STUDIES ON THE FUNGAL DISEASES OF ORNAMENTAL PLANTS

By

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THESIS

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DECLARATION

I hereby declare that this thesis entitled "STUDIES ON THE FUNGAL DISEASES OF ORNAMENTAL PLANTS" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis entitled "STUDIES ON THE FUNGAL DISEASES OF ORNAMENTAL PLANTS" is a record of research work done independently by Kum. P. Santha kumari under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship, or associateship to her.

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INTRODUCTION

INTRODUCTION

Gardening is now a recognised activity and an important item in town planning and urban development. Ornamental plants have a significant role in beautifying places, small or big, private or public. Also growing of ornamental plants is a part of human pleasures and flowers are symbolic of beauty, love and tranquility. Ornamental plants are being grown in India from time immemorial. Many people grow plants for the adornment of their homes and many others produce ornamental flowers commercially and earn their livelihood from floriculture. The sale of flowers as cut ones and as garlands form an important business in many of the Indian cities. Ornamental plants, their seeds, bulbs etc. are being exported and earn a good amount of foreign exchange to us. Besides their aesthetic value flowers are also important for their economic uses in medicine and extraction of perfumes etc.

Successful growing of ornamental plants like any other crop husbandry is often a difficult task, due to the incidence of diseases and insect pests. A working knowledge of the common diseases of the common ornamentals is desirable to pursue remedial measures intelligently and judiciously. No book in India is at present available that gives a comprehensive account of the diseases of the ornamentals we grow, although references about them are made occasionally.

whenever a new disease is observed.

A list of all the diseases occurring on common ornamental plants in India, when prepared would serve the need of scientists working on ornamental plants with the ultimate aim of identifying and controlling them. An attempt is made in the present study to identify the common fungal foliage diseases of the ornamental plants grown in and around the Campus of College of Agriculture, Vellayani, Trivandrum. Since Colletotrichum gloeosporioides forms a major pathogen, affecting a number of the common ornamentals a comparative morphological study of few different isolates of this pathogen were made.

Further detailed studies were carried out with an isolate of C. gloeosporioides from Hydrangea hortensia. The host-range of this organism, its nutrient requirement, role of toxin production by the organism in pathogenesis and changes in chemical constituents in Hydrangea leaves brought about by infection by the pathogen were studied. A fungicidal trial to find out the best fungicide for the control of the leaf spot on Hydrangea hortensia was also carried out. It is hoped that the present study will fill up this long felt need for detailed information on diseases of ornamental plants especially under conditions prevailing in our country to a certain extent.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Diseases and attack by insect pests are the important factors which result in the loss of aesthetic value of ornamental plants. Various workers have reported a number of diseases on ornamentals, some of them causing great economic loss. In the present study, a survey was conducted on the occurrence of fungal diseases on thirty five ornamental plants and detailed comparative studies of leaf spot diseases were carried out in the case of seven selected common ornamental plants. The occurrence of new diseases noted in the present study on the plants surveyed were discussed under the chapter 'Discussion' and a short review of the fungal leaf spot diseases on the seven selected ornamentals are summarised below.

Clitoria ternatea L.

Cercospora pentaleuca Syd. and *Cercospora ternatea* Petch. causing leaf spot on *Clitoria ternatea* were reported by Sydow (1913) and Sydow and McRae (1929). Patel *et al.* (1949) noticed the incidence of *Oidium* sp. on *C. ternatea*. A leaf spotting due to *Chaetosentoria wellmanii* Stev. was reported by Quiros Calvos (1950). Attack due to *Periconia byssoides*, *P. clitoriae* and *Dwayamala prathilomaka* Subram. were noticed by Subramanian (1955; 1956). *Colletotrichum dematium* f. sp. *clitoricola* causing anthracnose was reported by Pavgi and Mukhopadhyay (1965). Occurrence of *Alternaria alternata* was noticed by Jamaluddin and Tandon (1973).

Cosmos bipinnatus Cav.

Goto (1950) isolated Sclerotium rolfsii from C. bipinnatus. The disease is mainly manifested on the plants at the blossoming stage. Neergaard (1945) reported the association of Alternaria zinniae on C. bipinnatus. Chiddarwar (1959a) reported Cercospora cosmi on this ornamental.

Gardenia jasminoides Ellis

Khaiskar (1968) reported a Phyllostiota leaf spot due to Phyllostiota gardenicola Khaiskar from India.

Gerbera jamesonii Hook.

Park (1936) reported a leaf disease caused by Septoria sp. on G. jamesonii. Other fungi reported from this plant were Septoria gerberae, Ascochyta gerberae, Cercospora sp., Cystorus cubicus (Mattrass, 1950); Septoria gerberae (Gilman, 1952); Cercospora gerberae (Chiddarwar, 1959b); Phyllostiota gerbericola (Patil and Rao, 1974).

Hydrangea hortensia DC.

Mendoza and Leus-palo (1941) recorded the occurrence of Cercospora hydrangeana on H. hortensia from Philippines. Alternaria hortensiae producing numerous circular lesions on the leaves was reported by Umemuno (1944), Lucas and Decamara (1952) isolated Phomopsis hydrangeae from the branches of H. hortensia. A detailed study on the leaf spot due to a species of Colletotrichum was conducted by Wilson (1965). The symptoms observed as minute reddish brown specks on the leaves.

As the disease advances these necrotic specks enlarged with a grey centre and slightly raised dark brown margins measuring 1 - 4 mm in diameter. The diseased tissues yielded Colletotrichum which produce light grey aerial mycelium with numerous acervuli in artificial medium. The conidia were hyaline, non-septate, oblong with rounded ends, measuring 12.9 to 22.3 µm in length and 3.6 - 5.2 µm in breadth the average being 19.5 x 4.4 µm. Rangaswami et al. (1970) reported the occurrence of C. gloeosporioides on H. hortensia from India.

Jasminum sambac Soland

Mathur and Mathur (1959) isolated Curvularia prasadii from the leaves of J. sambac. Colletotrichum jasminicola Y Tilak causing leaf spot on J. sambac was reported by Tilak (1960). Other fungal pathogens reported were Bortalinia robillardes Tassi, Phoma jasminicola (Agarwal and Sahni, 1965); Phyllosticta jasminicola Vasant Rao (Rao, 1965); Chaetothyrium jasminicola (Sahni, 1966a); Lembertella brunneola (Pat.) Le Gal (Tewari and Pant, 1966); Colletotrichum dematium (Pers. ex.Fr.) Grove (Prasad and Achaya, 1967); Cercospora jasminicola (Dayal and Ram, 1967).

Jasminum sp.

Sydow and Sydow (1916) first reported Sirococcus butleri Syd. on Jasminum sp. Aschochyta jasminicola causing rounded zonate leaf spot with dark margins on an undetermined species of jasmine was reported by Canonaco (1936). Mitter (1938)

reported Phyllactinia corylea var. subspiralis Salm. on jasmine leaves. Cercospora jasminicola Muller and Chupp was noticed by Thirumalachar and Chupp (1948). Puccinia chrysopogi Barclay and Puccinia exhaurians Thum. were reported by (Sydow and Sydow, 1912; Ramakrishnan and Ramakrishnan, 1949); Colletotrichum sp. (Venkatarayan, 1956); Torula herbarum (Subramanian, 1956) were the other fungal pathogens noticed.

Colletotrichum gloeosporioides

Since Colletotrichum gloeosporioides was observed as a major pathogen on majority of the plants surveyed further studies were made on this pathogen.

The genus Colletotrichum was described by Corda (1831-32) under the name Colletotrichum with a single species. He, later on (Corda, 1837) changed the name as Colletotrichum. Saccardo (1884) placed Colletotrichum in Melanconiales and in Acervulales by Potebnia (1910). Arx (1957) made a detailed study on the species of the genus Colletotrichum and assigned the ascogenous state of C. gloeosporioides as Glomerella cingulata. The designation of C. gloeosporioides is selected from about 600 names to indicate the conidial state of C. cingulata. The variability of the species is attributed to the easy and regular occurrence of mutations. It is pathogenic to numerous plants causing fruit rot, wilt, leaf spots, necrosis and anthracnose and may also be harboured in a symptomless form.

I. Morphology.

(a) Conidial state

The morphological characters of the conidial state varies and various workers gave different measurements for the various isolates of this organism.

Prasad and Singh (1960) described the Dioscorea isolate of Colletotrichum gloeosporioides as follows.

The mycelium olive green to dark grey in colour, hyphae hyaline to olive, guttulate, measuring 2.9 to 6.5 µm in diameter. Acervuli slight pink to brown, globose to saucer shaped on culture media, without setae. On the leaves and stem these were erumpent, variously shaped. Conidia borne singly on conidiophores, oval to oblong or cylindrical 11 to 18.5 µm by 3.7 to 6.4 µm; average 15.6 x 4.9 µm in size, non-septate guttulate usually with one or two oil drops, hyaline singly but pink in masses. Conidiophores simple and hyaline.

Prasad (1962) reported that the Nephelium litchi isolate of C. gloeosporioides have a deep brown multicellular setae having a swollen basal cell. Cylindrical and oblong one celled straw berry pink conidia (17.28 to 18.42 µm x 3.93 to 6.14 µm) with granular contents are produced in large numbers on simple conidiophores (17.68 to 24.68 µm x 3.45 to 6.00 µm). The hyphae, branched, septate and 1.31 to 2.62 µm broad.

Varadharajan (1964) described the Rauwolfia serpentina isolate as having disc shaped acervuli with dark coloured 3 to 5 septate setae at the edge of the fruiting body. The setae

had a basal swollen cell and a rounded apex with 87 to 113 µm x 3 to 5 µm. Conidiophores are closely packed, 17 to 24 µm x 3 to 5 µm; conidia, numerous, simple, cylindrical, elongate and oblong 12 to 30 µm x 3 to 5 µm.

Agnihothrudu and Hafapa (1966) reported Avocado isolate of C. gloeosporioides as follows. The mycelium pseudoparenchymatous, sub-hyaline, conidiophores simple, cylindrical upto 20 µm long each producing conidia terminally and successively. No acervular setae were recorded. Conidia cylindrical, hyaline, continuous with rounded ends and measured 12 to 21 x 3 to 6 µm.

In culture medium, Plumeria acutifolia isolate produced acervuli which were 135 to 210 µm in diameter. Setae irregularly arranged, dark brown, septate with slightly pointed apex measured 38 to 113 µm in length. Conidiophores simple, hyaline, non-septate and closely packed together. Conidia oblong to cylindrical with rounded ends, hyaline, single but pink in masses, non-septate and granular with one or two vacuoles. They measured 12.0 to 17.5 µm in length and 4.0 to 5.5 µm in breadth (Wilson and Joshi, 1966).

Lele and Ram (1969) observed the loquat isolate of C. gloeosporioides having the following characters. Acervuli on culture media have 80.5 to 275.0 µm diameter, setae absent, conidia 7.0 to 17.5 x 3.5 µm, oblong, hyaline, single celled and often bearing oil drops.

Mordue (1971) enumerated the following characters for this organism.

The acervuli are usually setose, sometimes sparsely setose or glaborous, rounded, elongated or irregular in shape and may attain as much as 500 µm in diameter. Setae variable in length, rarely more than 200 µm long, 4 to 8 µm wide, 1 to 4 septate, brown, slightly swollen at the base and tapered to the apex on which conidia are occasionally borne. Conidia hyaline, cylindrical, aseptate, uninucleate, 9 to 24 x 3 to 6 µm, formed on unicellular hyaline or faintly brown cylindrical phialidic conidiophores.

On PDA culture the conidia varied in size and shape than those on the host. The conidia are formed on setose or glaborous acervuli on solitary phialides on mycelium, usually pale salmon in mass.

(b) Perfect state

Mordue (1971) gave the following descriptions for the perfect state, Glomerella cingulata.

Perithecia on host is solitary or aggregated, globose or obpyriform, dark brown to black 85 to 300 µm diameter, wall upto 8 cells thick. The wall may be sclerotial outside, pseudoparenchymatous within, ostiole slightly papillate, circular, canal lined with paraphysis. The asci are eight spored, clavate to cylindrical, thickened at the apex, 55 to 80 x 8 to 40 µm, interspersed with paraphysis. The ascospores are narrowly oval to cylindrical to fusiform, slightly curved, unicellular, hyaline and uniseptate prior to germination.

II. Physiology

Miller (1955) noticed V-8 juice agar as a general purpose

medium for Glomerella cingulata and Colletotrichum minoides along with several other fungi. Increase in growth and sporulation was observed with sucrose at pH 3.5 to 5.5 (Sitterly, 1958). Chandra and Tandon (1962) observed glucose as the best carbon source for C. gloeosporioides along with several other fungi. Utilisation of maltose, lactose and raffinose by C. gloeosporioides was reported by Tandon and Chandra (1962a,b). They also observed glucose, galactose and sucrose as the best carbon source, $MgSO_4$ as the good sulphur source, glutamine as the best amino acid. Prasad (1965) observed glucose as a good source of carbohydrate. Lal and Tandon (1968) reported good growth of C. gloeosporioides (Glomerella cingulata) on glucose.

Sitterly (1958) recorded an increase in growth of Glomerella cingulata at pH 3.5 to 5.5. Best growth and sporulation of Glomerella cingulata at 3 to 8.5 was reported by Tandon and Chandra (1962b). Verma (1969) recorded a pH of 6 as the optimum for the growth of C. gloeosporioides.

III. Host range

The pathogen C. gloeosporioides has a wide host range. In India alone, a number of workers reported this organism on a wide variety of host plants as enumerated by Bilgrami et al. (1979).

Albizzia lebbeck, Allamanda cathartica, Aloe vera, Alstonia scholaris, Anona squamosa, Arachis hypogea, Aralia bipinnatifida, A. balfornii, A. cordo, Aralia sp., Artocarpus

incisa, Averrhoea carambola, Bougainvillaea spectabilis, Bredelia retusa, Buchanania lanzona, Canna zebrina, Cassava esculenta, Carica papaya, Celosia argenta var. cristo, Citrus acida, C. aurantium, C. medica, C. sinensis, Coccinia indica, Cinnamon tenala, Ceiba pentandra, Dalbergia sisoo, Dieffenbachia amoena, Dodonae viscosa, Dracaena terniflora, Eriobotrya japonica, Eugenia dalbergioides, Ficus asperima, F. bengalensis, Flacourtia inermis, Gomphrena globosa, Glyricidia maculata, fruits of guava, Hippeastrum johsonii, Hydrangea hortensia, Inga macrophylla, Ixora sp., Jasminum pubescens, Jasminum sambac, Jatropha curcas, J. gossypifolia, J. podagrica, Mangifera indica, Manihot sp., Marsdenia volubilis, Murraya koenigii, Pemmonia tithymoides, Pilea macrophylla, Pithecolobium dulce, Persea gratissima, Plumeria rubra, Polyalthia longifolia, Psium arvense, Prunus persica, Ricinus communis, Samania saman, Sansoveera roxburgiana, Santalum album, Seraca indica, Schefflera stellata, Swietenia macrophylla, S. mahogoni, Theobroma cacao and Vitis vinifera are some of the important host plants for this pathogen.

Toxin production.

Production of toxic metabolites by species of Colletotrichum was reported by various workers. Lin (1948) reported the production of a powerful toxic substance by Glomerella cingulata. Production of toxin by tobacco anthracnose organism (Colletotrichum nicotianae) was observed by Wolf and Flowers (1957). They obtained anthracnose symptoms

on the leaves and petioles with the sterile filtrates of cultures of the organism thus revealing the secretion of a toxic metabolite into the growing medium. Goodman (1960) isolated Colletotin from C. fuscum. Han (1968) isolated a toxic metabolite from the culture filtrates of C. musae. Lycomarasmic acid production by Colletotrichum gloeosporioides Penz. was reported by Ballio et al. (1969). The production of a toxic metabolite causing citrus die-back in India was reported by Sharma and Sharma (1969). Detailed studies on the toxin produced by C. capsici associated with turmeric leaf spot disease were done by Nair (1972).

(a) Effect of substrate on toxin production

The effect of various media supporting toxin production was studied by various workers. Brain et al. (1949) proved that Czapek's medium containing 10 to 15 per cent sucrose was suitable to Alternaria solani for the greatest toxin activity and production of toxic substance. Best toxin production in Czapek's medium was also reported by Nair (1969) for Trichoconis padwickii. Sharma and Sharma (1969) observed good toxin production by C. gloeosporioides in Richard's medium.

The type of carbon sources supplied greatly affects the synthesis of toxin production. Brain et al. (1951) showed that the media containing high concentration of sugar proved to be best suited for the growth and activity of metabolic substance produced by Alternaria solani. Graniti and De Leo (1964) reported the best yield of toxin by Fusarium amygdali

with arabinose, glucose or sucrose. Increased toxin production with glucose or sucrose as carbon source was observed by Nair (1972).

(b) Bioassay techniques

Luke and Wheeler (1955) revealed that the susceptible varieties of oats when treated with the toxic metabolite produced striking reduction in the growth of both roots and shoots. The activity of non-specific phyto-toxins can be evaluated by the inhibition of growth of seedlings. Ludwig (1957) used seed inhibition as a method of bioassay for testing the toxicity of the culture filtrate of Helminthosporium sativum. Krishnaswamy et al. (1969) tested the toxin production by different isolates of Pyricularia oryzae by plumule and radicle inhibition bioassays. Effect of toxin in seed germination inhibition by the toxin produced by the fungus associated with paddy seeds was demonstrated by Vidhyasekharan et al. (1970). Nair (1972) used the host leaves itself for the bioassay of toxin produced by Colletotrichum capsici isolated from turmeric.

(c) Specificity of toxic substance produced by the fungus in culture filtrate

Brain et al. (1949) produced wilting of radish, cabbage and carrot seedlings with the culture filtrate of Alternaria solani. Litzenberger (1949) found that the susceptible Victoria variety of oats rapidly developed chlorosis and necrosis accompanied by wilting when treated with the culture filtrate of Helminthosporium victoriae. Goodman (1960)

reported the wilting of tomato seedlings, with the culture filtrate of Collototrichum fuscum. The effect of culture filtrate of Periconia circinata was tested by Scheffer and Pringle (1961) against the new hosts like rye, barley, wheat, oats, tomato etc., but no toxic effect was produced on them. Graniti (1964) demonstrated the wilting of tomato cuttings with culture filtrate of Fusicoccum amygdali. Pringle and Scheffer (1967) found that toxin isolated from the culture filtrates of Helminthosporium carbonum as a host specific one. Wilting of the cut twigs of citrus immersed in culture filtrates was demonstrated by Sharma and Sharma (1969).

(d) Effect of temperature and dilution on the toxic activity of culture filtrate

The toxic effect of the culture filtrate boiled at 100°C for 10 minutes was demonstrated by Sharma and Sharma (1969) for C. gloeosporioides. They also reported the reduction in the toxic activity of the culture filtrate when sterilised at 10 lb pressure for 15 minutes.

Decrease in toxic activity of culture filtrate with increase in dilution was reported by Sharma and Sharma (1969) for C. gloeosporioides.

III. Fungicidal control.

(a) In vitro evaluation

Sijpesteijn and Janssen (1958) found that C. cingulata to be sensitive to 1:2 copper dimethyl dithiocarbamates. Dosiljka and Ristanovic (1962) reported dihydro streptomycin

to be inhibitory on Colletotrichum lini. Anti-fungal activity of 2-deoxy-D-glucose on C. circulata was reported by Alin et al. (1964). C. circulata was sensitive to Zineb, Thiram, bulbocan, captan and triphenyl tin acetate (Eiklenboom, 1964). Kothari and Bhatnagar (1966) reported complete inhibition of spore germination of C. capsici with Ferbam even at the lowest concentration tried (2 ppm). Fytolan and Dithane Z-78 showed total inhibition of spore germination at 64 and 128 ppm respectively.

Beccari et al. (1969) showed the inhibition of growth of Colletotrichum musae with Benomyl at 500 ppm and Thiabendazole at 450 ppm. Aureofungin at 50 ppm were effective in restricting the conidial germination of C. capsici (Narain and Panigrahi, 1971). Gupta (1974) reported Bresten and Aureofungin to be effective in inhibiting the spore germination of C. piperatum. One ppm concentration of Aureofungin gave complete inhibition of spore germination of this organism.

Saksena et al. (1975) showed Thiram as a best anti-respiratory fungicide for C. capsici. In vitro effect of Captan (0.25%) and Thiram (0.25%) on C. circulata were reported by Saraswathy et al. (1975). Singh (1976) obtained good inhibition of growth of C. falcatum with oils of Tagetus patula, sunflower, clove, cardamon and eucalyptus. Lima et al. (1978) reported the best toxic effect of Tecto 40 F, Daconil 2787 Vitavax, Orthodifolatan 4 F and Benomyl against C. gloeosporioides.

(b) Field evaluation

Anon (1941) reported the control of papaya anthracnose (C. gloeosporioides) by spraying the fruits at 14 days interval with Bordeaux mixture (4-4-50), cuprocide 54 or cuprocide 547. Howell and Howard (1948) demonstrated the control of bean anthracnose with Kyocton-4 and Onyx DL-1.

Effective control of C. capsici (turmeric leaf spot) with Bordeaux mixture (1 per cent) was reported by Govind Rao (1952). Effective control of bean anthracnose with four to six application of Dithane Z-78 was shown by Bruinsma and Labruyere (1953). Johnston (1953) reported the effect of Perenox and Bordeaux mixture (0.5 per cent) against bean anthracnose organism, C. lindemuthianum. Agnin Vooladkar (1953) obtained satisfactory control of the bean anthracnose with Bordeaux mixture or perenox. Zineb sprays or dusts gave effective control of cucumber anthracnose and tobacco anthracnose (Anon., 1955; 1956). McDonald et al. (1958) found methyl bromide to be better for the control of C. tabacum when given as a soil treatment. Satisfactory control of C. gloeosporioides was obtained with Orthodifolatan, Dithane M-45, Daconil, Cercobin and Benomyl against cashew anthracnoses (Menezes et al., 1975a,b).

Post-infection changes in tissue constituents of the host

Meneses (1957) reported that there is a correlation between sugar content and susceptibility of maize seedlings to Colletotrichum graminicola. The resistance to infection

occurred when the sugar content is over 5 per cent. High content of chlorogenic acid and other orthodihydric phenols in the scab resistant varieties of potato was observed by Johnson and Schaal (1957). An increase in the glutamic acid and a decrease in glutamine and citrulline contents were noticed in the anthracnose infected water melons by McComb and Winstead (1961). They also observed a decrease in the glucose and sucrose contents in the tissues as the disease severity increased. Chandrachan et al. (1967) observed a decrease in both O-D and total phenols in the diseased leaves of Amaranthus tricolor infected by Alternaria sp. Considerable decrease in the reducing, non-reducing and total soluble sugars in the infected tissues was reported by Ram Dayal and Joshi (1967). A decrease in ascorbic acid, amino acids and carbohydrates such as fructose and xylose were observed by Chahal and Grover (1972) in relation to Choanephora cucurbitarum. Nair (1972) reported a decrease in the phenolic content and an increase or decrease of certain sugars in turmeric plants infected by Colletotrichum capsici. Singh and Chohan (1977) recorded the decrease in amino acid and sugar contents in the various varieties of cucurbit fruits infected by Pythium butleri.

In leaf spot diseases, the various parts of the spots formed by infection of fungi on leaves, is known to vary in their content of various constituents. The detailed studies carried out by Main (1971) on the spots formed by

Alternaria tenuis on tobacco leaves has made him to suggest the apparently healthy area around the spots as prehalo, which was entirely different biochemically from the spot. Nair (1972) also made a detailed study of the contents of chemical constituents in the different parts of the spot formed by *Colletotrichum capsici* on turmeric leaves, and found that the central necrotic area is biochemically different from the yellow halo and from the surrounding apparently healthy area.

MATERIALS AND METHODS

MATERIALS AND METHODS

I. Survey on the occurrence of fungal diseases on common ornamental plants.

A survey was conducted for a period of one year in and around the campus of the College of Agriculture, Vellayani, Trivandrum to record the incidence of various fungal diseases of common ornamentals. Specimens showing disease symptoms were collected from the different plants, dried and preserved. Specimens were examined for the presence of any fungal pathogens and recorded.

II. Comparative studies on *Colletotrichum* leaf spot disease of seven common ornamental plants.

A comparative study of the leaf spot diseases caused by *C. gloeosporioides* on seven host plants, viz., *Cosmos bipinnatus* Cav., *Clitoria ternatea* L., *Gardenia jasminoides* Ellis., *Gerbera jamesonii* Hook., *Hydrangea hortensis* DC., *Jasminum Sambac* Soland., and *Jasminum* sp. were carried out. The symptoms caused by infection on all these hosts were studied in detail.

The naturally infected specimens of all the seven leaf spot diseases were collected from the College of Agriculture, Vellayani. The leaves showing the symptoms were collected and cut into small bits. They were then surface sterilised with 0.1 per cent mercuric chloride solution and then washed in three changes of sterile distilled water. These bits were

then transferred to sterile potato dextrose agar medium (PDA) contained in 90 mm sterilised petri dishes. The petri dishes were incubated at room temperature (28 ± 2°C). The isolate was purified by single spore isolation and the culture maintained on PDA slants by periodical subculturing. The growth habit of all the seven isolates on PDA were studied. Measurements of conidia collected from culture medium were also made by mounting them in 1:1 mixture of glycerine-water medium.

The pathogenicity of seven isolates of C. gloeosporioides were established by artificially inoculating them on their respective hosts. Freshly prepared spore suspensions in sterile water containing 10^6 spores per ml was used for artificially inoculating the hosts. Pathogenic reactions of all the seven isolates were tested by cross inoculating the seven host plants. The test plants were raised on earthern pots and they were sprayed with spore suspension. Inoculated plants were kept covered with polythene bags for three days for maintaining high relative humidity. The pathological reactions noted were recorded in each case.

III. Studies on the leaf spot disease of Hydrangea hortensia caused by Colletotrichum gloeosporioides.

The leaf spot disease of Hydrangea hortensia caused by Colletotrichum gloeosporioides was investigated in detail. The isolate of C. gloeosporioides used in the present study was obtained from naturally infected leaves of Hydrangea hortensia. The pathogen was isolated and purified as described earlier.

A. Morphological studies of the pathogen

Detailed study of the morphological characters of isolate was carried out. Measurements of mycelium, conidia and germ tube length were made by mounting the materials in 1:1 glycerine-distilled water mixture. Germination of spores were studied in sterile distilled water in hanging drops placed on clean grease free glass slides placed in sterile moist petri dishes. The slides were examined periodically and germination noted.

B. Host-range studies of the pathogen

To study the host range of C. gloeosporioides isolated from Hydrangea hortensis the following plants were artificially inoculated and the symptoms developed were observed and recorded. The inoculations were carried out by spraying spore suspensions as described earlier.

1.	Banana	Musa paradisiaca L.
2.	Betelvine	Piper betle L.
3.	Bread fruit	Artocarpus altilis (Park) Fosberg.
4.	Cashew	Anacardium occidentale L.
5.	Butterfly pea	Clitoria ternatea L.
6.	Castor	Ricinus communis L.
7.	Cardamom	Elettaria cardamomum (L.) Maton
8.	Clove	Eugenia caryophyllata Thumb.
9.	Cocoa	Theobroma cacao L.
10.	Cosmos	Cosmos bipinnatus Cav.
11.	Euphorbia	Euphorbia geniculata L.

12.	Gardenia	Gardenia jasminoides Ellis
13.	Gerbera	Gerbera jamesonii Hook.
14.	Ginger	Zingiber officinale Roscoe
15.	Guava	Psidium guajava L.
16.	Jack	Artocarpus heterophyllus Lam.
17.	Jasmine	Jasminum sambac Soland
18.	Lemon	Citrus aurantifolia L.
19.	Mango	Mangifera indica L.
20.	Nutmeg	Myristica fragrans Houtt.
21.	Papaya	Carica papaya L.
22.	Pithecolobium	Pithecolobium dulce (Roxb.)Benth.
23.	Rose	Rosa sp.
24.	Sapota	Achras sapota L.
25.	West Indian Cherry	Malphigia punicifolia. L.

C. Growth and sporulation of C. gloeosporioides on different solid and liquid media

To study the influence of different solid and liquid media on the growth and sporulation of the pathogen, the following eight solid media and six liquid media were tried.

Solid medium:

1. Potato dextrose agar
2. Oat meal agar
3. Czapek's agar
4. Host leaf extract agar
5. Bean juice agar
6. Richard's agar

7. Coon's agar
8. Sabouraud's agar

Liquid medium:

1. Ozapek's medium
2. Richard's medium
3. Coon's medium
4. Host leaf extract dextrose medium
5. Host leaf extract medium
6. Frie's medium

(The composition of the media are given in Appendix I).

The above media were prepared and sterilised by autoclaving at 1.05 kg/cm^2 for 20 minutes.

(i) Growth on solid media

An aliquot of 15 ml of each of the sterilised media were transferred into sterilised 90 mm petri dishes. After solidification of the media, 5 mm discs from an actively growing zone of the fungus on PDA were carried out by a sterile cork borer and transferred into the centre of the plates containing the respective media aseptically. The plates were incubated at room temperature (28 ± 2°C) and radial measurements of the colony were taken when the maximum growth was attained in any one of the media tested. Three replications were kept for each treatment.

(ii) Growth on liquid media

In studies with liquid media, 30 ml of each of the

media were taken in 250 ml Erlenmayer's flasks and sterilised by autoclaving. The medium was inoculated with 5 mm disc cut out from an actively growing zone of the fungus culture (on PDA) by a sterile cork borer. The flasks were then incubated at room temperature (28 ± 2°C). Three replications for each treatment were maintained. After ten days, the cultures were filtered through previously weighed filter paper (Whatman No.1). The biomass on the filter paper was washed with sterile distilled water for three times and dried at 60°C till a constant weight was obtained.

D. Influence of various carbon sources on the growth of the pathogen.

For the studies on the utilization of different carbon sources, Czapek's medium was used and the sucrose was substituted separately with different carbon sources. The necessary quantities of the various carbon sources were so calculated as to give a carbon equivalent to that in the Czapek's medium. Controls were kept without adding any sugar and the medium autoclaved and inoculated with fungal culture disc as earlier. Three replications were kept for each treatment.

Carbon source tested:

Sucrose, Dextrose, Glucose and Soluble starch.

E. Influence of various nitrogen sources on the growth of the pathogen.

The different sources of nitrogen were so selected as to include nitrite, nitrate and organic form of nitrogen (Sodium nitrite, Potassium nitrate and peptone respectively).

The quantities of nitrogen sources were adjusted in all cases except in peptone in such a way as to contain 0.2 per cent nitrogen equivalent as in Czapek's medium. Three replications were kept for each treatment.

The pH of the medium was adjusted between 6.0 and 6.5. The medium was autoclaved at 1.05 kg/cm^2 for 20 minutes. They were then inoculated with 5 mm discs of fungal culture and incubated at room temperature (28 ± 2°C). After ten days of incubation the biomass was harvested and dried at 60°C and the dry weight recorded.

F. Influence of different pH levels of the medium on the growth of the pathogen.

pH of the Czapek's medium was adjusted with 0.1 N NaOH or 0.1 N HCl to obtain pH levels of 4, 5, 6, 7, 8, 9 and 10. After autoclaving at 1.05 kg/cm^2 pressure for 20 min. the medium was inoculated with 5 mm mycelial discs of fungus growth. The flasks were inoculated at room temperature (28 ± 2°C) and the biomass seperated by filtration, dried at 60°C and the dry weight of the mycelium was noted.

G. Influence of trace (micro) elements on the growth of the pathogen.

The combined effect of the following four micro elements on the growth of the fungus was studied as per the method described by Granitti (1954).

Trace element	Concentration
Ferrous sulphate	5, 10 and 20 ppm
Manganese sulphate	5, 10 and 20 ppm

Copper sulphate	5, 10 and 20 ppm
Zinc sulphate	5, 10 and 20 ppm

The semi synthetic medium (Appendix I) suggested by Graniti (1964) was used with the addition of the above four trace elements at three concentrations. Controls were also kept without adding any trace elements. Sterilisation and inoculation of the media were done as in previous cases. After incubation at room temperature (28 ± 2°C) for ten days, the biomass was separated and the dry weight of the mycelium was recorded.

IV. Studies on toxin production by Colletotrichum gloeosporioides.

The following bioassay techniques were used in assaying the toxin production by C. gloeosporioides, isolated from Hydrangea hortensis.

(i) Inhibition of seed germination

(ii) Inhibition of plumule and radicle elongation

(iii) Bioassay using host leaves

(i) Inhibition of seed germination

The method described by Vidhyasekharan et al. (1970) was followed. Seeds of bhindi, cucumber and cowpea were surface sterilised with 0.1 per cent mercuric chloride and were then washed in three changes of sterile distilled water. They were then spread in Whatman No.1 filter paper placed in sterile 90 mm petri dishes at the rate of 5 seeds per plate.

An aliquot of 5 ml of the test solution was poured into the plates over the filter paper and the dishes were incubated at room temperature. Controls were run simultaneously with sterile water. The number of seeds germinated on the third day in each of the treatments were counted and the percentage germination over control were calculated.

(ii) Inhibition of radicle and plumule elongation

The seeds of cucumber, bhindi and cowpea were first surface sterilised with. 0.1 per cent mercuric chloride solution and then washed repeatedly in sterile distilled water. They were then allowed to germinate in sterile petri dishes over moist filter paper for three days and those with uniform length of plumule and radicle were selected and placed in sterile filter paper in sterile petri plates. The filter paper in each set was wetted with 10 ml of the test solution. Controls were maintained with sterile water. After 48 hr. of incubation at room temperature (28 ± 2°C) the length of plumule and radicle were measured and per cent inhibition over control were calculated.

(iii) Bioassay using host leaves

Healthy vigorous young leaves of Hydrangea were cut and surface sterilised with. 0.1 per cent mercuric chloride and washed in sterile water. They were then placed in moist petri dishes and the test solution was then placed at the rate of 0.05 ml per spot on one half of the leaf after slightly injuring the surface while on the other half an equal quantity

of sterile water was kept in a similar manner. The culture filtrate were placed at three spots on one side of a single leaf.

A. Influence of various media on toxin production

Various liquid media, viz., Czapek's medium, Coon's medium, Host leaf extract without dextrose and Host leaf extract with 2 per cent dextrose, Richard's medium and Fric's medium were used for the study. Forty ml each of the different media were taken in 250 ml flasks and sterilised at 1.05 kg/cm^2 pressure for 20 minutes. The flasks were then inoculated with 5 mm mycelial discs of an actively growing culture of the organism on PDA. They were then incubated at room temperature (28 ± 2°C) for 14 days. The cultures were then filtered and the culture filtrate was assayed for toxin production by the seed germination inhibition bioassay and also by assay on host leaves as described earlier.

B. Influence of various carbon sources on toxin production

Czapek's medium was prepared and the sucrose was substituted with various carbon sources as described earlier. The sterilisation and inoculation of the media were done as in other cases and the inoculated flasks were incubated at room temperature (28 ± 2°C). After 14 days of incubation the culture was filtered and the culture filtrate was assayed for toxin production by the plumule-radicle elongation inhibition bioassay and also by assaying on host leaves.

C. Partial purification of toxin

Czapek's medium was taken at the rate of 30 ml in 250 ml flasks, autoclaved and inoculated with 5 mm mycelial discs of the pathogen as described earlier and incubated at room temperature (28 ± 2°C) for 14 days. The growth in the flasks were then filtered through Whatman No.1 filter paper and the toxic activity of both mycelial extract and culture filtrate were assayed by following the method described by Nair and Ramakrishnan (1973).

The mycelium was homogenised with five volume (v/v) sterile water in a homogeniser. The homogenate was centrifuged at 1000 rpm for 15 minutes and the pellets discarded. The supernatant liquid was reduced to one-tenth of the original volume under reduced pressure, combined with equal volume of methanol, stirred well, kept over night at 5°C, filtered and methanol removed at reduced pressure. The concentrated solution was adjusted to pH 3.5 with 0.1 N HCl and shaken well with equal volume of diethyl ether in a separating funnel. Ether phase separated and mixed well with equal volume of 5 per cent aqueous solution of sodium bicarbonate and again the aqueous phase discarded. The ether solution evaporated to dryness under vacuum which left an ash coloured precipitate. The toxin in culture filtrate was also purified by the above procedure, except the preliminary homogenation. The residue was dissolved separately in 2 ml of distilled water and neutralised with 0.1 N HCl and or 0.1 N NaOH. The concentrated

toxin prepared from the mycelium is designated as endotoxin and that from culture filtrate as exotoxin. These were bioassayed on Hydrangea leaves as described earlier. Controls were maintained using sterile distilled water. The observations on the symptom development were recorded.

D. Translocation of toxin

The ability of the toxic metabolite produced by C. gloeosporioides to translocate in the host tissue was studied following the method described by Sharma and Sharma (1969) with slight modifications.

The culture filtrate of the pathogen grown in Czapek's medium for fourteen days was separated by filtration and centrifuged at 1000 rpm for 15 minutes. The supernatent was taken in sterilised test tube. Healthy and vigorous growing tender twigs of Hydrangea were cut and transferred to the test tubes containing the filtrate and was kept in position by using cotton. Similar twigs kept in sterile water served as control. After 72 hrs the twigs were observed for any abnormality.

E. Physical properties of toxin

(1) Effect of heat on the culture filtrate

The method described by Sharma and Sharma (1969) was employed for the study. The culture filtrate of the pathogen grown in Czapek's medium for fourteen days was used. An

aliquot of 5 ml of the culture filtrate was taken in a sterile test tube and flamed it just to warm it (at 50°C). Another set was heated to 100°C and a third set was autoclaved at 1.05 kg/cm^2 pressure for 20 minutes. The toxic effect of the treated culture filtrate was assayed using healthy host leaves.

(ii) Effect of dilution of culture filtrate

The dilution end-point of the toxin in the culture filtrate was assayed following the method described by Sharma and Sharma (1969). The culture filtrate was prepared by growing the organism in Czapek's medium as described earlier. It was then diluted with sterile water in the proportions of 1:10 and 1:100. The toxicity of the diluted culture filtrate was assayed on host leaves.

V. Evaluation of fungicides against the pathogen.

(a) In vitro evaluation of fungicides against the pathogen

The effect of the following twelve fungicides on the growth of the fungus was tested by poisoned food technique described by Bentmeyer (1955).

1.	Aureofungin-sol.	(N-Methyl-P-amino aceto phenone mycosamine heptane)
2.	Bavistin	2(Methoxy-carbamoyl)-benzimidazole
3.	Blitox	Copper oxychloride
4.	Captan	N-(trichloromethyl thio)-tetrahydrophthalimide
5.	Daconil	Chlorothalonil (Tetrachloro isophthalnitrile)

6. Difolatan	Cis N-(1, 1,2,2-tetrachloroethyl thio)-4-Cyclohexane-1, 2, dicarboximide
7. Dithane ^{Z},45	Zinc ion and manganese ethylene bisdithiocarbamate
8. Hitazin	O, O-di isopropyl-S-benzyl thiophosphate
9. Mildothane	Thiophanate-methyl (1,2,-bis(3 methoxy carbonyl-2-thio uroido) benzene
10. Rovral	3-(3,5-dichlorophenyl)-1-isopropyl carbamoyl hydrantion
11. Thiride	Tetramethyl thiuram disulphide
12. Zirlde	Zinc dimethyl dithiocarbamate

Stock solution of the fungicides were prepared and the requisite quantity of each was added separately to 50 ml of PDA so as to get the desired concentrations of the fungicides. Fifty ml of the poisoned medium was poured in sterile petri dishes and after solidification 5 mm disc from a seven day old culture of the fungus was cut out by a sterile cork borer and were placed at the centre of the agar plates. The plates were then incubated at room temperature (28 $\pm$ 2°C). PDA without any fungicide served as control. The radial growth of the colony was taken when maximum growth in control plates were noted. The percentage inhibition over control was calculated following the formula

$$\text{Percentage inhibition I} = \frac{C-T \times 100}{C}$$

C - radial growth in control

T - radial growth in treatment

(b) Inhibition of spore germination

The method used by Khare and Singh (1979) was followed with slight modification. Spores obtained from ten day old cultures of *C. gloeosporioides* in PDA were used for the study. The spores were harvested and a suspension was made in sterile distilled water. The concentration was adjusted to contain 50 to 60 spores under the low power of the microscope. Drops of 0.05 ml of the spore suspension was placed on a clean slide and mixed thoroughly with 0.05 ml of the fungicidal solution. They were then kept in moist chamber. The per cent inhibition of spore germination was recorded after 24 hr.

(c) Field evaluation of fungicides against the leaf spot disease of *Hydrangea hortensia*

A pot culture experiment was laid out with a view to assess the efficacy of different fungicides on the control of leaf spot disease of *H. hortensia*.

Lay out	CRD
Treatments	6(5 fungicides and 1 control)
Replication	3
Fungicide	Dose
Dithane M-45	2 g/l
Blitox	5 g/l

Bavistin	1 g/l
Cayton	2 g/l
Thiride	2 g/l

The severity of the disease was observed after the South West monsoon from November to February. So the plants were sprayed at an interval of 30 days, the first spraying was given by the middle of November. The intensity of the leaf spot disease was recorded before the spraying and thirty days after each spraying. The intensity of the disease at each observation was calculated using the following score chart (Fig.1).

Grade	Disease intensity	Description
0	0	No spots
1	5 - 10 per cent	2 - 10 spots
2	10 - 25 per cent	10 or more spots
3	26 - 50 per cent	Half of the leaf area infected
4	51 - 75 per cent	Half to three fourth of the leaf area infected
5	76 and above	Almost complete infection of leaf

All the leaves of the plants under each treatment were observed and the intensity of the disease was recorded. The disease index for each treatment was calculated from the observations.

Fig.1

Disease scale of the leaf spot due to *Colletotrichum gloeosporioides* on *Hydrangea hortensia*.

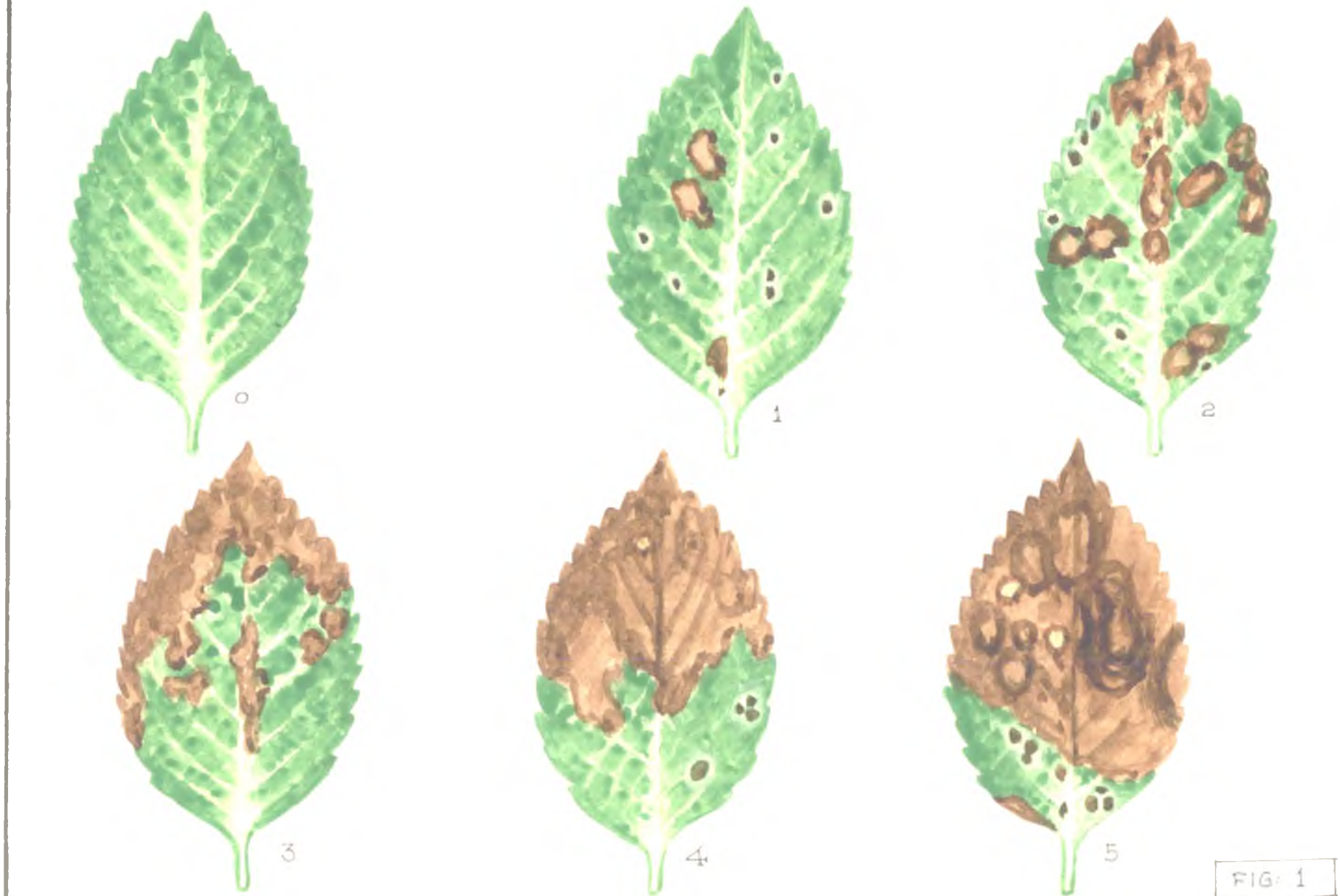


FIG. 1

$$= \frac{\text{Sum of grades of each leaf}}{\text{Total number of leaves}}$$

VI. Carbohydrates and phenolic contents of the various parts of the leaf spot formed by *C. gloeosporioides* on *H. hortensia*.

The various parts of the spot formed by *C. gloeosporioides* on Hydrangea leaves were arbitrarily grouped into the central fungal invaded portion consisting of a brownish necrotic area, the surrounding halo and apparently healthy area around the halo designated as prehalo following Main (1971). The different tissues were sampled separately and used for the study. Small bits of the different areas were cut out from the surface sterilised naturally infected leaves of uniform age and analysed for total carbohydrates and total phenolics.

(a) Total carbohydrates

The total carbohydrates in the various parts were determined by following the method described by Yemm and Willis (1954). One gram of the leaf sample was ground with 70 per cent ethanol and dried in vacuum. It was then mixed with warm water and dried with aluminium hydroxide.

Five ml of anthrone reagent (Anthrone 0.2 g was dissolved in 100 ml dilute H_2SO_4, 5:2 acid and water) was pipetted out into a thick walled pyrex tube and chilled in ice water. One ml of the test solution was layered on the acid, cooled for a further 5 minutes and then thoroughly mixed, while still immersed in cool water. The tubes were

loosely fitted with corks, heated for 2 minutes in a boiling water bath and then cooled in water. Distilled water was used as blank. Readings were taken using a Spectronic 20 Spectrophotometer at 590 mμ. The quantity of the total carbohydrates in the samples were expressed as μg/g of sample as glucose equivalent.

Total phenolics

The total phenolics was determined by the method of Bray and Thrope (1954). One gram of fresh leaf sample was ground with 80 per cent hot ethanol, boiled and filtered. The filtrate evaporated to dryness and dissolved in 1 ml of 80 per cent ethanol. An aliquot of 0.1 ml of this solution was taken in a boiling tube and made upto 70 ml with water. One ml of Folin Ciocalteus reagent and 2 ml of saturated sodium bicarbonate were added and the tube heated for one minute in a boiling water bath, cooled and the colour read in Spectronic 20 Spectrophotometer with red filter (645 mμ). The total phenolics expressed as μg/g of the leaf sample as pyrogallol equivalent.

RESULTS

RESULTS

I. SURVEY ON THE OCCURRENCE OF FUNGAL DISEASES ON COMMON ORNAMENTAL PLANTS.

A one year survey conducted on the occurrence of the fungal diseases of ornamental plants grown in the College of Agriculture Campus, Vellayani, Trivandrum, revealed that almost all of them are affected by one or other type of fungal infection. The list of fungal pathogens observed on few common ornamental plants and their frequency of occurrence are presented in Table 1. It is seen that Colletotrichum glocosporioides occurs as the major pathogen in the locality infecting the majority of the ornamentals surveyed. This is followed by species of Cercospora. The other fungal pathogens observed were species of Pestalotia, Corynespore, Curvularia and sooty mould. Red rust is also recorded on two ornamental plants.

II. COMPARATIVE STUDIES ON COLLETOTRICHUM LEAF SPOT DISEASES OF THE SEVEN ORNAMENTAL PLANTS.

A. Symptomatology.

1. Clitoria ternatea

The symptoms were observed severely on mature leaves. On young leaves they appeared as isolated small brown or grey specks about 0.1 mm in diameter. As the leaves matured, these specks coalesced together and formed greyish papery areas often covering the whole lamina. Complete defoliation occurred when the infection was severe (Fig.2).

Fig.2.

Leaf spot due to *C. glocosporioides* on *Clitoria ternatea*.

Fig.2

Fig.3.1. Leaf spot due to *C. gloeosporioides* on *Cosmos bipinnatus*.

Fig.3.2. Portion of the affected leaf let enlarged showing the symptoms.

Fig.3.1

2. Cosmos bipinnatus

The initial symptoms appeared as small brown pin head dots on the pinnately compound leaves. Infection is also evident as tip blight resulting in the withering of the affected parts (Fig.3.1 and 3.2).

3. Gardenia jasminoides

The symptoms appeared as very small dots, which soon enlarged to attain a size of 2 to 4 mm in diameter. The centre of the spots turned greyish white surrounded by a light brown margin. The greyish white centre of the spots were studded with acervuli of the organism. Symptoms were more evident on the upper surface of the leaves (Fig.4.1 and 4.2).

4. Gerbera jamesonii

The older leaves were found to be easily affected by the pathogen. Isolated brown to black circular spots appeared on the leaves which varied in size from 2 to 5 mm in diameter. These spots were studded with the acervuli of the organism. If infection is severe, the affected area dropped off resulting in shot hole symptom (Fig.5.1 and 5.2).

5. Hydrangea hortensia

The symptom appeared on the leaves as minute pinkish black spots with grey white centre. The spots varied in size from small specks upto 5 mm in diameter. In severe infection the spots coalesced to form large irregular patches and resulted

Fig.4.1. Leaf spot due to *C. gloeosporioides* on *Gardenia jasminoides*.

Fig.4.2. Portion of the leaf showing the sympton (enlarged

Fig.4.1

Fig.4.2

Fig.5.1. Leaf spot due to *C. gloeosporioides* on *Gerbera jamesonii*.

Fig.5.2. Portion of the leaf enlarged showing the symptom.

Fig.5.1

Fig.5.2

Fig.6.1. Hydrangea plant infected with *C. gloeosporioides*.

Fig.6.2. A single leaf of *H. hortensis* showing symptom of infection by *C. gloeosporioides*.

Fig.6.1

Fig.6.2.

Fig.7.1. Leaf spot due to *C. gloeosporioides* on *Jasminum sambac*.

Fig.7.2. Portion of the leaf showing the sympton.

Fig.7.1

Fig.7.2

in the drying up of the leaves. Often an yellowish halo was observed around the necrotic area. Young and older leaves were found to be affected. In advanced stages the central necrotic area became grey white and was found to be studded with minute black pin heads, the acervuli of the organism. Severely affected leaves wither, droop down and dried up prematurily (Fig.6.1 and 6.2).

6.Jasminum sambac

The symptoms developed as pronounced irregular areas. The affected area become greyish white which were studded with black acervuli of the fungus. The older leaves were more severely affected presenting a crinkled and distorted appearance (Fig.7.1 and 7.2).

7. Jasminum sp.

The symptoms appeared on mature leaves as small circular specks to irregular areas. The individual spots measured upto 5 mm in diameter. These spots had a characteristic pinkish boundary and a white centre. In advanced stages these areas turned brown with characteristic concentric rings of acervuli and the affected leaves became twisted and malformed (Fig.8).

B. Morphology

Colletotrichum gloeosporioides was isolated from all the seven hosts mentioned above, purified and maintained in PDA slants. The morphological characters of these seven isolates were studied in detail. The data are tabulated and presented in Table 2. The general growth habits of all the

Fig.8.

Leaf spot due to C. *gloeosporioides* on *Jasminum* sp.

Fig.8.

Table 2. Comparative morphological characters of seven different isolates of *Colletotrichum gloeosporioides* from seven ornamental plants.

Host	Growth characters on PDA	Conidial measurements (µm)	
		Range (LxB)	Average (LxB)
Clitoria ternatea	Greyish white to black, highly branched and septate hyphae with an average width of 3.57 µm.	14.28-17.85 x 3.57	15.34 x 3.57
Cosmos bipinnatus	do	5.36-14.28 x 3.57	13.49 x 3.57
Gardenia jasminoides	do	5.36-14.28 x 3.57	10.30 x 3.57
Gerbera jamesonii	do	11.71-14.28 x 3.57	13.08 x 3.57
Hydrangea hortensia	do	5.36-14.28 x 3.57	13.49 x 3.57
Jasminum sambac	do	7.14-14.28 x 3.57	11.85 x 3.57
Jasminum sp.	do	5.36-17.75 x 3.57	8.85 x 3.57

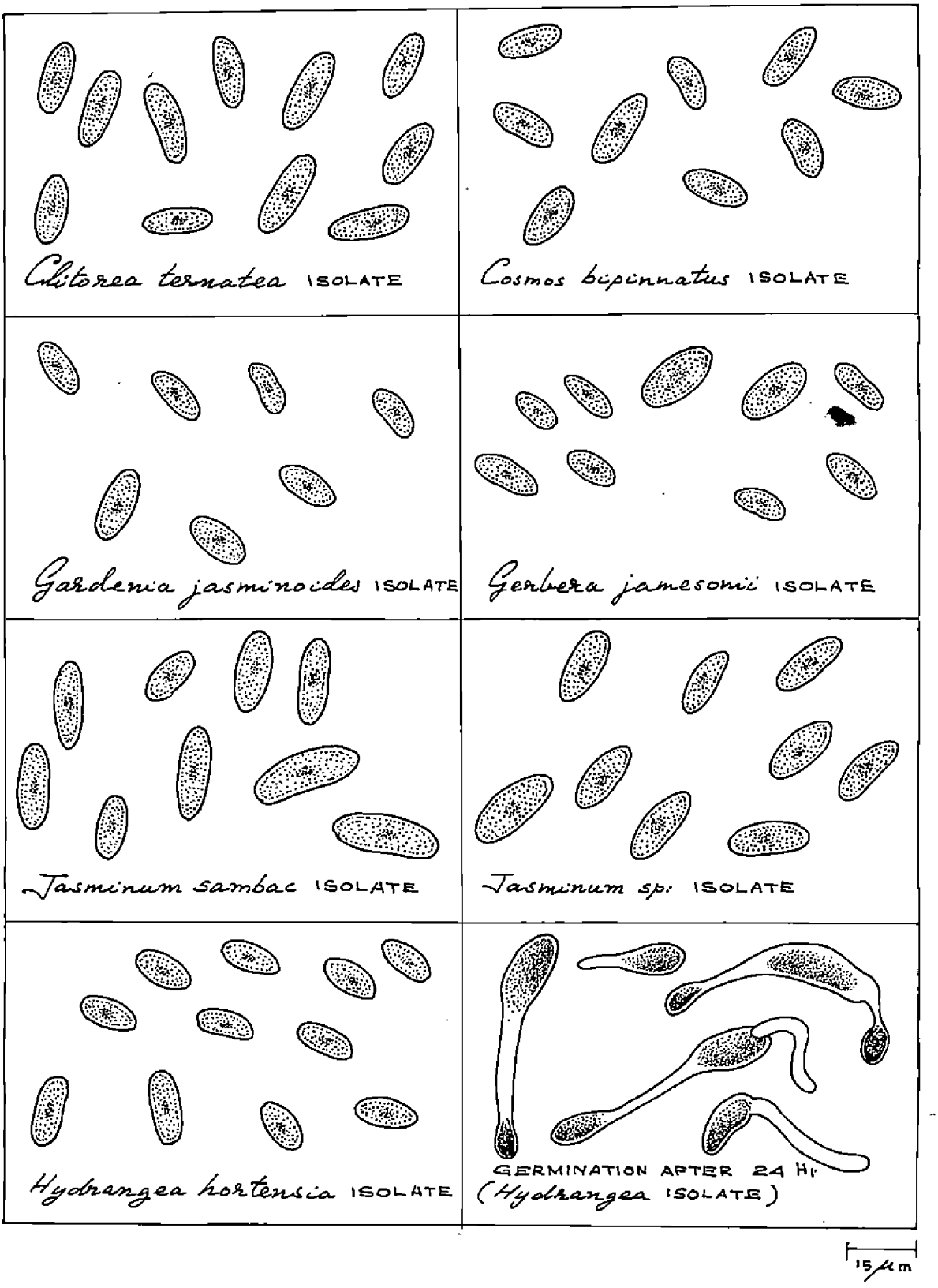


FIG: 9 CONIDIA OF *C. gloeosporioides* FROM SEVEN DIFFERENT HOST PLANTS AND GERMINATING CONIDIA OF THE *Hydrangea* ISOLATE

seven different isolates were more or less similar. It consisted of greyish white to blackish mycelial growth and the mycelium has an average width of 3.57 µm. The conidial measurements varied in different isolates. Conidia of the organism isolated from Jasminium sp. were shorter than the isolates from other hosts studied, while those from Clitoria ternatea were longer than all the other isolates. The maximum conidial length viz., 15.34 µm was observed in the case of the isolate from Clitoria ternatea. The conidia were non-septate, hyaline, oblong or cylindrical with rounded ends (Fig.9).

C. Pathogenicity

All the seven isolates of C. gloeosporioides obtained from the seven different host plants gave positive results when inoculated on the respective host plants. Cross inoculation studies with the isolates of the pathogen gave positive results except on Gerbera jamesonii. The Gerbera isolate of C. gloeosporioides has not cross infected other host plants indicating its specificity to infection by the particular isolate of the pathogen only. The severity of symptoms produced on the different host plants are tabulated in Table 3.

Table 3. Results of the cross inoculation studies with the seven different isolates of *C. gloeosporioides*.

Isolate from	Host plant						
	Hydrangea hortensia	*Clitoria ternatea*	*Gardenia jasminoides*	*Gerbera jamesonii*	*Jasminum sambac*	*Jasminum* sp.	*Cosmos bipinnatus*
Hydrangea hortensia	+++	++	+++	-	+++	+++	++
Clitoria ternatea	++	+++	++	-	+++	+++	++
Gardenia jasminoides	+++	++	+++		+++	+++	+++
Gerbera jamesonii	++	++	++	+++	++	++	++
Jasminum sambac	+++	++	+++	-	+++	+++	++
Jasminum sp.	++	++	+++	-	+++	+++	++
Cosmos bipinnatus	+++	++	+++	-	++	++	++

+++ Well developed necrotic area

++ Sparsely developed necrotic area

\+ No symptom

III. STUDIES ON THE LEAF SPOT DISEASE OF HYDRANGEA HORTENSIA CAUSED BY COLLETOTRICHUM GLOEOSPORIOIDES.

The causal organism was isolated on potato dextrose agar medium, purified by single spore isolation and maintained on PDA slants.

A. Morphological studies of the pathogen

Mycelium septate, profusely branched, greyish white with an average thickness of 3.57 µm. The conidia were oblong or cylindrical, hyaline, non-septate with rounded ends. The conidial measurements are given in Table 4. Conidia collected from PDA culture measured 5.36 to 14.28 x 3.57 µm (average 13.49 x 3.57 µm) while those from host tissue were longer than those on PDA and measured 14.28 to 21.40 µm (average 17.14 µm). Setae were observed only on acervuli formed on host and were long, slightly swollen at the base and measured 185.90 to 177.00 µm in length (average 153.38 x 3.57 µm). Setae were found to bear solitary conidium in rare cases.

The spore suspensions in sterile water kept on clean glass slides were observed for the germination. It was found that the spores germinated readily and in 24 hours the germ tubes attained an average length of 36.77 µm and in 48 hours 77.11 µm. The germ tube ended in the production of appressoria (Fig.8).

Table 4. Comparative measurements of conidia and setae of C. gloeosporioides from the host Hydrangea hortensia and culture (in µm).

	Conidia						Setae					
	Length			Breadth			Length			Breadth		
	Maximum	Minimum	Average	Maximum	Minimum	Average	Maximum	Minimum	Average	Maximum	Minimum	Average
PDA	14.28	5.36	13.49	3.57	3.57	3.57	-	-	-	-	-	-
Host	21.48	14.28	17.14	3.57	3.57	3.57	177.00	123.90	153.38	3.57	3.57	3.57

B. Host-range studies of the pathogen

Twenty five plants were tested for the host-range studies of the pathogen. The details of symptoms observed and the time taken for the symptom expression are given in Table 5. Except bread fruit, castor, cardamom, cocoa, jack, papaya and gerbera, all the other hosts gave positive results. The time taken for symptom expression varied with different hosts.

C. Growth and sporulation of C. gloeosporioides

(1) On different solid media

Eight solid media were tested for the growth and sporulation of the organism and the results are tabulated in Table 6 (Fig.10). The maximum growth was observed on potato dextrose agar medium. Growth was visible after two days of inoculation in the medium and the growth covered the petri dishes completely within seven days. Statistical analysis of the data showed that there is no significant difference in growth on potato dextrose agar, Czapek's agar and Bean juice agar. However, these media were superior to Sabouraud's medium, Host leaf extract agar, Oat meal agar, Richard's agar and Coon's agar medium. But there was no significant difference between Sabouraud's medium and Host leaf extract agar medium and Oat meal agar medium. But Sabouraud's medium was superior to Oat meal agar. The different media tested varied in their ability to support sporulation also. Sporulation was abundant on potato dextrose

Table 5. Host range studies of the pathogen *C. gloeosporioides* isolated from *Hydrangea hortensia*.

Host	Symptoms observed	Time taken for symptom expression
Banana	Circular to oval brown water soaked spots	++
Betelvine	Discoloured water soaked area which spread gradually and enlarged	+
Bread fruit	-	-
Butterfly bean	Brown water soaked areas	++
Cashew	Brown water soaked areas on the mature leaves, while the tender twigs showed a blighted appearance	+++
Castor	-	-
Cardamom	-	-
Clove	A blighting of the leaves from tips and margins	++
Cacao	-	-
Cosmos	Water soaked brown necrotic areas	++
Euphorbia	Brown water soaked areas	++
Gardenia	A blighting of the tips and margins of the leaves	++
Gerbera	-	-
Ginger	Yellowish discolouration of the leaves which spread gradually	+
Guava	Brown water soaked areas	++
Jack	-	-

(continued...)

Table 5 continued

-2-

Host	Symptoms observed	Time taken for sympton expression
Jasmine	Brown necrotic areas	++
Lemon	Blighting of the tips and margins of the leaves	+++
Mango	Water soaked areas	+++
Nutmeg	Water soaked areas	++
Papaya	-	-
Pithecollobium	Brown water soaked areas	+
Rose	Brown water soaked areas	+
Sapota	Brown water soaked areas	+
West Indian cherry	Brown water soaked areas	++

- No sympton
+ Sympton developed within three days after inoculation
++ Sympton developed within four to ten days after inoculation
+++ Sympton developed within eleven to seventeen days after inoculation

Table 6. Growth characteristics and sporulation of Hydrangea isolate of *C. gloeosporioides* on different solid media.

Medium	Mean colony diameter on the seventh day of incubation at room temperature (in mm)	Growth and colony characters	Sporulation
Potato dextrose agar	90.00	Colony at first white, floccose, gradually become blackish white with characteristic concentric rings.	+++
Oat meal agar	73.00	Colony white, adpressed floccose	-
Czapek's agar	83.30	Pure white, smooth, floccose colony with aerial mycelium	++
Host leaf extract	78.30	Mycelium scanty developed, submerged No aerial mycelium	-
Bean juice extract agar	86.80	Grey white mycelium sporulation abundant as a clear demarkation towards the periphery	+++
Richard's medium	72.50	Mycelium grey white with puffy growth	++
Coon's medium	63.80	Mycelium adpressed	-
Sabouraud's medium	81.50	Scanty developed mycelium	-

Average of 3 replications.

+++ Abundant sporulation
++ Good sporulation
- No sporulation

C.D. at (0.05) level = 5.28

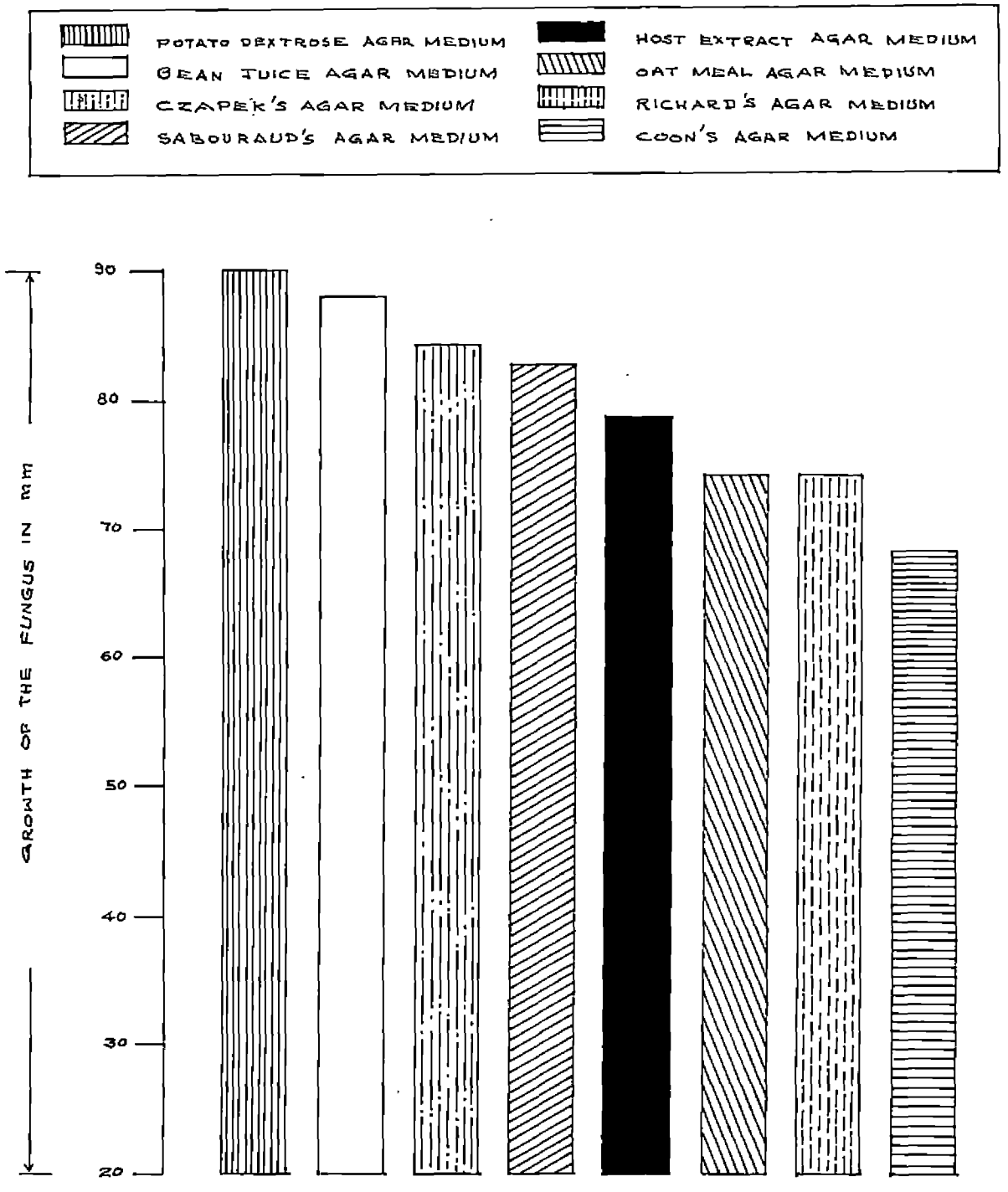


FIG: 10 GROWTH OF *Colletotrichum gloeosporioides* (*Hydrangea* ISOLATE) ON DIFFERENT SOLID MEDIA IN THE 7th DAY OF INOCULATION AT ROOM TEMPERATURE

agar and bean juice agar media and was found to be nil in the case of Oat meal agar, Host leaf extract, Coon's and Sabouraud's agar media.

(ii) On different liquid media

The fungus grew well on all the liquid media tested (Table 7). Significant differences in the dry weight of the mycelium was, however, noticed in the different liquid media. The maximum dry weight was noticed in Richard's medium followed by Coon's medium. Statistical analysis of the data showed that Richard's and Coon's medium were found to be superior to all other media. Fric's medium was on par with host leaf extract dextrose medium. Host leaf extract medium supported only very poor growth of the fungus.

D. Influence of various carbon sources on the growth of the pathogen.

Of the different carbon sources tried, dextrose was found to be the best for growth, closely followed by sucrose (Table 8). Statistical analysis of the data showed that there are no significant differences in the dry weight of mycelium in dextrose and sucrose but dextrose is superior to glucose and soluble starch.

E. Influence of various nitrogen sources on the growth of the pathogen.

Sodium nitrate, the normal constituent of Czapek's medium was found to support the maximum growth of the organism. But statistical analysis of the data showed that there are no

Table 7. Growth characteristics and sporulation of *Colletotrichum gloeosporioides* on different liquid media.

Medium	Dry weight of mycelium (mg)	Growth characteristics and sporulation
Coon's medium	283	Growth very poor, started on sixth day of inoculation sporulation poor.
Czapek's medium	279	Initial growth started on the fourth day of inoculation and attained maximum growth on tenth day.
Host leaf extract	45	Very poor growth, no sporulation.
Host leaf extract dextrose	161	Growth very poor, no sporulation.
Richard's medium	364	Initial growth started on the fourth day of inoculation and attained maximum growth on the tenth day of inoculation. Mycelium white, sporulation abundant.
Frie's medium	172	Poor growth, no sporulation

Average of 3 replications

C.D. at (0.05) level = 71.30

Table 8. Influence of various carbon sources on the growth of C. gloeosporioides.

Carbon sources	Dry weight of mycelium (mg)*
Dextrose	349
Starch	260
Glucose	275
Sucrose	316
No carbon source	-

*Average of 3 replications C.D. at (0.05 level = 58.13

Table 9. Effect of various nitrogen sources on the growth of C. gloeosporioides.

Nitrogen sources	Dry weight of mycelium (mg)*
Sodium nitrate	397
Potassium nitrite	302
Peptone	212
No nitrogen	-

*Average of 3 replications C.D.at (0.05) level = 42.14

significant differences in the dry weight of mycelium in the three nitrogen sources tested (Table 9).

F. Influence of different pH levels of medium on the growth of the pathogen

Of the various pH levels tested ranging from 4 to 10, the maximum dry weight of mycelium was obtained in the medium with pH 10. Statistical analysis showed that there is no significant difference between pH 8 and 6 and 8 and 7 and pH 7 and 5 and pH 9 and 4 (Table 10).

G. Influence of trace (micro) elements on the growth of the pathogen.

The various trace elements used at different concentrations showed that they favoured the growth of the pathogen. The maximum dry weight of mycelium was obtained with 10 ppm followed by 20 ppm of the trace elements. However, there is no significant difference between the levels tested (Table 11).

IV. STUDIES ON TOXIN PRODUCTION BY COLLETOTRICHUM GLOEOSPORIOIDES.

A. Influence of various media on toxin production.

The effect of various media on toxin production by the organism was studied following two bioassay technique, viz., inhibition of seed germination and effect on host leaf.

(1) Inhibition of seed germination

Of the six media tested, all of them supported toxin production, though their ability to support toxin production varied. The sensitivity of the bioassay technique also varied with different seeds used.

Table 10. Effect of pH of the medium on the growth of *C. gloeosporioides*.

pH	Dry weight of mycelium (mg)*
10	304
9	190
8	255
7	299
6	268
5	227
4	171

*Average of 3 replications

C.D. at (0.05) level = 28.70

Table 11. Effect of various trace elements on the growth of the pathogen

ppm	Dry weight of mycelium (mg)*
5	495
10	620
20	560

*Average of 3 replications

C.D. at (0.05) level = 17.43

In general, the toxic effect was maximum with the culture filtrate obtained from Frie's medium, followed by Richard's and host leaf extract medium. The data are tabulated in Table 12 (Fig.11).

With regard to bhindi seeds the inhibition of germination of seeds was uniform with the culture filtrate of the above three media, the per cent inhibition being 78.59. Cent per cent inhibition of cucumber seeds was observed with culture filtrate of Frie's medium 70.00 per cent inhibition with Richard's medium and 39.00 per cent for host leaf extract medium. In the case of cowpea seeds the per cent inhibition observed were 73.40, 66.60 and 53.40 per cent respectively for the above three media. The least toxic effect was noticed with culture filtrate from Coon's medium (Fig.11).

(ii) Toxic activity bioassayed on host leaf surface

A water soaked necrotic area was observed on the leaves when inoculated with the culture filtrate. The observations are presented in Table 13. Statistical analysis of the data revealed that necrotic areas produced by the culture filtrates obtained by growing the organisms in Czapek's, Frie's and Richard's media were not significantly different and they were on par. They are significantly superior to culture filtrates from other media. Control (sterile water) has not produced any necrotic spots.

Table 12. Per cent inhibition of germination of vegetable seeds by the toxin produced by *C. gloeosporioides* in different media (Observation taken 72 hr after incubation).

Medium	Bhindi	Cucumber	Cowpea
Coon's medium	46.67	6.60	20.00
Czapek's medium	50.11	46.67	40.00
Erie's medium	78.59	100.00	73.40
Host leaf extract medium	78.59	39.60	53.40
Richard's medium	78.59	70.00	66.60
Control (Sterile water)	-	-	-

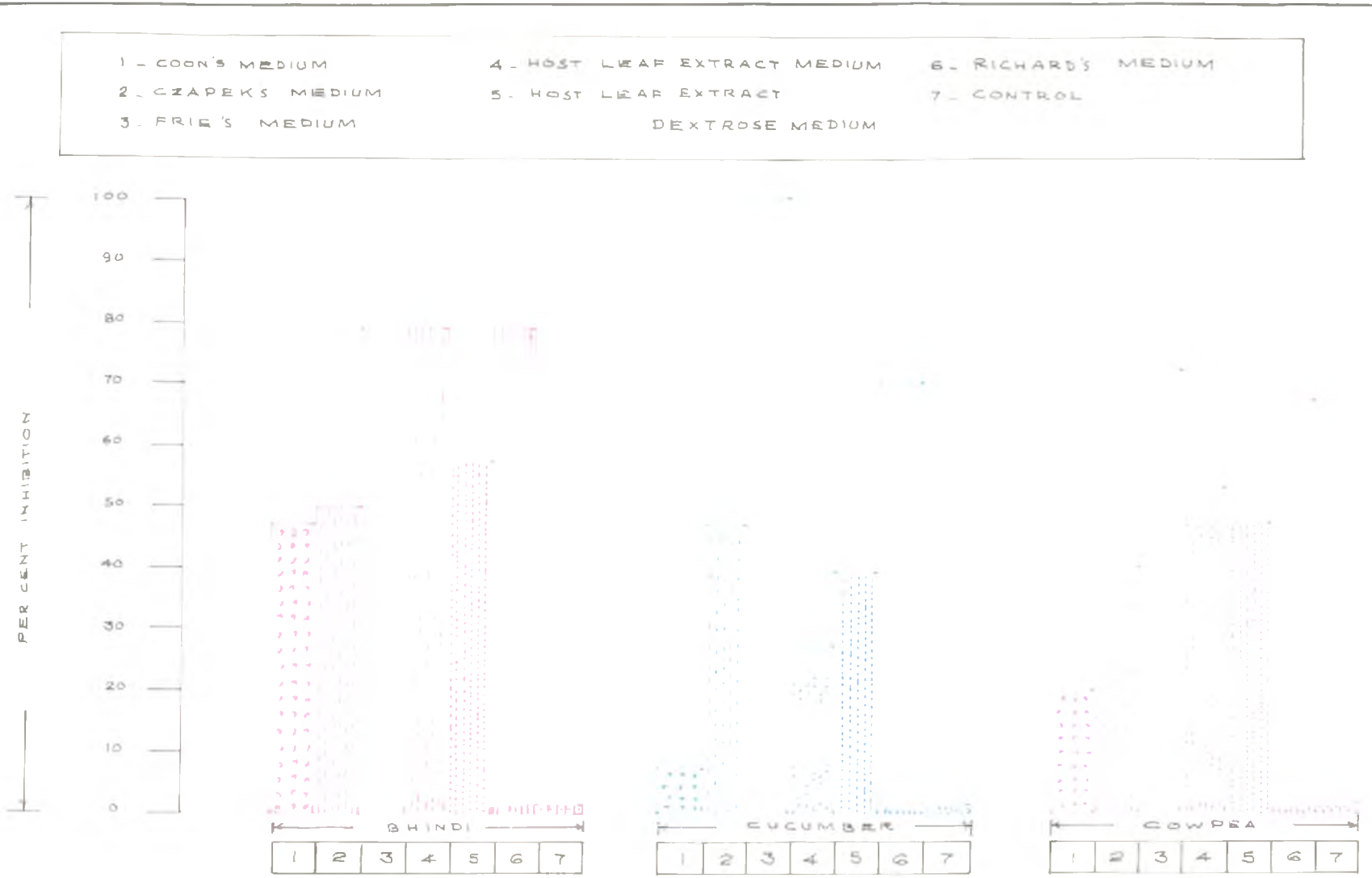


FIG II PER CENT INHIBITION OF GERMINATION OF VEGETABLE SEEDS BY THE TOXIN PRODUCED BY *C glocosporioides* IN DIFFERENT MEDIA (OBSERVATION AFTER 12 HR.)

Table 13. Effect of toxin produced by C. *piaeogmorioides* in different media bioassayed on host leaf (Diameter of the necrotic area produced 24 hr after incubation).

Medium	Diameter of necrotic area (mm)*
Coon's medium	2.67
Czapek's medium	8.83
Host leaf extract medium	5.83
Host leaf extract dextrose	2.50
Frio's medium	8.00
Richard's medium	7.83
Control (Sterile water)	-

*Average of 6 spots

C.D. at (0.05) level = 1.95

B. Influence of various carbon sources on toxin production.

The effect of different carbon sources on toxin production by the organism was studied following the bioassay techniques, viz., effect on plumule and radicle elongation and also by its effect on host leaf.

(i) Effect on radicle and plumule elongation

The different carbon sources tried varied in their capacity to support the production of the toxic metabolite by the organism.

Culture filtrate from medium with dextrose as carbon source gave the maximum toxic activity as evidenced by the inhibition of radicle and plumule elongation in cucumber and cowpea seeds. The maximum toxic activity was detected with culture filtrate containing glucose as carbon source in the case of bhindi seeds. The data are presented in Table 14 (Fig.12).

(ii) Toxic activity bioassayed on host leaf surface

Water soaked necrotic areas developed in the inoculated host leaves. The average diameter of the necrotic area are tabulated in Table 15. All the carbon sources tested induced the excretion of toxin. The maximum necrosis was noticed with culture filtrate from media containing glucose and dextrose. But analysis of the data showed that the difference between the various treatments was not significant.

C. Partial purification of toxin

The partially purified exo- and endo-toxins

Table 14. Effect of various carbon sources on toxin production by C. gloeosporioides bioassayed by the radicle and plumule elongation inhibition of vegetable seeds.

Carbon sources	Bhindi				Cucumber				Cowpea			
	radicle		plumule		radicle		plumule		radicle		Plumule	
	Mean	Per cent inhibition over control	Mean	Per cent inhibition over control	Mean	Per cent inhibition over control	Mean	Per cent inhibition over control	Mean	Per cent inhibition over control	Mean	Per cent inhibition over control
Dextrose	1.86	60.45	1.11	25.50	0.45	71.34	0.40	78.84	3.00	36.84	0.51	59.20
Glucose	3.34	28.94	0.93	37.58	1.49	5.10	1.05	74.44	3.31	30.32	0.77	36.40
Sucrose	1.25	73.63	0.89	40.27	1.33	15.29	0.82	56.61	3.27	31.16	0.88	29.60
Control	4.70		1.49		1.57		1.09		4.75		1.25	

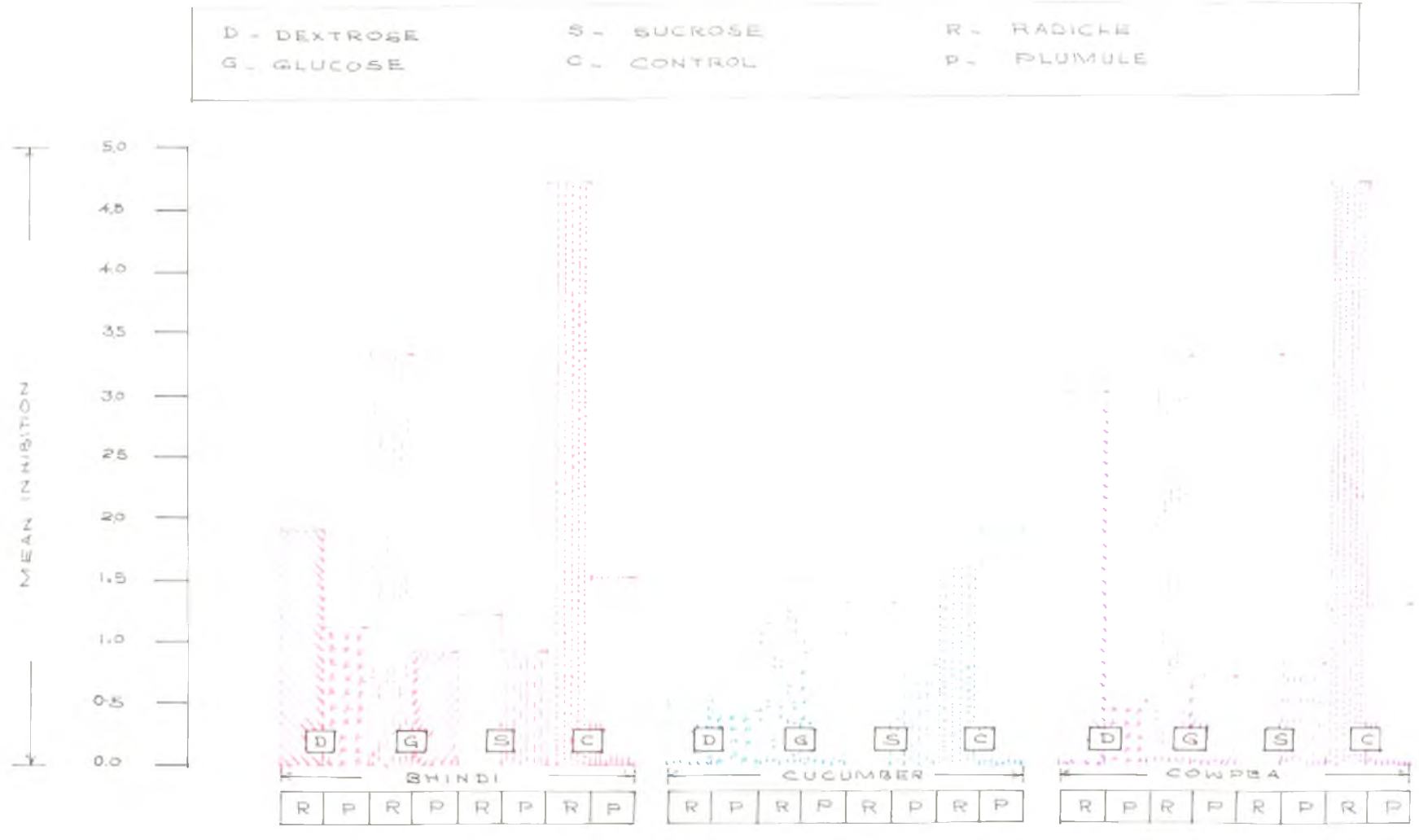


FIG 12 EFFECT OF VARIOUS CARBON SOURCES ON TOXIN PRODUCTION BY *Colletotrichum glocosporioides* BIOASSAYED BY THE RADICLE PLUMULE ELONGATION INHIBITION OF VEGETABLE SEEDS

Table 15. Influence of various carbon sources on toxin production (Observation after 24 hr).

Carbon source	Diameter of necrotic area (mm)*
Dextrose	6.83
Glucose	7.15
Sucrose	5.83
Control	0.00

*Average of 6 spots

C.D. at (0.05) level = 1.90

were bioassayed for their toxic effects. Both exo- and endo-toxins inoculated on healthy host leaves developed necrotic areas as in other cases. The endo-toxin gave the maximum necrotic reaction on host leaves. The diameter of the necrotic spots developed are given in Table 16.

D. Translocation of toxin

The cut twigs of Hydrangea dipped in the toxic metabolite of the fungus obtained by growing the organism on Czapek's medium, exhibited visible alterations in the morphology of the leaf. After 24 hr the tips and margins of the lower leaves wilted and drooped. The whole leaves collapsed gradually. After 48 hr complete wilting and defoliation of the twigs occurred. The base of the twigs dipped in culture filtrate also showed discoloration and rotting. The twigs in the control series remained as such even after 48 hr.

E. Physical properties of toxin.

(i) Effect of heat on culture filtrate

The necrotic area developed on Hydrangea leaves by samples of culture filtrate which were variously heated are presented in Table 17. It can be seen that culture filtrate even after boiling at 100°C developed necrosis on host leaf. However the symptoms were not so pronounced as with untreated filtrate. The toxic effect was maximum with untreated raw culture filtrate. The data

Table 16. Effect of Exo- and Endo-toxins on host leaves (Necrotic area on host leaf after 74 hr of incubation).

Toxin	Diameter of necrotic area (mm)*
Exo-toxin	4.50
Endo-toxin	6.83

*Average of 6 spots

Table 17. Effect of heat on the culture filtrate (Observation after 24 hr).

Samples	Diameter of necrotic area (mm)*
Raw toxin	8.67
At 50°C	6.17
At 100°C	4.67
Autoclaved	1.50

*Average of 6 spots

revealed that the toxic principle produced by the pathogen can withstand boiling.

(ii) Effect of dilution of culture filtrate

The toxic effect of the culture filtrate after dilution are presented in Table 18. The data showed that the toxic effect of the culture filtrate was completely lost with 1:100 dilution.

V. EVALUATION OF FUNGICIDES AGAINST THE LEAF SPOT DISEASE OF HYDRANGEA HORTENSIA CAUSED BY COLLETOTRICHUM GLOEOSPORIOIDES.

(a) In vitro evaluation of fungicides against the pathogen by poisoned food technique.

The results of the effect of various fungicides on the radial growth of the pathogen are tabulated in Table 19 (Fig.13). All fungicides tested were found to inhibit the growth of the pathogen. Complete inhibition of the growth of the fungus was observed with Bavistin, Kitazin, Mildothane, Thiride and Ziride at all concentrations. They were significantly superior to all the other fungicides tested. There is no significant difference between the above fungicides.

Cent per cent inhibition of growth was observed with Blitox at 7000 ppm and there was more than 90 per cent inhibition at all other concentrations tried. However, the effects of Blitox at 3000, 4000, 5000 and 6000 ppm were on par. The inhibitory effects of the other fungicides were not appreciable even at higher concentration. Among the fungicides tried Dithane M-45, Rovral and Daconil were the

Table 18. Intensity of necrotic area developed on Hydrangea leaves by bioassaying diluted culture filtrate (Observation after 24 hr).

	Intensity of necrotic area					
	Number of spots observed					
Dilution	1	2	3	4	5	6
Undiluted	++	++	++	++	++	++
1:10	+	+	+	-	-	-
1:100	-	-	-	-	-	-

\- No spots

\+ Necrotic area upto 5 mm

++ Necrotic area upto 5-10 mm

+++ Necrotic area > 10 mm

Table 19. *In vitro* effect of fungicides on the growth of *C. gloeosporioides* (Poisoned food technique).

Treatment	Concentration of fungicides (ppm)	Mean colony diameter* (in mm)	Per cent inhibition over control
1. Aureofungin Sol.	12.50	67.83	24.63
	25.00	64.17	28.72
	50.00	47.76	46.93
	100.00	14.67	83.70
	200.00	13.33	85.19
2. Bavistin	250	0.00	100.00
	500	0.00	100.00
	1000	0.00	100.00
	2000	0.00	100.00
	3000	0.00	100.00
3. Blitox	3000	4.70	94.77
	4000	19.06	97.93
	5000	3.33	97.30
	6000	3.33	97.30
	7000	0.00	100.00
4. Captan	500	42.20	53.11
	1000	35.80	60.22
	2000	33.80	62.44
	3000	30.70	65.88
	4000	30.70	65.88
5. Daconil	500	70.00	22.22
	1000	58.33	35.19
	2000	41.33	53.00
	3000	37.00	58.89
	4000	34.20	62.00

(Continued...)

Table 19 continued

-2-

Treatment	Concentration of fungicides (ppm)	Mean colony diameter* (in mm)	Per cent inhibition over control
6. Difolaton	500	48.00	46.67
	1000	22.67	74.81
	2000	11.33	87.43
	3000	10.00	88.89
	4000	7.83	91.30
7. Dithane M-45	500	90.00	0.00
	1000	90.00	0.00
	2000	90.00	0.00
	3000	88.33	18.56
	4000	87.17	31.44
8. Kitazin	25	0.00	100.00
	50	0.00	100.00
	100	0.00	100.00
	200	0.00	100.00
	300	0.00	100.00
9. Mildothane	500	0.00	100.00
	1000	0.00	100.00
	2000	0.00	100.00
	3000	0.00	100.00
	4000	0.00	100.00
10. Rovral	250	90.00	0.00
	500	76.33	26.30
	1000	67.83	36.30
	2000	67.83	36.30
	3000	61.13	43.19

(Continued...)

Table 19 continued

-3-

Treatment	Concentration of fungicides (ppm)	Mean colony diameter* (in mm)	Per cent inhibition over control
11. Thiride	500	0.00	100.00
	1000	0.00	100.00
	2000	0.00	100.00
	3000	0.00	100.00
	4000	0.00	100.00
12. Ziride	500	0.00	100.00
	1000	0.00	100.00
	2000	0.00	100.00
	3000	0.00	100.00
	4000	0.00	100.00
13. Control		90.00	

C.D. for comparison between fungicides = 7.59

C.D. for comparison between levels of fungicides or with control = 3.39

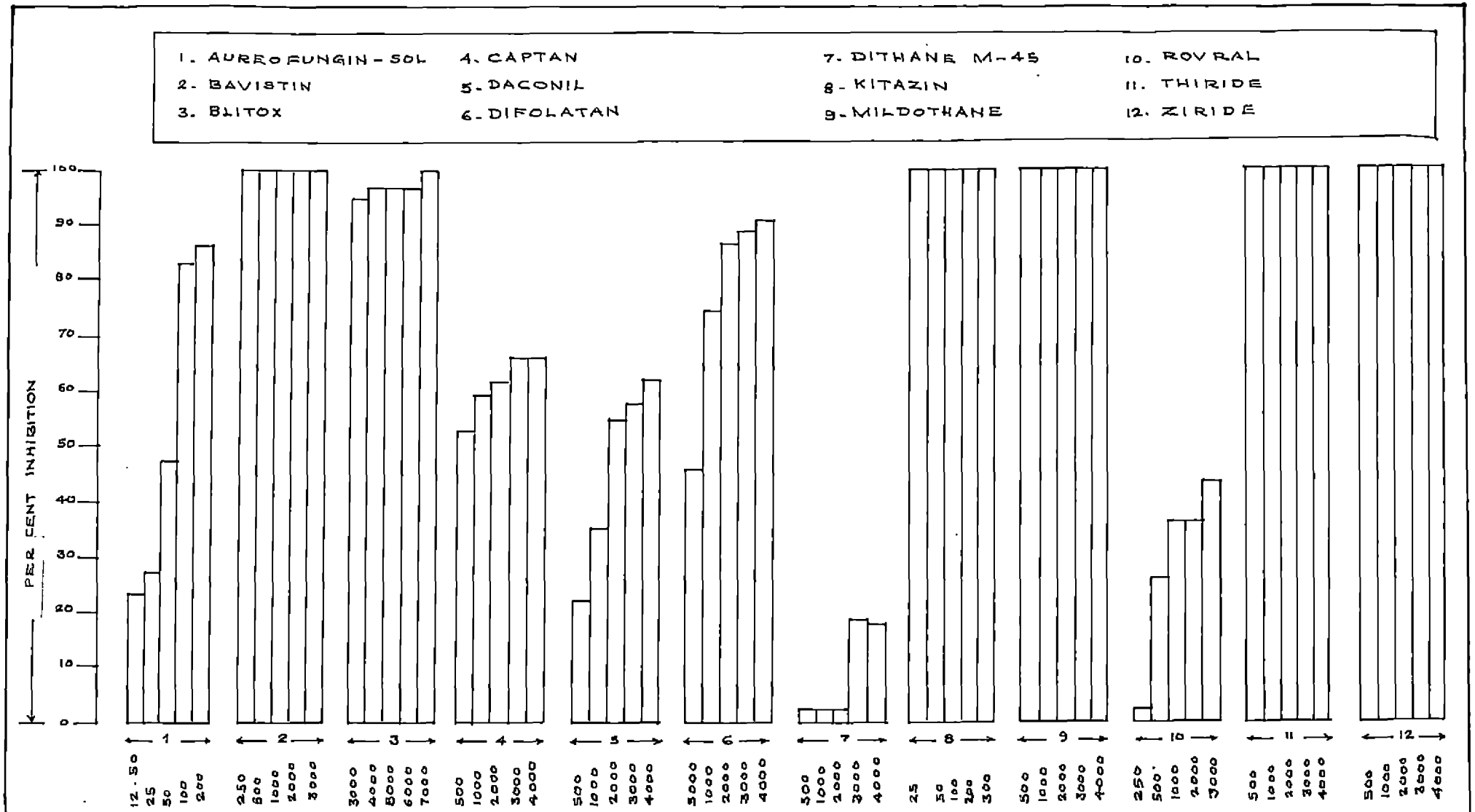


FIG: 13 EFFECT OF DIFFERENT FUNGICIDES ON THE RADIAL GROWTH OF *Colletotrichum gloeosporioides*.

least effective against the pathogen.

(b) Effect of fungicides on inhibition of spore germination.

All fungicides tested except Rovral and Ziride inhibited the spore germination of C. gloeosporioides (Table 20). Complete inhibition of spore germination was noticed with Blitox at 5000 and 6000 ppm. The per cent inhibition was zero and 12.50 with Rovral 500 and 1000 ppm respectively, while with Ziride the per cent inhibition was 7.75 and 12.50 at 2000 and 3000 ppm respectively.

(c) Field evaluation of fungicides against the leaf spot disease of Hydrangea hortensia.

The data on the first observation, prior to fungicidal application, on the leaf spot disease were not significant. The effect of fungicides on the intensity of the leaf spot-disease was taken thirty days after the first spraying and the data are given in Table 21. The data showed that all the treatments were better than control Blitox is found to have the least effect. Statistical analysis showed that there is no significant difference between captan, Thiride, Bavistin and Dithane M-45. They were on par, but they were superior to Blitox (Fig.14).

The observations taken thirty days after the second spraying showed that there was no significant difference between the different fungicides. However, Blitox is found to have the least effect. All these fungicides significantly reduced the intensity of disease as compared to control.

Table 20. Effect of fungicides on the inhibition of spore germination of C. gloeosporioides (Observation after 24 hr)

Sl. No.	Fungicides	Concentration (ppm)	Per cent inhibition over control
1.	Aureofungin sol.	25	98.35
		50	97.25
2.	Bavistin	1000	98.67
		2000	96.50
3.	Blitox	5000	100.00
		6000	100.00
4.	Captan	1000	90.05
		2000	92.27
5.	Daconil	2000	93.90
		3000	100.00
6.	Difolatan	1000	81.25
		2000	82.25
7.	Dithane M-45	2000	73.65
		3000	72.67
8.	Kitasin	50	94.00
		100	95.09
9.	Mildothane	1000	83.34
		2000	87.54
10.	Rovral	500	0.00
		1000	12.50
11.	Thiride	2000	81.54
		3000	92.00
12.	Zirido	2000	7.75
		3000	12.50
13.	Control		2.00

Table 21. Effect of fungicides on the intensity of the leaf spot disease of H. hortensia caused by C. gloeosporioides.

Sl. No.	Fungicides	Mean intensity of infection		
		Before spraying	30 days after first spraying	30 days after second spraying
1.	Dithane M-45	0.875	0.391	0.289
2.	Dlitox	0.966	0.596	0.367
3.	Bavistin	0.867	0.272	0.125
4.	Captan	0.812	0.166	0.111
5.	Thiride	0.803	0.266	0.194
6.	Control	0.907	0.974	1.359

C.D. at (0.05) level = 0.42
C.D. at (0.05) level = 0.42

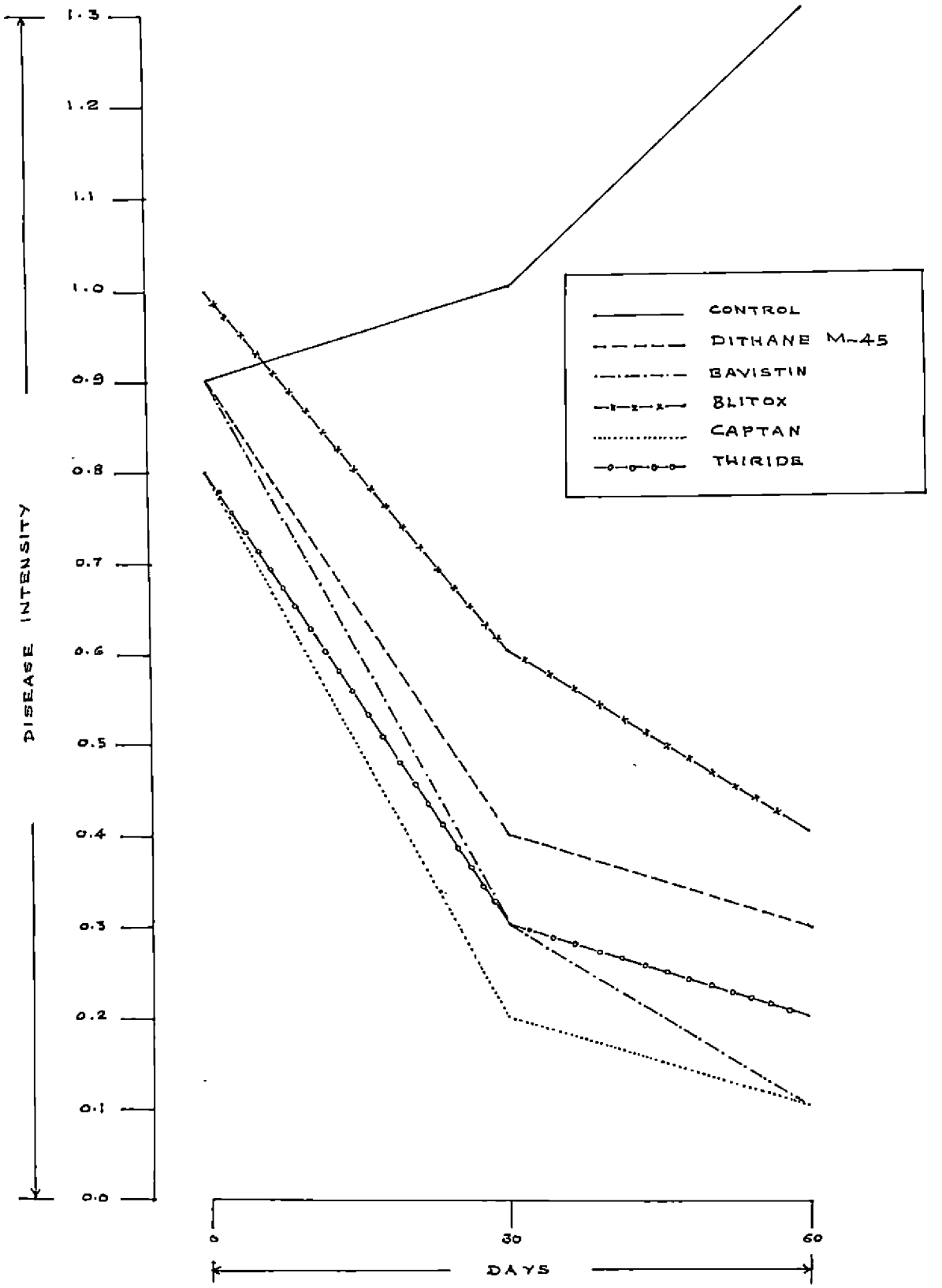


FIG: 14 EFFECT OF FUNGICIDES ON THE INTENSITY OF LEAF SPOT DISEASE CAUSED BY *Colletotrichum gloeosporioides* ON *Hydrangea hortensia*

VI. CARBOHYDRATES AND PHENOLIC CONTENTS OF VARIOUS PARTS OF THE LEAF SPOT FORMED BY *COLLETOTRICHUM GLOEOSPORIOIDES* ON *HYDRANGEA HORTENSIA*.

The changes in the chemical constituents, viz., total carbohydrates and total phenolics were estimated in the three regions of the leaf spot. The data showed that there was a reduction in both total carbohydrates and phenolics in the necrotic area when compared to the halo and prehalo region (Table 22). There was no change in the total carbohydrates in the halo and pre halo region. The phenolic content was maximum at the pre halo area.

Table 22. Chemical constituents of the various parts of the leaf spot caused by *C. gloeosporioides*.

Area affected	Chemical constituents (µg/g of leaf sample)	
	Total carbohydrates	Total phenolics
Necrotic	5.00	7.00
Halo	10.00	16.00
Pre-halo	10.00	22.00

DISCUSSION

DISCUSSION

The survey conducted on the occurrence of the various fungal diseases of different ornamental plants cultivated in the College of Agriculture, Vellayani revealed that Colletotrichum gloeosporioides (Penz.) and species of Cercospora, Alternaria, Pestalotia and Curvularia were the common pathogens which caused the different leaf spot and leaf blight diseases. The periodical observation made for a period of one year showed that out of the thirty five plants observed, twenty four were found to be infected by C. gloeosporioides (Table 1). Out of these twenty four plants, the following thirteen ornamental plants were new records for this organism. Alpinia sp. (Fig.15), Bauhinia tomentosa (Fig.16), Caesalpinia pulcherrima (Fig.17), Codiaeum variegatum (Fig.18), Cosmos bipinnatus, Clitoria ternatea, Gardenia jasminoides, Catharanthus roseus (Fig.19), Mussaenda philipica var. 'Aurorae', Mussaenda philipica var. 'Queen sirikit', Murraya exotica (Fig.20.1 and 20.2), Quisqualis indica (Fig.21) and Tabernaemontana coronaria. However, the perfect state of Colletotrichum gloeosporioides, viz., Glomerella cingulata Spauld and Schrenk has been recorded previously from India on Bauhinia variegata by Hasija (1964).

Species of Colletotrichum other than C. gloeosporioides were already known to occur on some of the host plants included in the present survey, such as Colletotrichum cajeni Rangel on

Fig.15.

Leaf spot due to C. gloeosporioides on Alpinia sp.

Fig.16.

Leaf spot due to C. gloeosporioides on Bauhinia tomentosa.

Fig.16

Fig.17.

Leaf spot due to *C. gloeosporioides* on *Caesalpinia pulcherrima*.

Fig.18.

Leaf spot due to *C. gloeosporioides* on *Codiaeum variegatum*.

Fig. 17

Fig.19.

Leaf spot due to *C. gloeosporioides* on *Catharanthus roseus*.

Fig.19

Fig.20.1 Leaf spot due to *C. gloeosporioides* on *Murraya exotica*.

Fig. 20.2. Leaf let showing the symptom on *M. exotica*.

Fig.20.1

Fig.20.2

Fig. 21.

Leaf spot due to C. gloeosporioides on Quisqualis indica.

Fig.22.

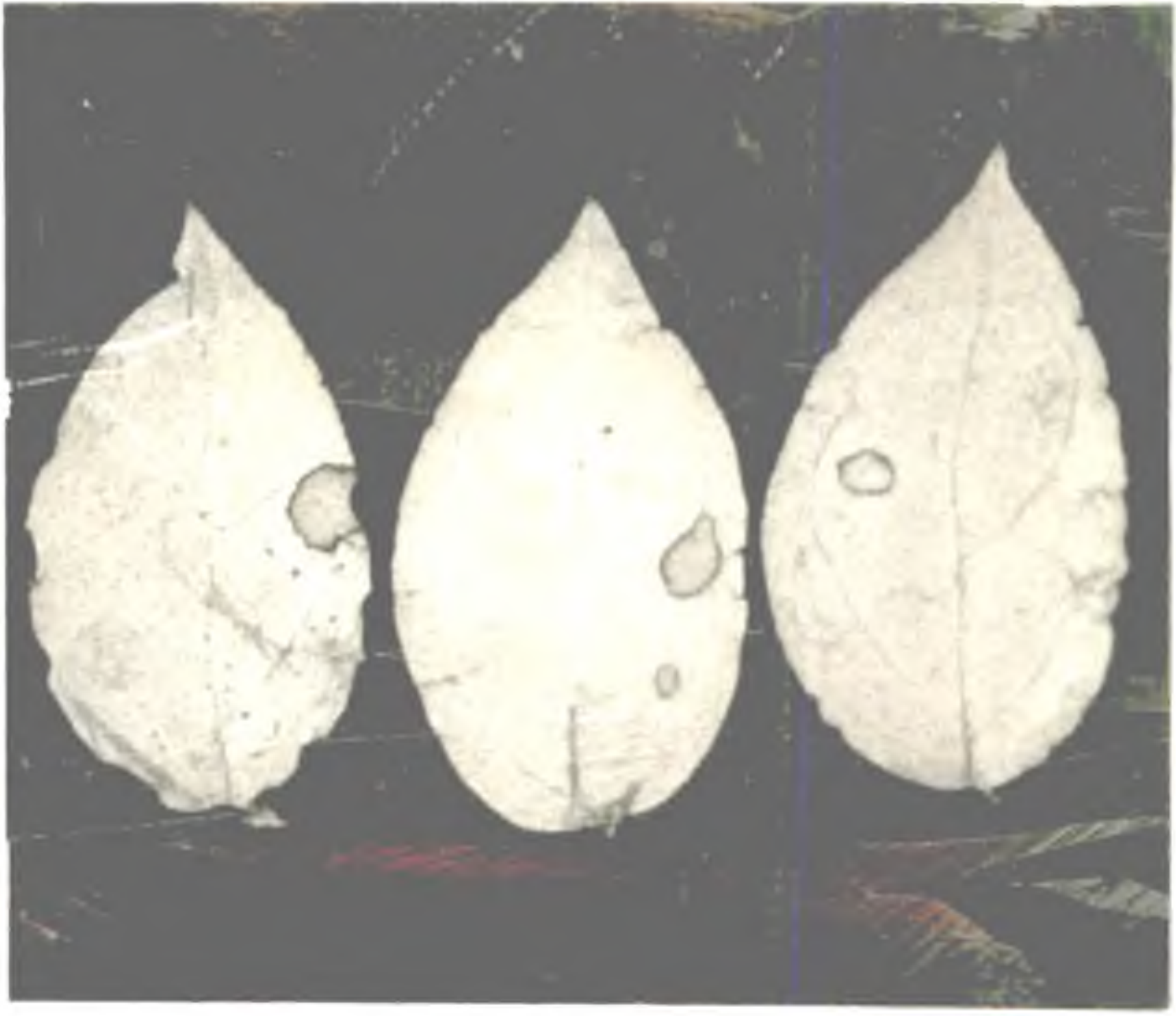
Leaf spot due to Pestalotia sp. on Gardenia jasminoides.

Fig.21.

Fig.22.

Fig.23.

Leaf spot due to *Corynespora* sp. on *Thunbergia erecta*.



Caesalpinia sepiaria Roxb. (Pavgi and Singh, 1964); Colletotrichum capsici (Syd.) Butler & Bisby on Sodiacum variegatum var. pictum Muell. and Croton sp. (Lal and Tandon, 1966; Singh and Khanna, 1970); Colletotrichum variegatum Singh & Upadhyay on Codiaeum variegatum L. (Pavgi and Upadhyay, 1964); Colletotrichum dematium f. sp. clitoricola Pavgi & Mak. on Clitoria ternatea (Pavgi and Mukhopadhyay, 1965); an unidentified species of Colletotrichum on Catharanthus roseus (Nambiar, 1967); Colletotrichum emiticum Pavgi & Singh on Mangifera emetica (Pavgi and Singh, 1964); Colletotrichum capsici Syd. on Dolichos lablab (Tandon and Bilgrami, 1961); Colletotrichum capsici Syd. on Tabernaemontana divaricata (Lal and Tandon, 1967); Colletotrichum dematium Pers. ex. Fr. and C. tabernaemontanae Pavgi on Tabernaemontana coronaria (Roy, 1966; Pavgi and Upadhyay, 1964).

Wilson (1965) reported a leaf spot disease of Hydrangea hortensia from India caused by a species of Colletotrichum. Sawada (1943) reported Colletotrichum hydrangeae as causing leaf spot disease on Hydrangea hortensia in Formosa. The Hydrangea isolate of Colletotrichum from India and Formosa came within the range of Colletotrichum gloeosporioides as identified by C.M.I. (Wilson, 1965). However, the correct identity of the Indian isolate has not been established by Wilson (1965) on the ground that no cross inoculation studies were carried out.

Fig.24.

Sooty mould on *Jasminum* sp.

Fig.25.

Sooty mould on *Quisqualis indica*

Fig.24

Attempts to identify species of other genera of fungi recorded on the various ornamental plants in the present study were not made since the study was mainly centred around C. gloeosporioides. Species of Cercospora were observed in the present survey on a number of ornamental plants such as Angelonia salicariaefolia, Bougainvillaea spectabilis, Clitoria ternatea, Gaillardia picta, Impatiens balsamina, Jasminum grandiflorum and Tabernaemontana coronaria. Among these plants, except on Angelonia salicariaefolia, and Ixora coccinea species of Cercospora have already been reported.

Cercospora bougainvilleae P.N. Rao on Bougainvillea spectabilis (Rao, 1962) and on Bougainvillea glabra Choic (Mehni, 1966); Cercospora nentaluca Syd. and Cercospora ternatea Petch. on Clitoria ternatea (Sydow, 1913; Sydow and McRae, 1929); Cercospora gaillardiae Chiddarwar on Gaillardia pulchella Foug. (Chidderwar, 1959b); Cercospora balsamina Syd.; C. fukushiana Matsumura and C. nojimai Cooke, on Impatiens balsamina (McRae, 1935; McRae, 1937; Chidderwar, 1959a; Govindu and Thirumalachar, 1955); Cercospora jasminicola Muller & Chupp. on Jasminum officinale (Parnekar, 1964); Cercospora tabernaemontanae Thirum & Govindu on Tabernaemontana coronaria (Chupp, 1953; Thirumalachar and Govindu, 1953; Ramaswami et al., 1970) and C. tabernaemontanae H & P. Syd. on T. hynoyana (Pande and Patwardhan, 1966).

Species of Alternaria were observed on Gerbera jamesonii, Gomphrena globosa, Mussaenda Philipica var. 'Aurorae' and Mussaenda philipica var. 'Queenoirikit'. A perusal of literature revealed that no species of Alternaria were recorded on the above two varieties of Mussaenda. Alternaria dauci, A. tenuis and A. alternata were recorded on Gerbera by Kulkarni (1972) and A. gomphrenae Togashi on Gomphrena globosa (Rao, 1964).

Species of Pestalotia on Gerbera jamesonii (Fig.22) and on Pentas lanceolata, Corynespora sp. on Thunbergia erecta (Fig.23) observed during the investigation were new host records for these organisms. Sooty mould recorded on Jasminum sp (Fig.24) and on Quisqualis indica (Fig.25) are also new records. Further studies to identify the causal organisms are necessary.

Since C. gloeosporioides was observed as a common pathogen on the majority of the ornamental plants in the locality, a detailed study on the comparative morphology and pathogenicity of this pathogen was carried out so as to gather detailed information on its inherent virulence potential. Moreover, C. gloeosporioides is known to cause diseases on a number of economically important crops resulting in great loss in Kerala. The studies conducted in this laboratory on the diseases of tree spices, viz., clove, nutmeg and cinnamon have revealed that C. gloeosporioides caused very serious diseases of these spice crops resulting in heavy loss

(Nair et al., 1978; Karunakaran and Nair, 1980a,b).

Seven isolates of C. gloeosporioides from different host plants, viz., Clitoria ternatea, Cosmos bipinnatus, Gardenia jasminoides, Gerbera jamesonii, Hydrangea hortensia, Jasminum sambac and Jasminum sp. were used for the study. Morphological studies on the growth characteristics and also on the mycelial and conidial measurements showed that all the seven isolates were more or less similar though slight variations were observed on the conidial size. Conidia from Jasminum sp. were the smallest (5.36 - 17.75 x 3.57 µm) average being 8.65 x 3.57 µm, while those from Clitoria ternatea were the largest (14.28 - 17.85 x 3.57 µm) average 15.24 x 3.57 µm (Table 2).

The pathogenicity trials showed that the isolates were pathogenic to their respective hosts. Cross inoculation studies revealed that the isolates could cross infect the hosts except that of Gerbera. The observations that the different isolates of C. gloeosporioides are more or less identical in their morphological characters but differ in their pathogenic reaction on G. jamesonii, indicate the possibility of the existence of strain variation in the organism. G. jamesonii was not infected by any of the isolates of the pathogen except from its own isolate(Table 3).

Hydrangea hortensia a popular ornamental shrub, known for its large showy flower heads, is found to be severely infected by C. gloeosporioides. A serious leaf spot and

blighting is found to occur almost throughout the year. Morphological study of the isolate of Hydrangea hortensia showed that the conidia measured on the average 13.49 x 3.57 µm. Sawada (1943) reported the conidial measurement of Colletotrichum hydrangeae as 13 - 15 x 5-7 µm while those of Colletotrichum sp. reported by Wilson (1965) have a conidial size of 12.9 - 22.3 x 3.6 - 5.2 µm) (19.5 x 4.4 µm). Both these isolates of C. gloeosporioides and those seven different isolates described in the present study also come within the range of C. gloeosporioides described by Mordue (1971) (9-24 x 3-6 µm) and other investigators (Prasad, 1962; Varadarajan, 1964; Agnihothrudu and Madapa, 1966; Wilson and Joshi, 1966; Lele and Rao, 1969 and Narendra et al., 1974). In its pathological reactions also the isolate behaved more or less in the same manner as other isolates of C. gloeosporioides. Hence the identity of the present isolate from Hydrangea was confirmed as C. gloeosporioides. Further detailed studies were carried out using the isolate of C. gloeosporioides from Hydrangea hortensia to bring out its biological and patholigical properties.

C. gloeosporioides is a versatile fungus, which has a wide host range. The Hydrangea isolate was artificially inoculated on twenty five common plants. All of them reacted positively except on Gerbera, bread fruit, castor, cacao, jack, cardamom and papaya. Sapota and West Indian

cherry which are artificially infected are new host records for this organism.

The fungus was able to grow well on a number of common culture media, both solid and liquid. Good growth and sporulation was observed with potato dextrose agar followed by Czapek's and bean juice agar medium (Table 6). However, there was no sporulation on oat meal, Host leaf extract, Coon's and Sabouraud's media. The best growth and abundant sporulation was observed in Richard's broth medium. In host leaf extract broth, the growth as well as sporulation of the fungus were poor. The studies, therefore reveal that the fungus requires certain specific nutrients for growth and sporulation which possibly are supplied in the synthetic medium and in nature are made available consequent to the advancement of infection.

Of the carbon sources tried, best growth was observed with dextrose and sucrose. Sucrose has been reported to be the best carbon source by Thomas (1939) for Colletotrichum falcatum and also for C. capsici (Durairaj, 1956; Nair, 1972). Best growth of the organism in the present study was recorded with sodium nitrate as the nitrogen source. Other nitrogen sources such as potassium nitrate and peptone also supported good growth. Peptone and potassium nitrate were reported to be best nitrogen source by Thomas (1939) and Ramakrishnan (1941) for C. falcatum. Nair (1972) reported ammonium nitrate

and sodium nitrate as the nitrogen sources which supported best growth of C. capsici.

Good growth of the fungus was observed with a pH ranging from 4 to 10. Growth was maximum at pH 10. Ramakrishnan (1941) recorded 4.5 to 5 as the optimum pH for C. gloeosporioides. A pH of 7 as the best condition for C. capsici was observed by Durairaj (1956). Sitterly (1958) obtained best growth of Glomerella cingulata at a pH ranging from 3.5 - 5.5. Tandon and Chandra (1962b) recorded good growth and sporulation at pH 3.5 to 8.5 for Glomerella cingulata. A pH of 5.5 to 6.5 was found to be optimum for growth and sporulation of C. falcatum (Ahmed and Divinagracia 1974). Though certain workers have reported good growth of C. falcatum, C. capsici and C. cingulata at acid ranges, the organism under the present study preferred neutral or alkaline range of the medium for good growth. The data also showed that the organism exhibits two peaks of growth i.e., pH 7 and 10.

The trace elements combinations of iron, manganese, copper and zinc sulphate at 10 ppm was found to be the best for the growth of the fungus. The growth was depressed at combinations of higher concentrations. The same effect was noticed by Singh and Prasad (1967) where a combined effect of zinc and manganese was found to influence the growth of C. gloeosporioides isolated from citrus and mango.

Various leaf spot fungi have been reported to produce toxins *in vitro* (Wheeler and Luke, 1963; Wood, 1967; Owens, 1969; Wood *et al.*, 1972). Species of *Colletotrichum* have also been reported to produce such toxic metabolites (Wolf and Flowers, 1957; Goodman, 1960; Hamin and Das, 1970; Nair and Ramakrishnan, 1973). Production of toxic metabolite by *C. gloeosporioides* was reported by Sharma and Sharma (1969); Janardhanan and Hussain (1970). An elaborative study on toxin production by species of *Colletotrichum* was done by Goodman (1960). He isolated a toxin from the culture filtrate of *Colletotrichum fuscum* and named it as Colletotin.

The fungus produced toxic metabolite when cultured on different broth medium. Czapek's, Fries's, Richard's and Host leaf extract broth were found to be good for the production of toxic metabolites. The culture filtrates showed toxic effects when bioassayed on the host leaf. Culture filtrate from Fries's medium showed maximum biological activity. In the present study it is noticed that in Fries's medium the growth of the fungus was scanty and its sporulation was poor. It is likely that media which are not favourable for growth and sporulation might be conducive for the production of toxic metabolites. However, this aspects needs further study for confirmation.

Different fungi are known to prefer different media for their growth and toxin production. Thus Chauhan (1961) and Chauhan (1964) reported Richard's medium was the best for the

production of toxic metabolite by Fusarium orthoceras var. ciceri and Ascochyta rabei. Nair (1972) reported Fries's medium as the best for the toxin production by Colletotrichum capsici. Sucrose and glucose as the carbon source supporting the best toxin production was observed with the present fungus. The same has been observed by Nair (1972) for C. capsici. The culture filtrate has been bioassayed by the radicle and plumule elongation inhibition bioassay and also by inoculating on host leaves. Inhibition of both germination of paddy seeds and elongation of radicle and plumule was observed by Nair (1969) with culture filtrates of Trichoconis padwickii.

The toxic principle in the culture filtrate was found to be thermostable. The toxin property was not completely destroyed even after autoclaving the filtrate. Thermostable toxins were observed in the culture filtrate of Fusarium solani (Fahmy, 1923); Helminthosporium victoriae (Litzenberger, 1949); Colletotrichum fuscum (Goodman, 1960); Trichoconis padwickii (Nair, 1969); C. gloeosporioides (Sharma and Sharma, 1969) and C. capsici (Nair, 1972).

Narain and Omprakash (1968) found that the culture filtrate of Aspergillus niger even after autoclaving, inhibited the germination of onion seeds suggesting that the toxic principle is highly thermostable. Culture filtrate of C. gloeosporioides boiled at 100°C for 10 min. was less toxic than unboiled culture filtrate whereas that sterilised at 10 lbs for 15 min. resulted in significant reduction in

toxicity (Sharma and Sharma, 1969).

Nair (1969) found that toxic principle in the culture filtrate of Trichocomis podvickii was active even after boiling at 100°C for 20 minutes or autoclaving at 15 lbs pressure for 20 minutes. The decrease in the toxic effect was noticed with dilution of the culture filtrate. Negligible or no effect was noticed by Narain and Das (1970) with decrease in the concentration of toxin. Visible effect of the translocated toxin was observed in the present study when the cut twigs were immersed in toxin solution. The uptake of toxin was observed by Narain and Das (1970); Sharma and Sharma (1969); Kuo and Scheffer (1969).

In vitro studies conducted on the effect of fungicides revealed that the complete inhibition of the radial growth of C. gloeosporioides was obtained with Daviotin, Kitasin, Mildothane, Thiride and Ziride (Table 19). The above fungicides were significantly superior to the other fungicides tested. All these fungicides were also effective in the inhibition of conidial germination of the fungus. Complete inhibition of the spore germination was obtained with Blitox, while with Ziride it was only 7.75 and 12.50 per cent at 3000 and 2000 ppm respectively. Eikelenboom (1964) reported fungitoxic activity of Dineb and Thiram for Colletotrichum lini. Lima et al. (1973) reported Daconil 2787 and Orthodifolatan to be toxic to C. gloeosporioides in in vitro evaluation. In the present study both these fungicides were

less effective in the inhibition of the growth of the fungus though Daconil is found to be fungi toxic in the spore germination test. Blitox was effective in the complete inhibition of spore germination in the present study. Aureofungin-sol, Bavistin, Captan, Daconil, Kitazin, Mildothane and Thiride were also effective in inhibiting the germination of spores. Narain and Panigrahi (1971) reported good inhibition of the conidial germination of C. capsici with Ziram even at a concentration of 5 ppm. They found that Aureofungin-sol and Thiram were effective only at 50 ppm. Gupta (1974) proved Aureofungin-sol and Brestan to be most efficatious in inhibiting the spore germination of C. piperatum. The in vitro effect of Thiram for the mycelial growth of C. gloeosporioides was reported by Saraswathy et al. (1975).

In field assay of fungicides the mean intensity of infection after each treatment was presented in Table 21. The data showed that all the fungicides tested were effective in decreasing the intensity of leaf spot when compared to control. The results after the first spraying showed that except Blitox all the other fungicides were effective in reducing the leaf spot. Captan is found to be the best. Statistical analysis showed that all of them were on par. Captan (0.2%), Thiride (0.2%), Bavistin (0.1%) and Dithane M-45 (0.2%). The results of the second spraying showed that there was no significant difference among these fungicides. All of them were

on par. McDonald et al. (1958) reported soil drenching with Thiram to reduce the chance of infection due to Colletotrichum tabaccum. For C. capsici Thiram was the best anti-respiratory fungicide (Sakaena et al., 1975). Chauhan and Duhan (1977) reported Difolatan and Bavistin followed by Dithane M-45 as the most effective fungicides for the field trials against C. capsici. Menezes et al. (1975b) reported Dithane M-45, Daconil and Benomyl to be satisfactory for controlling the disease incited by C. gloeosporioides.

Changes in total carbohydrates and total phenolics in the various regions of the spot formed by C. gloeosporioides on Hydrangea leaves showed marked decrease in phenolic content in the infected area. The apparently healthy area around the spots (the pre-halo) contained more than three times phenolic materials than the central necrotic area. The central portion contained only less carbohydrates than the other regions. Decrease in phenolic content and sugar contents in infected regions is known to be a common feature in a number of leaf spot diseases (Chandramohan et al., 1967; Ram Dayal and Joshi, 1967; Johnson and Schaal, 1957; Thind et al., 1977).

SUMMARY

SUMMARY

A survey for a period of one year was conducted to study the occurrence of fungal diseases on common ornamental plants grown in the gardens of the College of Agriculture, Vellayani, Trivandrum. Colletotrichum gloeosporioides forms the major pathogen affecting majority of the ornamental plants. Cercospora sp., Pestalotia sp., Curvularia sp., Corynespora sp., Sooty mould and red rust were the other fungal pathogens observed. Leaf spots and blight due to C. gloeosporioides were noted for the first time on the following thirteen ornamentals viz., Alpinia sp., Bauhinia tomentosa, Caesalpinia pulcherrima, Codiaeum variegatum, Cosmos bipinnatus, Clitoria ternatea, Gardenia jasminoides, Catharanthus roseus, Mussaenda philipica var. 'Aurorae', Mussaenda philipica var. 'Queen sirikit', Murraya exotica, Quisqualis indica and Tabernaemontana coronaria. Of the other fungal diseases noted in the present study leaf spot due to Cercospora sp. on Angelonia salicariaefolia, Alternaria sp. on Mussaenda philipica var. 'Aurorae', M. philipica var. 'Queen sirikit', Pestalotia sp. on Gardenia jasminoides and Pentas lanceolata and Corynespora sp. on Thunbergia erecta are new host records.

Symptomatology, morphology and cultural characters of seven different isolates of C. gloeosporioides from Clitoria ternatea, Cosmos bipinnatus, Gardenia jasminoides, Gerbera

jamesonii, Jasminum sambac, Jasminum sp. and Hydrangea hortensia were made. The pathogenicity of C. gloeosporioides was established on the respective host plants, by following Koch's postulates. C. gloeosporioides isolated from Gerbera jamesonii showed specificity in infection. All the different isolates of C. gloeosporioides used in the present study compared well in its growth habit, morphological, cultural and physiological characters with those of Colletotrichum gloeosporioides reported in literature.

An isolate of C. gloeosporioides, from Hydrangea was used for further studies. Host-range studies using twenty five different plants showed that pathogen was able to infect almost all the plants tested except seven of them. The possibility of the ornamental plants acting as collateral hosts for the pathogen was discussed. Of the host plants tested, sapota and West Indian Cherry form new host records for the pathogen.

Physiological studies of the Hydrangea isolate showed that the fungus was able to grow on a number of solid and liquid media. Potato dextrose agar, Czapek's agar and bean juice agar were found to be the best solid media, while Richard's broth was found to be the best liquid medium. Sucrose and dextrose as carbon sources and sodium nitrate as nitrogen source was found to be the best for the growth of the pathogen. A pH ranging from 4 to 10 was found to be conducive for the growth of the pathogen and combinations of

iron, manganese, copper and zinc sulphate at 10 ppm supported good growth of the organism.

Studies on the production of toxic metabolites by C. gloeosporioides gave positive results. Of the different common media tested, Frie's, Richard's and host leaf extract broth were found to be good in supporting toxin production. Bioassay using vegetable seeds like bhindi, cucumber and cowpea seeds gave positive results and the toxic activity could be expressed quantitatively by studying its effect on inhibition of growth of plumule and radicle. Necrotic effect on host leaves were also observed when the toxic principle was inoculated on the detached host leaves, which also has been utilised as a bioassay technique. Toxic effect of the culture filtrate was retained even after autoclaving, though the effect was found to be slightly less showing the thermostability of the toxin. But on dilution the culture filtrate lost its toxic property.

In vitro evaluation of fungicides on inhibition of mycelial growth of the pathogen showed that complete inhibition was noticed with Bavistin, Kitazin, Mildothane, Thiride and Zixide. All these fungicides were also found to be fungitoxic to germinating conidia also.

A field trial conducted showed that Captan (0.2%) was the most effective fungicide in controlling the disease. On statistical analysis it was found that Captan, Thiride,

Bavistin, Dithane M-45 and Blitox were on par.

Studies on the content of chemical constituents like total sugars and total phenolics on the spots formed by infection by C. gloeosporioides on Hydrangea revealed that there was a marked decrease in the total sugars and phenolics in the necrotic area when compared to the apparently healthy region.

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*Originals not seen

APPENDICES

Appendix I

Bean juice agar medium

Bean juice	-	215.00 ml
Agar agar	-	10.00 g
Distilled water	-	1000.00 ml

Final pH ± 6.5

Coon's agar medium

Sucrose	-	7.20 g
Dextrose	-	3.60 g
Magnesium sulphate	-	1.23 g
Potassium dihydrogen phosphate	-	2.72 g
Potassium nitrate	-	2.02 g
Agar agar	-	20.00 g
Distilled water	-	1000.00 ml

Final pH ± 6.5

Czapek's agar medium (Thom, 1910)

Magnesium sulphate	-	0.50 g
Dipotassium hydrogen phosphate	-	1.00 g
Potassium chloride	-	0.50 g
Ferrous sulphate	-	0.01 g
Sodium nitrate	-	2.00 g
Sucrose	-	30.00 g

Appendix I continued

Agar agar	-	20.00 g
Distilled water	-	1000.00 ml

Final pH ± 6.5

Host extract agar medium

Hydrangea leaves	-	200.00 g
Agar agar	-	20.00 g
Distilled water	-	1000.00 ml

Final pH ± 6.5

Host extract dextrose agar medium

Hydrangea leaves	-	200.00 g
Dextrose	-	20.00 g
Agar agar	-	20.00 g
Distilled water	-	1000.00 ml

Final pH ± 6.5

Oat meal agar medium

Rolled oats	-	60.00 g
Agar agar	-	20.00 g
Distilled water	-	1000.00 ml

Final pH ± 6.5

Potato dextrose agar medium

Peeled and sliced potato	-	200.00 g
Dextrose	-	20.00 g
Distilled water	-	1000.00 ml

Final pH ± 6.5

Richard's agar medium (Fehmy, 1923)

Potassium nitrate	-	10.00 g
Potassium dihydrogen phosphate	-	5.00 g
Magnesium sulphate	-	2.50 g
Ferric chloride	-	0.20 g
Sucrose	-	50.00 g
Distilled water	-	1000.00 ml

Final pH ± 6.5

Sabouraud's agar medium

Glucose	-	40.00 g
Peptone	-	10.00 g
Agar agar	-	20.00 g
Distilled water	-	1000.00 ml

Final pH ± 6.5

Source: Source book of laboratory exercises in Plant Pathology. Source book committee of the American Phytopathological Society, pp.366-368.

Erie's medium (Pringle and Scheffer, 1963)

Ammonium tartarate	-	5.00 g
Ammonium nitrate	-	1.00 g
Potassium dihydrogen phosphate	-	1.00 g
Magnesium sulphate	-	0.50 g
Sodium chloride	-	0.10 g
Calcium chloride	-	0.13 g
Sucrose	-	30.00 g
Manganese sulphate	-	0.01 g
Ferrous sulphate	-	0.02 g
Boric acid	-	0.001 g
Copper sulphate	-	0.0001 g
Zinc sulphate	-	0.0001 g
Distilled water	-	1000.00 ml

Final pH ± 6.5

Semi synthetic medium (Granit, 1964)

Sucrose	-	30.00 g
Diammonium phosphate	-	2.50 g
Dipotassium phosphate	-	3.00 g
Ferrous sulphate	-	0.005 g
Manganese sulphate	-	0.005 g
Copper sulphate	-	0.005 g

Zinc sulphate	-	0.005 g
Calcium carbonate	-	5.00 g
Malt extract	-	5.00 g
Distilled water	-	1000.00 ml

pH - 6.4 - 6.7

Appendix II

Analysis of variance table
(Growth on different solid media)

Source	S.S.	df	MS	F.value	Whether significant or not
Total	1885.33	23			
Treatment	1739.00	7	248.43	27.21	Significant
Error	146.33	16	9.13		

Ranking T_1 T_3 T_5 T_8 T_4 T_2 T_6 T_7

Appendix III

Analysis of variance table
(Growth on liquid media)

Source	S.S.	df	MS	F.value	Whether significant or not
Total	232368.00	17			
Treatment	213111.33	5	42622.27	26.56	Significant
Error	19256.67	12	1604.72		

Ranking T_5 T_1 T_2 T_6 T_4 T_3

Appendix IV

Analysis of variance table

(Growth on various carbon sources)

Source	S.S.	df	MS	F.value	Whether significant or not
Total	423794.00	24			
Treatment	385108.00	4	96277.00	49.77	Significant
Error	38686.00	20	1934.30		

Ranking $\overline{T_1 \quad T_4 \quad T_3 \quad T_2 \quad T_5}$

Appendix V

Analysis of variance table

(Growth on various nitrogen source)

Source	S.S.	df	MS	F.value	Whether significant or not
Total	38176.95	19			
Treatment	30186.30	3	10062.10	7.56	Significant
Error	7990.65	6	1331.77		

Ranking $\overline{T_3 \quad T_2 \quad T_1 \quad T_4}$

Appendix VI

Analysis of variance table

(Growth on different pH levels)

Source	S.S.	df	MS	F.value	Whether significant or not
Total	410309.52	20			
Treatment	372696.19	6	62116.03	23.12	Significant
Error	37613.33	14	2686.67		

RANKING T_1 T_5 T_3 T_4 T_6 T_2 T_7

Appendix VII

Analysis of variance table

(Growth on various trace elements)

Source	S.S.	df	MS	F.value	Whether significant or not
Total	33931.66	11			
Treatment	31520.66	2	15760.33	58.83	Significant
Error	2411.00	9	267.88		

RANKING T_2 T_3 T_1

Appendix VIII

Analysis of variance table

(Effect of different media on toxin production)

Source	S.S.	df	MS	F.value	Whether significant or not
Total	510.40	41			
Treatment	412.57	6	68.76	24.60	Significant
Error	97.83	35	2.79		

Ranking $\overline{T_2 \quad T_5 \quad T_6} \quad T_3 \quad \overline{T_1 \quad T_4} \quad T_7$

Appendix IX

Analysis of variance table

(Effect of various carbon sources on toxin production)

Source	S.S.	df	MS	F.value	Whether significant or not
Total	246.96	23			
Treatment	196.46	3	65.48	25.93	Significant
Error	50.50	20	2.52		

Ranking $\overline{T_2 \quad T_1} \quad T_3 \quad T_4$

Appendix X

Analysis of variance table

(*In vitro* evaluation of fungicides on the growth of *C. gloeosporioides*)

Source	S.S.	df	MS	F.value	Whether significant or not
Total	187813.94	182			
Treatment	184226.44	60	3070.44	104.41	Significant
Between fungicides	153518.45	11	13956.22	474.60	"
Treatment Vs Control	11663.21	1	11663.21	3.71	"
Error	3587.50	122	29.40		

Ranking $\overline{T_2 \quad T_8 \quad T_9 \quad T_{11} \quad T_{12}} \quad T_3 \quad T_6 \quad T_4 \quad T_1 \quad T_5 \quad T_{10} \quad T_7$

Appendix XI

Analysis of variance table

(30 days after the first spraying)

Source	S.S.	df	MS	F.value	Whether significant or not
Total	3.217	17			
Treatment	2.535	5	0.507	8.89	Significant
Error	0.682	12	0.057		

Ranking $\overline{T_4 \quad T_5 \quad T_3 \quad T_1}$ T_2 T_6

Appendix XII

Analysis of variance table

(30 days after the second spraying)

Source	S.S.	df	MS	F.value	Whether significant or not
Total	4.249	17			
Treatment	3.562	5	0.712	12.49	Significant
Error	0.687	12	0.057		

Ranking $\overline{T_4 \quad T_5 \quad T_3 \quad T_1 \quad T_2}$ T_6

STUDIES ON THE FUNGAL DISEASES OF ORNAMENTAL PLANTS

By

P. SANTHA KUMARI

ABSTRACT OF A THESIS

SUBMITTED IN PARTIAL FULFILMENT OF THE
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ABSTRACT

A survey was conducted to study the occurrence of fungal pathogens on common ornamental plants grown in the College campus. Collototrichum gloeosporioides, Cercospora sp., Pestalotia sp., Corynespora sp., Curvulgria sp., Sooty mould and red rust were the common pathogens noticed. Of these, leaf spot disease due to C. gloeosporioides forms the major disease affecting majority of the ornamental plants. Symptomatology, morphology and cultural characters of the seven isolates of C. gloeosporioides were made. The characteristics of these isolates compares well and agreed with those reported in literature.

Among the fungi recorded on the various ornamental plants, thirteen form new host records for C. gloeosporioides, one for Cercospora sp., two for Alternaria sp., two for Pestalotia sp. and one for Corynespora sp. Comparative study on the morphological and pathological characters of the seven different isolates of C. gloeosporioides were made. Detailed studies were conducted with an isolate of C. gloeosporioides from Hydrangea, a common ornamental shrub. Host-range studies conducted with this isolate showed that the pathogen can infect a number of economically important crop plants. Sapota and West Indian cherry which infected when artificially inoculated were new host records for this pathogen.

Potato dextrose agar, Czapek's agar and bean juice agar were found to be the best solid media for the growth of the pathogen. Among the liquid media tested, best growth was observed with Richard's broth. Best growth was observed with sucrose as carbon source, sodium nitrate as nitrogen source and a 10 ppm concentration of trace elements such as iron, manganese, copper, and zinc sulphate in combinations. A pH ranging from 4 to 10 supported good growth of the organism.

Toxic metabolite produced by the pathogen was able to inhibit the germination percentage of vegetable seeds like bhindi, cucumber, and cowpea. Reduction in radicle and plumule length in these seeds were also observed by the toxin treatment. The culture filtrate applied on host leaves also produced necrotic lesions. Toxic effect of the culture filtrate was noticed even in autoclaved samples, while with increasing dilution the effect decreased.

In the in vitro assay of fungicides, Bavistin, Kitazin, Mildothane, Thiride and Ziride showed complete inhibition of mycelial growth. Conidial germination inhibition was also noticed with these fungicides.

A field assay of fungicides on leaf spot of Hydrangea hortensia showed that Captan was the best in reducing the intensity of the disease. Statistical analysis showed that Captan, Thiride, Bavistin, Dithane M-45 and Blitox were on par.

Considerable reduction in the total carbohydrates and phenolics were noticed in the diseased areas when compared to the apparently healthy areas.