
| 6. | <i>Anthurium andracanum</i> (Lind). Schott. | Minute pinhead like spots which turned dark | 4 days |
| 7. | <i>Anthurium grande</i> Lind | Water soaked spots developed which turned dark | 4 days |
| 8. | <i>Antigonon leptopus</i> Endl. | Minute spots developed | 7 days |
| 9. | <i>Asplenium nidus</i> Linn. | Water soaked dark brown lesions resulted in shot hole | 5 days |
| 10. | <i>Bauhinia purpurea</i> Linn. | Water soaked lesions enlarged soon to 2.5 x 2cm on 5th day with visible fungal growth and resulted in defoliation | 4 days |
| 11. | <i>Begonia 'rex'</i> Linn. | Water soaked lesions resulted in complete destruction of leaf on 5th day | 3 days |
| 12. | <i>Begonia sachsen</i> Linn. | Brown water soaked lesions resulted in defoliation | 6 days |
| 13. | <i>Begonia semperflorens</i> Link. & Otto. | Minute spots which coalesced and turned brown | 4 days |
| 14. | <i>Bougainvillea spectabilis</i> Willd. | Water soaked spots of .5 x .5cm on 5th day and turned brown | 3 days |
| 15. | <i>Calathea acuminata</i> G.F.W.Mey. | Water soaked spots turned brown | 6 days |
| 16. | <i>Calathea insignis</i> G.F.W.Mey. | Water soaked spots which turned brown | 6 days |
| 17. | <i>Calathea kegeliana</i> G.F.W.Mey. | Water soaked spots turned brown | 6 days |

Table 2. (Contd...)

| Sl. No. | Name of Host Plant | Symptoms observed | Incubation period |
|---------|---|--|-------------------|
| 18. | <i>Calathea leuconeura</i> G.F.W.Mey. | Light brown spots with yellow halo | 5 days |
| 19. | <i>Calathea ornata</i> G.F.W.Mey. | Water soaked brown spots | 6 days |
| 20. | <i>Calathea picturata</i> G.F.W.Mey. | Water soaked brown spots | 6 days |
| 21. | <i>Calathea splendida</i> 'hort' G.F.W. Mey | Water soaked brown spots | 6 days |
| 22. | <i>Calathea zebrina</i> G.F.W.Mey. | Water soaked brown spots | 6 days |
| 23. | <i>Canna indica</i> Linn. | Dark brown lesions of 2 x 1.5cm formed with yellow halo | 4 days |
| 24. | <i>Chrysothemis pulchella</i> Linn. | Water soaked areas turned dark and rotted completely | 4 days |
| 25. | <i>Colocasia antiquorum</i> Schott | Water soaked spots with yellow halo. Spots measured 1 x 1cm on 5th day | 4 days |
| 26. | <i>Costus sanguineus</i> Linn. | Brown lesions enlarged and the whole leaf rotted on 6th day | 3 days |
| 27. | <i>Costus malortieanus</i> Linn. | Water soaked lesions formed | 5 days |
| 28. | <i>Dieffenbachia</i> 'Exotica alba' | Water soaked areas developed .5 x .5cm and soon enlarged | 4 days |
| 29. | <i>Dieffenbachia maculata</i> 'Rud Roehrs' Schott | Necrotic brown spots of .5 x .5cm were formed | 4 days |
| 30. | <i>Dioscorea alata</i> Linn. | Water soaked areas of 2.5 x 1.5cm formed on 6th day | 4 days |
| 31. | <i>Dracaena deremensis</i> Linn. | Water soaked areas turned to severe wet rot symptoms | 5 days |
| 32. | <i>Dracaena fragrans</i> 'Victoriae' Linn. | Brown water soaked lesions of 1 x 2cm developed on 6th day | 4 days |
| 33. | <i>Dracaena godseffiana</i> Linn. | Water soaked brown areas formed and rotted with shredding symptoms of infected portion | 5 days |
| 34. | <i>Dracaena marginata</i> Linn. | Small brown spots with shredding symptoms | 6 days |
| 35. | <i>Dracaena reflexa</i> Linn. | Small pinhead like spots | 5 days |

Table 2. (Contd....)

| Sl. No. | Name of Host Plant | Symptoms observed | Incubation period |
|---------|---|--|-------------------|
| 36. | <i>Dracaena sanderiana</i> Linn. | Water soaked spots of 1 x .7cm | 4 days |
| 37. | <i>Duranta plumieri</i> Linn. | Water soaked spots formed. followed by defoliation | 4 days |
| 38. | <i>Eurycles amboinensis</i> Linn. | Water soaked areas formed | 8 days |
| 39. | <i>Gerbera jamesonii</i> L.Bolus | Water soaked small spots | 5 days |
| 40. | <i>Hamelia patens</i> Jacq. | Rotting of leaves, twigs and defoliation | 6 days |
| 41. | <i>Hedychium coronarium</i> Koenig | Water soaked spots turned light brown | 4 days |
| 42. | <i>Heliconia metallica</i> Linn. | Small brown spots formed | 5 days |
| 43. | <i>Iris bicus rosa-sinensis</i> Linn. | Water soaked brown lesions resulted in shot hole and defoliation | 5 days |
| 44. | <i>Hippeastrum reticulatum</i> Herb. | Chlorotic spots of 1 x .5cm formed which turned brown | 4 days |
| 45. | <i>Hippeastrum</i> sp. | Brown water soaked spots enlarged rapidly and leaves rotted | 4 days |
| 46. | <i>Homalomena miamiensis</i> Schott | Water soaked brown areas with yellow halo which enlarged and leaves rotted | 3 days |
| 47. | <i>Hydrangea hortensia</i> DC. | Brown spots which enlarged to 1 x 1cm on 6th day | 4 days |
| 48. | <i>Hymenocallis lillioasafoetida</i> Salisb. | Water soaked areas developed wet rot of infected leaves on 5th day | 4 days |
| 49. | <i>Iris tectorum</i> Max. | Small pinhead like spots formed | 5 days |
| 50. | <i>Ixora singaporensis</i> Linn. | Brown necrotic spots formed on the 6th day | 5 days |
| 51. | <i>Kopsia fruticosa</i> A.DC. | Water soaked spots which turned dark | 5 days |
| 52. | <i>Lantana camara</i> Linn. | Water soaked areas developed | 7 to 8 days |
| 53. | <i>Maranta arundinacea 'Variegata'</i> Linn. | Brown spots on leaves and leaves turned yellow | 4 days |
| 54. | <i>Mussaenda erythrophylla</i> Schum and Thonn. | Severe water soaked spots, enlarged covered whole leaf and resulted in defoliation | 3 days |

Table 2. (Contd....)

| Sl. No. | Name of Host Plant | Symptoms observed | Incubation period |
|---------|---|---|-------------------|
| 55. | <i>Neomarica gracilis</i> Linn. | Withering and blighting of leaves | 5 days |
| 56. | <i>Ochna squarrosa</i> Linn. | Water soaked spots which turned brown | 5 days |
| 57. | <i>Philodendron goldieana</i> Kunth. & Bouche | Brown spots with yellow halo enlarged in size and turned yellow | 3 days |
| 58. | <i>Pleomele reflexa</i> Linn. | Water soaked brown spots with shredding symptoms | 4 days |
| 59. | <i>Plumeria acutifolia</i> Poir | Rotting and defoliation | 3 days |
| 60. | <i>Polyscias paniculata</i> "Variegata" Forst & Forst. f. | Brown water soaked spots on leaves | 4 days |
| 61. | <i>Pothos scandens</i> Linn. | Minute spots developed on leaves | 5 days |
| 62. | <i>Quisqualis indica</i> Linn. | Water soaked spots developed and defoliation followed | 4 days |
| 63. | <i>Sansevieria 'Hahnii'</i> . | Water soaked brown spots were formed which turned black | 4 days |
| 64. | <i>Sansevieria trifasciata</i> Prain | Necrotic spots with grey centre of 5 x .5cm formed | 4 days |
| 65. | <i>Spathiphyllum commutatum</i> L. | Water soaked spots turned brown and rotted | 5 days |
| 66. | <i>Syngonium podophyllum</i> L. | Brown water soaked spots and later shot holes formed | 4 days |
| 67. | <i>Tagetes erecta</i> Linn. | Water soaked spots enlarged and rotting of leaves and twigs | 4 days |
| 68. | <i>Tecomaria capensis</i> (Thumb) Spach. | Water soaked brown lesion were formed | 4 days |
| 69. | <i>Thunbergia erecta</i> Retz. | Water soaked brown spots followed by defoliation | 4 days |

Great variability has been observed in different isolates. Among the 69 isolates tested, isolates 11, 14, 26, 46, 54, 57, 59 and 67 were found to be highly pathogenic and caused infection within three days. The isolates 1, 3, 6, 7, 10, 13, 23, 24, 25, 28, 29, 30, 32, 36, 37, 41, 44, 45, 47, 48, 53, 58, 60, 62, 63, 64, 66, 68 and 69 initiated symptoms in four days. The other isolates viz. 2, 4, 5, 8, 9, 12, 15, 16, 17, 18, 19, 20, 21, 22, 27, 31, 33, 34, 35, 38, 39, 40, 42, 43, 49, 50, 51, 52, 55, 56, 61 and 65 produced symptoms in five to eight days.

The pathogens reisolated from their respective hosts were found to be identical with the original isolates used as inoculum, proving the pathogenicity of the causal organism.

The isolates 5, 7, 23, 25, 26, and 46 produced spots of different sizes with yellow halo. The isolates 1, 10, 12, 37, 40, 43, 62 and 69 were found to produce necrotic patches of variable sizes and caused defoliation. Rotting symptoms were observed with isolates 24, 26, 31, 33, 40, 48, 59 and 67. Isolates 34 and 66 produced shot-hole symptoms. Necrotic lesions were formed with isolates 5, 29, 50 and 64.

4.3 Cultural characters of isolates

The cultural characters of the isolates are presented (Tables 3, 4, 5, 6 and 7).

Table 3. Colony diameter of *Colletotrichum gloeosporioides* isolates on three different periods of growth (in cm.)

| Isolate | | 4th day | 6th day | 8th day |
|---------|---|---------|---------|---------|
| No. | Name of Host Plant | | | |
| 1. | <i>Acalypha hispida</i> Linn. | 6.0 | 8.8 | 9.0 |
| 2. | <i>Aglaonema commutatum</i> Schott | 6.7 | 7.9 | 9.0 |
| 3. | <i>Allamanda cathartica</i> Linn. | 3.9 | 6.8 | 9.0 |
| 4. | <i>Alpinia purpurata</i> Roxb. | 5.7 | 7.1 | 9.0 |
| 5. | <i>Amorphophallus campanulatus</i> Blume | 5.8 | 8.1 | 9.0 |
| 6. | <i>Anthurium andraeanum</i> (Lind) Schott | 6.3 | 8.0 | 9.0 |
| 7. | <i>Anthurium grande</i> (Lind). | 5.0 | 8.1 | 9.0 |
| 8. | <i>Antigonon leptopus</i> Endl. | 5.6 | 7.6 | 9.0 |
| 9. | <i>Asplenium nidus</i> Linn. | 5.8 | 8.5 | 9.0 |
| 10. | <i>Bauhinia purpurea</i> Linn. | 7.5 | 9.0 | 9.0 |
| 11. | <i>Begonia 'rex'</i> Putz. | 6.4 | 8.5 | 9.0 |
| 12. | <i>Begonia sachsen</i> Linn. | 6.3 | 7.6 | 8.5 |
| 13. | <i>Begonia semperflorens</i> Link. & Otto | 6.6 | 9.0 | 9.0 |
| 14. | <i>Bougainvillea spectabilis</i> Willd. | 6.9 | 8.2 | 9.0 |
| 15. | <i>Calathea acuminata</i> G.F.W.Mey. | 5.7 | 7.8 | 9.0 |
| 16. | <i>Calathea insignis</i> G.F.W.Mey. | 4.2 | 8.0 | 9.0 |
| 17. | <i>Calathea kegeliana</i> G.F.W.Mey. | 4.6 | 7.8 | 9.0 |
| 18. | <i>Calathea leuconeura</i> G.F.W.Mey. | 6.3 | 7.8 | 9.0 |
| 19. | <i>Calathea ornata</i> G.F.W.Mey. | 7.3 | 8.8 | 9.0 |
| 20. | <i>Calathea picturata</i> G.F.W.Mey. | 4.2 | 8.2 | 9.0 |
| 21. | <i>Calathea splendida 'hort'</i> G.F.W.Mey. | 2.9 | 7.8 | 9.0 |
| 22. | <i>Calathea zebrina</i> G.F.W.Mey. | 5.2 | 8.0 | 9.0 |
| 23. | <i>Canna indica</i> Linn. | 4.5 | 7.8 | 9.0 |
| 24. | <i>Chrysothemis pulchella</i> Linn. | 5.0 | 8.4 | 9.0 |
| 25. | <i>Colocasia antiquorum</i> Schott | 3.5 | 6.8 | 9.0 |
| 26. | <i>Costus sanguineus</i> Linn. | 6.1 | 7.9 | 9.0 |
| 27. | <i>Costus malorteanus</i> Linn. | 5.0 | 6.2 | 9.0 |
| 28. | <i>Dieffenbachia 'Exotica alba'</i> | 6.3 | 9.0 | 9.0 |
| 29. | <i>Dieffenbachia maculata 'Rud Roehrs'</i> Schott | 6.1 | 8.7 | 9.0 |
| 30. | <i>Dioscorea alata</i> Linn. | 6.1 | 6.7 | 7.0 |
| 31. | <i>Dracaena deremensis</i> Linn. | 7.1 | 9.0 | 9.0 |
| 32. | <i>Dracaena fragrans 'Victoriae'</i> Linn. | 6.9 | 7.7 | 9.0 |
| 33. | <i>Dracaena godseffiana</i> Linn. | 6.2 | 8.2 | 9.0 |
| 34. | <i>Dracaena marginata</i> Linn. | 6.6 | 9.0 | 9.0 |
| 35. | <i>Dracaena reflexa</i> Linn. | 7.1 | 9.0 | 9.0 |

Table 3. (Contd.....)

| Isolate | | | | |
|---------|---|---------|---------|---------|
| No. | Name of Host Plant | 4th day | 6th day | 8th day |
| 36. | <i>Dracaena sanderiana</i> Linn. | 6.1 | 9.0 | 9.0 |
| 37. | <i>Duranta plumieri</i> Linn. | 8.2 | 9.0 | 9.0 |
| 38. | <i>Euryeles amboinensis</i> Linn. | 1.7 | 4.2 | 5.6 |
| 39. | <i>Gerbera jamesonii</i> L.Bolus | 2.8 | 6.2 | 8.6 |
| 40. | <i>Hamelia patens</i> Jacq. | 6.4 | 9.0 | 9.0 |
| 41. | <i>Hedychium coronarium</i> Koenig | 5.6 | 8.3 | 9.0 |
| 42. | <i>Heliconia metallica</i> Linn. | 6.5 | 8.6 | 9.0 |
| 43. | <i>Hibiscus rosa-sinensis</i> Linn. | 6.3 | 8.1 | 9.0 |
| 44. | <i>Hippeastrum reticulatum</i> Herb. | 4.3 | 6.3 | 9.0 |
| 45. | <i>Hippeastrum</i> sp. | 6.8 | 9.0 | 9.0 |
| 46. | <i>Homalomena miamiensis</i> Schott | 6.1 | 8.7 | 9.0 |
| 47. | <i>Hydrangea hortensia</i> DC. | 6.0 | 6.9 | 7.0 |
| 48. | <i>Hymenocallis lilloasafotida</i> Salish. | 8.5 | 9.0 | 9.0 |
| 49. | <i>Iris tectorum</i> Max. | 5.7 | 8.5 | 9.0 |
| 50. | <i>Ixora singaporensis</i> Linn. | 5.9 | 7.7 | 9.0 |
| 51. | <i>Kopsia fruticosa</i> A.DC. | 6.4 | 8.7 | 9.0 |
| 52. | <i>Lantana camara</i> Linn. | 3.8 | 6.3 | .0 |
| 53. | <i>Maranta arundinacea</i> 'Variegata' Linn. | 5.7 | 8.0 | 9.0 |
| 54. | <i>Mussaenda erythrophylla</i> Schum and Thonn. | 4.5 | 7.1 | 9.0 |
| 55. | <i>Neomarica gracilis</i> Linn. | 6.3 | 8.0 | 9.0 |
| 56. | <i>Ochna squarrosa</i> Linn. | 6.1 | 7.8 | 8.6 |
| 57. | <i>Philodendron goldieana</i> Kunth. & Bouche | 4.6 | 7.2 | 8.1 |
| 58. | <i>Pleomeli reflexa</i> | 6.2 | 9.0 | 9.0 |
| 59. | <i>Plumeria acutifolia</i> Poir | 5.3 | 9.0 | 9.0 |
| 60. | <i>Polyscias paniculata</i> 'Variegata' Forst & Forst. f. . | 5.8 | 8.2 | 9.0 |
| 61. | <i>Pothos scandens</i> Linn. | 3.4 | 6.3 | 9.0 |
| 62. | <i>Quisqualis indica</i> Linn. | 6.4 | 8.8 | 9.0 |
| 63. | <i>Sansevieria 'Hahnii'</i> Prain | 5.7 | 9.0 | 9.0 |
| 64. | <i>Sansevieria trifasciata</i> Prain | 3.3 | 5.4 | 8.1 |
| 65. | <i>Spathiphyllum commutatum</i> Linn. | 5.8 | 8.3 | 9.0 |
| 66. | <i>Syngonium podophyllum</i> Linn. | 5.9 | 6.9 | 7.1 |
| 67. | <i>Tagetes erecta</i> Linn. | 6.4 | 9.0 | 9.0 |
| 68. | <i>Tecomaria capensis</i> (Thumb) Spach. | 5.6 | 7.0 | 8.7 |
| 69. | <i>Thunbergia erecta</i> Retz. | 5.7 | 8.1 | 9.0 |

4.3.1 Rate of growth

The colony diameter was measured at four, six and eight days of growth on PDA after inoculation and data are presented (Table 3). The colony diameter on the 4th day varied from 1.7 cm (isolate No.38) to 8.5cm (isolate No.48). The colony diameter of the isolates 48, 37, 10, 19, 31 and 35 was 8.5, 8.2, 7.5, 7.3 and 7.1 cm respectively. The colony diameter was 6.0 to 6.9cm in isolates 1, 2, 6, 11, 12, 13, 14, 18, 26, 28, 29, 30, 32, 33, 34, 36, 40, 42, 43, 45, 46, 47, 51, 55, 56, 58, 62 and 67. The average growth was 5 to 5.9cm in isolates 4, 5 7, 8, 9, 15, 22, 24, 27, 41, 49, 50, 53, 59, 60, 63, 65, 66, 68 and 69. The colony diameter was 4 to 4.9cm in isolates 16, 17, 20, 23, 44, 54 and 57. In isolates 3, 21, 25, 38, 39, 52, 61 and 64, the colony diameter was very less.

The colony diameter of the isolates 10, 13, 28, 31, 34, 35, 36, 37, 40, 45, 48, 58, 59, 63 and 67 was 9cm (maximum) on the 6th day of inoculation. It was 6.2 to 6.9cm in isolates 3, 25, 27, 30, 39, 44, 47, 52, 53, 61 and 66. A slow growth of 4.2 and 5.4cm was observed in isolates 38 and 64.

The colony diameter of the isolates was 9cm on the 8th day in almost all the isolates except in 12, 30, 38, 39, 47, 56, 57, 64, 66 and 68.

Thus in 59 isolates out of a total of 69, the colony diameter was the highest and reached the maximum of 9cm on the 8th day of inoculation.

It was also observed that the colony diameter was the highest in isolates 10, 13, 21, 22, 28, 31, 34, 35, 36, 37, 40, 45, 48, 58, 59,

63 and 67, as they reached full growth of 9cm on the 6th day itself. A poor growth of 5.6cm was observed in isolate 38 and 7.0cm in isolate 47 on the 8th day.

The rate of growth of each isolate was determined by using the formula given in 3.3.1 and the data are presented (Table 4). There was variation in the rate of growth of the isolates. The rate of growth of the isolate varied from 0.47cm/day (isolate 21) to 1.1cm/day (isolate 48). In 47 isolates out of 69, the rate of growth was above 0.90cm/day showing that these were fast growers. In 20 isolates, growth rate was 0.70 to 0.9cm/day and in the remaining two isolates (21 and 38) a poor growth rate of less than 0.70cm/day was observed.

4.3.1.1 Growth in liquid media

The mycelial weight of different isolates of *C. gloeosporioides* after 10 days growth on potato dextrose broth (semi synthetic medium) and Richard's medium (synthetic medium) was determined and the data are presented (Table 5 and Figs. 1 and 2). The results showed that there was wide variation in the weight of mycelium also in both the media used. On PDB, the maximum dry weight of 537mg was obtained in isolate 58 while the minimum dry weight of 124mg in isolate 7. In Richard's medium, the maximum dry weight of 798mg was recorded in isolate 17 while the minimum dry weight of 116mg in isolate 55. The rest of the isolates had moderate dry weight in both the media used. The data also indicated the superiority of Richard's medium in supporting the mycelial growth of *C. gloeosporioides*, giving the maximum dry weight of 798mg as compared to PDB with 537 mg only.

Table 4. Growth rates on different isolates on PDA

| Isolate No. | Name of Host Plant | Growth rate |
|-------------|---|-------------|
| 1. | <i>Acalypha hispida</i> Linn. | 0.70 |
| 2. | <i>Aglaonema commutatum</i> Schott | 0.98 |
| 3. | <i>Allamanda cathartica</i> Linn. | 0.82 |
| 4. | <i>Alpinia purpurata</i> Roxb. | 0.90 |
| 5. | <i>Amorphophallus campanulatus</i> Blume | 0.95 |
| 6. | <i>Anthurium andraeanum</i> (Lind) Schott | 0.97 |
| 7. | <i>Anthurium grande</i> (Lind). | 0.92 |
| 8. | <i>Antigonon leptopus</i> Endl. | 0.92 |
| 9. | <i>Asplenium nidus</i> Linn. | 0.97 |
| 10. | <i>Bauhinia purpurea</i> Linn. | 1.06 |
| 11. | <i>Begonia 'rex'</i> Putz. | 0.89 |
| 12. | <i>Begonia sachsen</i> Linn. | 0.93 |
| 13. | <i>Begonia semperflorens</i> Link. & Otto | 1.02 |
| 14. | <i>Bougainvillea spectabilis</i> Willd. | 1.02 |
| 15. | <i>Calathea acuminata</i> G.F.W.Mey. | 0.94 |
| 16. | <i>Calathea insignis</i> G.F.W.Mey. | 0.88 |
| 17. | <i>Calathea kegeliana</i> G.F.W.Mey. | 0.89 |
| 18. | <i>Calathea leuconeura</i> G.F.W.Mey. | 0.96 |
| 19. | <i>Calathea ornata</i> G.F.W.Mey. | 1.04 |
| 20. | <i>Calathea picturata</i> G.F.W.Mey. | 0.89 |
| 21. | <i>Calathea splendida 'hort'</i> G.F.W.Mey. | 0.62 |
| 22. | <i>Calathea zebrina</i> G.F.W.Mey. | 0.92 |
| 23. | <i>Canna indica</i> Linn. | 0.88 |
| 24. | <i>Chrysothemis pulchella</i> Linn. | 0.93 |
| 25. | <i>Colocasia antiquorum</i> Schott | 0.80 |
| 26. | <i>Costus sanguineus</i> Linn. | 0.95 |
| 27. | <i>Costus malorteanus</i> Linn. | 0.84 |
| 28. | <i>Dieffenbachia 'Exotica alba'</i> | 1.01 |
| 29. | <i>Dieffenbachia maculata 'Rud Roehrs'</i> Schott | 0.99 |
| 30. | <i>Dioscorea alata</i> Linn. | 0.82 |
| 31. | <i>Dracaena deremensis</i> Linn. | 1.04 |
| 32. | <i>Dracaena fragrans 'Victoriae'</i> Linn. | 0.98 |
| 33. | <i>Dracaena godseffiana</i> Linn. | 0.97 |
| 34. | <i>Dracaena marginata</i> Linn. | 1.02 |
| 35. | <i>Dracaena reflexa</i> Linn. | 1.04 |

Contd..

Table 4. (Contd.....)

| Isolate No. | Name of Host Plant | Growth rate |
|-------------|---|-------------|
| 36. | <i>Dracaena sanderiana</i> Linn. | 1.00 |
| 37. | <i>Duranta plumieri</i> Linn. | 1.09 |
| 38. | <i>Eurycles amboinensis</i> Linn. | 0.47 |
| 39. | <i>Gerbera jamesonii</i> L.Bolus | 0.73 |
| 40. | <i>Hamelia patens</i> Jacq. | 1.01 |
| 41. | <i>Hedychium coronarium</i> Koenig | 0.95 |
| 42. | <i>Heliconia metallica</i> Linn. | 1.00 |
| 43. | <i>Hibiscus rosa-sinensis</i> Linn. | 0.97 |
| 44. | <i>Hippeastrum reticulatum</i> Herb. | 0.81 |
| 45. | <i>Hippeastrum</i> sp. | 1.03 |
| 46. | <i>Homalomena miamiensis</i> Schott | 0.99 |
| 47. | <i>Hydrangea hortensia</i> DC. | 0.82 |
| 48. | <i>Hymenocallis lillioasafoetida</i> Salish. | 1.10 |
| 49. | <i>Iris tectorum</i> Max. | 0.96 |
| 50. | <i>Ixora singaporensis</i> Linn. | 0.94 |
| 51. | <i>Kopsia fruticosa</i> A.DC. | 1.01 |
| 52. | <i>Lantana camara</i> Linn. | 0.79 |
| 53. | <i>Maranta arundinacea</i> 'Variegata' Linn. | 0.94 |
| 54. | <i>Mussaenda erythrophylla</i> Schum and Thonn. | 0.85 |
| 55. | <i>Neomarica gracilis</i> Linn. | 0.97 |
| 56. | <i>Ochna squarrosa</i> Linn. | 0.93 |
| 57. | <i>Philodendron goldieana</i> Kunth. & Bouche | 0.82 |
| 58. | <i>Pleomeli reflexa</i> | 1.00 |
| 59. | <i>Plumeria acutifolia</i> Poir | 0.97 |
| 60. | <i>Polyscias paniculata</i> 'Variegata' Forst & Forst. f. . | 0.95 |
| 61. | <i>Pothos scandens</i> Linn. | 0.77 |
| 62. | <i>Quisqualis indica</i> Linn. | 1.00 |
| 63. | <i>Sansevieria 'Hahnii'</i> Prain | 0.98 |
| 64. | <i>Sansevieria trifasciata</i> Prain | 0.70 |
| 65. | <i>Spathiphyllum commutatum</i> Linn. | 0.96 |
| 66. | <i>Syngonium podophyllum</i> Linn. | 0.82 |
| 67. | <i>Tagetes erecta</i> Linn. | 1.01 |
| 68. | <i>Tecomaria capensis</i> (Thumb) Spach. | 0.88 |
| 69. | <i>Thunbergia erecta</i> Retz. | 0.95 |

Three different groups of isolates could be distinguished based on the dry mycelial weight after 10 day's growth in Richards medium (Table 5). The isolates producing a dry mycelial weight of 300mg or below were grouped as slow growers (isolates 3, 7, 14, 15, 21, 27, 29, 32, 33, 35, 37, 38, 40, 43, 47, 50, 55, 57, 64 and 68); isolates producing a dry mycelial weight of 300-500 mg were grouped as medium growers (isolates 1, 4, 5, 6, 8, 9, 10, 12, 13, 16, 20, 22, 24, 30, 31, 34, 36, 39, 41, 42, 44, 46, 49, 52, 53, 54, 56, 58, 59, 60, 65, 66 and 69) and isolates producing a dry mycelial weight of above 500mg were grouped as fast growers (isolates 2, 11, 17, 18, 19, 23, 25, 26, 28, 45, 48, 51, 61, 62, 63 and 67).

Similarly three groups of isolates could be distinguished based on the dry mycelial weight when grown in potato dextrose broth (Table 5).

The isolates producing a dry mycelial weight of 300mg or below were grouped as slow growers (isolates 7, 11, 12, 13, 14, 22, 26, 32, 38, 55 and 61); isolates producing a dry mycelial weight of 300-400mg were grouped as moderate growers (isolates 1, 2, 3, 5, 6, 9, 16, 18, 21, 23, 25, 27, 29, 31, 35, 37, 41, 42, 43, 45, 48, 49, 50, 51, 52, 53, 57, 62, 64, 66, 67 and 69) and isolates producing a dry mycelial weight of above 400mg were grouped as fast growers (isolates 4, 8, 10, 15, 17, 19, 20, 24, 28, 30, 33, 34, 36, 39, 40, 44, 46, 47, 54, 56, 58, 59, 60, 63, 65 and 68).

4.3.2 Colony characters

Eight-day-old colonies of all the 69 isolates maintained on PDA were used to study the colony characters.

Table 5. Dry weight of mycelium of isolates in mg. after 10 days growth

| Isolate No. | Name of Host Plant | Media | |
|----------------|---|-------|-----------|
| | | PDB | Richard's |
| 1. | <i>Acalypha hispida</i> Linn. | 389 | 411 |
| 2. | <i>Aglaonema commutatum</i> Schott | 394 | 663 |
| 3. | <i>Allamanda cathartica</i> Linn. | 372 | 227 |
| 4. | <i>Alpinia purpurata</i> Roxb. | 438 | 338 |
| 5. | <i>Amorphophallus campanulatus</i> Blume | 301 | 423 |
| 6. | <i>Anthurium andraeanum</i> (Lind). | 393 | 387 |
| 7. | <i>Anthurium grande</i> (Lind). | 124 | 128 |
| 8. | <i>Antigonon leptopus</i> Endl. | 458 | 408 |
| 9. | <i>Asplenium nidus</i> Linn. | 376 | 449 |
| 10. | <i>Bauhinia purpurea</i> Linn. | 422 | 399 |
| 11. | <i>Begonia 'rex'</i> Linn. | 296 | 613 |
| 12. | <i>Begonia sachsen</i> Linn. | 206 | 364 |
| 13. | <i>Begonia semperflorens</i> Link. & Otto | 292 | 489 |
| 14. | <i>Bougainvillea spectabilis</i> Willd. | 209 | 245 |
| 15. | <i>Calathea acuminata</i> G.F.W.Mey. | 446 | 259 |
| 16. | <i>Calathea insignis</i> G.F.W.Mey. | 324 | 381 |
| 17. | <i>Calathea kegeliana</i> G.F.W.Mey. | 486 | 798 |
| 18. | <i>Calathea leuconeura</i> G.F.W.Mey. | 363 | 518 |
| 19. | <i>Calathea ornata</i> G.F.W.Mey. | 420 | 576 |
| 20. | <i>Calathea picturata</i> G.F.W.Mey. | 463 | 408 |
| 21. | <i>Calathea splendida 'hort'</i> G.F.W.Mey. | 376 | 211 |
| 22. | <i>Calathea zebrina</i> G.F.W.Mey. | 300 | 452 |
| 23. | <i>Canna indica</i> Linn. | 350 | 698 |
| 24. | <i>Chrysothemis pulchella</i> Linn. | 466 | 304 |
| 25. | <i>Colocasia antiquorum</i> Schott | 340 | 690 |
| 26. | <i>Costus sanguineus</i> Linn. | 238 | 599 |
| 27. | <i>Costus malortieanus</i> Linn. | 383 | 237 |
| 28. | <i>Dieffenbachia 'Exotica alba'</i> Schott | 435 | 548 |
| 29. | <i>Dieffenbachia maculata 'Rud Roehrs'</i> Schott | 305 | 186 |
| 30. | <i>Dioscorea alata</i> Linn. | 528 | 461 |
| 31. | <i>Dracaena deremensis</i> Linn. | 348 | 323 |
| 32. | <i>Dracaena fragrans victoricae</i> Linn. | 281 | 161 |
| 33. | <i>Dracaena godseffiana</i> Linn. | 462 | 238 |
| 34. | <i>Dracaena marginata</i> Linn. | 459 | 499 |
| 35. | <i>Dracaena reflexa</i> Linn. | 385 | 216 |

Contd ..

Table 5. (Contd..)

| Isolate | | Media | |
|---------|---|-------|-----------|
| No. | Name of Host Plant | PDB | Richard's |
| 36. | <i>Dracaena sanderiana</i> Linn. | 404 | 454 |
| 37. | <i>Duranta plumieri</i> Linn. | 385 | 246 |
| 38. | <i>Eurycles amboinensis</i> Linn. | 154 | 179 |
| 39. | <i>Gerbera jamesonii</i> L.Bolus | 434 | 439 |
| 40. | <i>Hamelia patens</i> Jacq. | 411 | 227 |
| 41. | <i>Hedychium coronarium</i> Koenig | 386 | 409 |
| 42. | <i>Heliconia metallica</i> Linn. | 362 | 499 |
| 43. | <i>Hibiscus rosa-sinensis</i> Linn. | 356 | 139 |
| 44. | <i>Hippeastrum reticulatum</i> Herb. | 474 | 367 |
| 45. | <i>Hippeastrum</i> sp. | 331 | 653 |
| 46. | <i>Homalomena miamiensis</i> Schott | 428 | 433 |
| 47. | <i>Hydrangea hortensia</i> DC. | 426 | 241 |
| 48. | <i>Hymenocallis lillioasafotida</i> Salish. | 327 | 521 |
| 49. | <i>Iris tectorum</i> Max. | 374 | 390 |
| 50. | <i>Ixora singaporensis</i> Linn. | 394 | 222 |
| 51. | <i>Kopsia fruticosa</i> A.DC. | 378 | 568 |
| 52. | <i>Lantana camara</i> Linn. | 374 | 364 |
| 53. | <i>Muraeta arundinacea</i> 'Variegata' Linn. | 396 | 395 |
| 54. | <i>Mussaenda erythrophylla</i> Schum and Thonn. | 485 | 421 |
| 55. | <i>Neomarica gracilis</i> Linn. | 202 | 116 |
| 56. | <i>Ochna squarrosa</i> Linn. | 410 | 374 |
| 57. | <i>Philodendron goldieana</i> Kunth. & Bouche | 386 | 233 |
| 58. | <i>Pleomele reflexa</i> Linn. | 537 | 463 |
| 59. | <i>Plumeria acutifolia</i> Poir | 442 | 482 |
| 60. | <i>Polyscias paniculata</i> 'Variegata' Forst & Forst. f. . | 445 | 338 |
| 61. | <i>Pothos scandens</i> Linn. | 168 | 554 |
| 62. | <i>Quisqualis indica</i> Linn. | 376 | 557 |
| 63. | <i>Sansieveria</i> 'Hahnii' | 425 | 676 |
| 64. | <i>Sansevieria trifasciata</i> Prain | 326 | 150 |
| 65. | <i>Spathiphyllum commutatum</i> Linn. | 417 | 401 |
| 66. | <i>Syngonium podophyllum</i> Linn. | 379 | 437 |
| 67. | <i>Tagetes erecta</i> Linn. | 380 | 525 |
| 68. | <i>Tecomaria capensis</i> (Thumb) Spach. | 417 | 133 |
| 69. | <i>Thunbergia erecta</i> Retz. | 360 | 405 |

Isolates varied considerably in colony characters. Pure white to black colonies were observed. Dirty white growth was observed in isolates 8, 10, 11, 29, 31 and 34. Black with jelly like sporulation was observed in isolates 2, 4, 5, 50 and 59. Mixtures of black and white colours were observed in isolates 3, 26, 36, 68 and 69. Grey coloured growth was observed in isolates 40, 56, 58, 62 and 63. White growth was noted in isolates 49 and 52. White creamy abundant sporulation was observed in isolate 41. Isolates 7 and 51 showed blackish grey mycelium. Submerged mycelial growth was noted in isolates 13 and 23. Black growth was seen in isolates 12, 19, 22, 25, 30, 32, 38 and 65. Black with massive sporulation was observed in isolates 35 and 44. Isolates 33 and 59 exhibited ashy sporulation and isolate 67 had creamy honey dew sporulation.

4.3.3 Degree of sporulation

The comparative degree of sporulation of the isolates was determined from the average counts of conidia from 15 microscopic fields as described in 3.3.3 and presented (Table 6).

Average number of conidia per microscopic field varied from 0.66 (isolate 38) to 69 (isolate 5).

4.3.4 Relationship between rate of growth and sporulation

The relationship between rate of growth and sporulation was determined based on the index, vide 3.3.4 and is presented (Table 7).

Table 6. Degree of sporulation of different isolates

| Isolate No. | Name of Host Plant | No. of conidia/ microscopic field |
|-------------|---|--------------------------------------|
| 1. | <i>Acalypha hispida</i> Linn. | 3.33 |
| 2. | <i>Aglaonema commutatum</i> Schott | 5.33 |
| 3. | <i>Allamanda cathartica</i> Linn. | 1.66 |
| 4. | <i>Alpinia purpurata</i> Roxb. | 26.33 |
| 5. | <i>Amorphophalus companulatus</i> Blume | 69.00 |
| 6. | <i>Anthurium andraenum</i> (Lind). | 25.00 |
| 7. | <i>Anthurium grande</i> (Lind). | 11.66 |
| 8. | <i>Antigonon leptopus</i> Endl. | 4.66 |
| 9. | <i>Asplenium nidus</i> Linn. | 30.00 |
| 10. | <i>Bauhinia purpurea</i> Linn. | 2.00 |
| 11. | <i>Begonia 'Rex'</i> Linn. | 6.66 |
| 12. | <i>Begonia sachsen</i> Linn. | 7.33 |
| 13. | <i>Begonia semperflorens</i> Link. & Otto | 5.66 |
| 14. | <i>Bougainvillea spectabilis</i> Willd. | 16.33 |
| 15. | <i>Calathia accuminata</i> G.F.W.Mey. | 5.33 |
| 16. | <i>Calathia insignis</i> G.F.W.Mey. | 4.66 |
| 17. | <i>Calathia kegeliana</i> G.F.W.Mey. | 7.00 |
| 18. | <i>Calathia leuconeura</i> G.F.W.Mey. | 6.66 |
| 19. | <i>Calathia ornata</i> G.F.W.Mey. | 7.66 |
| 20. | <i>Calathia picturata</i> G.F.W.Mey. | 4.33 |
| 21. | <i>Calathia splendida 'hort'</i> G.F.W.Mey. | 7.66 |
| 22. | <i>Calathia zebrina</i> G.F.W.Mey. | 27.00 |
| 23. | <i>Canna indica</i> Linn. | 25.00 |
| 24. | <i>Chrysothemis pulchella</i> Linn. | 20.33 |
| 25. | <i>Colocasia antiquorum</i> Schott | 27.33 |
| 26. | <i>Costus sangiuneus</i> Linn. | 7.33 |
| 27. | <i>Costus malortieanus</i> Linn. | 5.00 |
| 28. | <i>Dieffenbachia amoena</i> Schott | 3.66 |
| 29. | <i>Dieffenbachia maculata altpicta</i> Schott | 2.33 |
| 30. | <i>Dioscorea alata</i> Linn. | 9.00 |
| 31. | <i>Dracaena deremensis</i> Linn. | 36.66 |
| 32. | <i>Dracaena fragrans Victoriae</i> Linn. | 7.00 |
| 33. | <i>Dracaena godseffiana</i> Linn. | 2.33 |
| 34. | <i>Dracaena marginata</i> Linn. | 19.66 |

Contd..

Table 6. (Contd...)

| Isolate No. | Name of Host Plant | No. of conidia/ microscopic field |
|-------------|---|--------------------------------------|
| 35. | <i>Dracaena reflexa</i> Linn. | 3.66 |
| 36. | <i>Dracaena sanderiana</i> Linn. | 5.66 |
| 37. | <i>Duranta plumieri</i> Linn. | 4.00 |
| 38. | <i>Eurycles amboinensis</i> Linn. | 0.66 |
| 39. | <i>Gerbera jamesonii</i> L.Bolus | 14.00 |
| 40. | <i>Hamelia patens</i> Jacq. | 8.00 |
| 41. | <i>Hedychium coronarium</i> Koenig | 11.00 |
| 42. | <i>Heliconia metallica</i> Linn. | 6.66 |
| 43. | <i>Hibiscus rosa-sinensis</i> Linn. | 4.00 |
| 44. | <i>Hippeastrum reticulatum</i> Herb. | 19.33 |
| 45. | <i>Hippeastrum</i> sp. | 12.66 |
| 46. | <i>Homalomena miamiensis</i> Schott | 3.00 |
| 47. | <i>Hydrangea hortensia</i> DC. | 25.33 |
| 48. | <i>Hymenocallis lillioasafotida</i> Salish. | 3.66 |
| 49. | <i>Iris tectorum</i> Max. | 5.66 |
| 50. | <i>Ixora singaporensis</i> Linn. | 13.66 |
| 51. | <i>Kopsia fruticosa</i> A.DC. | 11.00 |
| 52. | <i>Lantana camara</i> Linn. | 3.33 |
| 53. | <i>Maranta arundinacea</i> 'Variegata' Linn. | 8.00 |
| 54. | <i>Mussaenda erythrophylla</i> Schum and Thonn. | 2.33 |
| 55. | <i>Neomarica gracilis</i> Linn. | 3.00 |
| 56. | <i>Ochna squarrosa</i> Linn. | 5.00 |
| 57. | <i>Philodendron goldieana</i> Kunth. & Bouche | 8.33 |
| 58. | <i>Pleomele reflexa</i> Linn. | 7.66 |
| 59. | <i>Plumeria acutifolia</i> Poir | 4.33 |
| 60. | <i>Polyscias paniculata</i> 'Variegata' Forst & Forst. f. | 8.00 |
| 61. | <i>Pothos scandens</i> Linn. | 25.33 |
| 62. | <i>Quisqualis indica</i> Linn. | 8.66 |
| 63. | <i>Sansevieria 'Hahnii'</i> Prain | 24.00 |
| 64. | <i>Sansevieria trifasciata</i> Prain | 8.33 |
| 65. | <i>Spathiphyllum commutatum</i> Linn. | 2.00 |
| 66. | <i>Syngonium podophyllum</i> Linn. | 10.00 |
| 67. | <i>Tagetes erecta</i> Linn. | 6.66 |
| 68. | <i>Tecomaria capensis</i> (Thumb) Spach. | 8.00 |
| 69. | <i>Thunbergia erecta</i> Retz. | 26.00 |

Table 7. Sporulation class of different isolates

| Sl. No. | Name of Host Plant | Sporulation Class |
|---------|---|-------------------|
| 1. | <i>Acalypha hispida</i> Linn. | Average |
| 2. | <i>Aglaonema commutatum</i> Schott | Good |
| 3. | <i>Allamanda cathartica</i> Linn. | Average |
| 4. | <i>Alpinia purpurata</i> Roxb. | Abundant |
| 5. | <i>Amorphophallus campanulatus</i> Blume | Abundant |
| 6. | <i>Anthurium andraeanum</i> (Lind). | Abundant |
| 7. | <i>Anthurium grande</i> (Lind). | Good |
| 8. | <i>Antigonon leptopus</i> Endl. | Average |
| 9. | <i>Asplenium nidus</i> Linn. | Abundant |
| 10. | <i>Bauhinia purpurea</i> Linn. | Average |
| 11. | <i>Begonia 'rex'</i> Linn. | Good |
| 12. | <i>Begonia sachsen</i> Linn. | Good |
| 13. | <i>Begonia semperflorens</i> Link. & Otto | Good |
| 14. | <i>Bougainvillea spectabilis</i> Willd. | Very good |
| 15. | <i>Calathea acuminata</i> G.F.W.Mey. | Good |
| 16. | <i>Calathea insignis</i> G.F.W.Mey. | Average |
| 17. | <i>Calathea kegeliana</i> G.F.W.Mey. | Good |
| 18. | <i>Calathea leuconeura</i> G.F.W.Mey. | Good |
| 19. | <i>Calathea ornata</i> G.F.W.Mey. | Good |
| 20. | <i>Calathea picturata</i> G.F.W.Mey. | Average |
| 21. | <i>Calathea splendida 'hort'</i> G.F.W.Mey. | Good |
| 22. | <i>Calathea zebrina</i> G.F.W.Mey. | Abundant |
| 23. | <i>Canna indica</i> Linn. | Abundant |
| 24. | <i>Chrysothemis pulchella</i> Linn. | Very good |
| 25. | <i>Colocasia antiquorum</i> Schott | Abundant |
| 26. | <i>Costus sanguineus</i> Linn. | Good |
| 27. | <i>Costus malortieanus</i> Linn. | Average |
| 28. | <i>Dieffenbachia 'Exotica alba'</i> Schott | Average |
| 29. | <i>Dieffenbachia maculata 'Rud Roehrs'</i> Schott | Average |
| 30. | <i>Dioscorea alata</i> Linn. | Good |
| 31. | <i>Dracaena deremensis</i> Linn. | Abundant |
| 32. | <i>Dracaena fragrans Victoriae</i> Linn. | Good |
| 33. | <i>Dracaena godseffiana</i> Linn. | Average |
| 34. | <i>Dracaena marginata</i> Linn. | Very good |

Contd...

Table 7. (Contd...)

| Sl. No. | Name of Host Plant | Sporulation Class |
|---------|---|-------------------|
| 35. | <i>Dracaena reflexa</i> Linn. | Average |
| 36. | <i>Dracaena sanderiana</i> Linn. | Good |
| 37. | <i>Duranta plumieri</i> Linn. | Average |
| 38. | <i>Eurycles amboinensis</i> Linn. | Poor |
| 39. | <i>Gerbera jamesonii</i> L.Bolus | Good |
| 40. | <i>Hamelia patens</i> Jacq. | Good |
| 41. | <i>Hedychium coronarium</i> Koenig | Good |
| 42. | <i>Heliconia metallica</i> Linn. | Good |
| 43. | <i>Hibiscus rosa-sinensis</i> Linn. | Average |
| 44. | <i>Hippeastrum reticulatum</i> Herb. | Very good |
| 45. | <i>Hippeastrum</i> sp. (Red) | Good |
| 46. | <i>Homalomena miamiensis</i> Schott | Average |
| 47. | <i>Hydrangea hortensia</i> DC. | Abundant |
| 48. | <i>Hymenocallis lillioasafotida</i> Salish. | Average |
| 49. | <i>Iris tectorum</i> Max. | Good |
| 50. | <i>Ixora singaporensis</i> Linn. | Very good |
| 51. | <i>Kopsia fruticosa</i> A.DC. | Good |
| 52. | <i>Lantana camara</i> Linn. | Average |
| 53. | <i>Maranta arundinacea</i> 'Variegata' Linn. | Good |
| 54. | <i>Mussaenda erythrophytia</i> Schum and Thonn. | Average |
| 55. | <i>Neomarica gracilis</i> Linn. | Average |
| 56. | <i>Ochna squarrosa</i> Linn. | Good |
| 57. | <i>Philodendron goldieana</i> Kunth. & Bouche | Good |
| 58. | <i>Pleomele reflexa</i> Linn. | Good |
| 59. | <i>Plumeria acutifolia</i> Poir | Average |
| 60. | <i>Polyscias paniculata</i> 'Variegata' Forst & Forst. f. . | Good |
| 61. | <i>Pothos scandens</i> Linn. | Abundant |
| 62. | <i>Quisqualis indica</i> Linn. | Good |
| 63. | <i>Sanseveria</i> 'Hahnii' | Very good |
| 64. | <i>Sansevieria trifasciata</i> Prain. | Good |
| 65. | <i>Spathiphyllum commutatum</i> Linn. | Average |
| 66. | <i>Syngonium podophyllum</i> Linn. | Good |
| 67. | <i>Tagetes erecta</i> Linn. | Good |
| 68. | <i>Tecomaria capensis</i> (Thumb) Spach. | Good |
| 69. | <i>Thunbergia erecta</i> Retz. | Abundant |

Isolates 1, 3, 8, 10, 16, 20, 27, 28, 29, 33, 35, 37, 43, 46, 48, 52, 54, 55, 59 and 65 were categorised under group 'average' and isolates 2, 7, 11, 12, 13, 15, 17, 18, 19, 21, 26, 30, 32, 36, 39, 40, 41, 42, 45, 49, 51, 53, 56, 57, 58, 60, 62, 64, 66, 67 and 68 under group 'good' .

Isolates 14, 24, 34, 44, 50 and 63 were under group 'very good' and isolates 4, 5, 6, 9, 22, 23, 25, 31, 47, 61 and 69 were under 'abundant' group. Only one isolate (isolate 38) was under group 'poor'

Out of 69 isolates, one isolate was grouped under 'poor', 20 under 'average', 31 under 'good', six under 'very good' and 11 under 'abundant' group. Thus about 70 per cent of the isolates under 'good', 'very good' and 'abundant' groups.

4.3.5 Size of conidia

The length (L) and breadth (B) of 150 conidia (50 each from three replications) were measured for each isolate. Ratio L/B were calculated and are presented (Table 8).

Measurements of conidia of the 69 isolates showed marked differences in the length and breadth.

Length of conidia of isolates varied from a minimum of 7.0 μ m to a maximum for 24.5 μ m. Breadth of conidia varied from a minimum of 3.5 μ m to a maximum of 7.00 μ m. Average length varied from a minimum of 7.14 μ m to a maximum of 17.85 μ m with a grand average of 11.14 μ m. Average breadth varied from a minimum of 1.47 μ m to a maximum of 5.43 μ m with a grand average of 3.8 μ m. L/B ratio ranged from 1.76 to 7.38.

Table 8. Spore measurement

| Isolate No. | Name of Host Plant | Length µm | | Breadth µm | | Average length µm | Average breadth µm | Ratio (L/B) |
|-------------|---|--------------|---------|---------------|---------|----------------------|-----------------------|-------------|
| | | Minimum | Maximum | Minimum | Maximum | | | |
| 1. | <i>Acalypha hispida</i> Linn. | 10.50 | 10.50 | 3.50 | 3.50 | 10.50 | 3.50 | 3.00 |
| 2. | <i>Aglaonema commutatum</i> Schott | 10.50 | 14.00 | 4.38 | 6.13 | 11.90 | 5.43 | 2.19 |
| 3. | <i>Allamanda cathartica</i> Linn. | 10.50 | 12.25 | 5.25 | 5.25 | 10.85 | 1.47 | 7.38 |
| 4. | <i>Alpinia purpurata</i> Roxb. | 8.75 | 14.00 | 3.50 | 3.50 | 7.14 | 3.50 | 2.04 |
| 5. | <i>Amorphophallus campanulatus</i> Blume | 12.25 | 17.50 | 3.50 | 3.50 | 14.70 | 3.50 | 4.20 |
| 6. | <i>Anthurium andraeanum</i> (Lind). | 12.25 | 24.50 | 3.50 | 3.50 | 17.85 | 3.50 | 5.10 |
| 7. | <i>Anthurium grande</i> (Lind). | 7.00 | 17.50 | 3.50 | 3.50 | 13.30 | 3.50 | 3.80 |
| 8. | <i>Antigonon leptopus</i> Endl. | 10.50 | 10.50 | 3.50 | 3.50 | 10.50 | 3.50 | 3.00 |
| 9. | <i>Asplenium nidus</i> Linn. | 10.50 | 12.25 | 3.50 | 3.50 | 11.20 | 3.50 | 3.20 |
| 10. | <i>Bauhinia purpurea</i> Linn. | 10.50 | 12.25 | 3.50 | 3.50 | 11.34 | 3.50 | 3.24 |
| 11. | <i>Begonia 'rex'</i> Linn. | 10.50 | 10.50 | 3.50 | 3.50 | 10.50 | 3.50 | 3.00 |
| 12. | <i>Begonia sachsen</i> Linn. | 7.00 | 10.50 | 4.38 | 5.25 | 8.40 | 4.73 | 1.78 |
| 13. | <i>Begonia semperflorens</i> Link. & Otto | 10.50 | 21.00 | 3.50 | 3.50 | 13.65 | 3.50 | 3.90 |
| 14. | <i>Bougainvillea spectabilis</i> Willd. | 8.75 | 10.50 | 3.50 | 4.38 | 9.45 | 4.03 | 2.34 |
| 15. | <i>Calathea acuminata</i> G.F.W.Mey. | 10.50 | 17.50 | 3.50 | 5.25 | 12.77 | 4.11 | 3.11 |
| 16. | <i>Calathea insignis</i> G.F.W.Mey. | 7.00 | 21.00 | 3.50 | 7.00 | 16.45 | 4.73 | 3.48 |
| 17. | <i>Calathea kegeliana</i> G.F.W.Mey. | 10.50 | 12.25 | 3.50 | 3.50 | 11.55 | 3.50 | 3.30 |
| 18. | <i>Calatea leuconeura</i> G.F.W.Mey. | 7.00 | 14.00 | 3.50 | 3.50 | 11.73 | 3.50 | 3.35 |
| 19. | <i>Calathea ornata</i> G.F.W.Mey. | 7.00 | 17.50 | 3.50 | 5.25 | 11.73 | 4.03 | 2.91 |
| 20. | <i>Calathea picturata</i> G.F.W.Mey. | 14.00 | 14.00 | 3.50 | 3.50 | 14.50 | 3.50 | 4.00 |
| 21. | <i>Calathea splendida</i> hort G.F.W.Mey. | 7.00 | 12.25 | 3.50 | 3.50 | 10.01 | 3.50 | 2.86 |
| 22. | <i>Calathea zebrina</i> G.F.W.Mey. | 12.25 | 15.75 | 3.50 | 3.50 | 14.00 | 3.50 | 4.00 |
| 23. | <i>Canna indica</i> Linn. | 10.50 | 10.50 | 3.50 | 3.50 | 10.50 | 3.50 | 3.00 |

Contd...

Table 8. (Contd...)

| Isolate No. | Name of Host Plant | Length µm | | Breadth µm | | Average length µm | Average breadth µm | Ratio (L/B) |
|-------------|---|--------------|---------|---------------|---------|----------------------|-----------------------|-------------|
| | | Minimum | Maximum | Minimum | Maximum | | | |
| 24. | <i>Chrysothemis pulchella</i> Linn. | 7.00 | 12.25 | 3.50 | 4.38 | 9.80 | 4.20 | 2.33 |
| 25. | <i>Colocasia antiquorum</i> Schott | 8.75 | 12.25 | 3.50 | 3.50 | 10.85 | 3.50 | 3.10 |
| 26. | <i>Costus sanguineus</i> Linn. | 8.75 | 12.25 | 3.50 | 3.50 | 10.85 | 3.50 | 3.10 |
| 27. | <i>Costus malortieanus</i> Linn. | 7.00 | 14.00 | 3.50 | 5.25 | 11.03 | 4.73 | 2.33 |
| 28. | <i>Dieffenbachia 'Exotica alba'</i> Schott | 7.00 | 14.00 | 3.50 | 5.25 | 10.68 | 4.46 | 2.39 |
| 29. | <i>Dieffenbachia maculata 'Rud Roehrs'</i> Schott | 8.75 | 14.00 | 3.50 | 4.38 | 11.20 | 3.68 | 3.04 |
| 30. | <i>Dioscorea alata</i> Linn. | 10.50 | 12.25 | 3.50 | 3.50 | 11.55 | 3.50 | 3.30 |
| 31. | <i>Dracaena deremensis</i> Linn. | 10.50 | 14.00 | 3.50 | 3.50 | 11.90 | 3.50 | 3.40 |
| 32. | <i>Dracaena fragrans 'Victoriae'</i> Linn. | 7.00 | 10.50 | 3.50 | 5.25 | 8.93 | 5.08 | 1.76 |
| 33. | <i>Dracaena godseffiana</i> Linn. | 8.75 | 10.50 | 3.50 | 4.38 | 9.45 | 4.03 | 2.34 |
| 34. | <i>Dracaena marginata</i> Linn. | 7.00 | 10.50 | 4.38 | 4.38 | 8.61 | 4.38 | 1.97 |
| 35. | <i>Dracaena reflexa</i> Linn. | 8.75 | 10.50 | 4.38 | 5.25 | 9.80 | 4.73 | 2.07 |
| 36. | <i>Dracaena sanderiana</i> Linn. | 10.50 | 14.00 | 3.50 | 3.50 | 11.90 | 3.50 | 3.40 |
| 37. | <i>Duranta plumieri</i> Linn. | 7.00 | 17.50 | 3.50 | 5.25 | 10.50 | 4.64 | 2.26 |
| 38. | <i>Eurycles amboinensis</i> Linn. | 8.75 | 14.00 | 3.50 | 3.50 | 11.55 | 3.50 | 3.30 |
| 39. | <i>Gerbera jamesonii</i> L.Bolus | 10.50 | 14.00 | 3.50 | 3.50 | 12.95 | 3.50 | 3.70 |
| 40. | <i>Hamelia patens</i> Jacq. | 7.00 | 17.50 | 3.50 | 5.25 | 11.20 | 4.73 | 2.37 |
| 41. | <i>Hedychium coronarium</i> Koenig | 8.75 | 10.50 | 3.50 | 3.50 | 9.80 | 3.50 | 2.80 |
| 42. | <i>Heliconia metallica</i> Linn. | 8.75 | 10.50 | 3.50 | 3.50 | 10.15 | 3.50 | 2.90 |
| 43. | <i>Hibiscus rosa-sinensis</i> Linn. | 8.75 | 12.25 | 3.50 | 3.50 | 10.85 | 3.50 | 3.10 |
| 44. | <i>Hippeastrum reticulatum</i> Herb. | 8.75 | 12.25 | 3.50 | 5.25 | 10.85 | 4.55 | 2.38 |
| 45. | <i>Hippeastrum</i> sp. | 10.50 | 17.50 | 3.50 | 3.50 | 13.65 | 3.50 | 3.90 |
| 46. | <i>Homalomena miamiensis</i> Schott | 7.00 | 8.75 | 3.50 | 3.50 | 8.05 | 3.50 | 2.30 |
| 47. | <i>Hydrangea hortensia</i> DC. | 8.75 | 14.00 | 3.50 | 4.38 | 10.50 | 3.85 | 2.72 |

Table 8. (Contd...)

| Isolate No. | Name of Host Plant | Length μm | | Breadth μm | | Average length μm | Average breadth μm | Ratio (L/B) |
|---------------|---|----------------------|---------|-----------------------|---------|------------------------------|-------------------------------|-------------|
| | | Minimum | Maximum | Minimum | Maximum | | | |
| 48. | <i>Hymenocallis lillioasfoetida</i> Salish. | 7.00 | 12.25 | 3.50 | 3.50 | 9.80 | 3.50 | 2.80 |
| 49. | <i>Iris tectorum</i> Max | 7.00 | 10.50 | 3.50 | 5.25 | 8.93 | 4.46 | 2.00 |
| 50. | <i>Ixora singaporensis</i> Linn. | 10.50 | 15.75 | 3.50 | 3.50 | 13.30 | 3.50 | 3.80 |
| 51. | <i>Kopsia fruticosa</i> A.DC. | 7.00 | 17.50 | 3.50 | 3.50 | 12.78 | 3.50 | 3.65 |
| 52. | <i>Lantana camara</i> Linn. | 8.75 | 10.56 | 4.38 | 4.38 | 9.45 | 4.38 | 2.16 |
| 53. | <i>Maranta arundinacea</i> 'Variegata' Linn. | 7.00 | 17.50 | 3.50 | 5.25 | 10.15 | 4.29 | 2.37 |
| 54. | <i>Mussaenda erythrophylla</i> Schum and Thonn. | 7.00 | 17.50 | 3.50 | 3.50 | 11.03 | 3.50 | 3.15 |
| 55. | <i>Neomarica gracilis</i> Linn. | 7.00 | 14.00 | 3.50 | 5.25 | 10.85 | 3.68 | 2.95 |
| 56. | <i>Ochma squarrosa</i> Linn. * | 8.75 | 10.50 | 4.38 | 4.38 | 9.45 | 4.38 | 2.16 |
| 57. | <i>Philodendron goldieana</i> Kunth. & Bouche. | 8.75 | 12.25 | 3.50 | 5.25 | 10.15 | 4.55 | 2.23 |
| 58. | <i>Pleomele reflexa</i> Linn. | 7.00 | 12.25 | 3.50 | 4.38 | 9.80 | 4.20 | 2.33 |
| 59. | <i>Plumeria acutifolia</i> Poir | 7.00 | 24.50 | 3.50 | 5.25 | 15.93 | 3.59 | 4.44 |
| 60. | <i>Polyscias paniculata</i> 'Variegata' Forst & Forst. f. | 8.75 | 12.25 | 3.50 | 3.50 | 10.85 | 3.50 | 3.10 |
| 61. | <i>Pothos scandens</i> Linn. | 14.00 | 17.50 | 3.50 | 4.38 | 15.75 | 3.68 | 4.28 |
| 62. | <i>Quisqualis indica</i> Linn. | 8.75 | 10.50 | 3.50 | 3.50 | 10.15 | 3.50 | 2.90 |
| 63. | <i>Sansieveria</i> 'Hahnii' | 7.00 | 14.00 | 5.25 | 5.25 | 10.85 | 5.25 | 2.07 |
| 64. | <i>Sansevieria trifasciata</i> Prain | 10.50 | 17.50 | 3.50 | 5.25 | 12.78 | 5.08 | 2.52 |
| 65. | <i>Spathiphyllum commutatum</i> Linn. | 10.50 | 17.50 | 5.25 | 5.25 | 11.55 | 5.25 | 2.20 |
| 66. | <i>Syngonium podophyllum</i> Linn. | 7.00 | 17.50 | 3.50 | 5.25 | 12.08 | 4.11 | 2.94 |
| 67. | <i>Tagetes erecta</i> Linn. | 7.00 | 21.00 | 5.25 | 5.25 | 14.00 | 3.50 | 4.00 |
| 68. | <i>Tecomaria capensis</i> (Thumb) Spach. | 7.00 | 8.75 | 3.50 | 3.50 | 8.05 | 3.50 | 2.30 |
| 69. | <i>Thunbergia erecta</i> Retz. | 10.50 | 14.00 | 3.50 | 3.50 | 12.25 | 3.50 | 3.50 |
| Grand average | | | | | | 11.14(L) | 3.80(B) | |

Out of 69 isolates studied, 35 had below the grand average length of 11.14 μ m and 41 isolates had below the grand average breadth of 3.8 μ m.

4.3.6 Protein estimation

The soluble protein content in different isolates of *C. gloeosporioides* was estimated on the fifth and eighth day of incubation and presented (Table 9).

The protein value ranged from 0.04 to 1.45 mg/l on the fifth day of growth and 0.10 to 1.45 mg/l on the eighth day of growth.

Isolates producing a protein value of 0.4 and below are considered as low protein producers; isolates producing a protein value ranging from 0.4 to 1 are considered as moderate protein producers and isolates producing a protein value of above 1 are considered as high protein producers. (Figs. 3 and 4).

Based on the protein production by different isolates the following three different groups of isolates could be distinguished.

Low protein producers on the fifth day of growth were isolates 1, 2, 5, 6, 7, 10, 17, 18, 19, 21, 24, 26, 31, 32, 33, 35, 38, 39, 40, 41, 43, 44, 52, 56, 58, 61, 62, 63, 67 and 68.

Low protein producers on the eighth day of growth, were isolates 3, 4, 6, 7, 8, 9, 10, 17, 23, 26, 28, 29, 32, 33, 34, 35, 37, 38, 41, 43, 47, 49, 51, 53, 54, 57, 60 and 63.

Table 9. Protein content of isolates

| Isolate No. | Name of Host Plant | Protein content | |
|-------------|---|-----------------|-----------------|
| | | 5th day mg/l | 8th day mg/l |
| 1. | <i>Acalypha hispida</i> Linn | 0.14 | 0.53 |
| 2. | <i>Aglaonema commutatum</i> Schott | 0.04 | 0.66 |
| 3. | <i>Allamanda cathartica</i> Linn. | 1.15 | 0.35 |
| 4. | <i>Alpinia purpurata</i> Roxb. | 1.36 | 0.40 |
| 5. | <i>Amorphophallus campanulatus</i> Blume | 0.04 | 0.66 |
| 6. | <i>Anthurium andraeanum</i> (Lind) Schott | 0.14 | 0.11 |
| 7. | <i>Anthurium grande</i> (Lind) Schott | 0.18 | 0.18 |
| 8. | <i>Antigonon leptopus</i> Endl. | 0.68 | 0.13 |
| 9. | <i>Asplenium nidus</i> Linn. | 0.68 | 0.40 |
| 10. | <i>Bauhinia purpurea</i> Linn. | 0.18 | 0.25 |
| 11. | <i>Begonia 'rex'</i> Putz. | 0.84 | 0.97 |
| 12. | <i>Begonia sachsen</i> Linn. | 1.20 | 1.00 |
| 13. | <i>Begonia semperflorens</i> Link. & Otto | 0.94 | 0.97 |
| 14. | <i>Bougainvillea spectabilis</i> Willd. | 0.68 | 1.29 |
| 15. | <i>Calathea acuminata</i> G.F.W.Mey. | 0.66 | 1.34 |
| 16. | <i>Calathea insignis</i> G.F.W.Mey. | 0.50 | 0.73 |
| 17. | <i>Calathea kegeliana</i> G.F.W.Mey. | 0.05 | 0.22 |
| 18. | <i>Calathea leuconeura</i> G.F.W.Mey. | 0.27 | 0.70 |
| 19. | <i>Calathea ornata</i> G.F.W.Mey. | 0.14 | 0.66 |
| 20. | <i>Calathea picturata</i> G.F.W.Mey. | 1.25 | 0.48 |
| 21. | <i>Calathea splendida 'hort'</i> G.F.W.Mey. | 0.26 | 0.71 |
| 22. | <i>Calathea zebrina</i> G.F.W.Mey. | 0.45 | 1.33 |
| 23. | <i>Canna indica</i> Linn. | 0.52 | 0.13 |
| 24. | <i>Chrysothemis pulchella</i> Linn. | 0.32 | 0.46 |
| 25. | <i>Colocasia antiquorum</i> Schott | 0.85 | 0.47 |
| 26. | <i>Costus sanguineus</i> Linn. | 0.08 | 0.19 |
| 27. | <i>Costus malortieanus</i> Linn. | 0.56 | 0.74 |
| 28. | <i>Dieffenbachia 'Exotica alba'</i> | 0.90 | 0.23 |
| 29. | <i>Dieffenbachia maculata 'Rud Roehrs'</i> Schott | 0.74 | 0.40 |
| 30. | <i>Dioscorea alata</i> Linn. | 0.94 | 0.74 |
| 31. | <i>Dracaena deremensis</i> Linn. | 0.28 | 0.73 |
| 32. | <i>Dracaena fragrans 'Victoriae'</i> Linn. | 0.24 | 0.30 |
| 33. | <i>Dracaena godseffiana</i> Linn. | 0.22 | 0.24 |
| 34. | <i>Dracaena marginata</i> Linn. | 0.66 | 0.19 |

Contd...

Table 9. (Contd...)

| Isolate No. | Name of Host Plant | Protein content | |
|-------------|---|-----------------|--------------|
| | | 5th day mg/l | 8th day mg/l |
| 35. | <i>Dracaena reflexa</i> Linn. | 0.20 | 0.13 |
| 36. | <i>Dracaena sanderiana</i> Linn. | 0.74 | 0.77 |
| 37. | <i>Duranta plumieri</i> Linn. | 0.94 | 0.10 |
| 38. | <i>Eurycles amboinensis</i> Linn. | 0.10 | 0.39 |
| 39. | <i>Gerbera jamesonii</i> L.Bolus | 0.06 | 0.84 |
| 40. | <i>Hamelia patens</i> Jacq. | 0.14 | 0.83 |
| 41. | <i>Hedychium coronarium</i> Koenig | 0.04 | 0.17 |
| 42. | <i>Heliconia metallica</i> Linn. | 0.85 | 0.63 |
| 43. | <i>Hibiscus rosa-sinensis</i> Linn. | 0.15 | 0.15 |
| 44. | <i>Hippeastrum reticulatum</i> Herb. | 0.15 | 1.45 |
| 45. | <i>Hippeastrum</i> sp. | 0.92 | 1.54 |
| 46. | <i>Homalomena miamiensis</i> Schott | 1.45 | 0.73 |
| 47. | <i>Hydrangea hortensia</i> DC. | 1.15 | 0.40 |
| 48. | <i>Hymenocallis lillioasafoetida</i> Salish. | 1.10 | 0.67 |
| 49. | <i>Iris tectorum</i> Max. | 1.44 | 0.06 |
| 50. | <i>Ixora singaporensis</i> Linn. | 0.68 | 0.61 |
| 51. | <i>Kopsia fruticosa</i> A.DC. | 0.58 | 0.38 |
| 52. | <i>Lantana camara</i> Linn. | 0.02 | 0.50 |
| 53. | <i>Maranta arundinacea</i> 'Variegata' Linn. | 0.86 | 0.17 |
| 54. | <i>Mussaenda erythrophylla</i> Schum and Thonn. | 0.50 | 0.16 |
| 55. | <i>Neomarica gracilis</i> Linn. | 0.65 | 0.67 |
| 56. | <i>Ochna squarrosa</i> Linn. | 0.05 | 0.94 |
| 57. | <i>Philodendron goldieana</i> Kunth. & Bouche | 0.84 | 0.17 |
| 58. | <i>Pleomele reflexa</i> Linn. | 0.28 | 1.21 |
| 59. | <i>Plumeria acutifolia</i> Poir | 0.61 | 0.91 |
| 60. | <i>Polyscias paniculata</i> 'Variegata' Forst & Forst. f. | 0.65 | 0.38 |
| 61. | <i>Pothos scandens</i> Linn. | 0.31 | 0.67 |
| 62. | <i>Quisqualis indica</i> Linn. | 0.26 | 0.93 |
| 63. | <i>Sansevieria</i> 'Hahnii' | 0.15 | 0.10 |
| 64. | <i>Sansevieria trifasciata</i> Prain | 0.74 | 0.93 |
| 65. | <i>Spathiphyllum commutatum</i> Linn. | 0.72 | 0.78 |
| 66. | <i>Syngonium podophyllum</i> Linn. | 0.75 | 0.75 |
| 67. | <i>Tagetes erecta</i> Linn. | 0.15 | 0.63 |
| 68. | <i>Tecomaria capensis</i> (Thumb) Spach. | 0.26 | 1.45 |
| 69. | <i>Thunbergia erecta</i> Retz. | 1.20 | 0.72 |

Moderate protein producers on the fifth day of growth were isolates, 8, 9, 11, 13, 14, 15, 16, 22, 23, 25, 27, 28, 29, 30, 34, 36, 37, 42, 45, 50, 51, 53, 54, 55, 57, 59, 60, 64, 65 and 66.

Moderate protein producers on eighth day of growth, were isolates, 1, 2, 5, 11, 12, 13, 16, 18, 19, 20, 21, 24, 25, 27, 30, 31, 36, 39, 40, 42, 46, 48, 50, 52, 55, 56, 59, 61, 62, 64, 65, 66, 67 and 69.

High protein producers on the fifth day of growth, were isolates, 3, 4, 12, 20, 46, 47, 48, 49 and 69

High protein producers on the eighth day of growth, were isolates, 14, 15, 22, 44, 45, 58 and 68

4.3.7 Enzyme activity

Enzyme activity such as poly phenol oxidase and peroxidase in different isolates of *C. gloeosporioides* was determined and are presented (Table 10).

4.3.7.1 Polyphenol oxidase

An initial study with ten isolates, showed that polyphenol oxidase activity was the maximum on the fifth day of incubation. Based on the above result, polyphenol oxidase activity of 69 isolates was determined on the fifth day of incubation and expressed as units/10² protein per minute. It ranged from 1.87 to 143.10 (Table 10 and Fig. 5). The isolates were grouped under low enzyme activity (0-10); moderate enzyme activity (11-30) and high enzyme activity (above 31) as shown hereunder.

Table 10. Enzyme activity of isolates

| Isolate No. | Name of Host Plant | Polyphenol | |
|----------------|---|------------|------------|
| | | oxidase | Peroxidase |
| 1. | <i>Acalypha hispida</i> Linn. | 48.99 | 0.24 |
| 2. | <i>Aglaonema commutatum</i> Schott | 143.10 | 0.10 |
| 3. | <i>Allamanda cathartica</i> Linn. | 2.11 | 1.86 |
| 4. | <i>Alpinia purpurata</i> Roxb. | 3.79 | 0.30 |
| 5. | <i>Amorphophallus campanulatus</i> Blume | 95.85 | 0.20 |
| 6. | <i>Anthurium andraeanum</i> (Lind.) Schott | 59.01 | 1.55 |
| 7. | <i>Anthurium grande</i> Lind. | 13.50 | 0.67 |
| 8. | <i>Antigonon leptopus</i> Endl. | 7.15 | 0.81 |
| 9. | <i>Asplenium nidus</i> Linn. | 5.52 | 0.53 |
| 10. | <i>Bauhinia purpurea</i> Linn. | 26.55 | 0.42 |
| 11. | <i>Begonia 'rex'</i> Linn. | 7.65 | 0.21 |
| 12. | <i>Begonia sachsen</i> Linn. | 3.78 | 0.09 |
| 13. | <i>Begonia semperflorens</i> Link. & Otto. | 7.14 | 0.29 |
| 14. | <i>Bougainvillea spectabilis</i> Willd. | 10.01 | 0.12 |
| 15. | <i>Calathea acuminata</i> G.F.W.Mey. | 10.15 | 0.09 |
| 16. | <i>Calathea insignis</i> G.F.W.Mey. | 8.64 | 0.23 |
| 17. | <i>Calathea kegeliana</i> G.F.W.Mey. | 96.66 | 0.55 |
| 18. | <i>Calathea leuconoura</i> G.F.W.Mey. | 17.50 | 0.22 |
| 19. | <i>Calathea ornata</i> G.F.W.Mey. | 43.59 | 0.22 |
| 20. | <i>Calathea picturata</i> G.F.W.Mey. | 5.51 | 0.18 |
| 21. | <i>Calathea splendida 'hort'</i> G.F.W.Mey. | 18.10 | 0.27 |
| 22. | <i>Calathea zebrina</i> G.F.W.Mey. | 7.74 | 0.32 |
| 23. | <i>Canna indica</i> Linn. | 7.11 | 0.96 |
| 24. | <i>Chrysothemis pulchella</i> Linn. | 20.93 | 0.11 |
| 25. | <i>Colocasia antiquorum</i> Schott. | 11.43 | 0.33 |
| 26. | <i>Costus sanguineus</i> Linn. | 74.93 | 0.84 |
| 27. | <i>Costus malortieanus</i> Linn. | 14.18 | 0.67 |
| 28. | <i>Dieffenbachia 'Exotica alba'</i> Schott | 3.63 | 0.72 |
| 29. | <i>Dieffenbachia maculata 'Rub Roehrs'</i> Schott | 6.93 | 0.31 |
| 30. | <i>Dioscorea alata</i> Linn. | 8.90 | 0.09 |
| 31. | <i>Dracaena deremensis</i> Linn. | 15.62 | 0.43 |
| 32. | <i>Dracaena fragrans Victoriae</i> Linn. | 18.90 | 0.38 |
| 33. | <i>Dracaena godseffiana</i> Linn. | 14.97 | 0.52 |
| 34. | <i>Dracaena marginata</i> Linn. | 6.91 | 0.43 |
| 35. | <i>Dracaena reflexa</i> Linn. | 28.35 | 2.58 |

Contd

Table 10. (Contd...)

| Isolate | | Polyphenol | |
|---------|---|------------|------------|
| No. | Name of Host Plant | oxidase | Peroxidase |
| 36. | <i>Dracaena sanderiana</i> Linn. | 6.20 | 0.14 |
| 37. | <i>Duranta plumieri</i> Linn. | 1.87 | 1.35 |
| 38. | <i>Eurycles amboinensis</i> Linn. | 40.77 | 0.24 |
| 39. | <i>Gerbera jamesonii</i> L.Bolus | 54.90 | 0.04 |
| 40. | <i>Hamelia patens</i> Jacq. | 25.84 | 0.22 |
| 41. | <i>Hedychium coronarium</i> Koenig | 120.15 | 0.62 |
| 42. | <i>Heliconia metallica</i> Linn. | 8.74 | 0.11 |
| 43. | <i>Hibiscus rosa-sinensis</i> Linn. | 28.80 | 1.27 |
| 44. | <i>Hippeastrum reticulatum</i> Herb. | 23.94 | 0.14 |
| 45. | <i>Hippeastrum</i> sp. | 8.31 | 0.08 |
| 46. | <i>Homalomena miamiensis</i> Schott | 3.44 | 0.27 |
| 47. | <i>Hydrangea hortensia</i> DC. | 4.39 | 0.26 |
| 48. | <i>Hymenocallis lillioasafotida</i> Salisb. | 7.81 | 0.31 |
| 49. | <i>Iris tectorum</i> Max. | 3.75 | 1.75 |
| 50. | <i>Ixora singaporensis</i> Linn. | 11.83 | 0.47 |
| 51. | <i>Kopsia fruticosa</i> A.DC. | 5.35 | 0.82 |
| 52. | <i>Lantana camara</i> Linn. | 25.65 | 0.17 |
| 53. | <i>Maranta arundinacea</i> 'Variegata' Linn. | 2.61 | 0.65 |
| 54. | <i>Mussaenda erythrophylla</i> Schum & Thonn. | 11.66 | 0.75 |
| 55. | <i>Neomarica gracilis</i> Linn. | 4.52 | 0.23 |
| 56. | <i>Ochna squarrosa</i> Linn. | 77.22 | 0.67 |
| 57. | <i>Philodendron goldieana</i> Kunth. & Bouche | 3.95 | 0.88 |
| 58. | <i>Pleomele reflexa</i> Linn. | 10.32 | 0.11 |
| 59. | <i>Plumeria acutifolia</i> Poir | 10.31 | 0.41 |
| 60. | <i>Polyscias paniculata</i> 'Variegata' Forst & Forst. f. | 3.82 | 0.34 |
| 61. | <i>Pothos scandens</i> Linn. | 12.11 | 0.31 |
| 62. | <i>Quisqualis indica</i> Linn. | 15.99 | 0.04 |
| 63. | <i>Sansevieria</i> 'Hahnii' | 39.15 | 0.76 |
| 64. | <i>Sansevieria trifasciata</i> Prain | 14.10 | 0.61 |
| 65. | <i>Spathiphyllum commutatum</i> Linn. | 5.03 | 0.17 |
| 66. | <i>Syngonium podophyllum</i> Linn. | 3.89 | 0.22 |
| 67. | <i>Tagetes erecta</i> Linn. | 18.72 | 0.19 |
| 68. | <i>Tecomaria capensis</i> (Thumb) Spach. | 15.47 | 0.06 |
| 69. | <i>Thunbergia erecta</i> Retz. | 2.95 | 0.14 |

Isolates under 'low polyphenol oxidase' were 3, 4, 8, 9, 11, 12, 13, 16, 20, 22, 23, 28, 29, 30, 34, 36, 37, 42, 45, 46, 47, 48, 49, 51, 53, 55, 57, 60, 65, 66 and 69.

Moderate polyphenol oxidase-isolates were 7, 10, 14, 15, 18, 21, 24, 25, 27, 31, 32, 33, 35, 40, 43, 44, 50, 52, 54, 58, 59, 61, 62, 64, 67 and 68.

High polyphenol oxidase-isolates were 1, 2, 5, 6, 17, 19, 26, 38, 39, 41, 56 and 63.

4.3.7.2 Peroxidase activity

An initial study with ten isolates showed that peroxidase activity was maximum on eighth day of incubation. Based on the above result, peroxidase activity of the 69 isolates was determined on the eighth day of incubation and expressed as units /10² protein per minute. The value ranged from 0.04 and 2.58 (Table 10 and Fig. 6). Based on the peroxidase activity, the isolates were grouped under low peroxidase activity (0.4 and below); moderate peroxidase activity (0.41 to 1) and high peroxidase activity (Above 1).

'Low peroxidase activity' isolates were 1, 2, 4, 5, 11, 12, 13, 14, 15, 16, 18, 19, 20, 21, 22, 24, 25, 29, 30, 32, 36, 38, 39, 40, 42, 44, 45, 46, 47, 48, 52, 55, 58, 60, 61, 62, 65, 66, 67, 68 and 69.

Moderate peroxidase activity isolates were 7, 8, 9, 10, 17, 23, 26, 27, 28, 31, 33, 34, 41, 50, 51, 53, 54, 56, 57, 59, 63 and 64.

High peroxidase activity isolates were 3, 6, 35, 37, 43 and 49.

4.4 Studies on the effect of environment

Effect of environmental conditions on the growth and sporulation of the isolates was studied. In field conditions four types of disease symptoms were noticed viz.,

1. Leaf spot with yellow halo
2. Leaf spot without yellow halo
3. Leaf blight with yellow halo
4. Leaf blight without yellow halo

The host plants were grouped based on the above classification and named as G₁, G₂, G₃ and G₄ respectively. For further studies two isolates from each group were selected. These were :

| | | | |
|----------------|------------------------------------|------------------|---------------|
| G ₁ | <i>Amorphophallus campanulatus</i> | - I ₂ | } Leaf spot |
| | <i>Dioscorea alata</i> | - I ₄ | |
| G ₂ | <i>Hydrangea hortensia</i> | - I ₆ | |
| | <i>Ixora singaporensis</i> | - I ₇ | |
| G ₃ | <i>Homalomena miamiensis</i> | - I ₅ | } Leaf blight |
| | <i>Philodendron goldieana</i> | - I ₈ | |
| G ₄ | <i>Anthurium andraeanum</i> | - I ₃ | |
| | <i>Aglaonema commutatum</i> | - I ₁ | |

4.4.1 Effect of temperature

Observations on colony diameter were recorded on the fourth, sixth and eighth day of inoculation of culture discs in Petri plates incubated at 15, 20, 27, 33 and 38°C. The measurements were analysed statistically. The temperature treatments, isolates and interaction of temperature and isolates were statistically significant.

The data of fourth, sixth and eighth day are presented in tables 11.1, 11.2 and 11.3 respectively. On the fourth day, the maximum growth was observed at 27°C and it was followed by growth at 20, 33 and 38°C. The growth at 20, 33 and 38°C were on par. The minimum growth was recorded at 15°C.

On the fourth day, the isolate I₆ from *H. hortensia* (1.777) recorded the maximum growth followed by I₄ from *D. alata* (1.743). The isolate I₇ from *I. singaporensis* (1.665) recorded the minimum growth.

Isolate I₅ from *H. miamiensis* recorded the maximum growth (2.302) at 27°C and it was followed by isolate I₆ from *H. hortensia* (2.295).

On the sixth day, the maximum (2.359) growth was recorded at 20°C followed by growth at 27°C (2.195). In this case also the minimum (1.381) growth was recorded at 15°C.

Isolate I₆ from *H. hortensia* recorded the maximum growth on sixth day also (2.056) and is followed by isolate I₄ from *D. alata* (2.031). I₃ from *A. andraeanum* recorded the minimum growth (1.868).

11.1 Effect of temperature on colony diameter (cm) of isolates on 4th day of inoculation

| Temperature | Colony diameter of isolates | | | | | | | | |
|-------------|-----------------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| | I ₁ | I ₂ | I ₃ | I ₄ | I ₅ | I ₆ | I ₇ | I ₈ | Mean |
| 15°C | 0.599 (1.264) | 0.533 (1.238) | 0.599 (1.264) | 0.566 (1.252) | 0.633 (1.278) | 0.666 (1.291) | 0.600 (1.265) | 0.566 (1.252) | 0.595 (1.263) |
| 20°C | 1.798 (1.673) | 1.698 (1.642) | 2.148 (1.774) | 1.963 (1.721) | 1.865 (1.693) | 2.299 (1.817) | 1.793 (1.671) | 1.958 (1.720) | 1.938 (1.714) |
| 27°C | 3.999 (2.236) | 3.500 (2.121) | 3.200 (2.049) | 4.100 (2.258) | 4.299 (2.302) | 4.265 (2.295) | 3.699 (2.168) | 3.532 (2.129) | 3.818 (2.195) |
| 33°C | 1.965 (1.722) | 2.298 (1.816) | 1.432 (1.560) | 2.033 (1.742) | 1.599 (1.612) | 2.199 (1.789) | 1.666 (1.633) | 2.317 (1.821) | 1.931 (1.712) |
| 38°C | 1.799 (1.673) | 2.298 (1.816) | 1.931 (1.712) | 2.033 (1.742) | 2.032 (1.741) | 1.866 (1.693) | 1.523 (1.588) | 1.198 (1.483) | 1.826 (1.681) |
| Mean | 1.938 (1.714) | 1.983 (1.727) | 1.796 (1.672) | 2.038 (1.743) | 1.976 (1.725) | 2.158 (1.777) | 1.772 (1.665) | 1.826 (1.681) | |

C.D Temperature = 0.0350

C.D Isolate = 0.0442

C.D Interaction = 0.0989

11.2. Effect of temperature on colony diameter (cm) of isolates on 6th day of inoculation

| Temperature | Colony diameter of isolates | | | | | | | | |
|-------------|-----------------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| | I ₁ | I ₂ | I ₃ | I ₄ | I ₅ | I ₆ | I ₇ | I ₈ | Mean |
| 15°C | 0.666 (1.291) | 0.899 (1.378) | 0.699 (1.303) | 0.666 (1.291) | 0.961 (1.400) | 0.797 (1.341) | 0.699 (1.304) | 2.021 (1.738) | 3.907 (1.381) |
| 20°C | 4.765 (2.401) | 4.931 (2.435) | 4.999 (2.449) | 4.732 (2.394) | 4.732 (2.394) | 4.366 (2.317) | 3.999 (2.236) | 4.058 (2.249) | 4.565 (2.359) |
| 27°C | 3.998 (2.236) | 3.499 (2.121) | 3.199 (2.049) | 4.099 (2.258) | 4.298 (2.302) | 4.264 (2.295) | 3.698 (2.168) | 3.531 (2.129) | 3.823 (2.221) |
| 33°C | 2.733 (1.932) | 2.687 (1.920) | 2.333 (1.826) | 3.295 (2.072) | 2.100 (1.761) | 3.433 (2.106) | 2.400 (1.844) | 2.820 (1.954) | 2.713 (1.927) |
| 38°C | 3.300 (2.074) | 3.300 (2.074) | 1.932 (1.713) | 3.566 (2.137) | 2.266 (1.807) | 3.932 (2.221) | 2.499 (1.870) | 2.600 (1.897) | 2.897 (1.974) |
| Mean | 2.948 (1.987) | 2.944 (1.986) | 2.489 (1.864) | 3.125 (2.031) | 2.736 (1.933) | 3.227 (2.056) | 2.549 (1.884) | 2.976 (1.994) | |

C.D Temperature = 0.0776

C.D Isolate = 0.0982

C.D Interaction = 0.2197

11.3. Effect of temperature on colony diameter (cm) of isolates on 8th day of inoculation

| Temperature | Colony diameter of isolates | | | | | | | | |
|-------------|-----------------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| | I ₁ | I ₂ | I ₃ | I ₄ | I ₅ | I ₆ | I ₇ | I ₈ | Mean |
| 15°C | 1.497 (1.580) | 1.233 (1.494) | 1.098 (1.448) | 1.493 (1.581) | 1.699 (1.643) | 1.297 (1.516) | 1.299 (1.516) | 0.796 (1.340) | 1.295 (1.575) |
| 20°C | 8.000 (3.000) | 8.166 (3.028) | 8.361 (3.060) | 7.833 (2.972) | 7.633 (2.938) | 7.599 (2.932) | 6.763 (2.786) | 7.065 (2.840) | 7.673 (2.945) |
| 27°C | 8.733 (3.120) | 9.000 (3.162) | 8.000 (3.000) | 9.000 (3.162) | 9.000 (3.162) | 9.000 (3.162) | 9.000 (3.162) | 9.000 (3.162) | 8.841 (3.137) |
| 33°C | 3.267 (2.666) | 3.133 (2.033) | 2.364 (1.834) | 3.825 (2.197) | 2.333 (1.826) | 5.465 (2.543) | 2.996 (1.999) | 4.288 (2.300) | 3.410 (2.100) |
| 38°C | 3.832 (2.198) | 5.132 (2.476) | 2.733 (1.932) | 5.366 (2.523) | 3.433 (2.105) | 6.363 (2.713) | 3.333 (2.082) | 3.700 (2.168) | 4.176 (2.275) |
| Mean | 4.726 (2.393) | 4.949 (2.439) | 4.085 (2.255) | 5.185 (2.487) | 4.452 (2.335) | 5.520 (2.573) | 4.331 (2.309) | 4.479 (2.362) | |

C.D Temperature = 0.0342

C.D Isolate = 0.0433

C.D Interaction = 0.0967

Isolate I₃ from *A. andraeanum* recorded the maximum growth at 20°C (2.449) followed by I₂ from *A. campanulatus* at 20°C (2.435). The minimum growth was noted in I₁ from *A. commutatum* at 15°C (1.291).

The data for the eighth day's growth also revealed that the maximum growth was observed at 27°C followed by growth at 20°C and the minimum at 15°C.

On the eighth day also, *H. hortensia* recorded the maximum growth (2.553) followed by *D. alata* (2.487). The minimum was recorded by *A. andraeanum* (2.255).

At 27°C, isolates I₂, I₄, I₅, I₇ and I₈ from *A. campanulatus*, *D. alata*, *H. miamiensis*, *H. hortensia*, *I. singaporensis* and *P. goldieana* respectively recorded the maximum growth (9cm).

4.4.2 Effect of relative humidity

The selected isolates were incubated at 75.6, 82.9, 92.9 and 100 per cent relative humidity maintained in desiccators. The colony diameter was measured on fourth, sixth and eighth day and presented in tables 12.1, 12.2 and 12.3 respectively. On the fourth day, the effect of isolates and interaction was found to be significantly different. Isolate I₂ from *A. campanulatus* recorded maximum growth (2.027) followed by isolate I₅ from *H. miamiensis* (2.012). The minimum growth was recorded by isolate I₃ from *A. andraeanum* (1.883).

12.1 Effect of relative humidity on colony diameter (cm) of isolates on 4th day of inoculation

| R.H. | Colony diameter of isolates | | | | | | | | |
|-------|-----------------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| | I ₁ | I ₂ | I ₃ | I ₄ | I ₅ | I ₆ | I ₇ | I ₈ | Mean |
| 100% | 2.731 (1.932) | 2.932 (1.983) | 2.400 (1.844) | 2.800 (1.949) | 3.400 (2.098) | 3.300 (2.074) | 2.896 (1.974) | 2.600 (1.897) | 2.877 (1.969) |
| 92.9% | 2.733 (1.932) | 2.700 (1.923) | 2.400 (1.843) | 2.630 (1.905) | 3.000 (2.000) | 2.998 (1.999) | 2.800 (1.949) | 3.329 (2.080) | 2.818 (1.954) |
| 82.9% | 2.832 (1.957) | 3.697 (2.167) | 2.500 (1.871) | 2.500 (1.871) | 2.800 (1.949) | 2.998 (1.999) | 2.866 (1.966) | 2.360 (1.833) | 2.810 (1.952) |
| 75.0% | 3.162 (2.040) | 3.132 (2.033) | 2.900 (1.975) | 3.400 (2.098) | 2.998 (1.999) | 2.833 (1.958) | 2.700 (1.924) | 2.091 (1.758) | 2.893 (1.973) |
| Mean | 2.861 (1.965) | 3.109 (2.027) | 2.546 (1.883) | 2.826 (1.956) | 3.048 (2.012) | 3.032 (2.008) | 2.814 (1.953) | 2.580 (1.892) | |

C.D Relative Humidity = 0.0274

C.D Isolate = 0.0388

C.D Interaction = 0.0775

12.2 Effect of relative humidity on colony diameter (cm) of isolates on 6th day of inoculation

| R.H. | Colony diameter of isolates | | | | | | | | |
|-------|-----------------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| | I ₁ | I ₂ | I ₃ | I ₄ | I ₅ | I ₆ | I ₇ | I ₈ | Mean |
| 100% | 5.630 (2.575) | 6.494 (2.738) | 5.498 (2.549) | 6.000 (2.646) | 5.233 (2.497) | 5.996 (2.645) | 5.896 (2.626) | 6.766 (2.787) | 5.933 (2.633) |
| 92.9% | 6.466 (2.732) | 6.533 (2.745) | 5.000 (2.449) | 6.099 (2.664) | 6.000 (2.646) | 6.000 (2.646) | 5.767 (2.601) | 6.433 (2.726) | 6.028 (2.651) |
| 82.9% | 6.300 (2.702) | 7.498 (2.915) | 5.267 (2.503) | 5.830 (2.614) | 6.229 (2.689) | 6.194 (2.682) | 5.700 (2.588) | 5.799 (2.607) | 6.091 (2.663) |
| 75.0% | 6.965 (2.822) | 7.200 (2.864) | 6.099 (2.665) | 6.999 (2.828) | 6.598 (2.756) | 5.899 (2.627) | 5.700 (2.588) | 6.200 (2.683) | 6.447 (2.729) |
| Mean | 6.333 (2.708) | 6.924 (2.815) | 5.462 (2.542) | 6.225 (2.688) | 6.007 (2.647) | 6.023 (2.650) | 5.765 (2.601) | 6.295 (2.701) | |

C.D Relative Humidity = 0.0257

C.D Isolate = 0.0364

C.D Interaction = 0.0728

12.3 Effect of relative humidity on colony diameter (cm) of isolates on 8th day of inoculation

| R.H. | Colony diameter of isolates | | | | | | | | |
|--------|-----------------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| | I ₁ | I ₂ | I ₃ | I ₄ | I ₅ | I ₆ | I ₇ | I ₈ | Mean |
| 100.0% | 6.000 (2.646) | 9.000 (3.162) | 6.500 (2.739) | 6.399 (2.720) | 5.532 (2.556) | 9.000 (3.162) | 9.000 (3.162) | 9.000 (3.162) | 7.491 (2.914) |
| 92.9% | 9.000 (3.162) | 9.000 (3.162) | 6.700 (2.775) | 9.000 (3.162) | 9.000 (3.162) | 9.000 (3.162) | 8.500 (3.082) | 9.000 (3.162) | 8.635 (3.104) |
| 82.9% | 9.000 (3.162) | 9.000 (3.162) | 7.000 (2.828) | 9.000 (3.162) | 9.000 (3.162) | 9.000 (3.162) | 9.000 (3.162) | 9.000 (3.162) | 8.741 (3.121) |
| 75.0% | 9.000 (3.162) | 9.000 (3.162) | 8.223 (3.037) | 9.000 (3.162) | 9.000 (3.162) | 9.000 (3.162) | 8.300 (3.050) | 9.000 (3.162) | 8.816 (3.133) |
| Mean | 8.199 (3.033) | 8.998 (3.162) | 7.094 (2.845) | 8.315 (3.052) | 8.066 (3.011) | 8.998 (3.162) | 8.697 (3.144) | 8.998 (3.162) | |

C.D Relative Humidity = 0.0137

C.D Isolate = 0.0194

C.D Interaction = 0.0387

R.H. 75.6 per cent recorded the maximum growth (1.973) followed by 100 per cent R.H., (1.969) and the minimum growth was noted in 82.9 per cent R.H (1.952).

Isolate I₂ from *A. companulatus* recorded the maximum growth at 82.9% R.H. (2.167) followed by isolate I₅ from *H. miamiensis* at 100 per cent R.H. and I₄ from *D. alata* at 75.6 per cent R.H. (2.098).

The minimum growth (1.758) was recorded for *P. goldieana* isolate at 75.6 per cent R.H.

The data for the sixth day showed significant difference in growth for different isolates at different R.H.

The maximum growth was recorded at 75.6 per cent R.H. (2.729) followed by 82.9 per cent R.H. (2.663). The least growth was recorded at 100 per cent R.H. (2.633).

The growth of isolate I₂ from *A. campanulatus* (2.815) was the maximum followed by I₁ from *A. commutatum* (2.708). The minimum growth was recorded for isolate I₃ from *A. andraeanum* (2.542).

The maximum growth was recorded for isolate I₂ from *A. companulatus* at 82.9 per cent R.H. (2.915) followed by growth of the same isolate at 75.6 per cent R.H. (2.864).

The data for eighth day also showed significant difference for different isolates, different R.H. and interaction. The maximum growth was recorded at 75.6 per cent R.H. (3.133) followed by 82.9 per cent R.H.

(3.121). Growth of isolate I₂ from *A. companulatus*, I₆ from *H. hortensia* and I₈ from *P. goldieana* recorded the maximum (3.162). The minimum was recorded for isolate I₃ from *A. andraeanum* (2.845).

4.4.3 Effect of light

The selected isolates were incubated at different light treatments namely continuous light for 24 hours, (125 lux) alternative light and darkness for 12 hours each and complete darkness for 24 hours per day for eight days. The colony diameter was measured at fourth, sixth and eighth days (Tables 13.1, 13.2 and 13.3), and analysed statistically. The effect of light was not significant on fourth day though it was significant on sixth and eighth days. The isolates were found to be significantly different at fourth, sixth and eighth days.

On the fourth day I₂ from *A. companulatus* recorded the maximum growth (2.023) followed by growth of I₆ from *H. hortensia* (1.971). The least growth was recorded for I₈ from *P. goldieana*. (1.752). *A. campanulatus* recorded the maximum growth at complete darkness (2.121) followed by *H. hortensia* at continuous light (2.025).

The data on the sixth day showed that the effect of light, isolates and interactions was highly significant. Continuous light recorded the maximum growth (2.623) while alternate light and darkness recorded the least growth (2.544).

Isolate I₂ from *A. companulatus* recorded the maximum growth (2.719) followed by isolate I₄ from *D. alata* (2.659). Isolate I₈ from *P. goldieana* recorded the least growth (2.482).

13.1 Effect of light on colony diameter (cm) of isolates on 4th day of inoculation

| LIGHT | Colony diameter of isolates | | | | | | | | |
|------------------------------|-----------------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| | I ₁ | I ₂ | I ₃ | I ₄ | I ₅ | I ₆ | I ₇ | I ₈ | Mean |
| Alternate light and darkness | 2.496 (1.869) | 3.000 (2.000) | 2.667 (1.915) | 3.063 (2.016) | 1.984 (1.727) | 2.666 (1.915) | 1.761 (1.662) | 2.733 (1.932) | 2.534 (1.880) |
| Complete light | 2.200 (1.789) | 2.798 (1.949) | 2.000 (1.732) | 2.700 (1.924) | 2.767 (1.941) | 3.100 (2.025) | 2.400 (1.844) | 2.033 (1.742) | 2.489 (1.868) |
| Complete darkness | 2.332 (1.825) | 3.500 (2.121) | 2.699 (1.923) | 2.599 (1.897) | 3.000 (2.000) | 2.899 (1.975) | 2.965 (1.991) | 1.500 (1.581) | 2.663 (1.914) |
| Mean | 2.242 (1.828) | 3.093 (1.023) | 2.448 (1.857) | 2.783 (1.945) | 2.568 (1.889) | 2.885 (1.971) | 2.356 (1.832) | 2.070 (1.752) | |

C D Light = 0.0598
 C.D Isolate = 0.0977
 C.D Interaction = 0.1692

13.2 Effect of light on colony diameter (cm) of isolates on 6th day of inoculation

| LIGHT | Colony diameter of isolates | | | | | | | | |
|------------------------------|-----------------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| | I ₁ | I ₂ | I ₃ | I ₄ | I ₅ | I ₆ | I ₇ | I ₈ | Mean |
| Alternate light and darkness | 5.324 (2.515) | 5.388 (2.527) | 5.522 (2.554) | 5.755 (2.599) | 4.193 (2.279) | 5.824 (2.612) | 5.463 (2.542) | 6.397 (2.720) | 5.472 (2.544) |
| Complete light | 5.967 (2.639) | 6.364 (2.714) | 5.623 (2.573) | 6.466 (2.732) | 5.899 (2.627) | 5.932 (2.633) | 5.628 (2.575) | 5.200 (2.490) | 5.880 (2.623) |
| Complete darkness | 6.265 (2.695) | 7.499 (2.915) | 5.798 (2.607) | 6.000 (2.646) | 5.899 (2.627) | 5.899 (2.627) | 5.700 (2.588) | 4.000 (2.236) | 5.854 (2.618) |
| Mean | 5.849 (2.617) | 6.393 (2.719) | 5.646 (2.578) | 6.070 (2.659) | 5.305 (2.511) | 5.885 (2.624) | 5.595 (2.568) | 5.160 (2.482) | |

C.D Light = 0.0634
 C.D Isolate = 0.1036
 C.D Interaction = 0.1794

13.3 Effect of light on colony diameter (cm) of isolates on 8th day of inoculation

| LIGHT | Colony diameter of isolates | | | | | | | | |
|------------------------------|-----------------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| | I ₁ | I ₂ | I ₃ | I ₄ | I ₅ | I ₆ | I ₇ | I ₈ | Mean |
| Alternate light and darkness | 9.000 (3.162) | 8.660 (3.108) | 8.127 (3.021) | 7.941 (2.990) | 6.283 (2.699) | 7.428 (2.903) | 7.347 (2.889) | 8.660 (3.108) | 7.910 (2.985) |
| Complete light | 8.732 (3.120) | 9.000 (3.162) | 8.000 (3.000) | 9.000 (3.162) | 8.500 (3.082) | 9.000 (3.162) | 9.000 (3.162) | 9.000 (3.162) | 8.778 (3.127) |
| Complete darkness | 9.000 (3.162) | 9.000 (3.162) | 7.600 (2.933) | 9.000 (3.162) | 9.000 (3.162) | 9.000 (3.162) | 9.000 (3.162) | 6.000 (2.646) | 8.419 (3.069) |
| Mean | 8.910 (3.148) | 8.885 (3.144) | 7.910 (2.985) | 8.641 (3.105) | 7.886 (2.981) | 8.462 (3.076) | 8.431 (3.071) | 7.833 (2.972) | |

C.D Light = 0.0778
 C.D Isolate = 0.1271
 C.D Interaction = 0.2202

Isolate I₂ from *A. companulatus* under complete darkness produced the maximum growth (2.915) followed by isolate I₄ from *D. alata* under continuous light treatment (2.732).

The data for the eighth day showed that effect of light, isolates and interactions was significant. The maximum growth was observed in continuous light (3.127). Growth in continuous light and complete darkness was on par and significantly superior to alternate light and darkness.

Among the isolates, isolate I₁ from *A. commutatum* recorded the maximum growth (3.148) followed by isolate I₂ from *A. campanulatus* (3.144). Growth of isolate I₈ from *P. goldieana* was the least (2.646).

All the isolates under continuous light except I₅ (*H. miamiensis*), I₁ (*A. commutatum*) and I₃ (*A. andraeanum*) produced the maximum growth (9cm). All isolates except those from *A. andraeanum* and *P. goldieana* under I₃ (complete darkness) also produced the maximum growth (9cm).

4.5 Growth and sporulation of selected isolates

The variability in growth rate and degree of sporulation of eight selected isolates was studied on different solid and liquid media, under different relative humidity and under different periods of light.

4.5.1 Growth on solid media

The variability in growth rate of eight selected isolates was studied on oat meal agar (OMA), potato dextrose agar (PDA) and malt extract agar (MEA).



(i) Growth on oat meal agar

The growth rate of isolate No. 2 obtained from *A. campanulatus* and 4 from *D. alata* was the maximum (6.9 mm) followed by isolate No. 1 obtained from *A. commutatum* and No. 3 from *A. adraeanum* (6.6 mm). The minimum growth rate was exhibited by isolate No. 8 obtained from *P. goldieana* (4.9 mm) (Table 14).

(ii) Growth on potato dextrose agar

The growth rate of different isolates varied from 6.2 to 8.8 mm (Table 14). The growth rate was the maximum in isolate No. 1 obtained from *A. commutatum* (8.8 mm) and the minimum in isolate No. 8 from *P. goldieana* (6.2 mm).

(iii) Growth on malt extract agar

The rate of growth of different isolates varied from 5.5 to 7.9 mm (Table 14). The growth rate of the isolate No.2 obtained from *A. campanulatus* was the maximum followed by I₃ and I₄ from *A. andraeanum* and *D. alata* respectively (7.3 mm). The minimum growth rate was observed in isolate No. 8 from *P. goldieana* (5.5 mm).

There was variation in the rate of growth between isolates and also between media. All the isolates showed the maximum growth on PDA.

4.5.2 Growth in liquid media

The variability of the growth rate of the eight selected isolates was studied in Coons, Richard's, Basal and potato dextrose media.

Table 14. Growth of selected isolates in different solid media

| Isolate obtained from | Media | Growth | | | Rate of growth (cm/day) |
|------------------------------------|-------|-----------------|-----------------|-----------------|----------------------------|
| | | 4th day (cm) | 6th day (cm) | 8th day (cm) | |
| <i>Aglaonema commutatum</i> | | | | | |
| | OMA | 2.0 | 4.8 | 9.0 | 0.66 |
| | PDA | 4.3 | 7.8 | 9.0 | 0.88 |
| | MEA | 3.0 | 5.3 | 9.0 | 0.72 |
| <i>Amorphophallus campanulatus</i> | | | | | |
| | OMA | 2.5 | 5.0 | 9.0 | 0.69 |
| | PDA | 3.9 | 6.3 | 9.0 | 0.80 |
| | MEA | 3.7 | 6.3 | 9.0 | 0.79 |
| <i>Anthurium andraeanum</i> | | | | | |
| | OMA | 2.5 | 4.5 | 9.0 | 0.66 |
| | PDA | 3.7 | 7.0 | 9.0 | 0.82 |
| | MEA | 2.9 | 6.3 | 8.2 | 0.73 |
| <i>Dioscorea alata</i> | | | | | |
| | OMA | 2.6 | 5.1 | 8.8 | 0.69 |
| | PDA | 3.0 | 5.5 | 9.0 | 0.73 |
| | MEA | 2.9 | 5.5 | 9.0 | 0.73 |
| <i>Homalomena miamiensis</i> | | | | | |
| | OMA | 2.8 | 4.9 | 7.3 | 0.63 |
| | PDA | 2.8 | 5.0 | 8.5 | 0.68 |
| | MEA | 2.8 | 4.8 | 7.3 | 0.62 |
| <i>Hydrangea hortensia</i> | | | | | |
| | OMA | 2.4 | 5.0 | 8.0 | 0.64 |
| | PDA | 3.0 | 5.5 | 9.0 | 0.73 |
| | MEA | 2.5 | 4.6 | 8.2 | 0.64 |
| <i>Ixora singaporensis</i> | | | | | |
| | OMA | 1.9 | 6.3 | 7.0 | 0.63 |
| | PDA | 3.0 | 5.1 | 9.0 | 0.71 |
| | MEA | 3.0 | 6.0 | 8.0 | 0.70 |
| <i>Philodendron goldieana</i> | | | | | |
| | OMA | 2.0 | 3.0 | 6.8 | 0.49 |
| | PDA | 3.5 | 5.4 | 6.0 | 0.62 |
| | MEA | 2.5 | 3.6 | 7.0 | 0.55 |

OMA - Oatmeal agar

PDA - Potato dextrose agar

MEA - Malt extract agar

The growth of the isolates in liquid media varied between isolates (Table 15). Richard's medium supported the maximum growth. It was observed that the maximum growth was exhibited by *H. miamiensis* in Coon's medium (0.959g) followed by *P. goldieana* (0.915g). Minimum growth was reported by isolate 3 from *A. andraeanum* (0.0705g) in Basal medium.

Table 15. Mycelial weight of isolates grown in different liquid media

| Isolate obtained from | Liquid Media | | | | |
|------------------------------------|--------------|-----------|--------|----------------------------|--------|
| | Coons | Richard's | Basal | Potato dextrose broth (mg) | Mean |
| <i>Aglaonema commutatum</i> | 0.2131 | 0.6110 | 0.1331 | 0.3940 | 0.3378 |
| <i>Amorphophallus campanulatus</i> | 0.1465 | 0.4990 | 0.0950 | 0.3010 | 0.2604 |
| <i>Anthurium andraeanum</i> | 0.1052 | 0.2190 | 0.0705 | 0.3930 | 0.1969 |
| <i>Dioscorea alata</i> | 0.7180 | 0.3320 | 0.0907 | 0.5280 | 0.4171 |
| <i>Homalomena miamiensis</i> | 0.9590 | 0.4650 | 0.1084 | 0.4280 | 0.4901 |
| <i>Hydrangea hortensia</i> | 0.1601 | 0.5600 | 0.1541 | 0.4260 | 0.3251 |
| <i>Ixora singaporensis</i> | 0.1550 | 0.5680 | 0.1093 | 0.3940 | 0.3066 |
| <i>Philodendron goldieana</i> | 0.9150 | 0.3500 | 0.8520 | 0.3860 | 0.6258 |

4.5.3 Sporulation of selected isolates

Sporulation of selected isolates varied considerably when grown under different relative humidity, under different periods of light and in different liquid media.

4.5.3.1 Sporulation under different relative humidity

The degree of sporulation of selected isolates varied when grown under different relative humidity (Table 16). The variation in sporulation under different relative humidity was statistically significant. The highest sporulation was observed at 92.9 per cent relative humidity followed by 75.6 per cent relative humidity. The least sporulation was recorded at 100 per cent relative humidity.

Among the isolates I₅ obtained from *H. miamiensis* (1.405) exhibited the highest sporulation followed by I₈ from *P. goldieana* (1.145). The least sporulation was observed in isolate 6 from *H. hortensia* (0.782).

The highest sporulation was recorded in *P. goldieana* at 75.6 per cent R.H. (2.0687) followed by I₇ from *Ixora singaporensis* (1.9978) under 82.9 per cent R.H. The least sporulation was noted in I₆ from *H. hortensia* and I₈ from *P. goldieana* under 100 per cent R.H.

4.5.3.2 Sporulation under different light periods

The rate of sporulation of selected isolates varied when exposed under different periods of light (Table 17). The effect of light on sporulation when exposed at different periods was statistically significant.

Table 16. Degree of sporulation (number of conidia) of selected isolates under different relative humidity

| Isolate obtained from | Number of conidia per microscopic field | | | | |
|------------------------------------|---|---------------------|---------------------|----------------------|--------------------|
| | RH 100% | RH 92.9% | RH 82.9% | RH 75.6% | RH Mean |
| <i>Aglaonema commutatum</i> | 6.2144 (0.7934) | 13.9766 (1.1454) | 9.8651 (0.9941) | 27.6694 (1.442) | 12.4165 (1.094) |
| <i>Amorphophallus campanulatus</i> | 2.8847 (0.4601) | 8.9640 (0.9525) | 7.9579 (0.9008) | 7.9579 (0.9008) | 6.3680 (0.804) |
| <i>Anthurium andraeanum</i> | 1.7923 (0.2534) | 6.9518 (0.8421) | 12.6038 (1.1005) | 11.8878 (1.0751) | 6.5917 (0.819) |
| <i>Dioscorea alata</i> | 17.9267 (1.2535) | 13.6584 (1.1354) | 13.9766 (1.1454) | 17.0216 (1.1231) | 14.5881 (1.164) |
| <i>Homalomena miamiensis</i> | 9.253 (0.9663) | 70.9088 (1.8507) | 36.7744 (1.5536) | 17.4944 (1.2429) | 25.4097 (1.405) |
| <i>Hydrangea hortensia</i> | 1.000 (0.00) | 42.5991 (1.6294) | 1.5874 (0.2007) | 19.8343 (1.2996) | 6.0534 (0.782) |
| <i>Ixora singaporensis</i> | 1.2598 (0.1003) | 41.0393 (1.6132) | 99.4947 (1.9978) | 27.2145 (1.4348) | 19.3642 (1.287) |
| <i>Philodendron goldieana</i> | 1.000 (0.000) | 65.8415 (1.8185) | 4.9329 (0.6931) | 117.1385 (2.0687) | 13.9637 (1.145) |
| Mean | 3.0130 (0.479) | 23.6048 (1.373) | 11.857 (1.074) | 21.0863 (1.324) | |

C.D R.H. = 0.0521

C.D Isolate = 0.0736

C.D Interaction = 0.1473

Table 17. Degree of sporulation (number of conidia) of selected isolates under different periods of light

| Isolate obtained from | Number of conidia per microscopic field | | | |
|------------------------------------|---|----------------------------|---------------------|--------------------|
| | Normal light | Continuous light (125 lux) | Darkness | Mean |
| <i>Aglaonema commutatum</i> | 5.1928 (0.7154) | 47.0760 (1.6728) | 3.1747 (0.5017) | 9.1833 (0.963) |
| <i>Amorphophallus campanulatus</i> | 8.1433 (0.9108) | 22.9403 (1.3606) | 3.1747 (0.5017) | 8.3946 (0.924) |
| <i>Anthurium andraeanum</i> | 4.3793 (0.6414) | 11.5478 (1.0625) | 32.8398 (1.5164) | 11.8304 (1.073) |
| <i>Dioscorea alata</i> | 25.2058 (1.4015) | 30.3319 (1.4819) | 11.0357 (1.0428) | 20.3704 (1.309) |
| <i>Homalomena miamiensis</i> | 13.2770 (1.1231) | 7.2694 (0.8615) | 8.7599 (0.9425) | 9.4624 (0.976) |
| <i>Hydrangea hortensia</i> | 71.3674 (1.8535) | 47.2172 (1.6741) | 7.1138 (0.8521) | 28.8403 (1.460) |
| <i>Ixora singaporensis</i> | 13.3906 (1.1268) | 72.5605 (1.8607) | 5.2420 (0.7195) | 17.2187 (1.236) |
| <i>Philodendron goldieana</i> | 29.9020 (1.4757) | 106.6105 (2.0278) | 21.9381 (1.3412) | 41.2098 (1.615) |
| Mean | 14.3219 (1.156) | 31.6228 (1.500) | 8.4528 (0.927) | |

C.D Light = 0.0655

C.D Isolate = 0.1069

C.D Interaction = 0.1852

The highest sporulation was recorded when exposed for 24 h of continuous light 125 lux (1.500) and the least when exposed continuously under darkness for 24 h (0.927).

Among the isolates I₈ from *P. goldieana* was the highest in spore production (1.615) followed by I₆ from *H. hortensia* (1.460). The least sporulation was noted in I₂ from *A. campanulatus* (0.924).

The highest sporulation was recorded in isolate No.8 obtained from *P. goldieana* when exposed for 24 h of continuous light (2.0278) followed by I₇ from *I. singaporensis* (1.8607). The sporulation was poor in isolate No.1 obtained from *Aglanema commutatum* and I₂ from *A. campanulatus* when grown in darkness continuously for 24 hours.

Statistical analysis of the data also showed that there was no significant difference between isolates Nos. 2, 1 and 5 which were on par. It was also observed that full bright light was highly essential for the maximum sporulation.

4.5.3.3 Sporulation in different liquid media

The degree of sporulation of the isolates varied when grown in different media (Table 18) and was statistically significant. Among the media tested basal medium was found to favour high spore production (1.193) while sporulation was poor in Coon's medium (0.832).

Isolate No.3 obtained from *A. andraeanum* showed the highest (1.442) spore production followed by I₆ from *H. hortensia* (1.240). The least sporulation was observed in I₅ from *H. miamiensis* (0.387).

Table 18. Degree of sporulation (number of conidia) of selected isolates in different liquid media

| Isolate obtained from | Number of conidia per microscopic field | | | |
|------------------------------------|---|---------------------|---------------------|--------------------|
| | Coon's medium | Richard's medium | Basal medium | Mean |
| <i>Aglaonema commutatum</i> | 37.9665 (1.5794) | 11.2928 (1.0528) | 5.2420 (0.7195) | 13.0918 (1.117) |
| <i>Amorphophallus campanulatus</i> | 5.7690 (0.7611) | 15.9184 (1.2019) | 41.8794 (1.6220) | 15.6675 (1.195) |
| <i>Anthurium andraeanum</i> | 19.9343 (1.2996) | 27.8099 (1.4420) | 38.2472 (1.5826) | 27.6694 (1.442) |
| <i>Dioscorea alata</i> | 7.9579 (0.9008) | 7.1138 (0.8521) | 19.0502 (1.2799) | 10.2565 (1.011) |
| <i>Homalomena miamiensis</i> | 1.0000 (0.000) | 3.1747 (0.5017) | 4.5793 (0.6608) | 2.4378 (0.387) |
| <i>Hydrangea hortensia</i> | 8.9640 (0.9525) | 19.1646 (1.2825) | 30.4509 (1.4836) | 17.3780 (1.240) |
| <i>Ixora singaporensis</i> | 6.3168 (0.8005) | 7.2694 (0.8615) | 23.9442 (1.3792) | 10.3276 (1.014) |
| <i>Philodendron goldieana</i> | 2.2893 (0.3597) | 48.6071 (1.6867) | 6.6039 (0.8198) | 9.0157 (0.955) |
| Mean | 6.7920 (0.832) | 12.8825 (1.110) | 15.5955 (1.193) | |

C.D Media = 0.0509

C.D Isolate = 0.0832

C.D Interaction = 0.1440

The highest sporulation was observed in I₈ from *P. goldieana* in Richards medium (1.6867) followed by I₂ from *A. campanulatus* in basal medium (1.6220). The least sporulation was observed with I₅ from *H. miamiensis* in Coon's medium.

4.6 Host range studies

Host range studies with eight isolates selected from G₁, G₂, G₃, G₄ groups on 24 host plants indicated that all the eight isolates exhibited varying degrees of infection (Table 19). However, 76-100 per cent infection was recorded on *Mussaenda erythrophylla* by all isolates except I₆ obtained from *Hydrangea hortensia*.

Isolates I₁, I₆ and I₇ obtained from *Aglaonema commutatum*, *H. hortensia* and *Ixora singaporensis* could produce the maximum infection on *Hibiscus rosasinensis*. The same level of infection was produced by isolates I₂, I₄ and I₈ obtained from *Amorphophallus campanulatus*, *Dioscorea alta* and *Philodendron goldieana* on *Sauropus androgynous*. The maximum infection was observed in *Piper nigrum* by the isolates I₅ and I₈ obtained from *Homalomena miamiensis* and *P. goldieana* respectively. On *Talinium triangulare* also same level of infection was produced by the isolate I₃ and I₈ obtained from *Anthurium andraeanum* and *P. goldieana* respectively.

Infection of 76-100 per cent was observed on *Amaranthus gangeticus* and *Mangifera indica* produced by only one isolate viz. I₁ from *A. commutatum* and I₂ from *A. campanulatus* respectively.

Table 19. Host range

| Sl. No. | Hosts | Isolate obtained from | | | | | | | |
|---------|--|-----------------------------|------------------------------------|-----------------------------|------------------------|------------------------------|----------------------------|----------------------------|-------------------------------|
| | | <i>Aglaonema commutatum</i> | <i>Amorphophallus campanulatus</i> | <i>Anthurium andraeanum</i> | <i>Dioscorea alata</i> | <i>Homalomena miamiensis</i> | <i>Hydrangea hortensia</i> | <i>Ixora singaporensis</i> | <i>Philodendron goldieana</i> |
| 1. | <i>Amaranthus gangeticus</i> Linn. | ++++ | - | + | ++ | +++ | + | +++ | + |
| 2. | <i>Anthurium grande</i> Lind. | + | + | - | + | +++ | - | + | ++ |
| 3. | <i>Artocarpus integrifolia</i> Linn. | - | - | ++ | +++ | ++ | ++ | + | - |
| 4. | <i>Bauhinia purpurea</i> Linn. | +++ | +++ | ++ | + | ++ | + | ++ | ++ |
| 5. | <i>Canna indica</i> Linn. | + | ++ | ++ | ++ | ++ | + | ++ | +++ |
| 6. | <i>Capsicum annuum</i> Linn. | + | ++ | + | ++ | ++ | + | + | + |
| 7. | <i>Carica papaya</i> Linn. | +++ | + | ++ | ++ | ++ | - | ++ | + |
| 8. | <i>Cinnamomum zeylanicum</i> Breyn. | + | + | + | + | + | + | + | + |
| 9. | <i>Citrus aurantifolia</i> (Christm.) Swingle | ++ | - | ++ | + | + | + | ++ | - |
| 10. | <i>Colocasia antiquorum</i> Schott | + | +++ | ++ | + | + | + | ++ | ++ |
| 11. | <i>Eugenia caryophyllata</i> Thumb | ++ | ++ | +++ | +++ | + | + | + | ++ |
| 12. | <i>Hibiscus rosa-senensis</i> Linn. | ++++ | - | - | ++ | - | ++++ | ++++ | ++ |
| 13. | <i>Jasminum sambae</i> (Linn.) Ait. | - | + | +++ | - | - | - | +++ | - |
| 14. | <i>Lycopersicon esculentum</i> Mill | + | +++ | +++ | +++ | +++ | +++ | + | ++ |
| 15. | <i>Mangifera indica</i> Linn. | + | ++++ | +++ | + | + | ++ | + | + |
| 16. | <i>Mirabilis jalapa</i> Linn. | +++ | - | +++ | + | +++ | +++ | +++ | ++ |
| 17. | <i>Mussaenda erythrophylla</i> Schum. & Thonn. | ++++ | ++++ | ++++ | ++++ | ++++ | +++ | ++++ | ++++ |
| 18. | <i>Myristica fragrans</i> Houtt | + | + | + | + | + | + | ++ | ++ |
| 19. | <i>Piper nigrum</i> Linn. | +++ | + | - | ++ | ++++ | ++ | ++ | ++++ |
| 20. | <i>Psidium guajava</i> Linn. | + | ++ | ++ | ++ | ++ | + | ++ | ++ |
| 21. | <i>Sauropus androgynous</i> Merrill | +++ | ++++ | +++ | ++++ | - | - | - | ++++ |
| 22. | <i>Solanum melongena</i> Linn. | + | ++ | +++ | + | - | +++ | + | ++ |
| 23. | <i>Talinum triangulare</i> Willd. | +++ | +++ | +++ | +++ | - | - | - | ++++ |
| 24. | <i>Theobroma cacao</i> Linn | +++ | ++ | + | + | -- | -- | +++ | ++ |

- : No infection

++++ : 76-100% infection

+++ : 51-75% infection

++ : 26-50% infection

+ : 1-25% infection

In *Mirabilis jalapa* 51-75 per cent infection was produced by isolates I₁, I₃, I₅, I₆ and I₇ from *A. commutatum*, *A. andraeanum*, *H. miamiensis*, *H. hortensia* and *I. singaporensis*. Infection of the same level was observed on *Lycopersicon esculentum* by I₂, I₃, I₄, I₅ and I₆.

Infection of 51-75% was observed on *A. gangeticus*, *Bauhinia purpurea*, *Eugenia caryophyllata*, *Jasminum sambac*, *S. androgynous*, *Solanum melongena* and *Theobroma cacao* by any of the two isolates. Hosts namely *Colocasia antiquorum*, *Carica papaya*, *Artocarpus integrifolia*, *Mangifera indica* and *Canna indica* exhibited 51-75% infection by any one of the isolates.

Psidium guajava indicated 26-50% infection by all isolates except I₁ obtained from *A. commutatum* and I₆ from *H. hortensia*.

Infection of 1-25% was recorded by all the isolates in *Cinnamomum zeylanicum* followed by *Miristica fragrans* where all the isolates except I₇ showed 1-25% infection:

Only isolates I₂, I₃ and I₇ could produce infection on *J. sambac*. No infection was recorded on *T. triangulare* and *S. androgynous* with three isolates viz. I₅, I₆ and I₇.

4.6.1 Cross inoculation studies

Cross inoculation studies indicated that the isolate I₆ showed varying degrees of infection in all the hosts except *Dioscorea alata* (Table 20).

Table 20. Cross infectivity

| Isolate obtained from | Test Plants | | | | | | | |
|------------------------------------|-----------------------------|------------------------------------|-----------------------------|------------------------|------------------------------|----------------------------|----------------------------|-------------------------------|
| | <i>Aglaonema commutatum</i> | <i>Amorphophallus campanulatus</i> | <i>Anthurium andraeanum</i> | <i>Dioscorea alata</i> | <i>Homalomena miamiensis</i> | <i>Hydrangea hortensia</i> | <i>Ixora singaporensis</i> | <i>Philodendron goldieana</i> |
| <i>Aglaonema commutatum</i> | ++ | + | + | + | + | ++ | + | ++ |
| <i>Amorphophallus campanulatus</i> | - | ++++ | +++ | +++ | ++++ | ++ | ++ | - |
| <i>Anthurium andraeanum</i> | ++ | + | + | + | ++ | + | + | + |
| <i>Dioscorea alata</i> | ++ | ++ | +++ | +++ | +++ | ++ | + | ++ |
| <i>Homalomena miamiensis</i> | + | + | ++ | ++ | +++ | + | + | ++ |
| <i>Hydrangea hortensia</i> | + | + | ++ | - | + | ++ | ++ | + |
| <i>Ixora singaporensis</i> | + | - | + | + | - | - | + | + |
| <i>Philodendron goldieana</i> | +++ | ++ | ++ | ++ | + | ++ | + | +++ |

- : No infection + : 1 to 25% infection ++ : 26 to 50% infection +++ : 51 to 75% infection ++++ : 76 to 100% infection

Isolate I₂ obtained from *Amorphophallus campanulatus* showed no infection on *Aglaonema commutatum* and *Philodendron goldieana*. However, this isolate produced the maximum infection on *A. campanulatus* and *Homalomena miamiensis*. Isolate I₇ recorded no infection on *A. campanulatus*, *H. miamiensis* and *Hydrangea hortensia*.

Isolates I₁, I₃, I₄, I₅ and I₈ from *A. commutatum*, *Anthurium andraeanum*, *D. alata*, *H. miamiensis* and *Philodendron goldieana* recorded varying degrees of infection on test plants.

The pathogen re-isolated from the respective hosts were found to be identical with the original isolates used as inoculum.

4.7 Studies on toxin production

Four isolates of *Colletotrichum gloeosporioides* causing leaf spot of *Amorphophallus campanulatus*, *Dioscorea alata*, *Hydrangea hortensia* and *Ixora singaporensis* and four isolates causing leaf blight of *Aglaonema commutatum*, *Anthurium andraeanum*, *Homalomena miamiensis* and *Philodendron goldieana* were used.

4.7.1 Effect of different media and incubation periods on toxin production

The effect of various media and incubation periods in the production of toxins was studied with the eight selected isolates.

The exotoxin (culture filtrate) obtained from eight selected isolates in different media and incubation periods, was utilized for studying their effect on inhibition of germination of two vegetable seeds, cowpea (*Vigna*

unguiculata) and amaranthus (*Amaranthus gangeticus*) and seeds of three ornamental plants, balsam (*Impatiens balsamina*), Vinca (*Vinca rosea*) and Caesalpinia (*Caesalpinia pulcherrima*) as per method described in 3.7.1 and the results are presented in Tables 21.1, 21.2, 21.3, 21.4 and 21.5.

Table 21.1 shows the inhibition of germination due to exotoxin of different isolates obtained from different media and different incubation periods in PDB, on cowpea seeds. The highest inhibition of germination was in toxin elaborated in Richard's medium and the lowest in PDB for 25 days incubation.

Among the isolates the highest suppression was in I₆, isolate obtained from *Hydrangea hortensia* and lowest in I₃, isolate obtained from *Anthurium andraeanum* with significant difference between the two.

Fries', Czapek's and Coon's media were significantly superior to PDB of 25 days incubation.

Culture filtrates of I₆ from *H. hortensia* in Fries' medium was the highest inhibitor of germination followed by I₈, from *P. goldieana*, I₆ from *H. hortensia* and I₅ from *H. miamiensis* in Richard's medium. The lowest inhibition was by I₃ from *A. andraeanum* in PDB.

Table 21.2 shows the inhibition of germination on amaranthus seeds due to exotoxin of different isolates obtained from different media and PDB for different periods of incubation. The highest inhibition of germination was in Richard's medium and the lowest in Fries' medium, with significant difference between Richard's medium and PDB of all days and Fries' medium.

Table 21.1. Inhibition of germination due to exotoxin produced by different isolates on cowpea seeds (*Vigna unguiculata*)

| | Isolates | | | | | | | | Mean |
|----------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| | I ₁ | I ₂ | I ₃ | I ₄ | I ₅ | I ₆ | I ₇ | I ₈ | |
| PDB 15 days | 5.28 (13.28) | 11.27 (19.61) | 0 (0.00) | 11.27 (19.61) | 5.28 (13.28) | 5.28 (13.28) | 11.27 (19.61) | 5.28 (13.28) | 5.84 (13.99) |
| PDB 20 days | 0 (0.00) | 5.28 (13.28) | 0 (0.00) | 0 (0.00) | 0 (0.00) | 11.27 (19.61) | 5.28 (13.28) | 5.28 (13.28) | 1.67 (7.43) |
| PDB 25 days | 0 (0.00) | 5.28 (13.28) | 0 (0.00) | 0 (0.00) | 5.28 (13.28) | 0 (0.00) | 0 (0.00) | 0 (0.00) | 0.34 (3.32) |
| Richard's 20 days | 18.38 (25.37) | 60.96 (51.31) | 20.00 (26.55) | 50.00 (44.98) | 70.50 (57.08) | 70.50 (57.08) | 50.00 (44.98) | 70.50 (57.08) | 50.98 (45.56) |
| Coon's 20 days | 20.00 (26.55) | 18.38 (25.37) | 5.28 (13.28) | 5.28 (13.28) | 11.27 (19.61) | 29.50 (32.89) | 0 (0.00) | 27.64 (31.70) | 12.08 (20.34) |
| Czapek's 20 days | 39.04 (38.65) | 0 (0.00) | 0 (0.00) | 20.00 (26.55) | 5.28 (13.28) | 0 (0.00) | 50.00 (44.98) | 11.27 (19.61) | 9.43 (17.88) |
| Fries' 20 days | 5.28 (13.28) | 5.28 (13.28) | 5.28 (13.28) | 0 (0.00) | 0 (0.00) | 72.39 (58.28) | 11.27 (19.61) | 11.27 (19.61) | 8.71 (17.17) |
| Mean | 8.29 (16.73) | 11.09 (19.45) | 1.74 (7.59) | 6.63 (14.92) | 8.21 (16.65) | 19.05 (25.88) | 12.09 (20.35) | 14.13 (22.08) | |

C.D Interaction = 37.566

CD Media = 13.281

CD Isolates = 14.198

Table 21.2. Inhibition of germination due to exotoxin produced by different isolates on *Amaranthus* seeds (*Amaranthus gangeticus*)

| | Isolates | | | | | | | | Mean |
|----------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| | I ₁ | I ₂ | I ₃ | I ₄ | I ₅ | I ₆ | I ₇ | I ₈ | |
| PDB 15 days | 44.97 (42.10) | 8.17 (16.60) | 40.06 (39.22) | 40.00 (39.22) | 44.97 (42.10) | 39.78 (39.09) | 34.19 (35.77) | 44.97 (42.10) | 36.25 (37.02) |
| PDB 20 days | 40.00 (39.22) | 0 (0.00) | 20.00 (26.55) | 18.38 (25.37) | 50.00 (44.98) | 55.24 (47.99) | 14.64 (22.49) | 30.00 (33.20) | 24.97 (29.98) |
| PDB 25 days | 24.83 (29.88) | 34.19 (35.77) | 18.97 (25.81) | 20.00 (26.55) | 39.78 (39.09) | 34.19 (35.77) | 44.76 (41.97) | 24.83 (29.88) | 29.81 (33.09) |
| Richard's 20 days | 39.78 (39.09) | 39.04 (38.65) | 39.78 (39.09) | 34.92 (36.21) | 50.03 (45.00) | 72.39 (58.28) | 81.65 (64.61) | 81.65 (64.61) | 55.56 (48.19) |
| Coon's 20 days | 34.92 (36.21) | 44.97 (42.10) | 14.64 (22.49) | 27.65 (31.70) | 34.92 (36.21) | 39.04 (38.65) | 23.26 (28.82) | 27.64 (31.71) | 30.45 (33.49) |
| Czapek's 20 days | 39.78 (39.09) | 39.04 (38.65) | 18.97 (25.81) | 14.64 (22.49) | 32.25 (34.59) | 14.64 (22.49) | 44.76 (41.97) | 57.07 (49.05) | 31.71 (34.27) |
| Fries' 20 days | 23.26 (28.82) | 11.27 (19.61) | 8.17 (16.60) | 18.97 (25.81) | 5.28 (13.28) | 72.39 (58.28) | 40.00 (39.22) | 11.27 (19.61) | 21.54 (27.65) |
| Mean | 35.11 (36.34) | 21.09 (27.34) | 21.95 (27.94) | 24.43 (29.62) | 35.31 (36.46) | 46.41 (42.94) | 40.05 (39.26) | 38.91 (38.59) | |

C.D Interaction = 39.717

CD Media = 14.042

CD Isolate = 15.012

Among isolates, the highest suppression was in I₆, isolate obtained from *Hydrangea hortensia* and the lowest in I₂, isolate obtained from *Amorphophallus campanulatus*, with significant difference between I₂ isolate and I₆ isolate.

The highest suppression was observed in I₈ isolate obtained from *Philodendron goldieana* and I₇ isolate from *Ixora singaporensis* in Richard's medium and the lowest in I₂ obtained from *A. campanulatus* in PDB incubated for 20 days.

Table 21.3 shows the inhibition of germination on balsam seeds due to exotoxin of different isolates obtained from different media and PDB for different periods of incubation. The highest inhibition of germination was in Richard's medium and the lowest in PDB after 25 days. There was significant difference between PDB of 25 days and Fries', Coon's, Czapek's, and Richard's media.

Among isolates, the highest suppression was in I₁ isolate obtained from *Aglaonema commutatum* followed by I₃ isolate obtained from *Anthurium andraeanum*. The lowest suppression was in I₄ isolate obtained from *Dioscorea alata*.

Interaction between isolates and media was found to be significant. The highest suppression was noted in all isolates in Richard's medium and for I₇ from *Ixora singaporensis* and I₈ from *Philodendron goldieana* in Czapek's medium. The lowest inhibition was noted in I₄ from *D. alata* in PDB of 25 days and I₆ from *Hydrangea hortensia* in PDB of 20 days incubation.

Table 21.3. Inhibition of germination due to exotoxin produced by different isolates on Balsam seeds (*Impatiens balsamina*)

| | Isolates | | | | | | | | Mean |
|----------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| | I ₁ | I ₂ | I ₃ | I ₄ | I ₅ | I ₆ | I ₇ | I ₈ | |
| PDB 15 days | 29.50 (32.89) | 40.00 (39.22) | 40.00 (39.22) | 18.38 (25.37) | 50.00 (44.98) | 50.00 (44.98) | 50.00 (44.98) | 27.64 (31.70) | 37.77 (37.92) |
| PDB 20 days | 72.39 (58.28) | 11.27 (19.61) | 40.00 (39.22) | 50.00 (44.98) | 18.38 (25.37) | 5.28 (13.28) | 11.27 (19.61) | 39.04 (38.65) | 28.66 (32.37) |
| PDB 25 days | 20.00 (26.55) | 20.00 (26.55) | 60.96 (51.31) | 5.28 (13.28) | 11.27 (19.61) | 11.27 (19.61) | 18.38 (25.37) | 0 (0.00) | 15.00 (22.79) |
| Richard's 20 days | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) |
| Coon's 20 days | 60.00 (50.75) | 50.00 (44.98) | 39.04 (38.65) | 40.00 (39.22) | 50.00 (44.98) | 94.74 (76.71) | 60.96 (51.31) | 60.96 (51.31) | 58.24 (49.74) |
| Czapek's 20 days | 88.75 (70.37) | 72.39 (58.28) | 94.74 (76.71) | 60.96 (51.31) | 70.50 (57.08) | 81.65 (64.61) | 100.00 (90.00) | 100.00 (90.00) | 88.06 (69.79) |
| Fries' 20 days | 81.65 (64.61) | 50.03 (45.00) | 29.50 (32.89) | 11.27 (19.61) | 60.00 (50.75) | 27.64 (31.71) | 40.00 (39.22) | 50.03 (45.00) | 43.21 (41.10) |
| Mean | 69.07 (56.21) | 52.15 (46.23) | 63.06 (52.57) | 42.25 (40.54) | 54.43 (47.54) | 56.44 (48.70) | 61.25 (51.50) | 57.86 (49.52) | |

C.D Interaction = 47.985

CD Media = 16.965

CD Isolate = 18.137

Table 21.4 shows the inhibition of germination on periwinkle seeds due to exotoxin of different isolates obtained from different media and PDB of different days of incubation. The highest inhibition of germination was in PDB at 20 days incubation and Coon's medium and the lowest in Fries' medium, with significant difference.

Among the isolates, the highest suppression was in I₄, isolate obtained from *Dioscorea alata* and lowest in I₁, I₅, I₆ and I₇.

The highest inhibition was noted in all isolates in PDB of 20 days incubation and Coon's medium. The lowest inhibition was observed in I₇ isolate from *Ixora singaporensis* in Fries' medium.

Table 21.5, shows the inhibition of germination on *Caesalpinia* seeds due to exotoxin of different isolates obtained from different media and PDB at different days incubation. The highest inhibition was in Richard's medium while the lowest in PDB at 25 day's incubation with significant difference between the two.

Among the isolates the highest suppression was in I₇, isolate obtained from *I. singaporensis* and the lowest in I₆, isolate obtained from *H. hortensia*.

The highest inhibition was noted for I₁, I₂, I₃ and I₄ isolates from *A. commutatum*, *A. campanulatus*, *A. andraeanum* and *D. alata* in Richard's medium and the lowest in I₂, I₃, I₄ and I₆ isolates in Fries' medium, I₄ isolate from Czapek's medium, I₂ isolate from Coon's medium; I₅ isolate in Richard's medium and I₂ in PDB at 25 days incubation.

Table 21.4. Inhibition of germination due to exotoxin produced by different isolates on periwinkle seeds (*Catharanthus roseus*)

| | Isolates | | | | | | | | Mean |
|----------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| | I ₁ | I ₂ | I ₃ | I ₄ | I ₅ | I ₆ | I ₇ | I ₈ | |
| PDB 15 days | 100.00 (90.00) | 100.00 (90.00) | 88.75 (70.37) | 100.00 (90.00) | 88.75 (70.37) | 88.75 (70.37) | 88.75 (70.37) | 94.74 (76.70) | 96.05 (78.53) |
| PDB 20 days | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) |
| PDB 25 days | 94.94 (76.70) | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 94.74 (76.70) | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 99.96 (86.68) |
| Richard's 20 days | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 94.74 (76.70) | 100.00 (90.00) | 100.00 (90.00) | 99.92 (88.34) |
| Coon's 20 days | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) |
| Czapek's 20 days | 94.74 (76.70) | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 99.92 (88.34) |
| Fries' 20 days | 88.75 (70.37) | 94.74 (76.70) | 94.74 (76.70) | 100.00 (90.00) | 94.74 (76.70) | 94.74 (76.70) | 81.65 (64.60) | 100.00 (90.00) | 95.48 (77.73) |
| Mean | 98.68 (83.40) | 99.89 (88.10) | 99.33 (85.30) | 100.00 (90.00) | 98.68 (83.40) | 98.68 (83.40) | 98.68 (83.40) | 99.89 (88.10) | |

C.D Interaction = 24.38

CD Media = 8.62

CD Isolates = 9.21

Table 21.5. Inhibition of germination due to exotoxin produced by different isolates on caesalpinia seeds (*Caesalpinia pulcherrima*)

| | Isolates | | | | | | | | Mean |
|----------------------|-------------------|-------------------|-------------------|-------------------|------------------|------------------|------------------|------------------|------------------|
| | I ₁ | I ₂ | I ₃ | I ₄ | I ₅ | I ₆ | I ₇ | I ₈ | |
| PDB 15 days | 70.50 (57.08) | 60.00 (50.75) | 60.00 (50.75) | 70.50 (57.08) | 80.00 (63.41) | 70.50 (57.08) | 70.50 (57.08) | 70.50 (57.08) | 69.20 (56.29) |
| PDB 20 days | 60.00 (50.75) | 70.50 (57.08) | 60.00 (50.75) | 60.00 (50.75) | 70.50 (57.08) | 70.50 (57.08) | 70.50 (57.08) | 60.00 (50.75) | 65.30 (53.91) |
| PDB 25 days | 70.50 (57.08) | 50.00 (44.98) | 80.00 (63.41) | 80.00 (63.41) | 60.00 (50.75) | 60.00 (50.75) | 80.00 (63.41) | 29.50 (32.89) | 64.33 (53.33) |
| Richard's 20 days | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 100.00 (90.00) | 50.00 (44.98) | 70.50 (57.08) | 60.00 (50.75) | 80.00 (63.41) | 90.48 (72.03) |
| Coon's 20 days | 60.00 (50.75) | 50.00 (44.98) | 88.75 (70.37) | 81.65 (64.61) | 94.74 (76.70) | 88.75 (70.37) | 88.75 (70.37) | 94.74 (76.70) | 82.95 (65.61) |
| Czapek's 20 days | 70.50 (57.08) | 80.00 (63.41) | 70.50 (57.08) | 50.00 (44.98) | 94.74 (76.70) | 70.50 (57.08) | 94.74 (76.70) | 94.74 (76.70) | 80.40 (63.72) |
| Fries' 20 days | 60.00 (50.75) | 50.00 (44.98) | 50.00 (44.98) | 50.00 (44.98) | 70.50 (57.08) | 50.00 (44.98) | 94.74 (76.70) | 94.74 (76.70) | 67.35 (55.15) |
| Mean | 73.58 (59.07) | 69.70 (56.60) | 76.57 (61.05) | 74.69 (59.40) | 76.44 (60.96) | 69.30 (56.35) | 81.59 (64.59) | 78.00 (62.03) | |

C.D interaction = 23.74

CD Media = 8.39

CD Isolates = 8.97

4.7.2 Partial purification of toxin

The toxins produced by the eight isolates were partially purified and bioassayed on two broad leaved ornamental plants *Philodendron goldieana* and *Aglaonema commutatum*.

4.7.2.1 Effect of various media on toxin production

Visible necrotic lesions were observed on the host leaves when bioassayed with the endotoxin obtained from the eight isolates (Plates 32, 33 and 36). The dimensions of the necrotic lesions produced by the endotoxin obtained from various media are presented (Tables 22.1 and 22.2).

In all the treatments, symptoms were initiated in six to eight hours after inoculation. The necrotic area gradually enlarged and fully matured spots were noticed after 72 hours. The results revealed that the necrotic lesions produced by the endotoxin obtained from the Coon's medium was found to be larger when compared to those from other media. It ranged from 1.383 to 1.498 on the leaves of *Philodendron goldieana* (Table 22.1) and 1.333 to 1.389 on the leaves of *Aglaonema commutatum* (Table 22.2).

Statistical analysis of the data (Table 22.1) revealed that Coon's medium was superior to PDB (1.498), though Czapek's, Richard's, Fries' and Coon's media were on par when bioassayed on the leaves of *Philodendron goldieana*.

Statistical analysis also revealed that the necrotic lesions produced by eight isolates were significantly different. The necrotic lesions produced by the I₈ were bigger in size (1.511) and that produced by the I₇ were smaller (1.400). Isolates I₇,

Table 22.1. Effect of endotoxin produced in different media on *Philodendron goldieana* leaf

| MEDIA | Necrotic lesion (cm) | | | | | | | | |
|-----------|----------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| | I ₁ | I ₂ | I ₃ | I ₄ | I ₅ | I ₆ | I ₇ | I ₈ | Mean |
| PDB | 1.232 (1.494) | 0.915 (1.384) | 0.580 (1.257) | 0.850 (1.360) | 0.866 (1.366) | 0.865 (1.366) | 0.713 (1.309) | 1.345 (1.531) | 0.913 (1.383) |
| Richard's | 1.365 (1.538) | 0.999 (1.414) | 1.016 (1.420) | 1.017 (1.420) | 1.649 (1.628) | 1.200 (1.483) | 0.960 (1.400) | 1.389 (1.546) | 1.193 (1.481) |
| Coon's | 0.736 (1.318) | 0.999 (1.414) | 1.178 (1.476) | 1.800 (1.673) | 1.464 (1.570) | 1.482 (1.575) | 1.080 (1.442) | 1.290 (1.513) | 1.244 (1.498) |
| Fries' | 1.266 (1.505) | 1.079 (1.442) | 1.331 (1.527) | 1.270 (1.507) | 1.050 (1.432) | 1.516 (1.586) | 0.865 (1.366) | 1.214 (1.488) | 1.196 (1.482) |
| Czapek's | 1.299 (1.516) | 0.899 (1.378) | 1.054 (1.433) | 1.300 (1.517) | 1.110 (1.453) | 1.268 (1.506) | 1.195 (1.481) | 1.181 (1.477) | 1.161 (1.470) |
| Mean | 1.173 (1.474) | 0.977 (1.406) | 1.022 (1.422) | 1.235 (1.495) | 1.220 (1.490) | 1.259 (1.503) | 0.960 (1.400) | 1.283 (1.511) | |

C.D Media = 0.0427

C.D Isolate = 0.0540

C.D Interaction = 0.1208

Table 22.2. Effect of endotoxin produced in different media on *Aglaonema commutatum* leaf

| MEDIA | Necrotic lesion (cm) | | | | | | | | |
|-----------|----------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| | I ₁ | I ₂ | I ₃ | I ₄ | I ₅ | I ₆ | I ₇ | I ₈ | Mean |
| PCP | 0.765 (1.329) | 0.749 (1.323) | 0.715 (1.310) | 0.992 (1.411) | 0.766 (1.329) | 0.817 (1.348) | 0.715 (1.310) | 0.699 (1.304) | 0.777 (1.333) |
| Richard's | 0.716 (1.310) | 0.683 (1.297) | 0.566 (1.252) | 1.249 (1.500) | 0.865 (1.366) | 1.394 (1.547) | 0.799 (1.341) | 0.500 (1.225) | 0.836 (1.355) |
| Coon's | 1.315 (1.522) | 0.550 (1.245) | 0.599 (1.265) | 0.731 (1.316) | 1.665 (1.633) | 1.148 (1.465) | 0.857 (1.363) | 0.693 (1.301) | 0.929 (1.389) |
| Czapek's | 0.747 (1.322) | 1.232 (1.494) | 0.882 (1.372) | 0.583 (1.258) | 0.698 (1.303) | 0.766 (1.329) | 1.114 (1.454) | 0.844 (1.358) | 0.852 (1.361) |
| Fries' | 0.831 (1.353) | 1.047 (1.431) | 0.797 (1.341) | 0.550 (1.245) | 0.777 (1.333) | 1.229 (1.493) | 0.733 (1.317) | 0.666 (1.296) | 0.823 (1.350) |
| Mean | 0.869 (1.367) | 0.844 (1.358) | 0.711 (1.308) | 0.812 (1.346) | 0.940 (1.393) | 1.065 (1.437) | 0.841 (1.357) | 0.680 (1.296) | |

C.D Media = 0.0357

C.D Isolate = 0.0452

C.D Interaction = 0.1011

I₂ and I₃ were on par. Eventhough isolates, I₁, I₅, I₄, I₆ and I₈ were on par, these were superior to I₇, I₂ and I₃ (Table 22.1).

The endotoxin obtained from the isolate I₄ produced the maximum necrotic area of 1.673 when incubated in Coon's medium and toxin from the isolate I₃ produced minimum necrotic lesions of 1.257 when incubated in potato dextrose broth.

The statistical analysis of the data on the effect of endotoxin on the leaves of *Aglaonema commutatum* is presented (Table 22.2). The data revealed that the necrotic lesion produced by the endotoxin obtained from Coon's medium was bigger in size (1.389) followed by Czepek's and Richard's medium. The spots formed by the endotoxins obtained from potato dextrose broth were found to be the smallest (1.333).

Statistical analysis also revealed that the necrotic lesions produced by the eight isolates were significantly different. The necrotic lesions produced by the isolate I₆ were found to be bigger in size (1.437) followed by I₅ (1.393) and I₁ (1.367). The smallest necrotic lesions were produced by the isolate I₈ (1.296) (Table 22.2).

4.7.2.2 Effect of incubation periods on toxin production

The optimum incubation periods of the cultures for the maximum toxin production were assessed. The toxic activity was measured by bioassay techniques with endotoxin on the leaves of *Philodendron goldieana* and *Aglaonema commutatum*. In all the cultures toxin production

started on the 15th day of incubation. The toxic activity increased up to a maximum incubation period of 25 days, after which it declined slowly. The maximum toxicity was observed on the 25th day of incubation at room temperature (27°C) (Table 23.2 and 23.2).

Statistical analysis of data revealed that the necrotic spots produced on *P. goldieana* leaves at the 25th day of incubation were bigger in size (1.462) and at the 15th day of incubation, the spots were smaller (1.356). Incubation for 25 days (I_3) was significantly superior to incubation for 15 (I_1) and 20 days (I_2) (Table 23.1).

Effect of different isolates incubated at different periods was also found to be statistically significant. The maximum necrotic lesions (1.596) were produced by the isolate I_8 and the minimum (1.287) by the isolate I_3 (Table 23.1).

On *P. goldieana* leaves necrotic lesions with yellow halos were observed 48 h after inoculation and 'shot-hole' symptoms were noticed after 72 hours (Plate 36).

On *A. Commutatum* leaves, the necrotic lesions produced at 25th day of incubation were bigger in size (1.423 cm) while at the 15th day of incubation, the spots were smaller (1.291) (Table 23.2).

The variation in the number of necrotic lesions produced by different isolates incubated at different periods was found to be statistically significant. The maximum number of necrotic lesions was produced on *A. commutatum* leaves by the isolate I_4 (1.403) and the minimum (1.279) by the isolate I_2 (Table 23.2).

Table 23.1. Effect of endotoxin produced under different periods of incubation on *Philodendron goldieana* leaf

| Incubation | Necrotic lesion (cm) | | | | | | | | |
|------------|----------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| | I ₁ | I ₂ | I ₃ | I ₄ | I ₅ | I ₆ | I ₇ | I ₈ | Mean |
| 15 Days | 0.633 (1.278) | 0.867 (1.366) | 0.550 (1.245) | 0.749 (1.323) | 1.148 (1.465) | 0.765 (1.329) | 0.650 (1.284) | 1.432 (1.560) | 0.839 (1.356) |
| 20 Days | 1.232 (1.494) | 0.916 (1.384) | 0.580 (1.257) | 0.850 (1.360) | 0.867 (1.366) | 0.865 (1.366) | 0.713 (1.309) | 1.345 (1.531) | 0.913 (1.383) |
| 25 Days | 1.066 (1.437) | 0.898 (1.378) | 0.848 (1.360) | 1.265 (1.505) | 1.049 (1.431) | 0.662 (1.289) | 1.549 (1.597) | 1.880 (1.697) | 1.137 (1.462) |
| Mean | 0.968 (1.403) | 0.893 (1.376) | 0.656 (1.287) | 0.949 (1.396) | 0.881 (1.421) | 0.764 (1.328) | 0.952 (1.397) | 1.547 (1.596) | |

C.D Duration of incubation = 0.0340
 C.D Isolate = 0.0555
 C.D Interaction = 0.0962

Table 23.2. Effect of endotoxin produced under different periods of incubation on *Aglaonema commutatum* leaf

| Incubation | Necrotic lesion (cm) | | | | | | | | |
|------------|----------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| | I ₁ | I ₂ | I ₃ | I ₄ | I ₅ | I ₆ | I ₇ | I ₈ | Mean |
| 15 Days | 0.597 (1.264) | 0.665 (1.290) | 0.598 (1.264) | 0.550 (1.245) | 0.533 (1.238) | 0.682 (1.297) | 0.514 (1.230) | 1.250 (1.500) | 0.667 (1.291) |
| 20 Days | 0.765 (1.329) | 0.749 (1.323) | 0.715 (1.310) | 0.992 (1.411) | 0.766 (1.329) | 0.817 (1.348) | 0.715 (1.310) | 0.699 (1.304) | 0.777 (1.333) |
| 25 Days | 1.128 (1.459) | 0.500 (1.225) | 1.219 (1.490) | 1.409 (1.552) | 1.366 (1.538) | 0.500 (1.225) | 1.366 (1.538) | 0.850 (1.360) | 1.025 (1.423) |
| Mean | 0.823 (1.350) | 0.636 (1.279) | 0.836 (1.355) | 0.968 (1.403) | 0.871 (1.368) | 0.664 (1.290) | 0.847 (1.359) | 0.926 (1.388) | |

C.D Duration of incubation = 0.0336
 C.D Isolate = 0.0548
 C.D Interaction = 0.0950

The toxins of each isolate, when bioassayed on the leaves of other plants produced necrotic symptoms. Such effect of the toxins from the eight isolates was obtained on *Costus malortieanus* (Plate 29), *Acalypha hispida* (Plate 30), *Dracaena sanderiana* (Plate 31), *Syngonium podophyllum* (Plate 34), *Colocasia antiquorum* (Plate 35) and *Begonia semperflorens* (Plate 37). This showed that the toxins were non-host specific.

4.8 Biological grouping of different isolates

Biological grouping of the 69 isolates of *C. gloeosporioides* was done based on the rate of growth in liquid media, biochemical traits like protein production and enzyme activity and on the different types of symptoms produced on the host leaves. The grouping was done based on the Adensonian classification employing the key enumerated by Bradbury (1970).

The first type of classification was based on the dry weight of the mycelium when grown in Richard's medium. The isolates were grouped into three viz., slow growers, moderate growers and fast growers (Table 5 and Fig. 1.).

The second type of classification was based on the dry weight of the mycelium when grown in potato dextrose broth. The isolates were grouped into three viz. slow growers, medium growers and fast growers (Table 5 and Fig. 2.).

The third type of classification was based on the soluble protein content in the different isolates on the fifth day of incubation. The isolates



Plate 29

Necrotic lesions formed on *Costus malortianus*
when bioassayed with the toxins



Plate 30

Necrotic lesions formed on *Acalypha hispida*

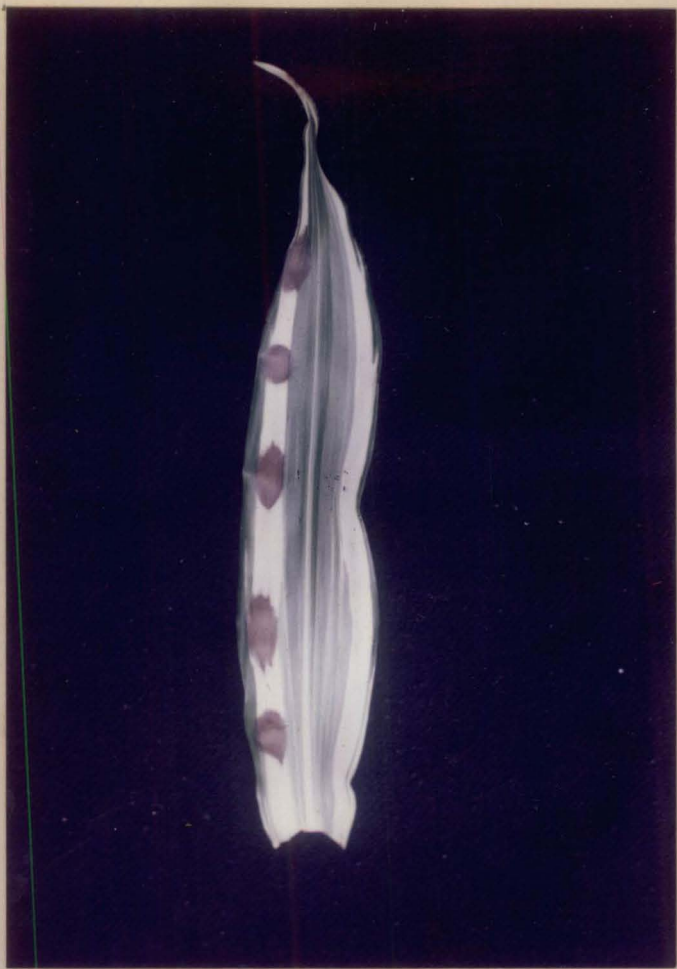


Plate 31
Necrotic lesions formed on
Dracaena sanderiana
when bioassayed with the toxins



Plate 32
Necrotic lesions formed on
Anthurium andraeanum
when bioassayed with the toxins

Plate 33
Necrotic lesions formed on
Homalomena miamiensis
when bioassayed with the toxins
when bioassayed with the toxins



Plate 34
Necrotic lesions formed on
Syngonium podophyllum
when bioassayed with the toxins

Plate 35
Necrotic lesions formed on
Colocasia antiquorum
when bioassayed with the toxins

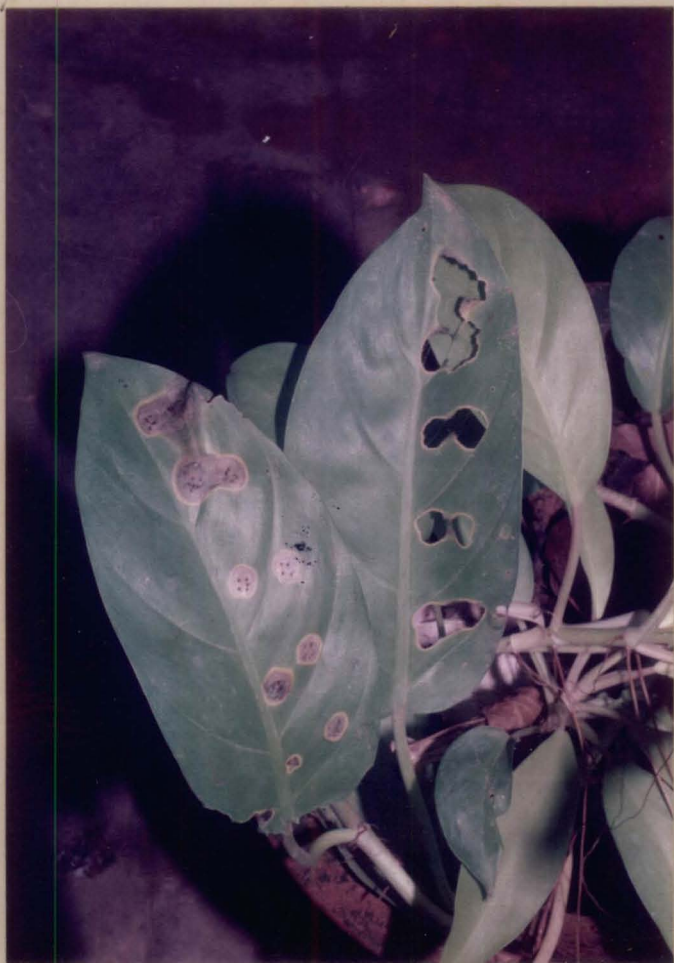


Plate 36
Shot holes formed on
Philodendron goldieana
when bioassayed with the toxins



Plate 37

Necrotic lesions formed on *Begonia semperflorens*
when bioassayed with the toxins

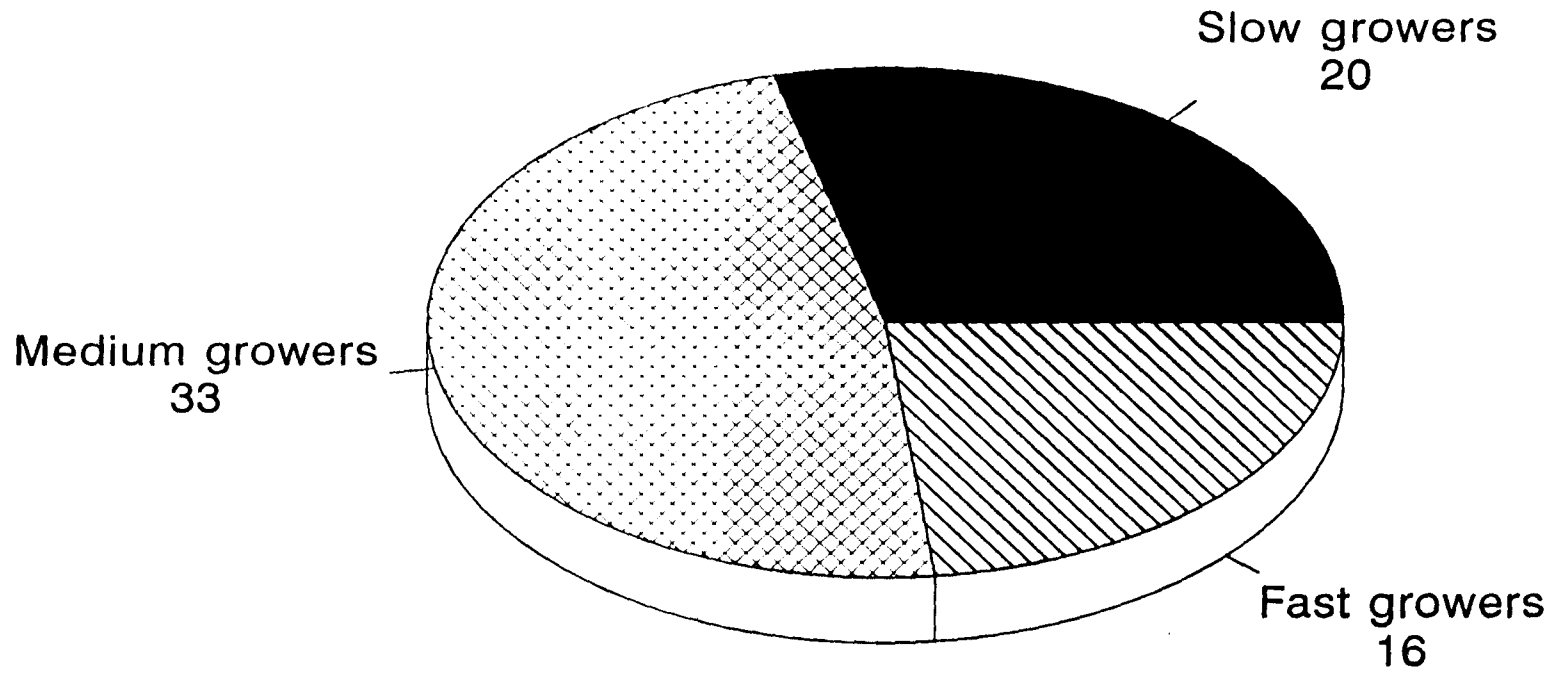


Fig. 1. Growth of *C. gloeosporioides* in Richard's medium

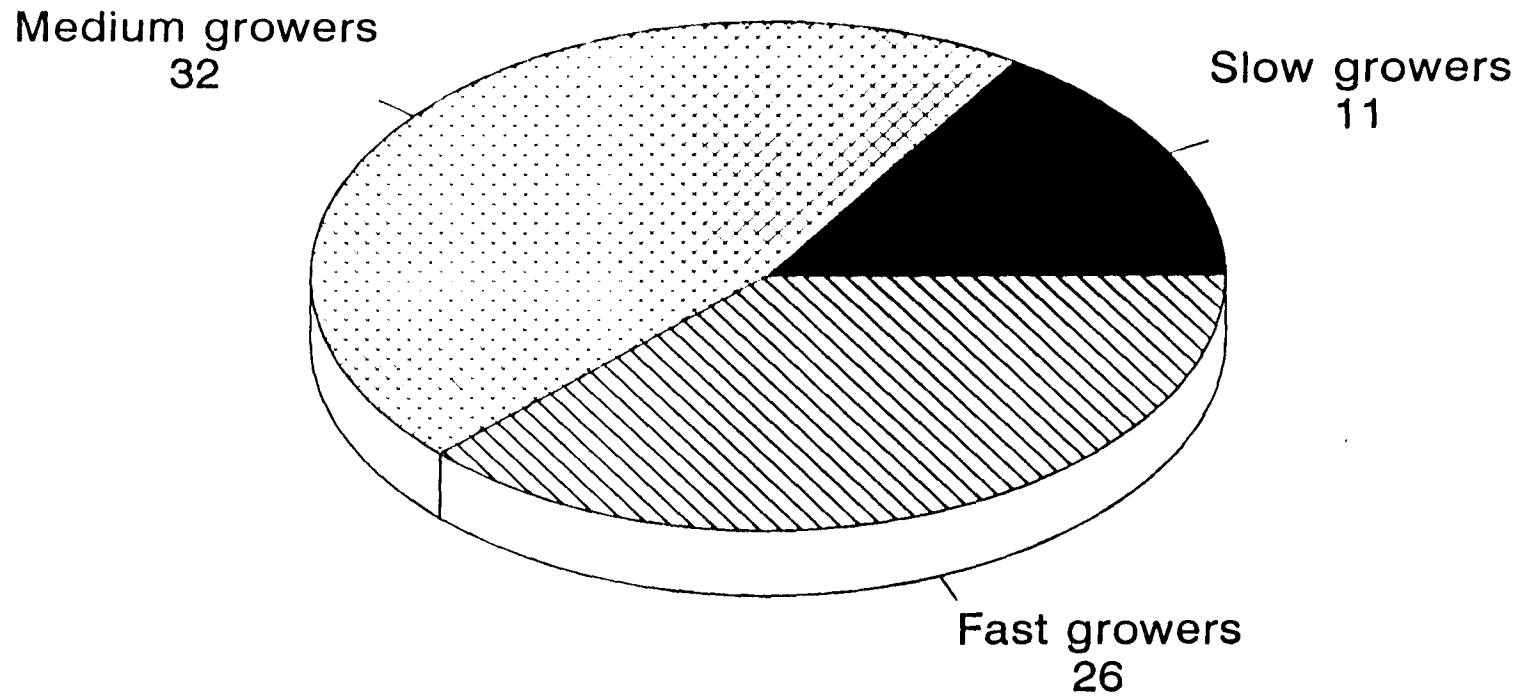


Fig. 2. Growth of *C. gloeosporioides* in Potato dextrose broth (PDB)

were grouped into three viz. low protein producers, moderate protein producers and high protein producers (Table 9 and Fig. 3.).

The fourth type of classification was based on the soluble protein content in different isolates on the eighth day of incubation. The isolates were grouped into three viz. low protein producers, moderate protein producers and high protein producers (Table 9 and Fig. 4.).

The fifth type of classification was based on the polyphenol oxidase enzyme activity. Based on the enzyme activity on the fifth day of incubation the isolates were grouped under low polyphenol oxidase activity, moderate polyphenol oxidase activity and high polyphenol oxidase activity (Table 10 and Fig. 5.).

The sixth type of classification was based on the peroxidase enzyme activity on the eighth day of incubation. The isolates were grouped under low peroxidase activity, moderate peroxidase activity and high peroxidase activity (Table 10 and Fig. 6.).

The seventh type of classification was based on the similarity in symptom expressions produced on the host leaves by different isolates (Table 24).

The schematic profile of some of the important characters were used for grouping of different isolates. The final classification of the isolates into different groups was based on the correlation analysis of different characters enumerated in the seven types of classification already described. Based on the correlation matrix eight different groups of isolates could be distinguished and designated as G_1 , G_2 , G_3 , G_4 , G_5 , G_6 , G_7 and G_8 strains (Figs. 7 and 8).

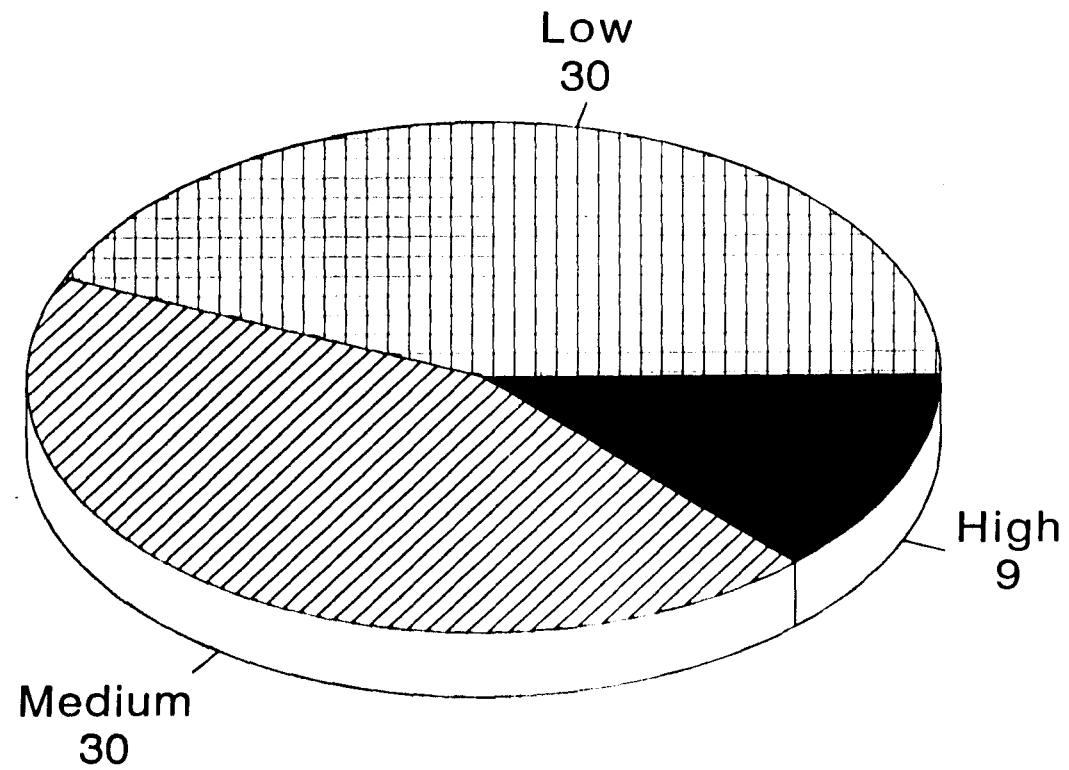


Fig. 3. Protein content of *C. gloeosporioides* on fifth day of inoculation

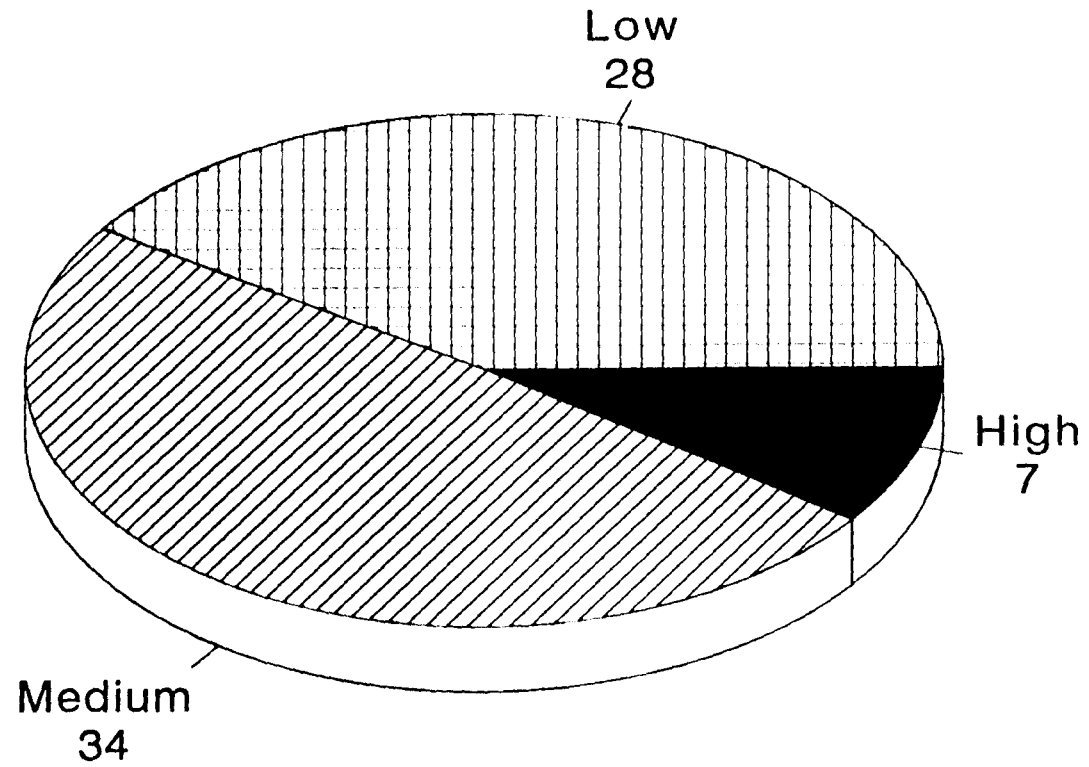


Fig. 4. Protein content of *C. gloeosporioides* on eighth day of inoculation

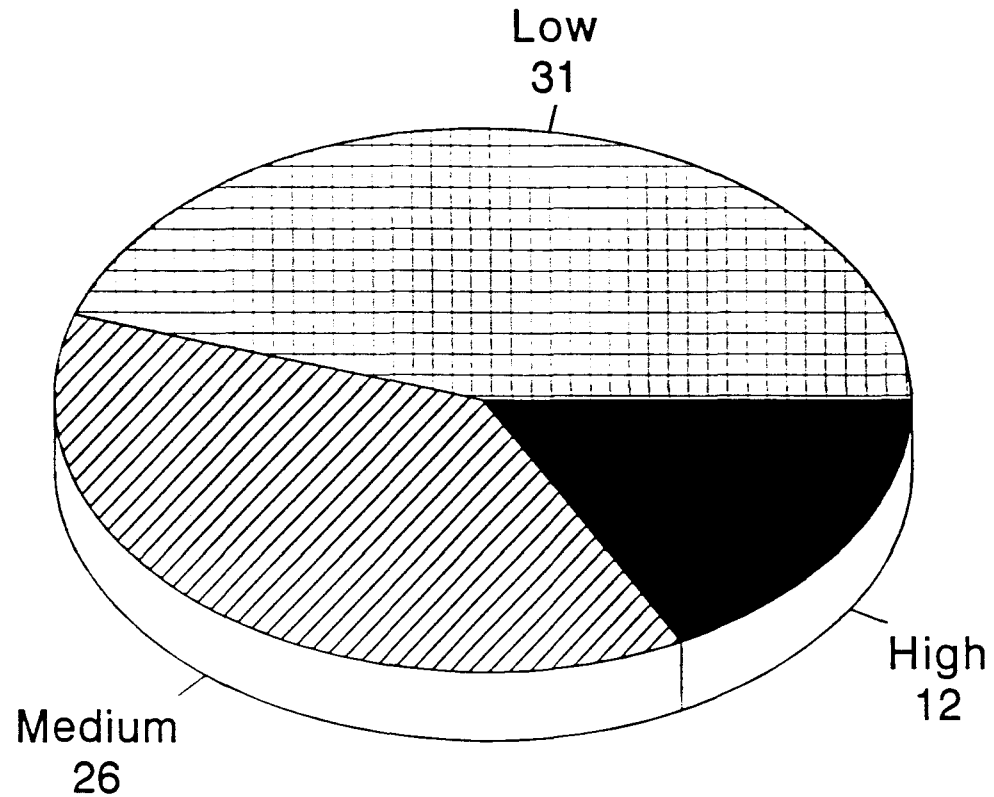


Fig. 5. Polyphenol oxidase activity of *C. gloeosporioides*

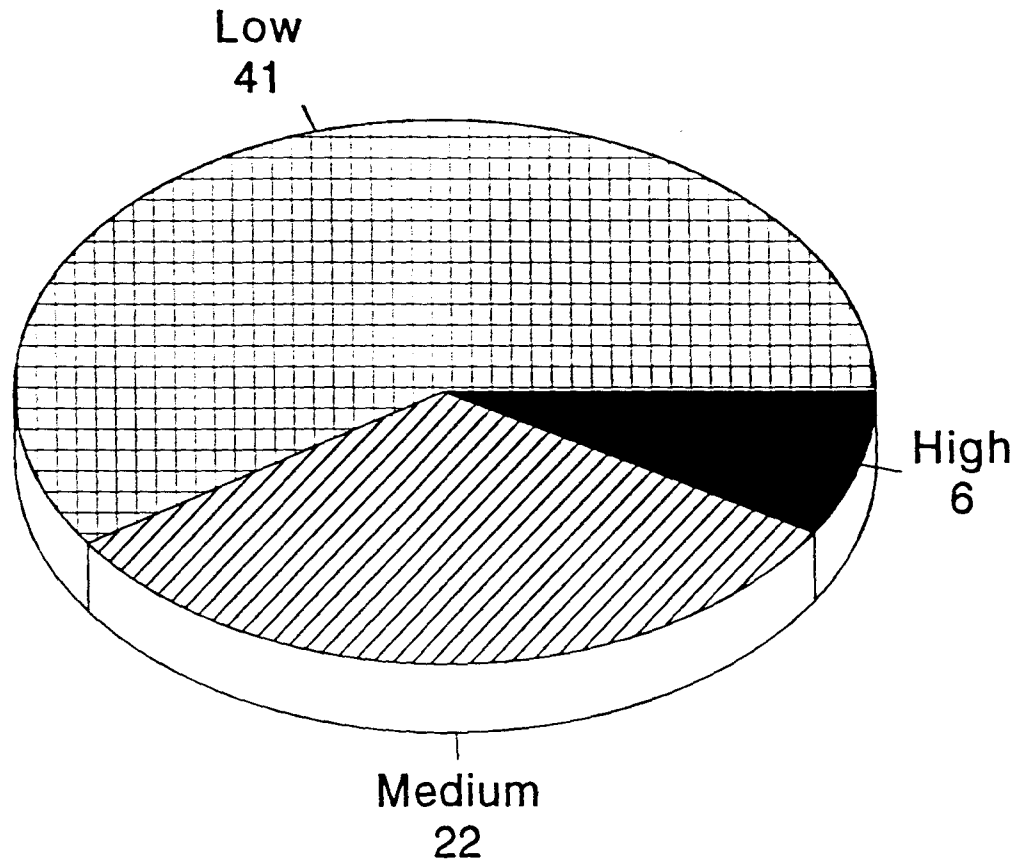


Fig. 6. Peroxidase activity of *C. gloeosporioides*

Table 24. Grouping of the isolates of *C. gloeosporioides* based on symptoms

| Sl. No. | Name of host plant from which the isolate obtained | Strain |
|---------|--|----------------|
| 1. | <i>Acalypha hispida</i> | G ₂ |
| 2. | <i>Aglaonema commutatum</i> | G ₄ |
| 3. | <i>Allamanda cathartica</i> | G ₂ |
| 4. | <i>Alpinia purpurata</i> | G ₂ |
| 5. | <i>Amorphophallus campanulatus</i> | G ₁ |
| 6. | <i>Anthurium andraeanum</i> | G ₄ |
| 7. | <i>Anthurium grande</i> | G ₁ |
| 8. | <i>Antigonon leptopus</i> | G ₈ |
| 9. | <i>Asplenium nidus</i> | G ₂ |
| 10. | <i>Bauhinia purpurea</i> | G ₈ |
| 11. | <i>Begonia 'rex'</i> | G ₈ |
| 12. | <i>Begonia sachsen</i> | G ₂ |
| 13. | <i>Begonia semperflorens</i> | G ₈ |
| 14. | <i>Bougainvillea spectabilis</i> | G ₂ |
| 15. | <i>Calathea acuminata</i> | G ₂ |
| 16. | <i>Calathea insignis</i> | G ₅ |
| 17. | <i>Calathea kegeliana</i> | G ₈ |
| 18. | <i>Calathea leuconeura</i> | G ₅ |
| 19. | <i>Calathea ornata</i> | G ₃ |
| 20. | <i>Calathea picturata</i> | G ₂ |
| 21. | <i>Calathea "splendida"</i> | G ₂ |
| 22. | <i>Calathea zebrina</i> | G ₆ |
| 23. | <i>Canna indica</i> | G ₃ |
| 24. | <i>Chrysothemis pulchella</i> | G ₂ |
| 25. | <i>Colocasia antiquorum</i> | G ₆ |
| 26. | <i>Costus sanguineus</i> | G ₂ |
| 27. | <i>Costus malortieanus</i> | G ₂ |
| 28. | <i>Dieffenbachia 'Exotica alba'</i> | G ₄ |
| 29. | <i>Dieffenbachia maculata 'Rud Roehrs'</i> | G ₄ |
| 30. | <i>Dioscorea alata</i> | G ₁ |
| 31. | <i>Dracaena deremensis</i> | G ₁ |
| 32. | <i>Dracaena fragrans 'Victoriae'</i> | G ₄ |
| 33. | <i>Dracaena godseffiana</i> | G ₃ |
| 34. | <i>Dracaena marginata</i> | G ₂ |

Contd...

Table 24. (Contd....)

| Sl. No. | Name of host plant from which the isolate obtained | Strain |
|---------|--|----------------|
| 35. | <i>Dracaena reflexa</i> | G ₂ |
| 36. | <i>Dracaena sanderiana</i> | G ₈ |
| 37. | <i>Duranta plumieri</i> | G ₂ |
| 38. | <i>Eurycles amboinensis</i> | G ₅ |
| 39. | <i>Gerbera jamesonii</i> | G ₂ |
| 40. | <i>Hamelia patens</i> | G ₂ |
| 41. | <i>Hedychium coronarium</i> | G ₃ |
| 42. | <i>Heliconia metallic:</i> | G ₈ |
| 43. | <i>Hibiscus rosa-sinensis</i> | G ₄ |
| 44. | <i>Hippeastrum reticulatum</i> | G ₂ |
| 45. | <i>Hippeastrum sp.</i> | G ₂ |
| 46. | <i>Homalomena miamiensis</i> | G ₃ |
| 47. | <i>Hydrangea hortensia</i> | G ₂ |
| 48. | <i>Hymenocallis lillioasafotida</i> | G ₅ |
| 49. | <i>Iris tectorum</i> | G ₇ |
| 50. | <i>Ixora singaporensis</i> | G ₂ |
| 51. | <i>Kopsia fruticosa</i> | G ₈ |
| 52. | <i>Lantana camara</i> | G ₂ |
| 53. | <i>Maranta arundinacea 'Variegata'</i> | G ₄ |
| 54. | <i>Mussaenda erythrophylla</i> | G ₈ |
| 55. | <i>Neomarica gracilis</i> | G ₁ |
| 56. | <i>Ochna squarrosa</i> | G ₈ |
| 57. | <i>Philodendron goldieana</i> | G ₃ |
| 58. | <i>Pleomele reflexa</i> | G ₂ |
| 59. | <i>Plumeria acutifolia</i> | G ₂ |
| 60. | <i>Polyscias paniculata 'Variegata'</i> | G ₂ |
| 61. | <i>Pothos scandens</i> | G ₄ |
| 62. | <i>Quisqualis indica</i> | G ₂ |
| 63. | <i>Sansevieria Hahnii</i> | G ₈ |
| 64. | <i>Sansevieria trifasciata</i> | G ₅ |
| 65. | <i>Spathiphyllum commutatum</i> | G ₈ |
| 66. | <i>Syngonium podophyllum</i> | G ₈ |
| 67. | <i>Tagetes erecta</i> | G ₂ |
| 68. | <i>Tecomaria capensis</i> | G ₂ |
| 69. | <i>Thunbergia erecta</i> | G ₈ |

Fig. 7. Schematic profile on the traits of isolates of *Colletotrichum gloeosporioides*

| Isolate Number | Growth in Richard's medium | Growth in PDB | Protein production on the 5th day | Protein production on the 8th day | P.P.O. activity | Peroxidase activity | Symptom score & Grouping |
|----------------|----------------------------|---------------|-----------------------------------|-----------------------------------|-----------------|---------------------|--------------------------|
| 61 | ● | ● | ● | | | ● | 4 |
| 53 | | | | ● | ● | | 4 |
| 28 | ● | ● | | ● | ● | | 4 |
| 29 | ● | | | ● | ● | ● | 4 |
| 32 | ● | ● | ● | ● | | ● | 4 |
| 43 | ● | | ● | ● | | ● | 4 |
| 6 | | | ● | ● | ● | ● | 4 |
| 2 | ● | | ● | ● | ● | ● | 4 |
| 19 | ● | ● | ● | ● | ● | ● | 4 |
| 46 | | ● | ● | ● | ● | ● | 3 |
| 23 | ● | | | ● | ● | | 3 |
| 57 | ● | | | ● | ● | | 3 |
| 33 | ● | ● | ● | ● | ● | | 3 |
| 17 | ● | ● | ● | ● | ● | | 3 |
| 63 | ● | ● | ● | ● | ● | | 2-4 |
| 51 | ● | | | ● | ● | | 2-4 |
| 36 | | ● | | | ● | ● | 2-4 |
| 8 | | ● | | ● | ● | | 2-4 |
| 10 | | ● | ● | ● | ● | | 2-4 |
| 54 | | ● | | ● | ● | | 2-4 |
| 65 | | ● | ● | | ● | ● | 2-4 |
| 56 | | ● | ● | | ● | ● | 2-4 |
| 66 | | | | | ● | ● | 2-4 |
| 42 | | | | | ● | ● | 2-4 |
| 69 | | | ● | | ● | ● | 2-4 |
| 11 | ● | ● | | | ● | ● | 2-4 |
| 13 | | ● | | | ● | ● | 2-4 |
| 48 | ● | | ● | | ● | ● | 2-4 |
| 38 | ● | ● | ● | | ● | ● | 1-3 |
| 64 | ● | | | | ● | ● | 1-3 |
| 16 | | | | | ● | ● | 1-3 |
| 18 | ● | | ● | | ● | ● | 1-3 |
| 26 | ● | ● | ● | ● | ● | ● | 2 |
| 62 | ● | | ● | ● | ● | ● | 2 |
| 68 | ● | ● | ● | ● | ● | ● | 2 |
| 34 | | ● | ● | ● | ● | ● | 2 |
| 59 | | ● | ● | ● | ● | ● | 2 |
| 58 | | ● | ● | ● | ● | ● | 2 |
| 9 | | | ● | ● | ● | ● | 2 |
| 39 | | ● | ● | ● | ● | ● | 2 |
| 67 | ● | ● | ● | ● | ● | ● | 2 |
| 20 | | ● | ● | ● | ● | ● | 2 |
| 40 | ● | ● | ● | ● | ● | ● | 2 |
| 1 | | | ● | ● | ● | ● | 2 |
| 12 | | ● | ● | ● | ● | ● | 2 |
| 52 | | ● | ● | ● | ● | ● | 2 |
| 60 | | ● | ● | ● | ● | ● | 2 |
| 24 | | ● | ● | ● | ● | ● | 2 |
| 15 | ● | ● | ● | ● | ● | ● | 2 |
| 14 | ● | ● | ● | ● | ● | ● | 2 |
| 37 | ● | | | ● | ● | ● | 2 |
| 27 | ● | | | ● | ● | ● | 2 |
| 50 | ● | | | ● | ● | ● | 2 |
| 35 | ● | | ● | ● | ● | ● | 2 |
| 3 | ● | | ● | ● | ● | ● | 2 |
| 21 | ● | | ● | ● | ● | ● | 2 |
| 4 | | ● | ● | ● | ● | ● | 2 |
| 44 | | ● | ● | ● | ● | ● | 2 |
| 45 | | ● | ● | ● | ● | ● | 2 |
| 47 | ● | ● | ● | ● | ● | ● | 2 |
| 5 | | | ● | ● | ● | ● | 2 |
| 31 | | ● | ● | ● | ● | ● | 1 |
| 7 | ● | ● | ● | ● | ● | ● | 1 |
| 41 | | ● | ● | ● | ● | ● | 1 |
| 55 | ● | ● | | | ● | ● | 1 |
| 25 | ● | | | | ● | ● | 1-4 |
| 30 | | ● | | | ● | ● | 1-4 |
| 22 | | ● | | | ● | ● | 1-4 |
| 49 | | | ● | ● | ● | ● | 2-3 |

● Fast/High

● Medium/Moderate

● Slow/Low

Group I (G₁ strain)

Isolates 5, 7, 30, 31 and 55 produced leaf spot symptoms with yellow halo and were designated as G₁ strain (Plates 1, 2, 3 and 4).

Group II (G₂ strain)

Isolates 1, 3, 4, 9, 12, 14, 15, 20, 21, 24, 26, 27, 34, 35, 37, 39, 40, 44, 45, 47, 50, 52, 58, 59, 60, 62, 67 and 68 produced leaf spot symptoms without yellow halo and were designated as G₂ strain (Plates 5, 6, 7, 8, 9 and 10).

Group III (G₃ strain)

Isolates 19, 23, 33, 41, 46 and 57 produced leaf blight symptoms with yellow halo and were designated as G₃ strain (Plates 18, 19, 20, 21, 22 and 23).

Group IV (G₄ strain)

Isolates 2, 6, 28, 29, 32, 43, 53 and 61 produced leaf blight symptoms without yellow halo and were designated as G₄ strain (Plates 24, 25, 26, 27 and 28).

Group V (G₅ strain)

Isolates 16, 18, 38, 48 and 64 produced leaf spot symptoms with yellow halo initially and later turned to leaf blight with yellow halo and were designated as G₅ strain.

Group VI (G₆ strain)

Isolates 22 and 25 produced leaf spot symptoms with yellow halo initially and later turned to leaf blight without yellow halo and were designated as G₆ strain.

Group VII (G₇ strain)

Isolates 49 produced leaf spot symptoms without yellow halo initially and later turned to leaf blight with yellow halo and was designated as G₇ strain.

Group VIII (G₈ strain)

Isolates 8, 10, 11, 13, 17, 36, 42, 51, 54, 56, 63, 65, 66 and 69 produced leaf spot symptoms without yellow halo initially and later turned to leaf blight without yellow halo and were designated as G₈ strain (Plates 11, 12, 13, 14 and 15).

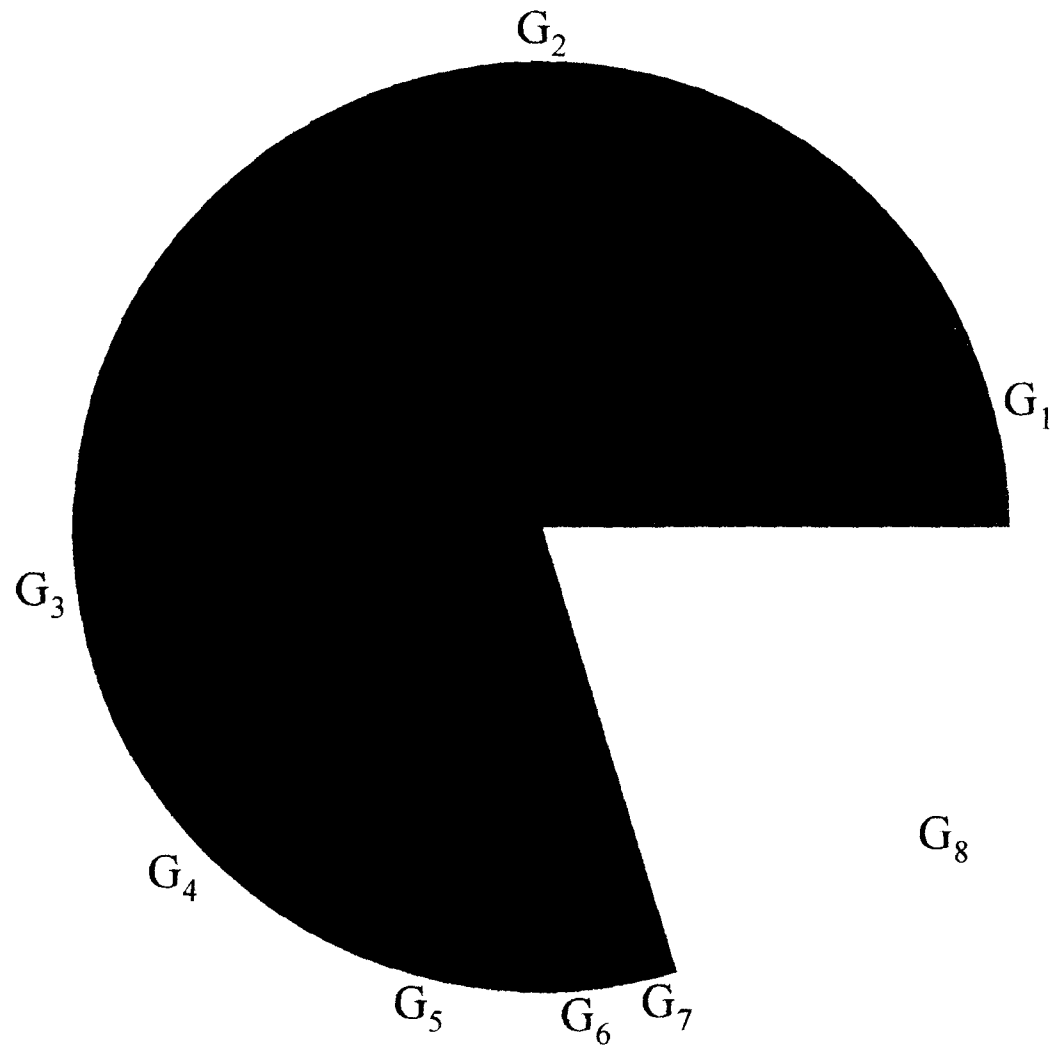


Fig. 8. Biological grouping of isolates of *C. gloeosporioides*

DISCUSSION

DISCUSSION

Colletotrichum gloeosporioides (Penz) Penz. and Sacc. was observed as a major pathogen infecting a number of plants including crop plants, ornamentals and weeds. Different isolates of *C. gloeosporioides* infecting different hosts showed great variability within the species consisting of different strain as recognised by Shear and Wood (1913), Andes and Keitt (1950), Von Arx (1957), Scharpf (1964), Souch and Corke (1979), Tan (1979) and Karunakaran (1981). Detailed studies of the variability of *C. gloeosporioides* infecting important vegetables and ornamental plants in different locations of Thiruvananthapuram and Thrissur were undertaken to distinguish different strains of the pathogen.

During the course of the present investigation attempts were made to isolate *C. gloeosporioides* associated with 150 ornamental plants and seven vegetables. On isolation only 66 ornamental plants belonging to 26 families and three vegetables belonging to two families yielded *C. gloeosporioides* (Penz) Penz. and Sacc. (Table 25). The maximum incidence of the disease was observed in the members of the family araceae followed by marantaceae.

The morphological characters of the pathogen isolated from the 66 ornamentals and three vegetables agreed with those enumerated by Von Arx (1957) and Mordue (1971) with reference to *C. gloeosporioides*.

Table 25. Occurrence of *C. gloesporioides* on various host plants

| Genus | Family | No. of plants |
|----------------|---------------|---------------|
| Aglaonema | Araceae | 12 |
| Amorphophallus | Araceae | |
| Anthurium | Araceae | |
| Colocasia | Araceae | |
| Dieffenbachia | Araceae | |
| Homalomena | Araceae | |
| Philodendron | Araceae | |
| Pothos | Araceae | |
| Spathiphyllum | Araceae | |
| Syngonium | Araceae | |
| Calathea | Marantaceae | 9 |
| Maranta | Marantaceae | |
| Dracaena | Lillaceae | 9 |
| Pleomele | Lillaceae | |
| Sansevieria | Lillaceae | |
| Eurycles | Amaryllaceae | 4 |
| Hippeastrum | Amaryllaceae | |
| Hymenocallis | Amaryllaceae | |
| Allamanda | Apocynaceae | 3 |
| Kopsia | Apocynaceae | |
| Plumeria | Apocynaceae | |
| Hamelia | Rubiaceae | 3 |
| Ixora | Rubiaceae | |
| Mussaenda | Rubiaceae | |
| Alpinia | Zingiberaceae | 4 |
| Costus | Zingiberaceae | |
| Hedychium | Zingiberaceae | |
| Begonia | Begoniaceae | 3 |
| Gerbera | Compositae | 2 |
| Tagetus | Compositae | |

Contd...

Table 25. (Contd....)

| Genus | Family | No. of plants |
|----------------|---------------|---------------|
| Iris | Iridaceae | 2 |
| Neomarica | Iridaceae | |
| Duranta | Verbenaceae | 2 |
| Lantana | Verbenaceae | |
| Quisqnqlis | Combretaceae | 1 |
| Dioscorea | Dioscoreaceae | 1 |
| Hydrangea | Saxuferaceae | 1 |
| Acalypha | Euphorbiaceae | 1 |
| Antigonon | Polygonaceae | 1 |
| Asplenium | Polypodiaceae | 1 |
| Bauhinia | Leguminaceae | 1 |
| Bougainvillae, | Myrtaceae | 1 |
| Canna | Cannaceae | 1 |
| Chrysothemis | Gesnariaceae | 1 |
| Heliconia | Musaceae | 1 |
| Hibiscus | Malvaceae | 1 |
| Ochna | Ochnaceae | 1 |
| Polyscias | Araliaceae | 1 |
| Tecomaria | Bignoniaceae | 1 |
| Thunbergia | Acanthaceae | 1 |

Extensive screening comprising 150 different species of ornamental plants and seven species of vegetable plants against *C. gloeosporioides* was done for the first time in Kerala State. Santhakumari and Nair (1981) recorded 13 ornamental plants as hosts of the pathogen, whereas Pratibha Shukla and Choudhury (1991) recorded *C. gloeosporioides* as one of the three fungi occurring on 150 cultivars of rose plants.

All the isolates of *C. gloeosporioides* obtained from their respective hosts were purified and maintained separately and were serially numbered from 1 to 69. These isolates were used for comparative studies.

Under natural conditions, there were variations in symptom expression in the 69 host plants caused by the respective isolates of *C. gloeosporioides*. Based on the symptoms observed on the host leaves, four major types of symptoms could be distinguished on the 69 host plants as detailed below :

- a) Leaf spots with yellow halo (G₁)
- b) Leaf spots without yellow halo (G₂)
- c) Leaf blight with yellow halo (G₃)
- e) Leaf blight without yellow halo (G₄)

Leaf spot symptoms surrounded by yellow halo were observed on *Amorphophallus campanulatus* (5), *Anthurium grande* (7), *Calathea insignis* (16), *C. leuconeura* (18), *C. zebrina* (22), *Colocasia antiquorum* (25), *Dioscorea alata* (30), *Dracaena deremensis* (31), *D. godseffiana* (33), *Eurycles amboinensis* (38) and *Neomarica gracilis* (55). (Plates 1, 2, 3 and 4).

Majority of isolates viz., 1, 3, 4, 8, 9, 10, 11, 12, 13, 14, 15, 17, 20, 21, 24, 27, 35, 36, 37, 39, 40, 43, 44, 45, 47, 49, 50, 51, 54, 56, 58, 59, 60, 62, 63, 64, 67, 68 and 69 produced leaf spot symptoms without yellow halos (Plates 5, 6, 7, 8, 9 and 10).

The isolates obtained from *Amorphophallus campanulatus* (5), *Asplenium nidus* (9), *Bauhinia purpurea* (10), *Begonia 'rex'* (11), *Begonia semperflorens* (13), *Bougainvillea spectabilis* (14), *Calathea kegeliana* (17), *Colocasia antiquorum* (25), *Costus sanguineus* (26), *Costus malortieanus* (27), *Duranta plumieri* (37), *Eurycles amboinensis* (38), *Hedychium coronarium* (40), *Hibiscus rosa-sinensis* (43), *Hippeastrum* sp (45), *Kopsia fruticosa* (51), *Mussaenda erythrophylla* (54), *Pleomeli reflexa* (58), *Polyscias paniculata 'Variegata'* (60), *Quisqualis indica* (62) and *Thunbergia erecta* (69) produced 'shot-hole' symptoms on the leaf lamina due to the dropping off of the central necrotic region (Plate 15, 16 and 17).

The isolates obtained from *Amorphophallus campanulatus* (5), *Anthurium grande* (7), *Calathea insignis* (16), *C. leuconeura* (18), *C. ornata* (19), *Canna indica* (23), *Costus sanguineus* (26), *Dioscorea alata* (30), *Dracaena godseffiana* (33), *Eurycles amboinensis* (38), *Hedychium coronarium* (41), *Homalomena miamiensis* (46), *Hymenocallis lillioasafoetida* (48), and *Philodendron goldieana* (57) produced blighting symptoms of variable sizes and shapes which were surrounded by yellow halo (Plates 18, 19, 20, 21, 22 and 23). Few isolates viz., 6, 28, 29, 32, 39, 53, 54, 61, 65 and 66 produced bigger patches covering a major portion of the leaf surface (Plate 24, 25, 26, 27, 28). The isolates 1, 10,

12, 30, 40, 43, 59, 62 and 69 produced defoliation symptoms. The isolates 7, 11, 17, 23, 29, 31, 32, 33, 34, 36, 38, 46, 53 and 65 produced leaf shredding symptoms. The isolates 32, 36 and 49 produced concentric zonations on the necrotic blotches. These zonations were mainly observed under alternate dry and wet conditions. Black acervuli of the organism were observed on the central region of the blighted area in majority of the host plants.

The results of the symptomatology on various host plants showed that there were variations in symptom expressions (Table 1). The results showed that different isolates of *C. gloeosporioides* infecting 69 ornamental and vegetable plants collected from different locations differed in symptom expression. The results also showed that different pathogenic isolates obtained from 69 different host plants were highly variable within the species. Tan (1979) also demonstrated that the pathogenicity of *C. gloeosporioides* infecting *Hevea brasiliensis* varied according to geographical region and also related to the severity of the disease. He observed that the most pathogenic isolates came from areas where severe leaf fall was of annual occurrence.

The concentric zonations in *Dracaena fragrans* 'Victoriae' (32), *Dracaena sanderiana* (36), and *Iris tectorum* (49) were mainly observed under alternate dry and wet conditions, possibly because of the erratic sporulation of the pathogen under varying climatic conditions.

The results of inoculation studies with the 69 isolates of *C. gloeosporioides* obtained from the respective hosts showed great variability

in the intensity of virulence in initiating the symptoms (Table 2). The isolates obtained from *Begonia 'rex'* (11), *Costus sanguineus* (26), *Mussaenda erythrophylla* (54), *Plumeria acutifolia* (59), *Bougainvillea spectabilis* (14), *Homalomena miamiensis* (46), *Philodendron goldieana* (57), and *Tagetes erecta* (67) were found to be virulent and caused infection in three days. The other isolates obtained from their respective host plants initiated infection in four to eight days (Table 2). The organisms reisolated from their respective host plants were found to be identical with the original isolates used as inoculum thereby the pathogenicity was proved. Santhakumari (1980) observed that the symptoms developed in 3-17 days after inoculation.

The results of the pathogenicity studies showed that different isolates of *C. gloeosporioides* could infect their respective host plants, indicating their susceptibility. Results also showed great variability in the intensity of virulence in initiating the symptoms on their respective host leaves.

Studies on the symptom expression as a result of inoculation with 69 isolates of *C. gloeosporioides* obtained from ornamental plants and vegetables showed a wide range of lesion types on their respective leaves (Table 2). The isolates obtained from *Calathea leuconeura* (18), *Amorphophallus campanulatus* (5), *Colocasia antiquorum* (25), *Canna indica* (23), *Philodendron goldieana* (57) and *Homalomena miamiensis* (46) produced leaf spots of different sizes with yellow halo. The isolates obtained from *Acalypha hispida* (1), *Duranta plumieri* (37), *Quisqualis indica* (62), *Bauhinia purpurea* (10), *Hamelia patens* (40), *Begonia sachsen*

(12), *Hibiscus rosa-sinensis* (43), *Plumeria acutifolia* (59), *Mussaenda erythrophylla* (54) and *Thunbergia erecta* (69) were found to produce necrotic patches of variable sizes and caused defoliation. Isolates obtained from *Asplenium nidus* (9) and *Syngonium podophyllum* (66) produced 'shot-hole' symptoms. The isolates obtained from *Chrysothemis pulchella* (24), *Costus sanguineus* (26), *Dracaena deremensis* (31), *Dracaena godseffiana* (33), *Hamelia patens* (40), *Hippeastrum* sp. (45), *Homalomena miamiensis* (46), *Hymenocallis lillioasafoetida* (48), *Plumeria acutifolia* (59), *Spathiphyllum commutatum* (65) and *Tagetes crecta* (67) produced rotting symptoms.

Cheema *et al.* (1976) reported similar variations in the pathogenicity of certain isolates of *C. gloeosporioides* in sweet orange. Verma (1982) obtained nine isolates of *Colletotrichum* infecting chillies showing various symptoms. Thind and Jhooty (1990) also concluded that significant difference existed in the pathogenic behaviour of different isolates of *Colletotrichum capsici* and *C. gloeosporioides*.

When the 69 selected isolates of *C. gloeosporioides* on ornamental and vegetables were grown on PDA, these showed great variability in growth rate, colony characters, degree of sporulation and size of conidia, indicating variation among the different isolates.

There was variation in the rate of growth among the isolates, which ranged from 0.4cm/day to 1.1cm/day (Table 3 and 4). However, the rate of growth in 45 isolates out of 69, was above 0.9cm/day showing that they were fast growers. Poor growth rate was recorded only in two isolates (15 and 38) where the growth rate was less than 0.70cm/day.

In the present studies the different isolates exhibited significant variation in mycelial growth in PDB and in Richard's medium (Table 5). Of these Richard's medium was found to be better for mycelial growth. In Richard's medium a maximum dry weight of 798mg (isolate 17) was recorded as against 537mg (isolate 58) in PDB.

Significant variations were recorded with regard to colony colour. Purple white to black, dirty white, mixtures of black and white, grey, blackish grey and ash colored colonies could be distinguished. However, based on the colour of the colony on PDA only white, dark and light types could be distinguished by earlier workers. (Chandra Mohanan *et al.*, 1989).

Sporulation studies showed that about 70 per cent of the isolates were found to be highly sporulating and grouped under 'good', 'very good', and 'abundant' (Table 7). Dasgupta (1981) also found that sporulation was very high in a few of the isolates of *Colletotrichum* studied.

The measurements of conidia of the 69 isolates showed significant variation in the size of the conidia (Table 8). The length varied from 7.0 μ m and 24.5 μ m and breadth varied from 3.5 μ m and 6.13 μ m. The range in length and breadth of each isolate is presented (Table 8). The size of the conidia of different isolates was also varied. Similar variations were observed by Andes and Keitt (1950), Scharf (1964) and Tan (1979) in their studies on *C. gloeosporioides* obtained from different hosts. In his classical attempt on the characterisation of species of *C. gloeosporioides* Von Arx (1957) proposed a key which was based on the

shape and size of the various morphological structures. He also attributed the variability of the species to the common and regular occurrence of mutation.

The different isolates of *C. Gloeosporioides* obtained from 69 host plants showed great variability in the production of soluble proteins. The protein values ranged from 0.04 to 1.45 mg/l on the fifth day of growth and from 0.10 to 1.45 mg/l on eighth day of growth. Based on the protein production three different groups of isolates could be distinguished viz., low protein producers, moderate protein producers and high protein producers (Table 9).

All the isolates of *C. Gloeosporioides* also varied markedly from each other in the enzyme activity such as polyphenol oxidase and peroxidase activity. The polyphenol oxidase activity of the 69 isolates ranged from 1.87 to 143.10/10² protein per minute. Based on the polyphenol oxidase activity, three different groups of isolates could be distinguished and were grouped under low polyphenol oxidase activity, moderate polyphenol oxidase activity and high polyphenol oxidase activity.

The peroxidase activity of the different isolates ranged from 0.04 to 2.58/10² protein per minute. Based on the peroxide activity three groups of isolates could be distinguished and were grouped under low peroxidase activity, moderate peroxidase activity and high peroxidase activity (Table 10).

Thus the 69 isolates of *C. Gloeosporioides* showed marked variability not only in their morphological, cultural and pathogenic

characters but also in the biochemical traits like protein production and enzyme activity. Kar and Mishra (1976), Agarwal and Agarwal (1979) and Zuber and Manibhushan Rao (1982) investigated the chemical constituents of the mycelium of pathogenic fungi in order to delineate the taxonomic groups and to study the complex metabolic processes of the fungi. It was also reported that the application of biochemistry to the systemics were more reliable than morphological characters. In the present study also variations among the 69 isolates were observed in their biochemical traits like protein production and enzyme activity.

Development and geographic distribution of plant diseases depend much upon climatic factors such as temperature, humidity, light etc. This is particularly true for those disease which affect aerial parts which are subjected to highly fluctuating aerial environments. *C. gloeosporioides* being a pathogen of the aerial plant parts, effect of temperature, relative humidity and light was studied.

Based on symptoms produced by the pathogen in field conditions, four groups were identified and two isolates from each group were selected for the studies. Such a classification based on symptom expression and grouping into four categories was done for the first time.

The effect of temperature on the selected isolates was studied using five different temperatures viz., 15, 20, 27, 33 and 38°C for a duration of four, six and eight days (Table 11.1, 11.2 and 11.3). The results were found to be statistically significant. It was seen that isolates of *C. gloeosporioides* showed the maximum growth at 27°C and the minimum

at 15°C. Good growth was also noted at 20, 33 and 38°C. This clearly showed that the fungus could survive at a wide temperature range of 20 to 38°C. Singh and Prasad (1967) also found that optimum temperature for growth of *C. gloeosporioides* was 26°C, with a range of 22 to 28°C. Abha Mishra and Om Gupta (1994) found (25 ± 1°C) as optimum temperature for *Colletotrichum dematium*, while Thakur and Khare (1991) found 27°C for *C. lindemuthianum* and 25°C for *C. dematium*. Thus the present finding is in agreement with the earlier reports.

The results showed that isolates varied in their growth under varying temperatures and varying duration. At 27°C, on the fourth day of incubation, isolate from *Hydrangea hortensia* (I₆) recorded the maximum growth followed by isolate from *Dioscorea alata* (I₄). Isolate from *Ixora singaporensis* (I₇) recorded the minimum growth. On the sixth day the maximum growth was recorded at 20°C followed by 27°C whereas on the eighth day the growth was maximum at 27°C followed by 20°C. Yun and Park (1990) observed that a duration of eighth h was required for infection by *C. gloeosporioides* at 25 to 30°C on grapes.

Among the different isolates studied, the maximum growth on all periods of duration viz., four, six and eight days was recorded by isolates from *H. hortensia* (I₆) and *D. alata* (I₄) while the least growth was exhibited by isolate from *Ixora singaporensis* (I₇), on the fourth day and isolate from *Anthurium andraeanum* (I₃) on sixth and eighth day.

The effect of relative humidity on the selected eight isolates was studied at 75.6, 82.9, 92.9 and 100 per cent. The relative humidity was

maintained in desiccators for a duration of four, six and eight days of incubation. (Table 12.1, 12.2 and 12.3). The results were found to be statistically significant. It was found that the maximum growth among various isolates was at 75.6 per cent RH. and the minimum growth at 100 per cent RH. Usually the RH in Kerala State varies from 70-75 per cent during all periods except during rainy seasons. This factor might account for the abundant growth of *C. gloeosporioides* in the state.

The effect of RH on all the three durations was found to be significantly different. On the fourth day the minimum growth was noted at 82.9 per cent RH and on sixth and eighth day at 100 per cent RH. Isolate from *Amorphophallus campanulatus* (I₂) had the maximum growth for all the four different RH for all the three durations, whereas isolate from *Anthurium andraeanum* (I₃) had the minimum growth. Thakur (1990) found RH 90-100 per cent most favourable for incidence of anthracnose of mungbean, whereas Sushil Sharma (1990) found RH 95 per cent favourable for bitter rot of pear. Abha Mishra and Om Gupta (1994) also found 100 per cent RH most suitable for *C. dematium* at room temperature. The reason for getting 75 per cent RH as most suitable during this study, may be due to the change in the temperature, as temperature influences the effect of RH.

The effect of light on the selected eight isolates was studied at different light treatments viz., continuous light for 24 h, alternate light and darkness for 12 h each and complete darkness for 24 h per day for a duration of eight days (Table 13.1, 13.2 and 13.3). The colony growth measured on the fourth, sixth and eighth day was analysed statistically. The effect of light was not significant on fourth day, but it was statistically significant on the sixth and eighth days.

On the fourth day isolate from *A. campanulatus* (I₂) recorded the maximum growth under complete darkness whereas isolate from *P. goldieana* (I₈) recorded the minimum growth. On the sixth day, isolate from *A. campanulatus* (I₂) recorded the maximum growth under complete darkness whereas isolate *P. goldieana* (I₈) recorded the minimum growth. On the eighth day, isolate from *A. campanulatus* (I₂) recorded the maximum growth under continuous light and complete darkness while minimum growth was recorded for isolate from *P. goldieana* (I₈) under complete darkness. Thus fluctuations with pattern of light was observed among the isolates studied. Complete darkness as well as continuous light favoured the growth of the fungus and the growth under these was on par statistically. Thus in general it could be assumed that light intensity had no effect on the growth of the pathogen, as it well survived in all intensities of light (Table 13.3). Thakur (1990) found that the maximum development of disease by *Colletotrichum* was recorded under conditions of continuous light as well as alternate light and darkness. Abha Mishra and Om Gupta (1994) also reported the maximum increase in lesion size by *C. dematium*, obtained with continuous light.

Thus considerable differences were observed in the rate of growth of *C. gloeosporioides* due to variations in environmental conditions studied, which are in agreement with the findings of Green and Simons (1991).

The variability in growth rate of eight selected isolates of *C. gloeosporioides* was studied in solid and liquid media. In oat meal agar the growth rate of different isolates varied from 4.9 to 6.9 mm. In PDA the growth rate ranged from 6.2 to 8.8 mm. In malt extract agar the rate of

growth varied from 5.5 mm to 7.3 mm (Table 14). Among the three solid media tried, all the isolates exhibited the maximum growth in PDA indicating that PDA was the best medium for mycelial growth (Table 14). Among the four liquid media tried, Richard's medium supported the maximum growth (Table 15). However, Singh and Jain (1979) found Czapek's (Dox) followed by Richard's medium supporting good growth of *C. lagenarium*. Singh and Shukla (1986) found Kirchoff's medium followed by Richard's medium supporting good growth of *C. truncatum*.

In liquid media the mycelial dry weight varied from 0.9590 mg in Coon's medium (isolate No. 5) to 0.0705 mg in basal medium (I₃).

C. gloeosporioides is a polyphagus fungus and variation within the species is common (Baker, 1940; Mordue, 1971). In the present studies all the isolates exhibited significant variations in the rate of growth. Such variations among isolates were also reported to be common in *C. gloeosporioides* pathogenic to different crop plants (Muthappa, 1974 and Singh *et al.*, 1966).

The degree of sporulation of selected isolates varied considerably when grown under different relative humidity, different periods of light and in different liquid media.

The variation in sporulation under different relative humidity was found to be statistically significant. The highest sporulation was observed at 92.9 per cent relative humidity and the least sporulation at 100 per cent

relative humidity. The highest sporulation was observed in I₈ at 75.6 per cent. The sporulation in general was poor in I₆ at 100 per cent relative humidity (Table 16). The present findings are in agreement with those of Sindhan (1983) on *C. lindemuthianum*.

The variation in sporulation when exposed at different periods of light was found to be statistically significant. The highest sporulation was in I₈ when exposed for 24 h of continuous light (125 lux). The sporulation was found to be poor in isolate No. 1 and 2 when exposed for 24 h darkness (Table 17). Increased light intensity is known to enhance sporulation of *Pyricularia oryzae* (Padhi, 1986).

Among the media used, basal medium was found to be the best for sporulation and sporulation was poor in Coon's medium. The highest sporulation was observed in I₈ grown in Richard's medium and the sporulation was very poor in I₅ in Coon's medium (Table 18). Singh and Shukla (1986) reported that Richards medium supported good growth and sporulation of *C. truncatum*.

The environmental conditions like relative humidity and light affects the pathogen, host and host pathogen system which inturn determine the sporulation of the fungus *Colletotrichum lindemuthianum* on Mung bean (Thakur and Khare, 1993). In the present studies all the eight isolates exhibited variations in sporulation under different relative humidity, different periods of light and in different media. Such variation among isolates was also reported by Chandra Mohanan *et al.* (1989).

The results of host range studies of eight isolates on 24 host plants (Table 19) revealed that all the isolates could cause 76 to 100 per cent

infection on *Mussaenda erythrophylla* indicating that this is the most susceptible host surveyed. A perusal of literature has revealed that *M. erythrophylla* was an important host of *C. gloeosporioides*.

The results of host range studies with eight selected isolates of *C. gloeosporioides* showed that the isolates were infectious on all the 24 host plants. However, great variability in the intensity of virulence in initiating symptoms was observed. All the eight isolates could cause the maximum infection (76-100%) on *Mussaenda erythrophylla*. Isolates I₂, I₄ and I₈, obtained from *Amorphophallus campanulatus*, *Dioscorea alata* and *Philodendron goldieana* could cause the maximum infection on *Sauropus androgynous*. The isolates I₃, I₅ and I₇ could cause only 51-75 per cent infection on the host plants. Only 1-25 per cent infection was recorded by all the isolates on *Cinnamomum zeylanicum*. Thus the results indicated that the eight isolates selected for the studies were not host specific.

The results of the studies also showed that different isolates of the pathogen *C. gloeosporioides* obtained from different host plants differed in pathogenicity indicating that these were highly variable.

The results showed that there were strain variations within the species as recognised by earlier workers like Santhakumari (1980) and Karunakaran (1981).

The results of cross inoculation studies with the eight isolates of *C. gloeosporioides* revealed that almost all isolates were able to cross infect the test plants. However a few isolates viz., I₂ could not cause infection on *Aglaonema commutatum* and *Philodendron goldieana*.

Similarly I₆ could not cause infection on *Dioscorea alata* and I₇ on *Amorphophallus campanulatus*, *Homalomena miamiensis* and *Hydrangea hortensia* (Table 20). The results indicated that the isolates were not host specific possibly because they might have certain physiological and biochemical properties in common as recognised by earlier workers like Stipes and Winstead (1964) and Scharpf (1964) and Karunakaran (1981).

Fungi are known to produce substances toxic to plants which affect adversely the seed germination and seedling vigour. Inhibitory effect on seed germination by the metabolites of fungi has been reported in the case of many crops by Nair, (1969). Naseema *et al.* (1982) and Jose Joseph (1986). The fungal metabolites have exerted toxic effects on the embryo resulting in its death (Brain *et al.* 1952). Naseema *et al.* (1982) observed reduction in germination of vegetable seeds like amaranthus, bhindi, brinjal, chillies, cowpea and cucurbits. In the present study also inhibition of germination by exotoxin was noted in vegetable seeds viz., cowpea, amaranthus and seeds of ornamental plants viz., balsam, periwinkle and caesalpinia (Tables 21.1, 21.2, 21.3, 21.4 and 21.5).

The results on the production of toxins by the eight isolates of *C. gloeosporioides* showed that the pathogen produced toxic metabolites when cultured in different media. The Coon's medium was found to be the best suitable medium for the production of the maximum toxin, based on the size of necrotic lesions produced on the test leaves. It ranged from 0.913 to 1.244cm on the leaves of *Philodendron goldieana* and 0.77 to 0.929cm on the leaves of *Aglaonema commutatum* (Table 22.1 and 22.2).

Statistical analysis of the data revealed that Coon's medium was found to be superior to PDB, though Czapek's, Richard's, Fries' and Coon's media were on par when bioassayed on the leaves of *Philodendron goldieana*. The results also revealed that the necrotic lesions produced by the isolate I₈ were found to be bigger (1.283cm) when compared to those of other isolates. The isolates I₁, I₅, I₄, I₆ and I₈ were found to be superior to I₇, I₂ and I₃ (Table 22.1).

When bioassayed on the leaves of *Aglaonema commutatum*, the toxin obtained from Coon's medium produced necrotic lesions bigger in size (0.929cm) followed by Czapek's and Richard's medium. The spots formed by the endotoxin obtained from PDB were found to be the smallest. Statistical analysis also revealed that the lesions produced by the isolate I₆ was bigger (1.065cm) followed by I₁ (0.869cm) and I₂ (0.844cm). The smallest spots were produced by I₈ (Table 22.2).

Different fungi are known to prefer different media for growth and toxin production. Sharma and Sharma (1969) observed that production of toxin by *C. gloeosporioides* was the maximum in Richard's medium. Nair and Ramakrishnan (1973), reported that Fries' medium was found to be the best for the production of toxin by *C. capsici*. Karunakaran (1981), observed that Richard's medium was found to be the best for the maximum toxin production. The toxin produced by all isolates were found to be the most potent. The purified toxin showed the same host specificity as does the causal organism and produced more or less identical symptoms. Even the yellow halos and 'shot-hole' symptoms were observed as seen under natural infection (Plate 36 and 34).

Production of toxins by different species of *Colletotrichum* was reported by earlier workers. Lin (1948) reported the production of a powerful substance by *Glomerella cingulata*, Wolf and Flowers (1957) by *C. nicotianae* from tobacco, Goodman (1960) by *C. fascum*, Sharma and Sharma (1969) by *C. gloeosporioides*, Nair and Ramakrishnan (1973) by *C. capsici* and Karunakaran (1981) by *C. gloeosporioides*.

The results on the effect of different incubation period on toxin production showed that the maximum toxic activity was observed on the 25th day of incubation, even though toxin production started on the 15th day of incubation. The results showed that the organism was capable of exerting toxins during its active growth stage (Table 23.1).

Effect of different isolates incubated at different periods was also found to be statistically significant when bioassayed on the leaves of *Philodendron goldieana* and *Aglaonema commutatum* (Tables 23.1 and 23.2).

Biological grouping of the 69 isolates of *C. gloeosporioides* was done based on the Adensonian classification employing the key enumerated by Bradbury (1970). The schematic profile of some of the important characters was used for grouping of different isolates. The classification was based on the rate of growth of the isolates in Richards and potato dextrose broths, biochemical traits like protein production and enzyme activity and on the similarity in symptom expression produced on the host leaves by different isolates. The final classification was based on the correlation analysis of such different characters. Based on the correlation

matrix, eight different groups of isolates could be distinguished and designated as G1, G2, G3, G4, G5, G6, G7 and G8 strains (Tables 1, 5, 9 and 10).

Group I (G1 strain)

The isolates obtained from *Amorphophallus campanulatus* (No. 5) *Anthurium grande* (7) *Dioscorea alata* (30) *Dracaena deremensis* (31) and *Neomarica gracilis* (55) agreed favourably in different characters and hence grouped together and designated as G1 strain. These isolates produced leaf spots with yellow halo (Plates 1, 2, 3 and 4).

Group II (G2 Strain)

The isolates obtained from *Acalypha hispida* (No. 1) *Allamanda cathartica* (3) *Alpinia purpurata* (4) *Asplenium nidus* (9), *Begonia sachsen* (12) *Bougainvillea spectabilis* (14), *Calathea acuminata* (15), *C. picturata* (20), *C. splendida* 'hort' (21), *Chrysothemis pulchella* (24), *Costus sanguineus* (26), *C. malortieanus* (27), *Dracaena marginata* (34), *D. reflexa* (35), *Duranta plumieri* (37), *Gerbera jamesonii* (39), *Hamelia patens* (40), *Hippeastrum reticulatum* (44), *Hippeastrum* sp. (45), *Hydrangea hortensia* (47), *Ixora singaporensis* (50), *Lantana camara* (52), *Pleomele reflexa* (58), *Plumeria acutifolia* (59), *Polyscias paniculata* 'Variegata' (60) *Quisqualis indica* (62), *Tagetes erecta* (67) and *Tecomaria capensis* (68) showed the same characters and hence grouped together and designated as G2 strain. These isolates produced leaf spots without yellow halo (Plates 5, 6, 7, 8, 9 and 10).

Group III (G3 strain)

The isolates obtained from *Calathea ornata* (19), *Canna indica* (23), *Dracaena godseffiana* (33), *Hedychium coronarium* (41) *Homalomena miamiensis* (46) and *Philodendron goldieana* (57) agreed in different characters and hence grouped together and designated as G3 strain. Those isolates produced leaf blights with yellow halo. (Plates 18, 19, 20, 21 and 22).

Group IV (G4 strain)

The isolates obtained from *Aglaonema commutatum* (2), *Anthurium andraeanum* (6), *Dieffenbachia* 'Exotica alba' (28), *D. maculata* 'Rud Roehrs' (29), *Dracaena fragrans Victoriae* (32), *Hibiscus rosa-sinensis* (43), *Maranta arundinacea* 'Variegata' (53) and *Pothos scandens* (61) agreed favourably in different characters and hence grouped together and designated as G4 strain. These isolates produced leaf blight symptoms without yellow halo (Plates 23, 24, 25, 26 and 27).

Group V (G5 strain)

The isolates obtained from *Calathea insignis* (16) *C. leuconeura* (18), *Eurycles amboinensis* (38) *Hymenocallis lillioasafoetida* (48) and *Sansevieria trifasciata* (64) were grouped together and designated as G5 strain. These isolates produced leafspots with yellow halo initially and later turned to leaf blights with yellow halo.

Group VI (G6 strain)

The isolates obtained from *Calathea zebrina* (22) and *Colocasia antiquorum* (25) produced leaf spots with yellow halo initially and later turned to leaf blight without yellow halo and hence designated as G6 strain.

Group VII (G7 strain)

The isolate obtained from *Iris tectorum* (49) produced leaf spot without yellow halo initially and later turned to leaf blight with yellow halo and hence designated as G7 strain.

Group VIII (G8 strain)

The isolates obtained from *Antigonon leptopus* (8), *Bauhinia purpurea* (10), *Begonia 'rex'* (11), *B. semperflorens* (13), *Calathea kegeliana* (17), *Dracaena sanderiana* (36), *Heliconia metallica* (42), *Kopsia fruticosa* (51), *Mussaenda erythrophylla* (54), *Ochna squarrosa* (56), *Sansevieria 'Hahnii'* (63), *Spathiphyllum commutatum* (65), *Syngonium podophyllum* (66) and *Thunbergia erecta* (69) produced leaf spots without yellow halo initially and later turned to leaf blight without yellow halo and hence designated as G8 strain (Plates 11, 12, 13, 14 and 15).

The eight different strains of *C. gloeosporioides* could be distinguished based on the correlation analysis of different characters. The results therefore showed that different isolates of *C. gloeosporioides*

obtained from 69 host plants are highly variable within the species consisting of different strains as recognised by Shear and Wood (1913) Andes and Keitt (1950) Von Arx (1957), Scharpf (1964), Souch and Corka (1979), Tan (1979) and Karunakaran (1981) on *C. gloeosporioides* (*Glomerella cingulata*) obtained from different hosts.

Bradbury (1970) had enumerated the key for classification of bacteria isolated from plants. (Adensionian classification) Mathew George (1983) had studied the strain variation of coconut Enterobacter associated with the root wilt affected coconut palms of Kerala. He conducted several biochemical tests of 80 isolates and finally arrived at seven groups.

In the present study 69 isolates of *C. gloeosporioides* obtained from three vegetable and 66 ornamental plants were subjected to various cultural, morphological and biochemical studies and grouped into eight. Such a classification of a fungus was done for the first time in Kerala.

SUMMARY

SUMMARY

A survey was conducted to study the variability of *Colletotrichum gloeosporioides* (Penz.) Penz. and Sacc. infecting important vegetables and ornamental plants in different locations of Thiruvananthapuram and Thrissur districts of Kerala in order to distinguish different strains of the pathogen. Attempts were made to isolate the pathogen associated with seven vegetables and 150 ornamental plants. On isolation three vegetables and 66 ornamental plants yielded *C. gloeosporioides*.

Studies on the symptoms showed that there were variations in the expression of symptoms on the host plants as detailed below :

- a) Leaf spot surrounded by yellow halo
- b) Leaf spot without yellow halo
- c) Shot-hole symptom due to dropping off of the central necrotic region
- d) Leaf blight surrounded by yellow halo
- e) Bigger patches covering a major portion of the leaf
- f) Appearance of concentric zonations on the necrotic blotches.
- g) Defoliation of infected plants

The symptoms caused by different isolates of *C. gloeosporioides* obtained from 69 different host plants were highly variable.

The results of inoculation studies with the 69 isolates showed great variability in the intensity of virulence in initiating symptoms on their respective host leaves. Studies on the symptom expression as a result of inoculation with the different isolates showed a wide range of lesion types on their respective host leaves indicating that significant differences existed in the pathogenic behaviour of different isolates of the pathogen.

The different isolates of the pathogen obtained from the 69 host plants showed great variability in growth rate, colony characters, degree of sporulation and size of conidia indicating that there were considerable variations among different isolates.

Among the media tried Richard's medium was found to be the best for mycelial growth. Sporulation studies showed that 70 per cent of the isolates were highly sporulating. Significant variations were also found in the colony colour. The measurements of conidia of different isolates showed significant variations.

All the 69 isolates of the pathogen showed great variability in the production of soluble proteins. Three different groups of isolates could be distinguished, viz. low protein producers moderate protein producers and high protein producers.

The different isolates of the pathogen also varied from each other in the enzyme activity such as polyphenol oxidase and peroxidase activity.

Based on the polyphenol oxidase activity three groups of isolates and based on the peroxidase activity three groups of isolates could be distinguished.

The effect of temperature, relative humidity and light on the selected isolates was studied. The maximum growth was recorded at 27°C and the minimum at 15°C. However, the isolates varied in their growth under varying temperatures and varying duration.

The maximum growth of the fungus was observed at 75.6 per cent RH and the minimum growth at 100 per cent RH. Fluctuations with pattern of light was observed among the isolates studied. Complete darkness as well as continuous light favoured the growth of the pathogen and these were on par statistically indicating that light intensity had no effect on the growth of the pathogen.

The variability in growth rate of eight selected isolates of the pathogen was studied on solid and in liquid media. Among the three solid media tried, all the isolates exhibited the maximum growth on PDA indicating that PDA was the best medium for mycelial growth. Richard's medium supported the maximum growth among the four liquid media tried. In the present studies all the isolates exhibited significant variations in the rate of growth.

The degree of sporulation of selected isolates varied considerably when grown under different relative humidity, different periods of light and in different media. The highest sporulation was observed at 92.9 per cent RH and least at 100 per cent RH. The highest sporulation was

observed when exposed for 24 h of continuous light (125 lux) and poor when exposed for 24 h darkness. Basal medium was found to be best for sporulation and sporulation was poor in Coon's medium.

Results of the host range studies showed that all the selected isolates were infectious on the host plants tested. However, great variability was observed in the intensity of virulence in initiating symptoms. All the isolates could cause 76-100 per cent infection on *Mussaenda erythrophylla* indicating that this was the most susceptible host surveyed.

Cross inoculation studies with eight selected isolates of the pathogen revealed that almost all the isolates were cross-infectious indicating that the isolates were not host specific.

All the eight selected isolates were found to produce toxic metabolites in different media. In the present studies inhibition of germination of vegetable seeds viz. cowpea and amaranthus and seeds of ornamental plants viz. balsam, vinca and caesalpinia was recorded when bioassayed with exotoxin.

Coon's medium was found to be the best medium for production of endotoxin. The purified toxins produced more or less identical symptoms as does the causal organism. The production of toxin in the media started on the 15th day of growth and reached the maximum on the 25th day of incubation.

Biological grouping of the 69 isolates of *C. gloeosporioides* was done based on the correlation analysis of different characters, viz. the rate

of growth, biochemical traits like protein production and enzyme activity and on the symptom expression by different isolates. Based on the correlation matrix, eight different groups of isolates could be distinguished and designated as G₁, G₂, G₃, G₄, G₅, G₆, G₇ and G₈ strains of the pathogen.

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APPENDIX

APPENDIX

COMPOSITION OF MEDIA USED

SOLID MEDIA

1. Potato dextrose agar

| | |
|--------------------------|---------|
| Potato peeled and sliced | 250 g |
| Dextrose | 20 g |
| Agar | 20 g |
| Distilled water | 1 litre |

2. Oat meal agar

| | |
|-----------------|---------|
| Oat meal | 30 g |
| Agar | 20 g |
| Distilled water | 1 litre |

3. Malt extract agar

| | |
|-----------------|---------|
| Malt extract | 20 g |
| Agar | 20 g |
| Distilled water | 1 litre |

LIQUID MEDIA

1. Coon's medium

| | |
|---------------------------------|---------|
| Potassium nitrate | 20.2 g |
| Magnesium sulphate | 1.23 g |
| Potassium di-hydrogen phosphate | 2.72 g |
| Sucrose | 7.2 g |
| Dextrose | 3.6 g |
| Distilled water | 1 litre |

2. Basal medium

| | |
|---------------------------------|---------|
| Glucose | 10.0 g |
| Potassium nitrate | 3.59 g |
| Potassium di-hydrogen phosphate | 1.75 g |
| Magnesium sulphate | 0.75 g |
| Distilled water | 1 litre |

APPENDIX (Contd....)

3. Richard's medium

| | |
|---------------------------------|---------|
| Potassium nitrate | 10.0 g |
| Potassium di-hydrogen phosphate | 5.0 g |
| Magnesium sulphate | 2.5 g |
| Ferric chloride | 0.02 g |
| Sucrose | 50.0 g |
| Distilled water | 1 litre |

4. Potato dextrose broth

| | |
|--------------------------|---------|
| Potato peeled and sliced | 200 g |
| Dextrose | 20 g |
| Distilled water | 1 litre |

5. Fries' medium

| | |
|------------------------------|---------|
| Ammonium tartarate | 5 g |
| Ammonium nitrate | 1 g |
| Potassium hydrogen phosphate | 1 g |
| Magnesium sulphate | 0.5 g |
| Calcium chloride | 0.1 g |
| Sucrose | 30 g |
| Distilled water | 1 litre |

6. Czapek's Dox Medium

| | |
|------------------------|---------|
| Sucrose | 30 g |
| Sodium nitrate | 2 g |
| Di-potassium phosphate | 1 g |
| Magnesium sulphate | 0.5 g |
| Potassium chloride | 0.5 g |
| Ferrous sulphate | 0.01 g |
| Distilled water | 1 litre |

**STRAIN VARIATION IN COLLETOTRICHUM
GLOEOSPORIODES (PENZ.) PENZ. & SACC.**

By

K. J. ALICE

ABSTRACT OF THE THESIS
SUBMITTED IN PARTIAL FULFILMENT OF
THE REQUIREMENT FOR THE DEGREE OF
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DEPARTMENT OF PLANT PATHOLOGY
COLLEGE OF AGRICULTURE
VELLAYANI, THIRUVANANTHAPURAM

1996

ABSTRACT

Colletotrichum gloeosporioides (Penz.) Penz. & Sacc. is observed as a major pathogen infecting a number of crop plants, ornamentals and weeds. Detailed studies on the variability of this pathogen on important vegetables and ornamental plants in different locations of Thiruvananthapuram and Thrissur districts of Kerala were undertaken to distinguish different strains of *C. gloeosporioides*.

Studies on the symptoms and pathogenicity tests of the 69 isolates showed that there were variations in the expression of symptoms and in initiating symptoms indicating that the isolates were highly variable.

Great variability in growth rate, colony characters, degree of sporulation and size of conidia were observed in different isolates.

Based on the protein production and enzyme activity three groups of isolates could be distinguished.

The isolates varied in their growth under varying temperature and relative humidity. Light intensity had no significant effect on the growth of the pathogen.

Among the solid media tested, all the selected isolates exhibited the maximum growth on PDA. Richard's medium was found to be the best liquid medium for mycelial growth.

The degree of sporulation of selected isolates was the highest at 92.9 per cent RH and the lowest at 100 per cent RH. The highest sporulation was observed when exposed for 24 h of continuous light (125 lux) and poor when exposed for 24 h darkness. Basal medium was found to be best for sporulation.

The results of host range studies and cross inoculation studies showed that the selected isolates were not host specific even though there were variations among the isolates in the intensity of infection.

Inhibition of germination of selected seeds was observed when bioassayed with exotoxin.

The purified toxin produced more or less identical symptoms as did by the causal organism. Coon's medium was found to be the best for production of endotoxin. The maximum production of toxin was observed in 25 day old cultures.

Biological groupings of all the 69 isolates studied were done based on the correlation matrix of different characters. Eight different groups of isolates could be distinguished and designated as G₁, G₂, G₃, G₄, G₅, G₆, G₇ and G₈ strains of *C. gloeosporioides*.