STUDIES ON THE LEAF BLIGHT DISEASE OF CLOVE CAUSED BY *Cylindrocladium sp.*

By

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THESIS

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DECLARATION

I hereby declare that this thesis entitled "Studies on the leaf blight disease of clove caused by *Cylindrocladium* sp." 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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INTRODUCTION

INTRODUCTION

Clove, the dried unopened flower buds of the tree Eugenia caryophyllata L. (Thunb.) is a much sought after spice of commerce especially in the orient. In India and China clove was very popular as a spice and also as an ingredient in some of the indigenous medicines. Today India is the second largest consumer of clove, in the world, and the internal production is far from satisfactory and we are importing clove to the tune of Rupees two million annually.

Clove is important as a spice and in medicine it is a stimulative, antispasmatic and carminative. Clove oil obtained by the distillation of flower buds, flower stalks and leaves are used in the manufacture of perfumes, soaps, bath salts etc. as flavouring agent in medicine and in dentistry, and as a clearing agent in microscopy.

The clove tree (Eugenia caryophyllata) is a tropical and subtropical plant, indigenous to the small volcanic islands of Ternate, Tidore, Mutir, Makyan and Bachian in the Moluccas. Several species of Eugenia have got edible fruits. It is an important crop yielding essential oils also.

In India it is cultivated in the States of Kerala and Tamil Nadu for the unopened dried flower buds and is a very remunerative cash crop. In recent years there is an increase in its area of cultivation in Kerala and Department of Agriculture is popularising its cultivation. Extensive

cultivation of clove in Kerala resulted in new disease problems also. A new leaf disease of clove is found to be prevalent in many parts near Trivandrum, Kerala from 1977 onwards. Seedlings and two to three year old plants were more easily affected by this leaf disease. The disease was severe during July to September, the period coinciding with the south-west monsoon, but is prevalent in a mild form throughout the year. No information is available on this disease of clove plants and the present study was undertaken on the symptomatology of the disease, isolation, morphology, pathogenicity and host range of the causal organism, toxin production by the organism and laboratory evaluation of few common fungicides against the pathogen.

The experimental results obtained are presented in this dissertation.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Infection of clove (Eugenia caryophyllata L.) by Cylindrocladium quinqueseptatum Boedijn et. Reitsma, was first reported from Indonesia by Sloof (1941). Later on Reitsma and Sloof (1950) reported leaf diseases of clove seedlings by C. quinqueseptatum and also by Gloeosporium piperatum. Artificial inoculation studies conducted by them indicated that C. quinqueseptatum was highly pathogenic to clove leaves, whereas G. piperatum attacked only plants that had been mechanically injured or weakened by infection with C. quinqueseptatum.

Boedijn et Reitsma (1950) recorded C. quinqueseptatum on clove seedlings in Java.

Rao (1971) reported C. quinqueseptatum from rubber for the first time from Malaya. In India, this fungus was reported on Eucalyptus grandis and on ten other species of Eucalyptus causing severe foliar damage in forest plantations in Kerala by Sehgal et al. (1969; 1975). A leaf rot (leaf spot) disease of clove in India caused by Cylindrocladium quinqueseptatum was first reported by Sarma and Nambiar (1978). The disease was observed both on seedlings and mature trees, but more severe on seedlings. During the early stages of infection, minute water-soaked spots appeared all over the leaf. The spots later became necrotic with a chlorotic halo. Mature lesions were about 1 to 3 mm in diameter and occasionally had a clear whitish centre. After coalescence the marginal lesions progressed

inward, exhibiting typical leaf rot symptoms. Rotting also appeared from the leaf tip downward, exhibiting a tip-burn appearance; which occasionally involved half of the leaf lamina. Under high humid conditions, the necrotic areas often showed faint whitish powdery sporulating masses, mostly on the lower side of the leaf. Leaf rot caused rapid defoliation. Spread of disease in nurseries was rapid during damp weather with intermittent rains. In severe cases, complete defoliation and subsequent death of seedlings occurred. In mature trees severely affected branches remained bare because of heavy defoliation. In artificial inoculation studies Sarma and Nambiar (1978) found that C. quinqueseptatum isolated from clove was pathogenic to other plants belonging to the family Myrtaceae, viz., Eugenia jambolana, Pimenta dioica, Eucalyptus grandis, E. maculata and E. globulus.

A leaf blight disease of clove caused by the mixed infection of C. quinqueseptatum and Colletotrichum capsici was reported from South India by Wilson et al. (1977). They found that seedlings as well as 2 to 3 year old plants were affected by the leaf spot and leaf blight during rainy months. The initial symptoms were manifested as minute chocolate coloured spots, usually surrounded by an yellow halo. The spots enlarged rapidly under humid conditions and appeared as greyish brown lesions, often involving large portions of the leaf lamina. A slight blistening at the infected region of the leaf lamina was often evident. Under dry conditions, the

lesions were ashy grey in colour. At times, more or less indistinct concentric zonations were visible on the upper side of the leaf. The yellow halo generally was not present around the larger lesions. Most of the infected leaves shed and eventually resulted in the death of the seedlings. Heavy rainfall and high humid conditions were found to be favourable for the development of the disease. Cylindrocladium caused dirty brown lesions on the leaves and Colletotrichum caused grey spots with dark brown margins. When these two were inoculated together, the characteristic greyish brown to ashy grey lesions were formed on the infected leaves.

The genus Cylindrocladium was erected by Morgan in 1892 with C. scoparium as the type species, with the following characters - "Hyphae steriles repentes, fertiles, erectae, dichotimice ramosae, septate, basidia in apice ramorum, subterna fusoidea, conidia cylindracea, 1 septata". C. scoparium was found on dead pods of Gleditschia triacanthos L. The same fungus was reported by Ellis and Everhart (1900) on dead leaves of Asimina triloba Dun. They considered it as an undescribed species of the genus Diplocladium and described it accordingly as D. cylindrosporum Ell. & Evrh. In 1912 Hawley erected the genus Candelospora with the type species C. ilicicola growing on dead leaves of Ilex aquifolium L. with the following characters - Hyphae steriles repentes, conidiophoris erectis, septatis, hyalinis, irregularitus, ramosis, veleitium simplicitus, supra penicillatium divisis, conidis singulis in ultimis ramulis ortis, hyalinis, multi

septatis".

Figueiredo and Namekata (1967) recorded Calonectria quinqueseptata as the perfect state of Cylindrocladium quinqueseptatum for the first time in Brazil. The organism was isolated from the leaf spots on Anona squamosa, Eugenia saligna, E. teretiocornis, and E. caryophyllata. The perfect state was obtained in old culture and on inoculated leaves kept for 5 to 6 days in humid chamber.

Peerally (1972) in CMI descriptions of pathogenic fungi and bacteria had given Caloncctria quinqueseptata Figueiredo & Namekata as the perfect state of C. quinqueseptatum.

Morphology of the fungus

Boedijn and Reitsma (1950) made detailed studies on the comparative morphology of Cylindrocladium and Candelospora and concluded that these two genera are the same, the only difference was in the conidia; which in Cylindrocladium are two celled while they are many celled in Candelospora. They gave detailed description of C. quinqueseptatum having the following characters. The conidiophore structure consisted of primary, secondary and tertiary sterigmata. The ultimate branch of tertiary sterigmata bears the structures-phialides. Conidiophores 5 to 6 µm, the primary branches measuring 22 to 31 x 3.5 to 5.0 µm, the secondary 17 to 21 x 3 to 3.5 µm and the tertiary 15 to 17 x 2 to 3 µm, the ultimate branches bearing 2 to 4 phialides, 11 to 12 x 2 to 3 µm, the main axis is about 2 µm in width, gradually elongating and becoming somewhat

clavate towards the apex. The straight cylindrical conidia produced at the ends, 75 to 106 x 5 to 7 µm, are provided with 3 to 6 (mostly 5) septa. The cultures are tawny turning to zinc orange, with a white edge, of loose texture, radially striate through darker fibres and with some indistinct concentric zones.

Peerally (1972) had given the details on the morphology of the fungus as follows. Perithecia superficial, oval to elliptical, orange to chestnut, 360 to 580 µm high, 300 to 400 µm diameter, perithecial wall roughened by masses of large pseudoparenchymatous cells, leaving a smooth papillate ostiolum. Asci club shaped, long stalked, hyaline, 8 spored, 76 to 126 x 13 to 22 µm, ascospores hyaline, variously curved, 1 to 6 septate, mostly 3 septate, 30 to 80 x 4 to 7 µm. Conidiophore branches arise laterally from a stipe; primary branches non-septate or septate, 8.4 to 26.6 µm long; secondary and tertiary branches nonseptate, 11.5 to 15.6 µm. Phialides hyaline, 13.0 to 15.6 µm long. Conidia hyaline straight, cylindrical, 1 to 6 septate, usually 5 septate, old conidia in culture may develop more than 6 septa, 59.8 to 104.6 x 5.2 to 7.0 µm *in vivo*, 72.8 to 119.6 x 5.2 to 7.8 µm *in vitro*. Sterile filament terminates in a narrowly clavate vesicle 2.5 to 3.0 µm wide. Sobers *et al.* (1975) had given the morphology of *C. quinqueseptatum* as follows. Conidiophores exhibit primary, secondary and tertiary branching having 19.5 to 30.1 x 4.7 to 7.7 µm, 17.1 to 24.2 x 4.1 to 6.5 µm and 14.2 to

23.1 x 4.1 to 5.9 µm, respectively. The phialides are reniform with 9.4 to 18.9 x 4.7 to 7.1 µm. Conidia are 5 septate, 62.6 to 95.2 x 4.7 to 7.8 µm in measurement. The main axis (stipe) of the conidiophore structure measured 126 to 299 x 3.5 to 6.5 µm with a clavate vesicle of 20.1 to 38.4 x 4.1 to 7.1 µm.

Sarma and Nambiar (1978) noticed the morphology of the organism as follows - the conidiophores were upright, septate, occurring in masses, often on the lower surface of the leaves, arising laterally from a stipe. They ranged from 110 to 484 µm in length and 4.7 to 9.5 µm in width at the base. The primary branches measured 11.9 to 28.5 x 2.3 to 4.7 µm and secondary branches 11.9 to 14.2 x 2.3 to 4.7 µm. Tertiary branches were rarely observed. Each branch had 2 to 4 phialides measuring 9.5 to 23.8 x 2.3 to 4.7 µm ultimately giving off conidia. The main axis of conidiophores became sterile threads, 187 to 473 µm in height, which terminated in a club shaped structure 2.3 to 4.6 µm in width. The conidia often appeared glued together in masses and were cylindrical, measuring 59.5 to 107.1 x 4.7 to 7.14 µm with 3 to 5 septa. Conidia with 3 to 5 septa, were seen more frequently and mature spores were strictly five septate. Spores germinated within 4 to 5 hours in free water on glass slides incubated in humid petri plates at 28 to 30°C. Germination was seen both from polar and intercalary cells.

Growth of the fungus in culture

Sarma and Nambiar (1978) recorded the growth of the fungus, Cylindrocladium quinqueseptatum, on potato sucrose agar (PSA). A fresh culture on PSA showed light brown and fast growing upright hyphae. Eight day-old mature colonies of the fungus showed dark brownish zones alternating with light brown to yellowish zones radiating from the centre. The outer border was always dull white, subtended by a narrow yellowish zone. Light brown to cream coloured aerial mycelium which later turned brown appeared uniformly over the entire colony in the beginning, but later zonate patterns appeared. The back side of the petri plate with a mature colony showed the zonate pattern clearly. Secretion of brown pigment was noted in the solid and liquid media. Sporulation was abundant on PSA as compared with oats agar medium. Formation of sclerotia like bodies were noticed in older cultures.

Host range

Peerally (1972) had reported that Cylindrocladium quinqueseptatum could infect Anona, Camellia sinensis, Eucalyptus, Eugenia caryophyllata and Hevea. Sarma and Nambiar (1978) conducted the host range studies of C. quinqueseptatum on a variety of plants, viz., Eugenia jambolana, Pimenta dioica, Eucalyptus grandis, E. maculata and E. globulus. Inoculated plants exhibited the first symptom of water soaked leaf spots in about 48 hours. Leaf rot symptoms were noticed after 4 to 6 days and sporulation occurred on the affected

patches, 6 to 8 days after inoculation, depending on climatic conditions. In Eugenia jambolana and Pimenta dioica, the initial symptoms started as minute water soaked spots which later coalesced to form tan coloured necrotic patches exhibiting typical leaf rot symptoms.

Toxin production by the fungus

Toxic effect of the culture filtrate of Cylindrocladium quinqueseptatum, the causal organism of seedling blight of Eucalyptus hybrid was demonstrated by Anahosur et al. (1976) in India. The fungus was grown in Czapeks', Richards' and potato dextrose broths in 250 ml conical flasks each containing 100 ml of the respective media. The culture filtrate was collected separately after 20, 30 and 41 days. Uninoculated media served as control. To assess the toxin production, healthy cuttings of Eucalyptus hybrid and Lycopersicon esculentus Mill were placed in culture filtrates in test tubes (10 ml in each test tube) separately. Healthy cuttings placed in uninoculated media and sterile water served as control.

Marginal necrosis of leaves, leaf spots and wilting were the characteristic symptoms observed in both Eucalyptus and tomato cuttings. With 20 day old culture filtrate, wilting was observed after 19 hours in Czapeks' medium whereas only after 40 hrs in Richards' and Potato broth. Application of culture filtrate obtained by growing the organism for 41 days in three different media, viz., Czapeks', Richards' and Potato broth was reported to differ in the toxic effect (Anahosur et al., 1976). Wilting was observed with culture filtrate

from Czapeks' and Richards' in 18 hrs while those of potato broth took 41 hrs to produce the same effect. The healthy cuttings of eucalyptus and tomato placed in sterile water and uninoculated solutions of Richards', Czapeks' and Potato broth remained unaffected. All these preliminary studies carried out by these workers led them to conclude that this organism is producing some toxic metabolite and the production was comparatively more in Czapeks' than in Richards' and Potato broth.

Katznelson and Richardson (1948) reported that the culture filtrates of Cylindrocladium sp. grown in culture media with acetic acid caused complete wilting of strawberry seedlings within 3 or 4 days. Production of toxic metabolites has been reported for a number of leaf spot fungi. Brian et al. (1949; 1952) found that alternaric acid produced by Alternaria solani prevented the spore germination of the fungus, Myrothecium verrucaria, at 1 per cent concentration. Ludwig (1955) observed that the spore germination of Sclerotinia fructicola was inhibited by the toxin produced by Helminthosporium sativum. Orschanskaya (1960) reported that the culture filtrate of Diplodia zeae inhibited the germination of maize seeds to a considerable extent.

Goodman (1960) reported colletotin, a toxin produced by Colletotrichum fuscum. In this assay, shoots bearing four terminal leaves of a tomato seedling is excised and the stem end of the cut tomato shoot is placed in a vial containing the

culture filtrate to be tested. Activity of the toxin is measured by intensity of the symptoms. Toxin production appeared on the 11th day of incubation and its effect on tomato foliage became more intense by the seventeenth and eighteenth days.

Scheffer and Pringle (1961) recorded the production of a selective toxin produced by Periconia circinata, the causal agent of the milo disease of certain cultivars of Sorghum vulgare var. subglabrescens (grain sorghum or milo). The culture filtrates were collected from cultures of various ages and tested for selective toxic action against seedlings of sorghum varieties, resistant and susceptible to Periconia circinata. They also found that toxin production apparently was correlated with pathogenicity and virulence of the fungus.

Govindan (1963) noted that metabolites of Pyricularia oryzae inhibited seed germination and plumule elongation of blast susceptible varieties of paddy.

Luke et al. (1966) reported the effects of the patho-toxin-victorin on the ultra structure of root and leaf tissue of Avena species. They showed that victorin causes permeability changes in susceptible oat plants which eventually lead to wilting.

Lakshmanan and Vanterpool (1967) reported that phomic acid produced by Phoma medicaginis inhibited spore germination of Penicillium notatum, Fusarium culmorum and Aspergillus niger at 50 ppm concentration. White and Starratt (1967) found that

zinniol produced by Alternaria zinniae inhibited germination of seeds of zinnia, tomato, lettuce and water melon.

Krishnaswamy et al. (1969) incubated cultures of different isolates of Pyricularia oryzae in Czapeks' Dox medium for a period of 15 days, to study the toxin production. Sharma and Sharma (1969) reported the toxic metabolite production by Colletotrichum gloeosporicides causing citrus die back in India. They found that the toxin production started at 8 days growth and the toxicity increased upto a maximum of 22 days growth, after which it declined slowly upto 30 days. Twigs kept in the filtrates showed mild symptoms in 8, 10 and 12 days old culture, moderate reaction in 16, 20, 26, 28 and 30 days old cultures and severe symptoms in 22 and 24 days old.

Kato et al. (1970) reported Cylindrochlorin as a new antibiotic produced by Cylindrocladium sp.

In 1970, Narain and Das reported the toxin production during pathogenesis of Colletotrichum capsici causing Anthracnose of chillies. They filtered two types of preparations. Assaying of both types of filtrates were done on seed germinability and also on detached leaves and fruits of chillies.

Vidhyasekharan et al. (1970) noted that the metabolites of certain seed borne fungi caused inhibition of seed germination and root and shoot elongation in paddy.

Studies conducted by Dwivedi and Singh (1971) on the percentage occurrence of rhizosphere microorganisms in the

presence of metabolites produced by certain fungi isolated from the rhizosphere of Andrographis paniculata, revealed that maximum inhibitory effect was exhibited by the metabolites of Aspergillus niger followed by that of Botrydiplodia theobromae. Rajagopalan (1971) observed that the culture filtrate of Diplodia natalensis caused considerable reduction in their per cent germination of cucumber and snakegourd seeds. The per cent spore germination of Gloeosporium psidii and Pestalotia psidii was also reduced to a great extent by the culture filtrate.

Nair and Ramakrishnan (1973) had reported the production of toxic metabolites by Colletotrichum capsici (Syd). Butl. & Bisby and its role in leaf spot diseases of turmeric. The concentrated toxic metabolite from mycelium (endotoxin) and that from culture filtrate (exotoxin) were bioassayed on turmeric leaves by employing drops of 0.05 ml on one half side of the leaves and lightly pricked under. On the other half of the same leaf sterile water was used as control and treated in the same way. In treatments with endotoxin and exotoxin solution, visible alternations in the inoculated areas were noted within 4 hrs after inoculation. The treated areas showed signs of necrosis. This necrotic area soon enlarged and within 24 hrs they developed an yellow halo around the necrotic spot. The spot found by the exotoxin was smaller in size than that by endotoxin, but both were similar in appearance.

Yang and Yu (1976) observed the inducement of toxic furanoterphenoids in sweet potato, in response to infection by Ceratocystis fimbriata, Fusarium oxysporum f. batatas, Diplodia tubericola and Alternaria sp.

Park et al. (1976) reported the effect of the host specific toxin from Alternaria kikuchiana on the ultra structure of plasmamembranes of cells in leaves of Japanese pear. The fungus produced a host specific toxin (AK-toxin) which caused immediate changes in permeability of susceptible leaf tissues.

Peterson (1977) reported the isolation of two toxins produced by Pyrenophora teres and their significance in disease development of net spot blotch of barley.

Effect of fungicides on Cylindrocladium quinqueseptatum

Anahosur et al. (1977) conducted a laboratory evaluation of fungicides against C. quinqueseptatum, the causal organism of seedling blight of Eucalyptus hybrid. The efficacy of 10 fungicides, at three concentration was investigated by adopting the poisoned-food technique. The fungicides used were Duter (Triphenyl tin hydroxide), Bavistin (2-Methoxy carbamyl)-benzimidazole), Dithane M-45 (Zinc and manganese ethylene bidithiocarbamate), Blitox (Copper oxychloride), Zincop (Copper oxychloride and zineb mixture), Hexaferb (Ferric dimethyl dithiocarbamate), Dithane Z-78 (Zinc ethylene bisdithiocarbamate), Captan (N-trichloro methyl mercapto 4-cyclo hexane 1,2-dicarboxinide), Thirum (Tetra methyl thirum disulphide) and Calixin (N-tridoyl (2-6 dimethyl morpholine). Bavistin and

Calixin were tested at 0.05%, 0.1% and 0.2% whereas other fungicides at 0.1%, 0.2% and 0.3%.

Bavistin and thiram at all concentrations and Hexaferb at 0.3% concentration completely inhibuted the growth of the fungus, whereas calixin and Dithane Z-78 showed least inhibition of growth in all the concentrations. Dithane M-45 and Blitox at 0.1% and 0.2% showed linear inhibition. At 0.3% concentration Dithane M-45 was found to be better than Blitox. Duter, Zincop and Captan at 0.2% and 0.3% were also found to give good inhibition. Bavistin (0.05%), followed by Thiram (0.2%), Hexaferb (0.3%) and Dithane M-45 (0.3%) were found to be effective fungicides in the control of seedling blight of Eucalyptus hybrid.

Horst and Boitink (1968) reported that C. scoparium and C. floridanum were completely controlled by Dupant fungicide 1991. Engelhard (1971) have reported that Benomyl drench could give complete control of Cylindrocladium scoparinum rot of cuttings rooting under mist. In 1971, Weaver reported that potassium nitrate killed microsclerotia of Cylindrocladium floridanum in artificially infested soil incubated at 5, 10 or 20°C. Roth et al. (1979) reported that low temperature induces decreased germinability of Cylindrocladium crotalariae microsclerotia.

MATERIALS AND METHODS

MATERIALS AND METHODS

Symptomatology

Symptoms of the disease were studied by observing the naturally infected clove plants and also following the course of development of the disease under artificial inoculation.

Isolation of the pathogen

Clove leaves showing initial stages of infection were collected from a plantation at Pattom, Trivandrum District and used for the isolation of the organism. The infected parts were cut into small bits, surface sterilized with 0.1 per cent mercuric chloride solution for one to two minutes and washed in three changes of sterilized distilled water. These bits were then placed in sterilized petri dishes previously poured with Czapeks' agar medium. The dishes were then incubated at room temperature (28 ± 2°C). After 2 to 3 days, when the growth of the fungus was visible, mycelial bits were transferred aseptically to Czapeks' agar slants. Culture was purified by single conidium isolation and the stock culture was maintained on slants of Czapeks' agar and potato dextrose agar at room temperature by periodical subculturing.

Morphology of the fungus

The morphology of the fungus was studied by growing it on Czapeks' agar medium.

Perfect state of the organism

For inducing the formation of perfect state of the organism, small twigs of 0.5 to 1.0 cm thickness were cut into bits of 8 to 10 cm length from clove (Eugenia caryophyllata L.), cashew (Anacardium occidentale L.) and anona (Anona squamosa L.) Three to four of these bits were taken in 250 ml flasks, 10 ml distilled water was poured and sterilized by autoclaving at 1.05 kg/cm^2 for 15 minutes. The bark of the twig was slightly injured before autoclaving and after sterilization the bits were inoculated by placing actively growing mycelial bits containing conidia of the fungus on the injured areas. Inoculated flasks were incubated under laboratory condition (28 ± 2°C), under complete darkness, inside laboratory cupboard and also at 4°C in a refrigerator. These bits were examined periodically for the presence of any perfect state of the organism.

Infected leaves at various stages of disease development was examined periodically for the presence of the perfect state. Severely affected stem and bark were also periodically examined for the presence of perfect state.

Pathogenicity tests

Pathogenicity of the fungus was tested by artificially inoculating two year old clove seedlings with 7 day old culture of the fungus. Branches of clove plants in the field were also inoculated in the same way. Inoculated plants and branches were covered with polythene bags to maintain a high percentage

of humidity. The leaves were surface sterilized by wiping with cotton dipped in 0.1 per cent mercuric chloride solution and repeatedly washed two to three times with sterilized distilled water. Inoculations were done with and without injury by applying the mycelial bit as well as by spraying the spore suspension. In the case of spray by spore suspension, the concentration of the spore in the spray suspension was adjusted to give approximately 10^4 per ml of the suspension in sterilized distilled water. Using an atomizer the suspension was sprayed on the test plants and covered with polythene bags for three days. Controls consisted of clove leaves which were surface sterilized, sprayed with sterilized distilled water and covered with polythene bags and maintained under identical conditions. Young detached twigs of clove also were inoculated in the same manner under laboratory conditions. The inoculated twigs were covered with bell jar lined with moist cotton. Suitable controls were also maintained.

Inoculated plants were observed for the symptom development and the observations recorded, 6 to 7 days after inoculation.

Host range of the pathogen

The host range of the pathogen was studied by artificially inoculating a total number of 16 host plants listed out below with culture bit as well as by spraying with the spore suspension of the fungus.

A. Inoculation using culture bit

All the 16 test plants were inoculated using the culture bit of the fungus with and without injury of the host tissues, both on cut twigs and on standing crop. The treated plants were covered with polythene bags. The observations were recorded 6 to 8 days after inoculation.

B. By spraying with the spore suspension of the fungus

Conidial suspension of the organism prepared in sterilized distilled water was sprayed on all the 16 test plants used in the above experiment and covered with polythene bags. Observations were taken 6 to 8 days after spraying. This study was also carried out on cut twigs and on standing crop.

Plants used for host range study

Common name	Botanical name
1. Tapioca	Manihot esculenta Crantz.
2. Cashew	Anacardium occidentale L.
3. Citrus	Citrus sinensis Osbeck.
4. Guava	Psidium guajava L.
5. Cherry	Malphigia punicifolia L.
6. Eucalyptus	Eucalyptus grandis Hill.
7. Nutmeg	Myristica fragrans Houtt.
8. Clerodendron	Clerodendron infortunatum Gaertn
9. Synedrella	Synedrella nodiflora L.
10. Lucas	Lucas aspera Spreng.
11. Croton	Croton sparsiflorus Morong.

12.	Ageratum	Ageratum conyzoides L.
13.	Sida	Sida acuta L.
14.	Euphorbia	Euphorbia hirta L.
15.	Anona	Anona squamosa L.
16.	Sapota	Achras sapota L.

Growth and sporulation of the fungus on different culture media

A. Solid media

The following culture media were used to study the growth of the fungus.

1. Potato dextrose agar
2. Czapeks' agar
3. Sabouraud's agar
4. Oat meal agar
5. Coon's agar
6. Host extract agar
7. Host extract dextrose agar
8. Richards' agar

The composition of the media used are given under Appendix I.

The media were prepared and sterilized by autoclaving at 1.05 kg/cm^2 for 15 minutes. It was melted and poured into sterilized petri dishes at the rate of 15 ml in each dish and allowed to solidify. Circular mycelial discs of 5 mm diameter were cut out by means of a sterile cork borer from the outer edge of 7 day old PDA culture of the fungus and placed in the centre of each dish. The plates were then incubated at room

temperature (28 ± 2°C). Observations were taken when full growth of the fungus was obtained in any one of the medium tested.

B. Liquid media

The following liquid media were used to study the growth of the fungus.

1. Fries' medium
2. Czapeks' medium
3. Coon's medium
4. Potato dextrose medium
5. Richards' medium

Composition of the media given under Appendix I.

Each medium was prepared and poured into 250 ml conical flasks at the rate of 30 ml and sterilized by autoclaving at 1.05 kg/cm^2 for a period of 15 minutes. Mycelial discs of 5 mm diameter were cut out from an actively growing culture of the fungus and inoculated into the flasks. The flasks were incubated at room temperature (28 ± 2°C). After 15 days of incubation, the culture was filtered through previously weighed Whatman No.1 filter paper and the dry weight of mycelium was determined. For each treatment three replications were maintained.

Effect of pH on the radial growth of the fungus

Czapeks' broth was prepared and before sterilization pH was adjusted using a Systronics pH meter. The pH range was adjusted by adding 0.1 N sodium hydroxide or 0.1 N

hydrochloric acid solution. The pH ranges tried were 3, 4, 5, 6, 7, 8 and 9. An aliquot of 30 ml each of the pH adjusted Czapeks' medium was transferred into 250 ml conical flasks and sterilized by autoclaving at 1.05 kg/cm^2 for 15 minutes. Three replications were maintained for each treatment. Mycelial discs of 5 mm diameter cut out from an actively growing culture of the fungus was inoculated into the flasks. They were incubated at room temperature (28 ± 2°C) for 15 days and the weight of the mycelial growth was determined.

Growth of the fungus on media incorporated with different oils

1. In solid media

The following oils were used for the study.

1. Clove oil
2. Coconut oil
3. Eucalyptus oil
4. Gingelly oil
5. Lemongrass oil

The oil was added into Czapeks' agar medium, at the rate of 1 ml per 60 ml of medium and thoroughly mixed by shaking for 20 minutes in a rotary shaker. The media were then sterilized by autoclaving at 1.05 kg/cm^2 for 15 minutes. After sterilization 15 ml each of the medium was transferred into sterilized petri dishes and allowed to solidify. Mycelial discs of 5 mm diameter were cut out from an actively growing culture of the fungus and placed in the centre of each dish. Controls were also run simultaneously using normal Czapeks'

agar medium without any oil. The petri dishes were incubated at room temperature (28 ± 2°C) and observations on the radial growth were taken on the third day of inoculation.

2. In liquid media

The effect of the above oils were tested in Czapeks' broth also. The concentration of the various oils added was the same as above. Suitable controls consisting of sterilized Czapeks' broth without any oils were also maintained. The flasks were incubated at room temperature. After 15 days incubation at room temperature (28 ± 2°C), the mycelial growth was filtered and the dry weight of the mycelium was determined.

Production of toxin by the pathogen

A preliminary study on the production of toxin by the pathogen was conducted.

A. Effect of various media on toxin production

The following liquid media were used to assess their comparative merits in supporting the production of toxic metabolites by the pathogen.

1. Host extract medium
2. Host extract dextrose medium
3. Richards' medium
4. Fries' medium
5. Czapeks' medium
6. Coon's medium

Composition of the media given in Appendix I.

Each medium was prepared and taken in 250 ml conical

flasks at the rate of 30 ml and sterilized by autoclaving at 1.05 kg/cm^2 for 15 minutes. The medium was inoculated with 5 mm diameter disc of 7 day old growth of the fungus on Czapeks' agar medium. For each treatment three replications were used. The flasks were then incubated at room temperature (28 ± 2°C). After 15 days of incubation, the culture was filtered through Whatman No.1 filter paper. The comparative toxic activity of each of the filtrate was studied by the following bioassay technique.

Bioassay using host leaf:

Clove leaves of uniform age were collected and placed inside sterilized petri dishes lined with moist cotton. On one half of the leaves 0.05 ml of the culture filtrate was placed and slightly pricked through the solution. On the other half of the same leaf sterilized water was applied in the same manner which served as control. The dishes were incubated at laboratory temperature and observations were recorded after 72 hours and expressed in four grades according to the necrotic area produced on each leaf.

B. Exo and Endo toxin production

The fungus was grown on liquid Czapeks' medium for a period of 15 days at room temperature and the mycelial growth was filtered through a previously weighed Whatman No.1 filter paper. The mycelium homogenised in a Homogeniser (Braun) by adding 5 times its weight of water (w/v). This homogenized mycelium was centrifuged at 1000 rpm for 15 minutes. The supernatant solution was taken and the pellets discarded and again

centrifuged at 1000 rpm for 15 minutes. The supernatant after second centrifugation was used as 'endotoxin' and the culture filtrate used as 'exo toxin'. These two test solutions (endo toxin and exo toxin) were assayed using clove leaves.

C. Properties of the toxin preparations

(i) Physical properties

(a) Effect of heating the culture filtrate and mycelial extract on lesion production

The effect of temperature on the activity of the toxic metabolite (both endo toxin and exo toxin prepared as described above) were studied by the following method. Five ml each of the test solutions (exo toxin and endo toxin) were taken in test tubes and autoclaved at 1.05 kg/cm^2 for 15 minutes. In another set 5 ml each of the toxin preparation was boiled in a water bath for 15 minutes. The toxic activities of these different preparations were bioassayed on the clove leaves by placing 0.05 ml of the test solutions on the surface of the leaves and slightly pricked through the solution. Uninoculated broth and sterile water served as control. The leaves were incubated in petri dishes lined with moist cotton for 48 hours and after that the observations were taken.

(b) Effect of dilution and sterilization of the preparation on lesion production

Culture filtrate used in the above test was used as the test solution for this purpose. One part of this culture filtrate was sterilized by autoclaving at 1.05 kg/cm^2 for 15 minutes. An aliquot of the sterilized and unsterilized

solutions were diluted four times its volume with sterilized water in order to reduce the concentration of the toxin to one-fourth of the original strength. The toxic activity of the solutions were assayed using the host leaf as described above.

(ii) Biological properties

(a) Translocation of the toxin

Actively growing young twigs of eucalyptus (Eucalyptus grandis Hill), cashew (Anacardium occidentale L.), tapioca (Manihot esculenta Crantz.) and clove (Eugenia caryophyllata L.) were detached and used in the study. Twigs were dipped in the toxin solution taken in 100 ml conical flasks at the rate of 50 ml per flask by just dipping the cut ends. The twig was kept in position using cotton plug and the whole thing was covered with bell jar lined with moist cotton wool and incubated under laboratory condition (Temperature 28± 2°C). All the cuttings were maintained under identical conditions. (temperature 28±2°C). Separate controls were kept with respective uninoculated media and sterile water. Observations were recorded at an interval of 24 hours after the treatment.

(b) Effect of culture filtrate on the germination of fungal spores

In this study, the fungus was grown on Czapeks' broth for 15 days and the culture filtrate was used. The effect of culture filtrate on the spore germination of Colletotrichum gloeosporioides and Curvularia sp. was investigated.

Spores were collected from 10 day old cultures of the fungi and spore suspensions were prepared in the test solution. The concentration of the spore suspensions was adjusted to 10^4 spores per ml of the solution. A small quantity - 0.05 ml of the spore suspension was placed on clean, grease-free sterile glass slide which was kept in petri dishes lined with moist cotton wool. Controls kept consisted of spore suspensions prepared in the broth and sterilized water and maintained in the same manner. All the dishes were incubated at room temperature (28 ± 2°C). Observations were taken 48 hours after incubation. The number of conidia germinated was counted from 10 random microscopic fields under low power of the microscope, and per cent inhibition was calculated.

(c) Effect of culture filtrate and mycelial extract on clove leaves of different maturity

This study was conducted by using the exo and endo toxin preparations after growing the fungus in Czapeks' broth for 15 days at laboratory temperature.

Three stages of growth of the clove leaves, viz., (1) tender (2) half mature (3) mature were collected, surface sterilized and kept in moist chambers. The test solution was applied on the leaves at the rate of 0.05 ml and they were slightly pricked. Leaves treated in the same manner with the broth and sterilized water were kept as control and were incubated at room temperature (28 ± 2°C). Observations were taken 72 hours after incubation.

Evaluation of fungicides against the pathogen

The following fungicides at different concentrations were used for laboratory evaluation against the pathogen.

Fungicide	Active ingredient	Concentration used (ppm)
Bavistin	2(methoxy-carbamyl)-benzimidazole	250, 500, 1000
Daconil-2787	Tetrachloroisophthalonitrile	1000, 2000 3000
Difolatan	Cis-N-(1-1-2 tetrachloro-ethylthio)-4-cyclohexene 1,2-dicarboximide	1000, 2000, 3000
Dithane M-45	Zinc ion and manganese ethylene bisdithiocarbamate	1000, 2000, 3000
Fytolan	Copper oxychloride	1000, 2000, 3000
Mildothane	Thiophanate-methyl (1,2-bis(3 methoxy carbomyl-2-thioureido) benzene)	500, 1000, 2000
Rovral	3-(3,5 dichloro phenyl)1-iso propyl carbamoyl hydantoin	500, 1000, 2000
Thiride	Tetra methyl thiuram disulphide	1000, 2000, 3000

1. Inhibition of spore germination of the fungus on glass slides

Conidia collected from 12 day old culture of the fungus grown in Czapeks' medium was used. Spore suspension was prepared in sterile distilled water. The concentration of the spore suspension was adjusted to 10^4 per ml. The required concentration of the fungicidal solutions were prepared in sterile distilled water. An aliquot of 0.05 ml of the fungicidal solution and 0.05 ml of the spore suspension were placed on sterile clean, grease-free glass slides and mixed well. The slides were kept in moist chambers in such a way that a hanging drop is formed with the spore suspension and fungicidal solution. Observations were taken at intervals of one, three, six, nine and twelve days after incubation and the per cent of inhibition of spore germination by each fungicide was determined.

2. Inhibition of growth of the fungus - poisoned food technique

(a) On solid medium

The 'poisoned food technique' described by Zentmyer (1955) was adopted in order to study the effect of different fungicides on the growth of the fungus. Weighed out the required quantity of each of the fungicide and added to 50 ml of sterilised Czapeks' agar medium to give the required concentration, mixed well and poured into sterile petri dishes at the rate of 15 ml per dish. After solidification, the dishes were inoculated by mycelial discs of 5 mm diameter cut out from an actively growing colony of the fungus. Controls consisted of unamended Czapeks' agar medium inoculated in the same way. All

the dishes were incubated at room temperature (28 ± 2°C). Observed daily for the growth of the fungus and observations were taken when the growth was completely covered the plates in the control. Per cent inhibition of growth over control was calculated by using the formula,

$$\text{Per cent inhibition} = \frac{C-T}{C} \times 100$$

Where C = radial growth in control

T = radial growth in treatment

(b) In liquid medium

The effect of fungicides on the growth of the fungus in liquid medium was studied. The required quantity of fungicides were weighed out in 250 ml conical flasks containing 50 ml sterilized Czapeks' broth. They were mixed well. The medium was inoculated with 5 mm diameter mycelial discs cut out from an actively growing culture of the fungus. The flasks were incubated at room temperature (28 ± 2°C). Controls were also maintained using unamended Czapeks' broth inoculated with the mycelial disc. Observations were taken on the 15th day of incubation. Cultures were filtered through previously weighed Whatman No.1 filter paper and the mycelial mat dried at 65°C, till a constant weight was obtained. Per cent inhibition of growth over control, on dry weight basis, was calculated by the following formula,

$$\text{Per cent inhibition of growth} = \frac{C-T}{C} \times 100$$

Where C = dry weight of mycelium in control

T = dry weight of mycelium in treatment

3. Effect of immersing culture discs in fungicidal solution on the viability of Cylindrocladium quinqueseptatum

The method followed by Sahai (1969) was adopted for this study. The fungicidal solutions at the required concentrations were prepared in 50 ml sterile distilled water contained in 250 ml conical flasks. Mycelial discs of 5 mm diameter were cut out from the actively growing culture of the fungus grown on Czapeks' agar medium were immersed in the fungicidal solutions. After adding the mycelial discs the flasks were rotated periodically. At intervals of 30 minutes, 1 hour, 2 hours and 24 hours the discs were removed from the fungicidal solution, rinsed in sterile water and placed on Czapeks' agar medium in petri dishes. Dishes were then incubated at room temperature (28 ± 2°C). Three replications were run for each treatment with suitable controls. Observations on the growth of the fungus were recorded at 24 and 48 hours after incubation.

Statistical analysis

Data relating to different experiments were analysed statistically following the methods of Snedecor and Cochran (1967). 'F' test was carried out by analysis of variance method and significant results were compared by working out the critical difference.

EXPERIMENTAL RESULTS

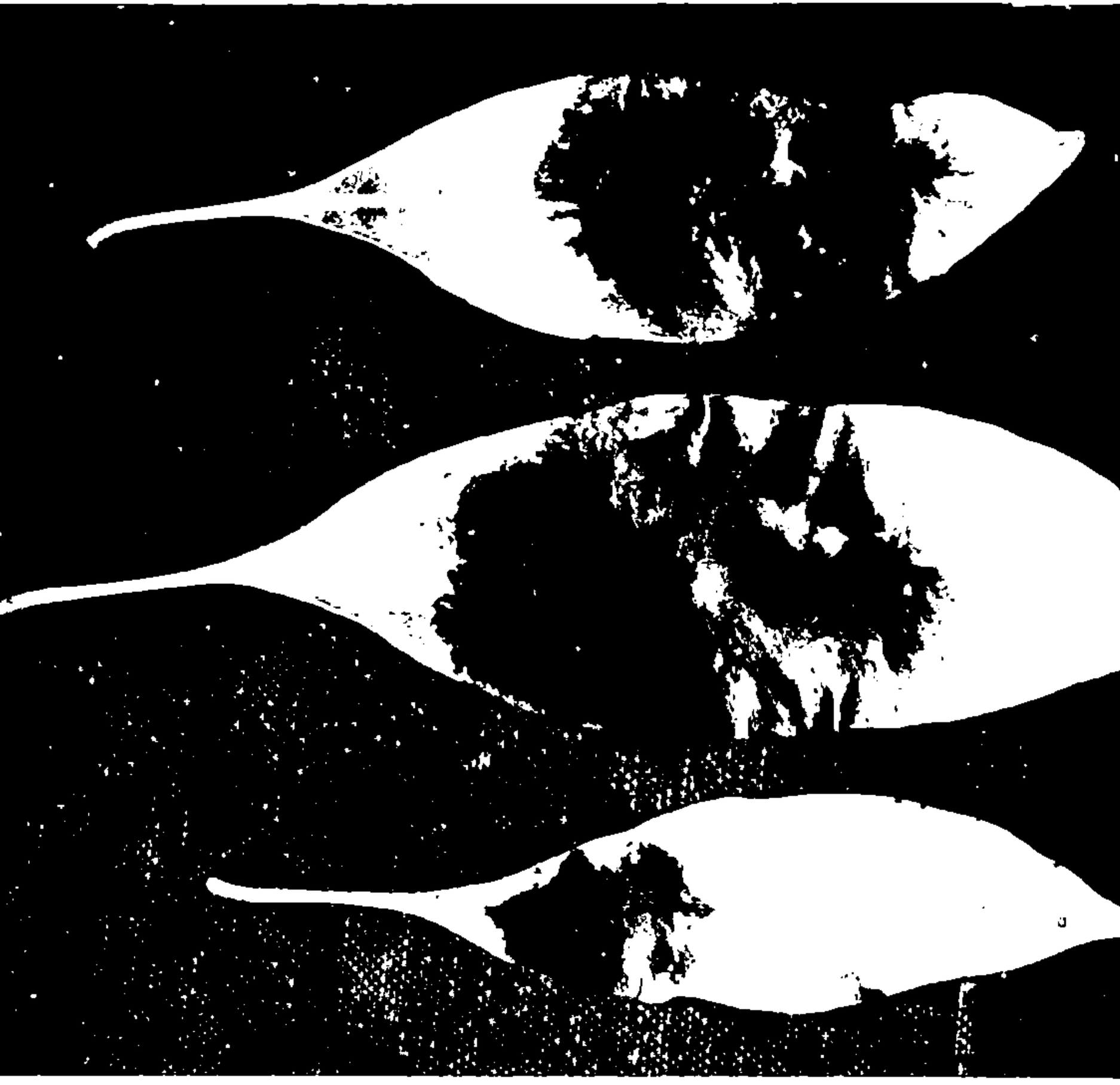
EXPERIMENTAL RESULTS

I. Symptomatology

The leaf blight disease of clove initiated as minute brown spots on the upper surface of the leaves. When fully mature they developed greyish white centre with distinct, well defined brown margins and measuring 2.85 to 4.85 mm in diameter. The number of spots on each leaf varied from 10 to 15. The spots developed on all parts of the leaf lamina. Several such spots coalesced forming large brown patches. Leaves of all stages of growth, both young and old were found to be equally susceptible to infection. In young leaves, downward rolling of the leaf margins were also noticed. In the advanced stages of infection, the spots developed all over and completely covered the surface of the lamina. As the spots grew older, the colour changed to dark brown (Plate 1 A,B) In severe cases, drying of twigs was also noticed. Leaves of the infected twigs became brown and eventually dried up.

The leaf blighting and shedding occurred in well advanced stages of the disease. Examination of the infected leaves and twigs revealed the presence of asexual fructification of the fungus.

During this investigation a mixed infection of *Cylindrocladium quinqueseptatum* and *Colletotrichum capsici* causing leaf blight of clove was also observed. The initial symptoms manifested as minute chocolate coloured spots



usually surrounded by an yellow halo. The spots enlarged rapidly during humid conditions and appeared as greyish brown lesions involving large areas of the leaf. Blistering at the infected region of the leaf lamina was evident. Under dry conditions, the lesions were ashy grey in colour. At times more or less indistinct concentric zonations were visible on the upper side of the lesion. Most of the infected leaves abscessed and eventually a number of seedlings died off. Over 50 per cent infection was noticed in the affected gardens.

Isolation of the pathogen

The causal organism, Cylindrocladium quinqueseptatum, was isolated from the tissues of infected leaves, brought into pure culture and maintained on Czapeks' and potato dextrose agar slants after purification by single spore isolation.

Morphology of the causal organism

Table 1 gives the morphology of the causal organism in detail.

Mycelium was found to be septate, highly branched and hyaline. Conidiophores were septate, branched, arise laterally from a stipe. Conidia were found to be produced on a broom shaped structure consisted of primary, secondary and tertiary sterigmata. Primary sterigmata septate, ranged from 10.20 to 21.66 µm; secondary and tertiary sterigmata nonseptate and measured 12.45 to 19.50 µm and 9.15 to 13.13 µm in length respectively. The tips of the tertiary sterigmata end in

Table 1. Morphology of the causal organism Cylindrocladium quinqueseptatum

Mycelium	Primary sterigmata	Secondary sterigmata	Tertiary sterigmata	Phialides	Conidia	Sterile filament
Septate, highly branched and hyaline. Conidiophore branches arise laterally from a stipe. Conidia were found to be produced on a broom shaped structure consisted of prinary, secondary and tertiary sterigmata. The tips of the teritiary sterigmata end in small phialides. Chlamydospores were formed with brown cells in chains	Septate 10.20 to 21.66 µm	Non-septate 12.45 to 19.50 µm	Non-septate 9.15 to 14.30 µm	Hyaline 7.30 to 15.45 µm	Hyaline, straight with rounded ends. 1 to 5 or 6 septate usually 5 septate. 36.4 to 39.0 x 4.0 to 7.0 µm	The main axis of the conidiophore structure continued to grow to form the sterile filament, which terminated in a narrowly clavate structure (vesicle) having 15.39 - 15.60 x 7.5 - 8.2 µm

small phialides, which measured 7.80 to 25.48 µm in length, on which the conidia were borne (Fig.1a). Conidia were hyaline, straight, cylindrical with the rounded ends and 1 to 6 usually 5 septate and measured 36.4 to 39.0 x 4.0 to 7.0 µm in size (Fig.1b). The main axis of the conidiophore structure continued to grow and formed the sterile filament, which terminated in a narrowly clavate structure which measured 15.39 to 15.60 x 7.5 to 8.25 µm in size. Chlamydospores were formed in chains as thick brown cells (Plate 2). Based on the above morphological features, the fungus was identified as Cylindrocladium quinqueseptatum Boedijn & Reitsma.

Perfect state of the organism

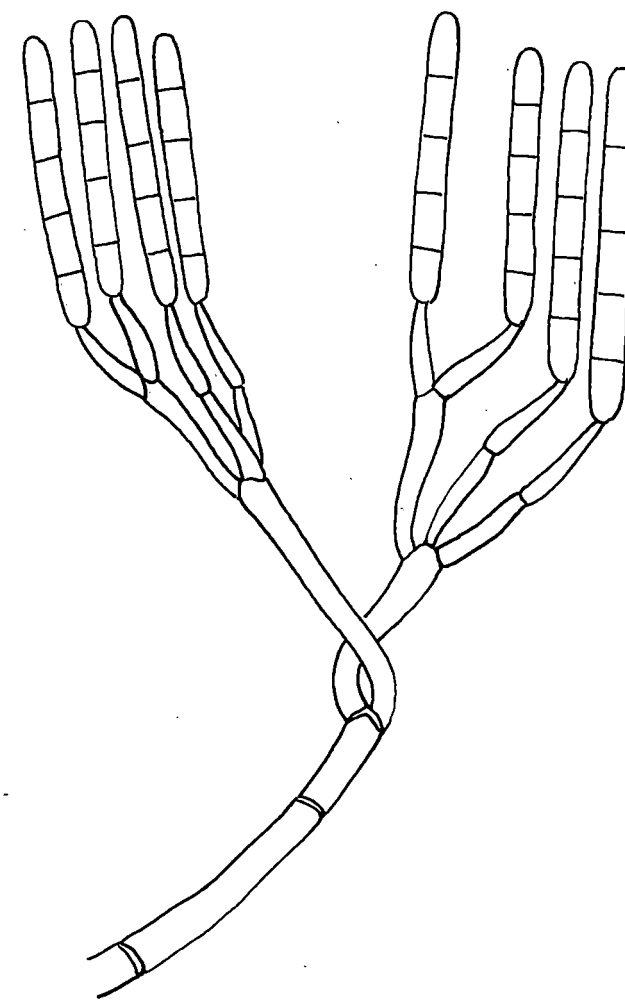
The perfect state of the fungus, viz., Calonectria quinqueseptata was obtained from the twigs of clove, cashew and anona when inoculated with the spore suspension and culture bits of the organism and incubated at laboratory conditions (Table 2). No perithecial state could be obtained from the infected twigs when kept at dark, inside laboratory cupboard and at 4°C. It was found to be produced only on twigs which were kept at laboratory condition.

Perithecia superficial, oval to elliptical, orange to chestunt, 320 to 450 µm high 237 to 383 µm diameter. Perithecial wall is roughened. Asci are club shaped, long stalked, hyaline (Fig.2a). The ascospores were hyaline, variously curved, irregularly crowded, fusoid, faintly bent, 1 to 5 septate, mostly 3 or 4 septate measured 21.0 to 26.0 x

Table 2. Morphological characters of Cylindrocladium quinqueseptatum - Perfect stage -

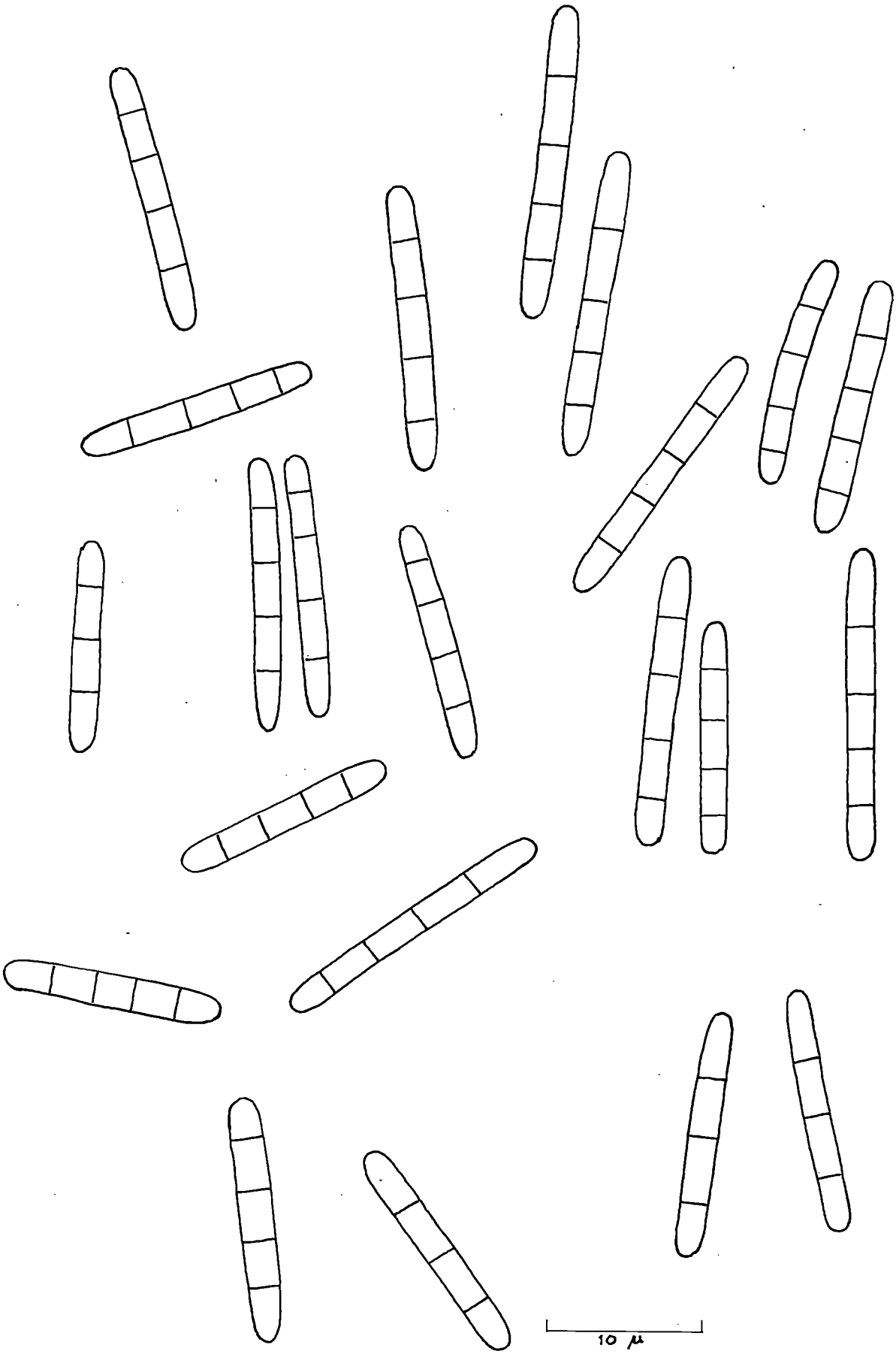
Perithecia	Asci	Ascospores
Perithecia, superficial, oval to elliptical, orange to chestnut 320-450 µm high, 237-383 µm diameter perithecial wall is roughened	Club shaped long stalked hyaline	Hyaline variously curved, irregularly crowded, fusoid, fairly bent 1 to 5 septate, mostly 3 or 4 septate measured 21.0 to 26.0 x 4.0 to 6.0 µm in size.

FIG. 1.a. CONIDIOPHORE OF *Cylindrocladium quinqueseptatum* BEARING CONIDIA AT THE TIP OF THE PHIALIDES SHOWING PRIMARY, SECONDARY AND TERTIARY STERIGMATA.



10 μ

FIG. 1.b. CONIDIA OF THE FUNGUS *Cylindrocladium quinqueseptatum*.



4.0 to 6.0 µm in size (Fig.2b, Plate 3).

Pathogenicity tests

Artificial inoculations revealed that the fungus could infect clove leaves of all stages of growth. The organism was found to infect both uninjured and injured leaves. But when injured, the spread of the disease was found to be more rapid (Table 3). Uninjured young leaves were more susceptible than uninjured mature leaves. Successful infection was obtained when inoculated with spore suspension as well as by culture bits of the organism. The symptoms produced in both cases were found to be identical to those produced under natural conditions. The initial symptoms were noticeable within 3 to 4 days after inoculation, but it took 6 to 7 days to produce the typical symptoms, characteristic of natural infections.

Host range of the pathogen

A. Inoculation using culture bit

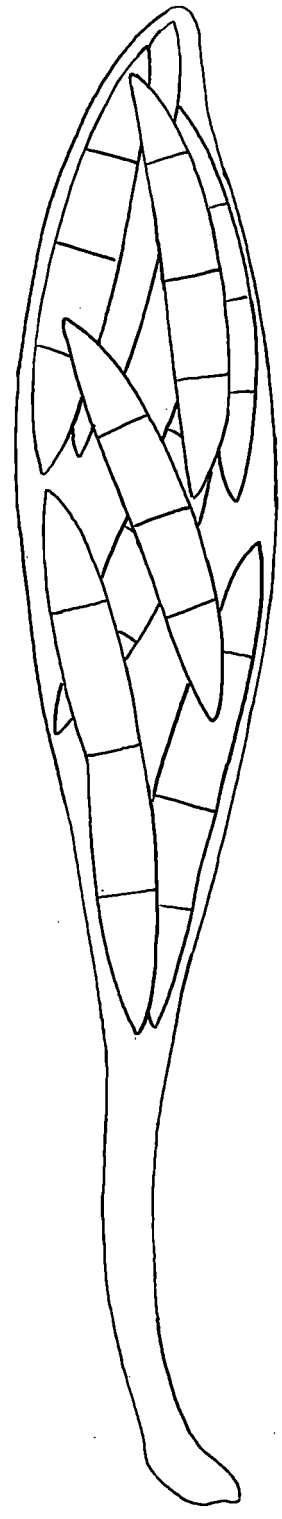
A total number of 16 hosts inoculated with culture bit of the fungus showed that all of them were infected and symptoms produced within 4 to 5 days after inoculation. It was noticed that injury of the tissues was a pre-requisite for the successful development of symptom expression in the case of mature tissues. Intensity of infection varied with the age of the tissue also. Injured young leaves were found to be more susceptible than injured mature ones. Host range studies were carried out using the spore suspension also, in

Table 3. Pathogenicity of *Cylindrocladium quinqueseptatum*

Method of inoculation of leaves	Observations after 10 days incubation	
	Injured leaves	Uninjured leaves
1. Inoculated with culture bit on the standing crop	++++	++
2. Inoculated with culture bit on cut twigs	++++	+++
3. Inoculated with spore suspension on standing crop	++++	++
4. Spore suspension sprayed on cut twigs	++++	++
5. Control - Sprayed with sterilized distilled water	-	-

-	No symptoms
++	Small minute spots
+++	Spots enlarged in size and spread
++++	Spots enlarged and coalesced with adjacent spots.

FIG. 2. a. ASCUS OF THE FUNGUS *Cylindrocladium quinqueseptatum*.



10 μ

Plate 2. Conidial state of the fungus *Cylindrocladium quinqueseptatum*.

Plate 3. Perfect state of the fungus - Ascospores *Calonectria quinquesentata*.

standing crop as well as on cut twigs with and without injury. Symptoms obtained on cut twigs and on standing crop were more or less similar.

Tapioca (Manihot esculenta)

Symptoms appeared on the third day of inoculation as a blighted area on the surface of the leaves which soon turned ashy grey and profuse white mycelial growth of the fungus was also observed on the affected leaf surface. Infection spread rapidly and covered about two third of the leaf area. A general chlorosis of the plant was also observed as a result of infection (Plate 4A).

Cashew (Anacardium occidentale)

Infection initiated on the third day of inoculation as small brown specks which soon enlarged. The spots varied in size, measuring 2.5 to 4.0 mm in diameter. These spots soon coalesced giving a blighted appearance of the affected area. Infection extended to the leaf stalk also resulting in defoliation. Twig drying was also noticed. On the infected twigs brown lesions with distinct margins were observed. Whitish mycelial growth was observed over the infected portions (Plate 4B).

Citrus (Citrus sinensis)

Necrotic area was observed on the fourth day around the inoculated region, which extended to two-third of the leaf surface. Infected portion turned dark brown in colour. Thick

whitish growth of the mycelium was observed on the under surface of the leaves.

Guava (Psidium guajava)

Symptoms were noticed on the next day of inoculation. Blighted areas were observed on the leaf surface which soon covered three-fourth of the lamina. Shedding of infected leaves within a few days was also observed.

Cherry (Malphigia punicifolia)

Small brown spots appeared on the leaves extending to large areas giving patchy brown appearance. Infections developed on the third day of inoculation and defoliation occurred on the sixth day.

Eucalyptus (Eucalyptus grandis)

Symptoms appeared on the third day of inoculation as black necrotic areas. Infected young leaves rolled downward. Thick white mycelium, was observed on the under surface of the leaves. Defoliation was also observed (Plate 4C).

Nutmeg (Myristica fragrans)

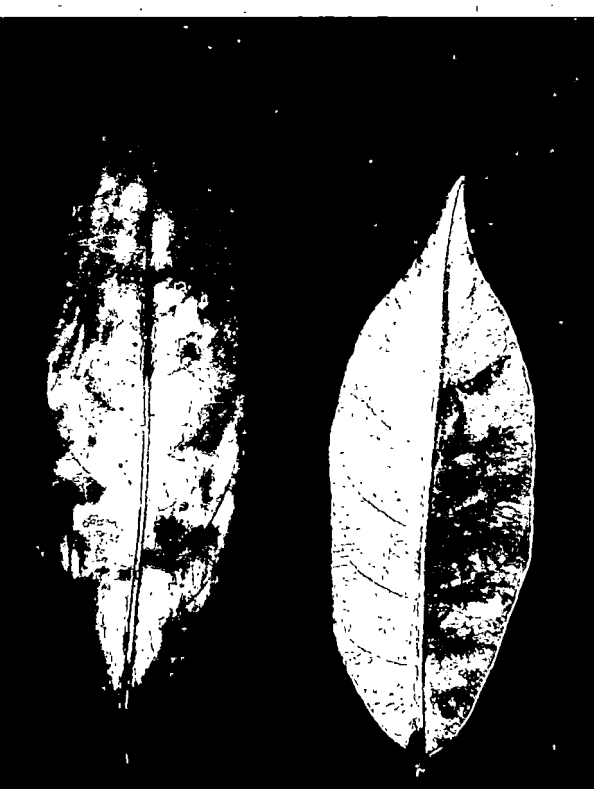
Blighted necrotic areas on the leaves were initiated within three to four days which later on enlarged and extended to about two-third of the leaf area (Plate 4D).

Clerodendron (Clerodendron infortunatum)

Symptoms developed on the fourth day of inoculation as dark brown blighted necrotic patch which later extends to the whole leaf surface. Shot hole symptoms were also observed.

Plate 4C. Eucalyptus leaf artificially inoculated in the culture bit of *Cylindrocladium quinqueseptatum*.

Plate 4D. Nutmeg leaf inoculated with culture bit of *Cylindrocladium quinqueseptatum*.



infection spread rapidly resulting in defoliation.

Synedrella (Synedrella nodiflora)

Dark brown spots appeared on the leaf surface which later coalesced with a blighted appearance. An yellowish green halo was also observed around the blighted portion.

Lucas (Lucas aspera)

Small brown necrotic areas surrounded by yellow halos were developed at first. This necrotic patches coalesced and later extended to the whole leaf.

Croton (Croton sparsiflorus)

Symptoms started as small brown necrotic specks on the leaf surface. These specks enlarged in size and colour turned to black. Leaves become blighted and lost the turgidity.

Ageratum (Ageratum conyzoides)

Brown necrotic areas were developed on the leaf surface by fourth day of inoculation. Around the necrotic area, an yellowish green halo was observed. After one week, the necrotic portions turned black and dried up.

Sida (Sida rhombifolia)

Dark coloured necrotic areas developed around the inoculated portion by the fourth day of inoculation. Later this necrotic area extended and covered the whole lamina. Whitish mycelial growth observed on the upper surface of the leaves. Severely infected leaves defoliated.

Euphorbia (Euphorbia hirta)

Symptoms developed on the fifth day of inoculation as small brown necrotic spots. These necrotic areas increased in size and covered nearly the entire lamina. Severely infected areas turned dark brown in colour.

Anona (Anona squamosa)

Infection initiated within five days of inoculation as blighted brown necrotic areas. This blighting soon extended and reached all over the leaf area.

Sapota (Achras sapota)

Infection started on the fourth day of inoculation as brown rotted areas which later turned black in colour. Rotting extended and covered the whole leaf, and the severely infected leaves defoliated.

B. By spraying with the spore suspension of the fungus

Separate lots of all the plants were sprayed with the spore suspension also. All the host plants sprayed gave positive results and the symptoms were identical to those described above. In this case also injury of the host tissues was found to be a pre-requisite for the development of symptom. Expression of symptom was observed on all hosts, ten to twelve days of spraying.

Growth and sporulation of the fungus on different culture media

A. Solid media

The effect of different solid media on the growth of the pathogen was studied. Czapeks' agar, potato dextrose agar,

Sabouraud's agar, oat meal agar, Coon's agar, host extract agar, host extract dextrose agar and Richards' agar were used. The mean radial growth and the growth characters of the organism in different media are presented in Table 4a. The results of the study revealed that potato dextrose agar was found to be the best medium for its growth followed by Coon's agar and Czapeks' agar (Fig.3). Good sporulation also was obtained on potato dextrose agar, Czapeks' agar and Coon's agar.

Analysis of the data revealed that potato dextrose agar was significantly superior to all other media used (Appendix II).

B. Liquid media

Of the five different liquid media used, maximum dry weight of the mycelium was obtained on potato dextrose broth, followed by Czapeks', Fries' and Richards' media. Coon's broth was found to be a poor substrate for the growth of the fungus. On all the media growth started on the third day of inoculation (Table 4b, Appendix III).

Effect of pH on the radial growth of the fungus

Among seven different pH levels tested ranging from 3 to 9 on the growth of the fungus, maximum dry weight of mycelium was obtained at pH 9 followed by pH 7 and pH 8 (Table 5). It is found that as the pH decreases (from 6 to 3) the mycelial dry weight was also found to be decreasing. The optimum pH range for the growth of the fungus was found

Table 4a. Growth and sporulation of *C. quinqueseptatum* on different culture media (Average of 3 replications)(Solid)

Sl. No.	Medium	Mean colony diameter in mm	Colony characters
1.	Potato dextrose agar	90.00	Cottony whitish grey mycelium at the edges and the remaining part brown with entire margins. Good sporulation. Concentric zones observed.
2.	Sabouraud's agar	48.33	Cottony white mycelium, sporulation moderate.
3.	Czapeks' agar	65.33	Mycelium white at the edges and the remaining part brown between lawny and zinc orgage. Some indistinct concentric zones observed, good sporulation.
4.	Oatmeal agar	16.33	Greyish white mycelium mingled with brown colour, fair sporulation.
5.	Coon's agar	66.67	Cottony white mycelium on the edges, brown between tawny and ochraceous tawny radially striate with concentric zones, good sporulation.
6.	Host extract agar	37.33	Growth was very thin, grey, white to brown colour, meagre sporulation.
7.	Host extract dextrose agar	26.00	Papery white to brown mycelium, fair sporulation.
8.	Richards' agar	17.70	Cottony white mycelium with entire margin, fair sporulation

FIG. 3. GROWTH OF *Cylindrocladium quinqueseptatum* ON DIFFERENT CULTURE MEDIA.

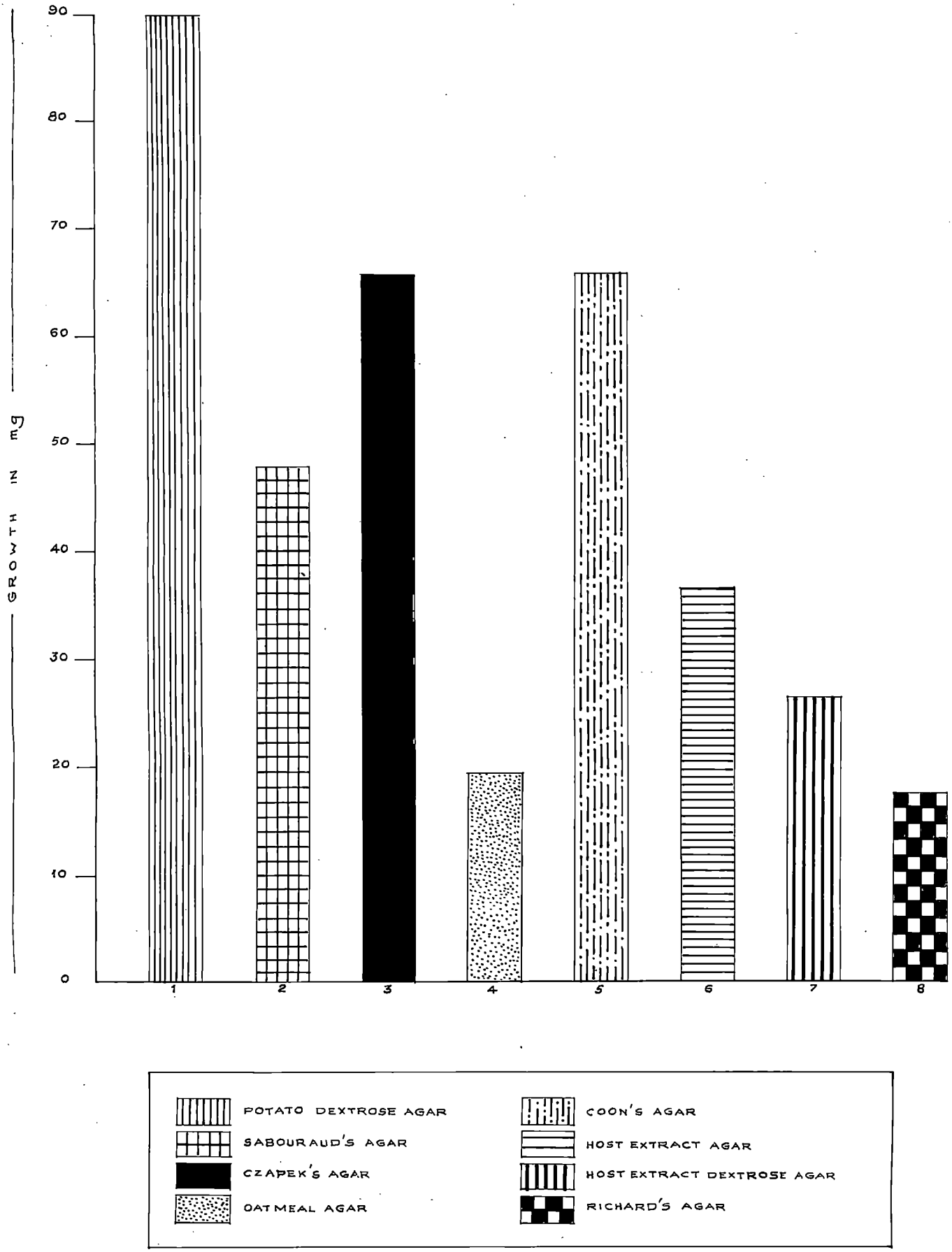


Table 4b. Growth of the fungus on different culture media (liquid).

No.	Medium	*Dry weight of mycelium in mg	Cultural characters
1	Czapek's medium or broth	402.50	Cottony white mycelium which covered the surface of the medium. The medium turned dark brown in colour within one week. Good sporuation.
2	Fries' medium	387.50	Initially cottony white mycelium on the surface and dark brown on the under surface of the flask. Liquid turned to dark brown and fair sporulation.
3	Coon's medium	168.50	Mycelium papery white in colour, medium turned to brown and sporulation occurred.
4	Potato dextrose medium	564.00	Abundant cottony white mycelial growth. Medium turned dark brown to red in colour. The mycelial mat turned into a hard structure. Excellent sporulation.
5.	Richards' medium	231.00	Papery white to cottony white mycelium on the upper surface and a light dark colour on the under surface. Fairly good sporulation.

*Average of 3 replications

Table 5. Effect of pH on the radial growth of the fungus

pH of medium	Dry weight of mycelium in mg			Mean
	R_1	R_2	R_3	
3	0.151	0.165	0.172	0.163
4	0.141	0.175	0.186	0.167
5	0.216	0.200	0.211	0.209
6	0.271	0.225	0.254	0.250
7	0.286	0.341	0.275	0.306
8	0.302	0.304	0.295	0.303
9	0.326	0.364	0.334	0.341

to be 7 to 9 (Fig.4).

The growth of the fungus at pH 9 was significantly superior to all other ranges. However, pH 7 was found to be superior to pH 8.

Growth of the fungus on media incorporated with different oils

1. On solid media

The average colony diameter of the fungus grown on solid medium amended with different oils are presented in Table 5.a. The fungus could grow on media amended with oils. Of the five different oils used, the maximum growth was obtained on medium amended with gingelly oil, followed by coconut and clove oils. The growth was least in medium with eucalyptus oil and there was no growth on medium with lemongrass oil (Plate 5).

The data revealed that the growth of the fungus on media amended with gingelly oil and coconut oil was not significantly different. The effect of clove oil and coconut oil was also on par (Appendix V).

2. In liquid media

Maximum dry weight of the mycelium was obtained in the medium amended with gingelly oil which was followed by coconut oil. The data are presented in Table 6.b. Coconut oil was followed by clove oil and eucalyptus oil. But there was no growth in the case of lemongrass oil. Statistical analysis revealed that treatments T_4 and control are on par and superior to other treatments. Clove and eucalyptus also are on par and superior to lemongrass oil in which there was

FIG. 4. GROWTH OF *Cylindrocladium quinqueseptatum* AT DIFFERENT pH LEVEL.

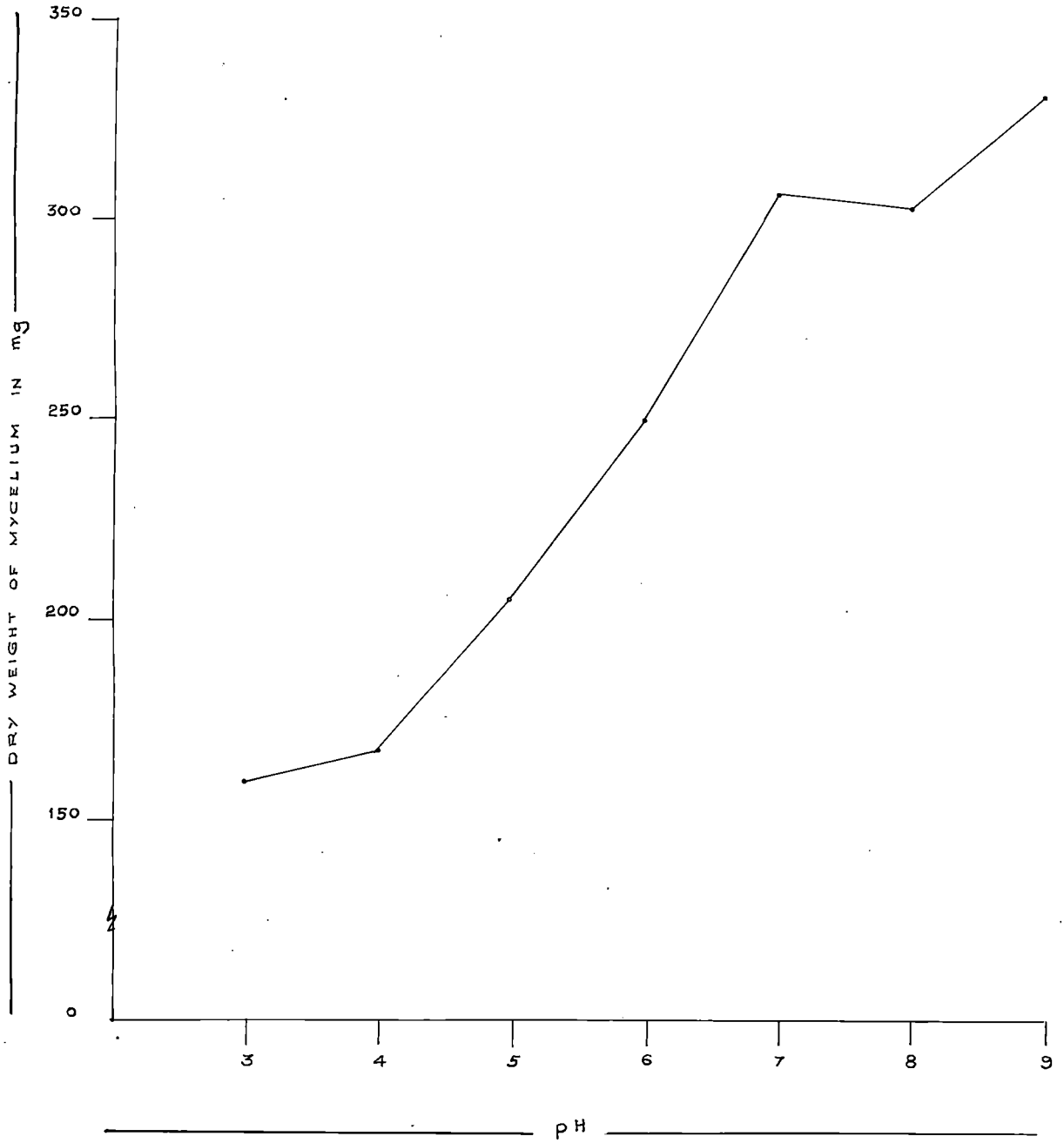


Table 6.a Growth of the fungus on media incorporated with different oils (1) On solid media

Czapeks' medium amended with different oils	*Radial growth of the fungus in mm	Colony characters
Clove oil	42.10	Whitish mycelium intermingled with brown colour. Concentric zones observed in the culture. Good sporulation.
Coconut oil	54.23	Cottony growth of the mycelium which turned brown in colour after 5 days of the growth, fair sporulation.
Lemongrass oil	6.00	No growth
Gingelly oil	61.25	Mycelium cottony white at the edges and the remaining part brown with concentric zones. Excellent sporulation.
Eucalyptus oil	15.45	Greyish white mycelial growth on the upper surface with brown colouration on the under surface of the plate. Fair sporulation.
Control	90.00	Good growth of the mycelium, cottony white in colour which turned to brown in due course, excellent sporulation.

*Average of 3 replications

no growth (Appendix VI).

Production of toxin by the pathogen.

A. Effect of various media on toxin production

Bioassay using host leaf: Observations on the toxin production of the fungus is presented in Table 7a. From the table it can be seen that maximum area of necrosis/lesion was produced in the case of Richards' and Czapeks' culture filtrates followed by Fries' and Host extract dextrose filtrates when compared with respective media and sterile water control.

B. Exo and Endotoxin production

The observations on the effect of exo and endotoxin on sympton/lesion development on host leaves are presented in Table 7b. It was observed that spotting of leaves was obtained with exotoxin (culture filtrate) after 72 hours of observation followed by endotoxin after 72 hours. It is seen that as the incubation period increases, there is a proportionate increase in the length of the lesion (Plate 6A and B).

C. Properties of the culture filtrate

(i) Physical properties

(a) Effect of heating the culture filtrate and mycelial extract on lesion production

The observations are presented in Table 8a. The untreated culture filtrate produced lesions of 6 cm length whereas those boiled for 15 minutes and autoclaved produced lesions of 4 to 6 cm. The treatments do not reduce the toxic effect of the culture filtrate appreciably. The mycelial

Table 7a. Effect of various media on toxin production (Bioassay using host leaf - Observation after 72 hours)

Treatment	Culture filtrate			Medium			Sterile water (Control		
	R_1	R_2	R_3	R_1	R_2	R_3	R_1	R_2	R_3
1. Host extract	++	+	++	-	-	-	-	-	-
2. Host extract dextrose	+++	+++	++	-	+	+	-	-	-
3. Richards'	+++++	+++++	++++	+	-	-	-	-	-
4. Fries'	+++	++++	+++	-	-	+	-	-	-
5. Czapeks'	+++++	++++	+++++	-	+	-	-	-	-
6. Coon's	+++	+++	++	+	-	-	-	-	-

-	No lesion produced
+	Lesion length less than 1 cm
++	Lesion length between 1-2 cm
+++	Lesion length between 2-3 cm
++++	Lesion length between 3-4 cm
+++++	Lesion length more than 4 cm

Table 7b. Effect of Exo and Endotoxin produced by C. quinqueseptatum on clove leaves

Treatment	Exotoxin after						Endotoxin after					
	48 hr			72 hr			48 hr			72 hr		
	R_1	R_2	R_3	R_1	R_2	R_3	R_1	R_2	R_3	R_1	R_2	R_3
Culture filtrate	++	+++	+++	+++++	++++	++++	++	+++	++	++++	+++	++
Medium (Control)	-	-	-	+	-	+	-	-	-	-	-	-
Sterile water (Control)	-	-	-	-	-	-	-	-	-	-	-	-

-	No symptoms/lesions
+	Symptoms/lesions upto 1 cm
++	Symptoms/lesions between 1 - 2 cm
+++	Symptoms/lesions between 2 - 3 cm
++++	Symptoms/lesions between 3 - 4 cm
+++++	Symptoms/lesions more than 4 cm

Table 8a. Effect of heating the culture filtrate and mycelial extract on lesion production on clove leaves.

Treatment	Production of lesion after 72 hr		
	R_1	R_2	R_3
Culture filtrate	+++++	+++++	+++++
Mycelial extract	+++++	+++	+++
Boiled culture filtrate	+++	+++	+++
Boiled mycelial extract	+++	+	++
Autoclaved culture filtrate	+++	+++	++
Autoclaved mycelial extract	++	++	++
Uninoculated broth	-	-	-
Sterile water	-	-	-

-	No symptoms
+	Necrotic area upto 1 cm
++	Necrotic area between 1 to 2 cm
+++	Necrotic area between 2 to 3 cm
++++	Necrotic area between 3 to 4 cm
+++++	Necrotic area more than 4 cm

extract of the fungus also produced lesions on leaves, but the effect was more with the culture filtrate. The treated mycelial extract also showed more or less similar effects as the treated culture filtrate. The results show that heating was not effective in eliminating the toxic effect of the extract and indicated that the toxic principle is thermostable.

(b) Effect of dilution and sterilization of the preparation on lesion production

The undiluted and unsterilized culture filtrate produced the maximum lesion after 48 hours. The diluted but unsterilized culture filtrate also produced lesion, the effect of which was more than the diluted and sterilized preparation. In all cases the lesion length increased as the incubation period, after inoculation was increased. Diluting the culture filtrate to 4 times its volume showed a reduction in the toxic effect; the reduction being more when the filtrate was diluted and sterilized. However, the treatments did not completely eliminated the toxic effect of the preparation (Table 8b).

(ii) Biological properties

(a) Translocation of the toxin

The toxic effect of the culture filtrate translocated and produced wilt symptoms followed by defoliation on the cut twigs of Eucalyptus (Eucalyptus grandis Hill) tapioca (Manihot esculenta Crantz), cashew (Anacardium occidentale L.) and clove (Eugenia caryophyllata L.). The leaves of the treated twigs of the plants lost turgidity and the young leaves showed a downward rolling. Young growing leaves turned pale and

Table 8b. Effect of dilution and sterilization of the culture filtrate on lesion production in clove leaves.

Treatment	Dilution	Production of lesion after 24 hr			48 hr		
		R_1	R_2	R_3	R_1	R_2	R_3
Unsterilized culture filtrate	1:0	+++	+++	++	+++	++	++
Unsterilized culture filtrate	1:4	++	++	-	+++	++	-
Sterilized culture filtrate	1:0	++	++	-	++	+++	-
Sterilized culture filtrate	1:4	-	+	-	++	-	++
Control (Sterilized water)	-	-	-	-	-	-	-

\- No lesion
\+ Lesion produced below 1 cm
++ Lesion produced in between 1 to 2 cm
+++ Lesion produced in between 2 to 3 cm

defoliated first followed by mature ones. Defoliation was found to be more in the case of clove followed by cashew and tapioca (Plate 7).

(b) Effect of culture filtrate on the germination of fungal spores

Spores of two fungi, viz., Colletotrichum gloeosporioides and Curvularia sp. were used in order to study the effect of culture filtrate on their germination. Germination of spores was recorded 48 hours after incubation and the results of the observation of ten microscopic field were recorded for each sample (Table 9). Eighty nine per cent inhibition was obtained in the case of Colletotrichum gloeosporioides and sixty one per cent inhibition in the case of Curvularia sp. on comparison with sterile water control. When compared to Czapek's broth, the per cent of inhibition was 88 in the case of C. gloeosporioides and 58 in Curvularia sp.

(c) Effect of culture filtrate and mycelial extract on clove leaves of different maturity

The observations on the effect of culture filtrate and mycelial extract on clove leaves of different maturity were given in Table 10. Both preparations produced lesions on leaves of different ages. The effect was more pronounced on tender leaves. The culture filtrate induced larger lesions than the mycelial extract in all types of leaves. Injurying the surface of leaves was conducive for the development of lesions.

Plate 6B. Effect of Endotoxin on clove leaf - lesions developed 72 hours after inoculation.

Plate 7. Translocation of toxin - wilting type symptoms on twigs of Eucalyptus - 72 hours after treatment.

Table 9. Effect of culture filtrate of C. quinqueseptatum on germination of spores of few other fungi.

Test fungus	Per cent inhibition over				
	Sterile water	Czapeks' broth	Culture filtrate	Czapeks' broth	Sterile water
Colletotrichum gloeosporioides	83.57	75	9	88.00	89.20
Curvularia sp.	93.02	86	36	58.15	61.30

Table 10. Effect of culture filtrate and mycelial extract on clove leaves of different maturity.

Stage of maturity of leaves	Production of lesion after 72 hours											
	Culture filtrate			Mycelial extract			Czapeks' broth (Control)			Sterile water (control		
	R_1	R_2	R_3	R_1	R_2	R_3	R_1	R_2	R_3	R_1	R_2	R_3
Tender	++++	++++	++++	+++	++	++	-	-	-	-	-	-
Half mature	+++	++	+	++	++	+	-	-	-	-	-	-
Mature	++	+	+	+	-	+	-	-	-	-	-	-

- No symptons
+ Necrotic area upto 1 cm
++ Necrotic area between 2 to 3 cm
+++ Necrotic area between 3 to 4 cm
++++ Necrotic area more than 4 cm

Evaluation of fungicides against the pathogen

1. Inhibition of spore germination of the fungus on glass slides

There was complete inhibition of germination of spores of the fungus with all the eight fungicides tested. Daconil-2787, Dithane M-45, Fytolan and Thiride were able to cause 94, 97, 94 and 95 per cent inhibition of spore germination respectively upto 12th day at maximum concentration tested (3000 ppm). But at 2000 ppm only Dithane M-45 and Thiride could cause 90 per cent inhibition upto twelth day, and at the minimum concentration (1000 ppm) none of them could inhibit 90 per cent germination on the twelth day. However at twelth day, Dithane M-45 exhibited maximum per cent inhibition at the highest concentration, followed by Thiride and Daconil-2787. The data are presented in Table 11.

Bavistin was able to cause more than 90 per cent inhibition only at the maximum concentration (1000 ppm) on the third day. Difolatan was able to cause 94 per cent inhibition of germination of the spores at all concentrations only upto third day. After that it was found that the per cent of inhibition was decreasing at all concentrations and reached a minimum of 49 per cent.

More or less similar results was observed in the case of Mildothane. Mildothane inhibited 95, 95 and 98 per cent germination at 1000, 2000 and 3000 ppm respectively upto third day, but at maximum concentration this inhibition was observed upto the sixth day which thereafter decreased.

Table 11. Effect of fungicides on the germination of spores of Cylindrocladium quinqueseptatum.

Fungicide	Concentration (ppm)	Per cent inhibition of spore germination Period of observation (in days)				
		1	3	6	9	12
Bavistin	250	100.00	83.79	62.40	41.80	24.75
	500	100.00	88.67	80.53	54.40	50.00
	1000	100.00	93.37	80.63	68.68	51.29
Daconil-2787	1000	100.00	91.15	85.48	81.32	56.00
	2000	100.00	94.39	88.53	81.63	72.35
	3000	100.00	97.84	97.00	95.35	94.07
Difolatan	1000	100.00	94.94	71.32	64.66	49.33
	2000	100.00	96.13	77.27	65.68	50.45
	3000	100.00	97.90	93.94	91.94	60.91
Dithane M-45	1000	100.00	91.72	89.46	87.24	73.46
	2000	100.00	98.68	96.56	95.66	92.06
	3000	100.00	94.42	98.86	96.88	96.50
Fytolan	1000	100.00	97.03	94.67	92.16	74.86
	2000	100.00	97.25	95.07	93.71	86.31
	3000	100.00	99.44	95.95	94.31	94.11
Mildothane	500	100.00	92.86	80.00	56.55	39.67
	1000	100.00	95.55	88.19	73.92	44.27
	2000	100.00	96.28	96.23	77.50	73.56

continued....

Table 11 continued

Fungicide	Concentration (ppm)	Per cent inhibition of spore germination Period of observation (in days)				
		1	3	6	9	12
Rovral	500	100.00	94.35	93.33	85.85	72.29
	1000	100.00	94.81	93.59	86.49	80.00
	2000	100.00	96.88	94.24	86.49	85.12
Thiride	1000	100.00	93.91	92.69	92.32	85.72
	2000	100.00	99.36	98.04	86.35	91.75
	3000	100.00	99.37	98.78	95.43	95.18
Control		29.09	29.09	2=.99	6.14	1.26

Rovral could cause more than 90 per cent inhibition upto sixth day at all concentrations tested i.e., 93, 94 and 94 per cent at 500, 1000 and 2000 ppm respectively.

2. Inhibition of growth of the fungus

Poisoned-food technique

(a) On solid medium

Of the eight fungicides tested, each at three concentrations, there was complete inhibition of growth of the fungus on Czapeks' agar medium containing Bavistin 250 ppm, 400 ppm and 1000 ppm; Dithane M-45 1000 ppm, 2000 ppm and 3000 ppm; Mildothane 500 ppm, 1000 ppm and 2000 ppm and Thiride 1000 ppm, 2000 ppm and 3000 ppm (Table 12a). They were found to be significantly superior to all other fungicides tested (Appendix VII). Of the remaining four fungicides, Difolatan was found to be superior to Fytolan, Daconil-2787 and Rovral.

Daconil-2787 was found to be superior to Rovral at 3000 ppm. Rovral was least effective in inhibiting the growth of the fungus (Plate 8(A),(B) and (C)).

(b) In liquid medium

Complete inhibition of growth of the fungus was obtained on Czapeks' solution containing Bavistin, Difolatan, Dithane M-45 and Mildothane at all concentrations tested (Table 12b). They are significantly superior over all other fungicides tried (Appendix VIII). Daconil-2787, Fytolan, Rovral and Thiride did not completely inhibit the growth of the fungus even at the maximum concentration tested.

Table 12a. Effect of different fungicides on the growth of the fungus (a) On solid medium.

Fungicide	Concentration (in ppm)	*Mean colony diameter (in mm)	Per cent inhibition over contrpl
Bavistin	250	0.00	100.00
	500	0.00	100.00
	1000	0.00	100.00
Daconil-2787	1000	43.30	51.88
	2000	44.30	50.77
	3000	24.00	73.33
Difolatan	1000	39.00	56.67
	2000	30.00	66.67
	3000	22.30	75.22
Dithane M-45	1000	0.00	100.00
	2000	0.00	100.00
	3000	0.00	100.00
Fytolan	1000	44.50	50.56
	2000	38.60	57.11
	3000	29.60	67.44
Mildothane	500	0.00	100.00
	1000	0.00	100.00
	2000	0.00	100.00
Rovral	500	56.60	43.78
	1000	48.00	57.78
	2000	37.30	58.56
Thiride	1000	0.00	100.00
	2000	0.00	100.00
	3000	0.00	100.00
Control	-	90.00	-

*Average of 3 replications

Plate 8A. Growth of Cylindrocladium quinqueseptatum in fungicide incorporated Czapeks' agar medium.

1. Bavistin
2. Daconil-2787
3. Difolatan
4. Dithane M-45
5. Fytolan
6. Mildothane
7. Rovral
8. Thiride

I - Control

250 ppm of Bavistin, 500 ppm of Mildothane and Rovral, 1000 ppm of Daconil-2787, Difolatan, Dithane M-45, Fytolan and Thiride

Plate 8B. 500 ppm of Bavistin, 1000 ppm of Mildothane and Rovral, 2000 ppm of Daconil-2787, Difolatan, Dithane M-45, Fytolan and Thiride.

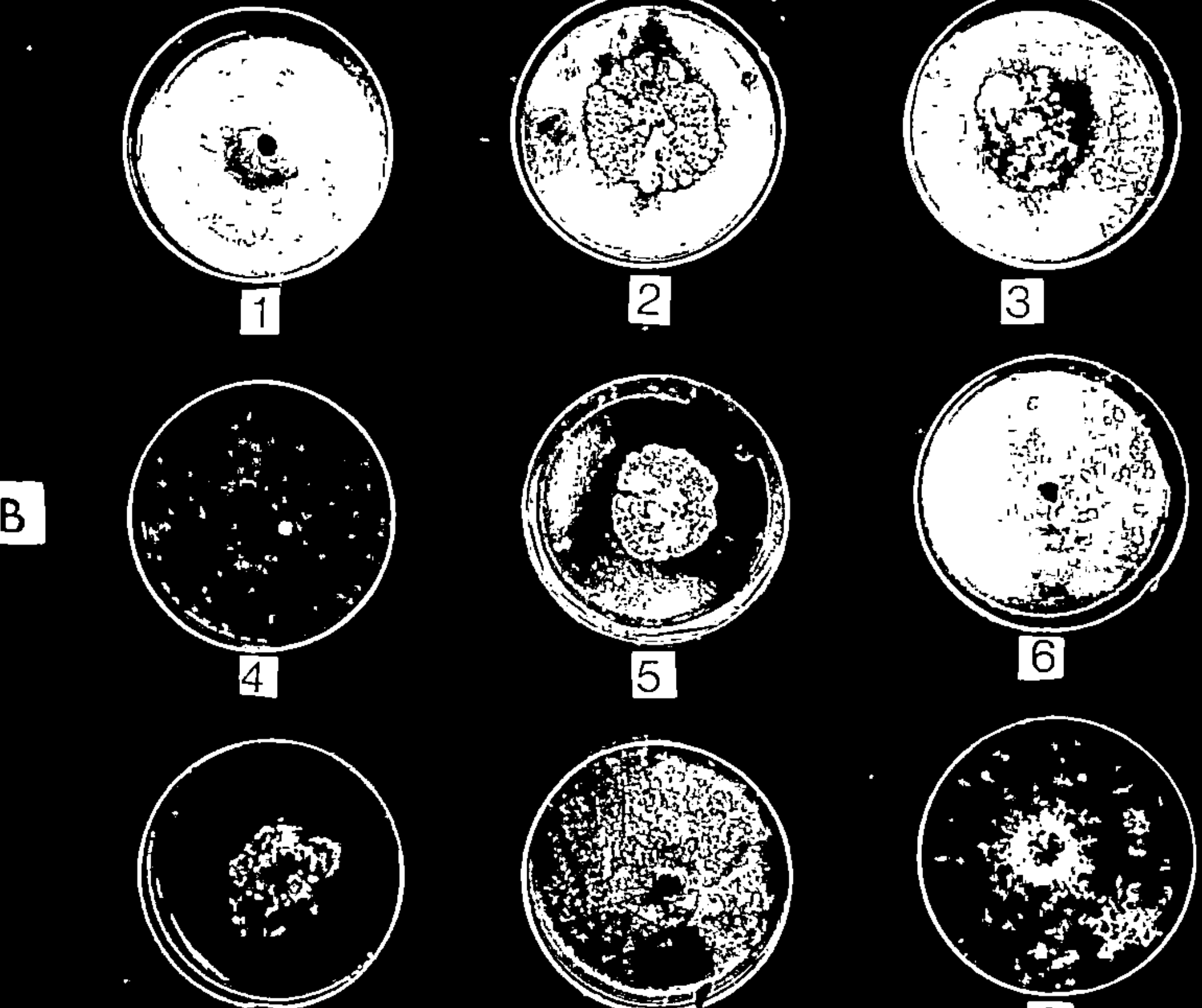
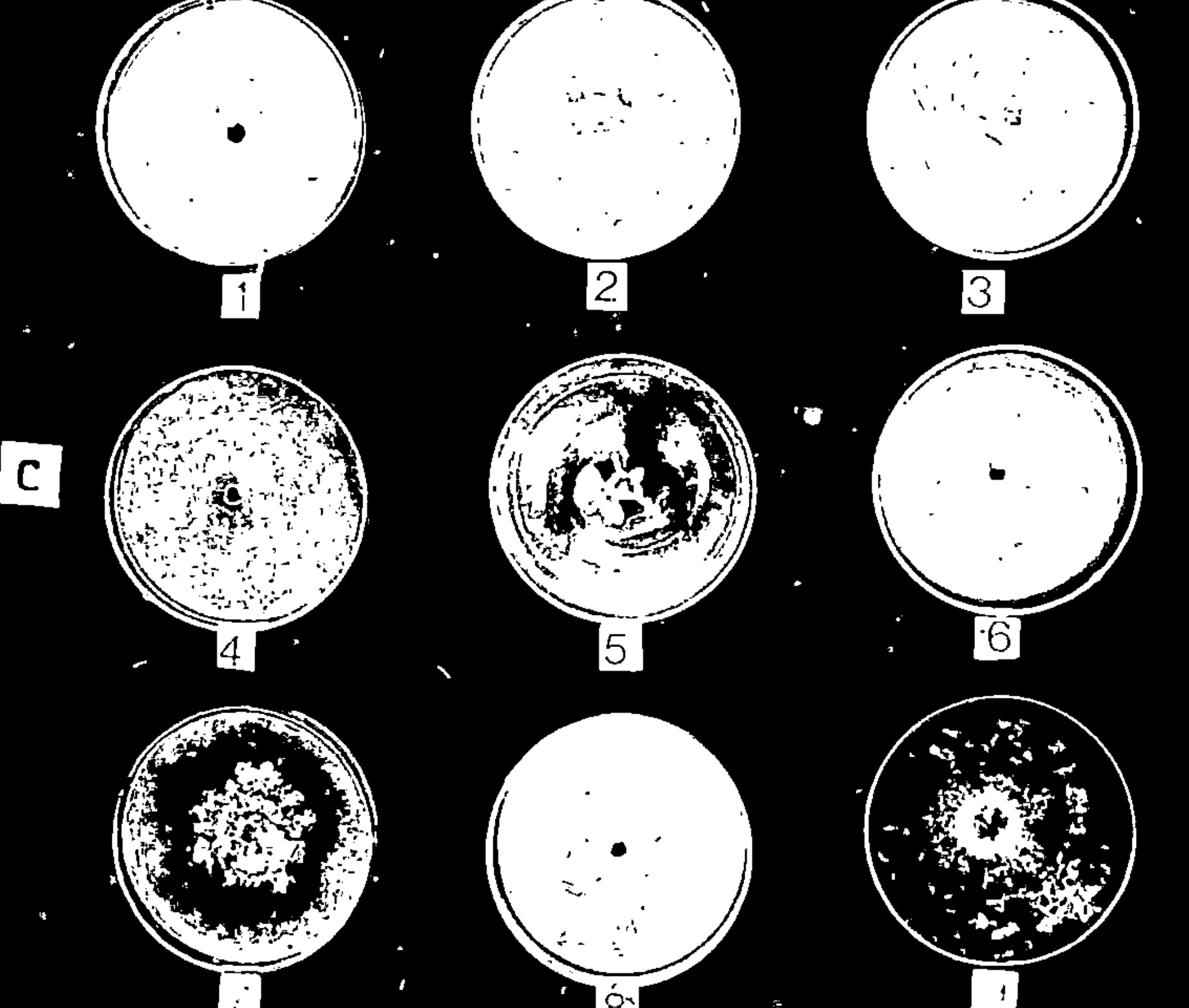

1
2
3
B
4
5
6

Plate 8(c). Growth of *Cylindrocladium quinqueseptatum* in fungicide incorporated Czapeks' agar medium.

1000 ppm of Bavistin, 2000 ppm of Mildothane and Rovral, 3000 ppm of Daconil-2787, Difolatan, Dithane M-45, Fytolan and Thiride.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6

Table 12b. Effect of fungicides on the growth of the fungus (b) In liquid medium.

Fungicide	Concentration (in ppm)	*Dry weight of mycelium (in mg)	Per cent inhibition over control
Bavistin	250	0.00	100.00
	500	0.00	100.00
	1000	0.00	100.00
Daconil-2787	1000	180.00	75.51
	2000	164.07	77.68
	3000	150.63	79.57
Difolatan	1000	0.00	100.00
	2000	0.00	100.00
	3000	0.00	100.00
Dithane M-45	1000	0.00	100.00
	2000	0.00	100.00
	3000	0.00	100.00
Fytolan	1000	143.33	80.50
	2000	104.31	85.81
	3000	96.68	86.85
Mildothane	500	0.00	100.00
	1000	0.00	100.00
	2000	0.00	100.00
Rovral	500	450.00	38.78
	1000	324.19	55.89
	2000	273.39	62.80
Thiride	1000	530.00	27.89
	2000	313.56	57.41
	3000	239.57	67.41
Control	-	735.00	-

*Average of 3 replications

3. Effect of immersing culture discs in fungicidal solutions on the viability of C. quinqueseptatum

Bavistin and Thiride were able to inhibit the growth of the fungus even at the minimum concentrations tested (250 ppm of Bavistin and 1000 ppm of Thiride), when the culture discs were tested for the viability of the fungus after immersion for a minimum period of 15 minutes in fungicidal solutions. Mildothane and Dithane-M-45 inhibited the growth of the fungus at the middle concentration (1000 ppm and 2000 ppm respectively), when the culture discs were tested for the viability of the fungus after immersion for one hour in fungicidal solution. Rovral was able to inhibit the growth (at 1000 and 2000 ppm concentration) after two hours immersion of the culture discs.

Fytolan and Difolatan were able to inhibit the growth of the fungus at the maximum concentration (both at 3000 ppm), only after 24 hours immersion in the fungicidal solution. The data are presented in Table 13.

Minimum concentration of Bavistin and Thiride (250 and 1000 ppm respectively) were found to be enough for the inhibition of growth of the fungus even after 48 hours incubation period on Czapeks' agar medium by immersing culture discs for 15 minutes in fungicidal solutions.

Mildothane and Dithane M-45 inhibited the growth of the fungus at the maximum concentration tested (2000 and 3000 ppm respectively). Mildothane inhibited the growth after 2 hours

Table 13. Effect of treating mycelial discs of the pathogen in fungicidal solutions on its viability

Fungicide	Concentration (in ppm)	Viability of the pathogen									
		After 24 hours					After 48 hours				
		Period of treatment of mycelial disc in fungicidal solution									
		15 m	30 m	1 h	2 h	24 h	15 m	30 m	1 h	2 h	24 h
Bavistin	250	-	-	-	-	-	-	-	-	-	-
	500	-	-	-	-	-	-	-	-	-	-
	1000	-	-	-	-	-	-	-	-	-	-
Daconil-2787	1000	+++	+++	++	++	+	+++	+++	+++	+++	++
	2000	++	++	+	+	-	+++	+++	++	+	+
	3000	+++	+++	++	+	-	++++	++++	+++	++	-
Difolatan	1000	+++	++	++	+	+	++++	++++	+++	++++	++
	2000	++	++	+	+	+	++++	+++	++	+	-
	3000	+++	+++	+	+	-	++++	+++	+++	++	-
Dithane M-45	1000	+++	++	+	+	+	+++	++	++	++	+
	2000	++	++	-	-	-	++++	+++	+	+	+
	3000	+++	++	-	-	-	+++	++++	++++	++	+
Fytolan	1000	++++	++++	+++	++	++	+++++	+++++	++++	+++	+++
	2000	+++	+++	++	+	+	+++	+++	+++	++	++
	3000	+++++	++++	+++	++	-	+++	+++	+++	++	-

Table continued...

Table 13 continued

Fungicide	Concentration (in ppm)	Viability of the pathogen									
		After 24 hours					After 48 hours				
		Period of treatment of mycelial disc in fungicidal solution									
		15 m	30 m	1 h	24 h	24 h	15 m	30 m	1 h	2 h	24 h
Mildothane	500	++	++	+	+	-	+++	+++	+++	++	-
	1000	+++	++	-	-	-	++++	++	++	+	+
	2000	+++	+++	-	-	-	++	+++	++	-	-
Rovral	500	+++	+++	++	++	+	+++	+++	+++	++	++
	1000	++++	+++	++	-	-	++++	++++	++++	+++	++
	2000	++++	++++	-	-	-	++	++	++	++	-
Thiride	1000	-	-	-	-	-	-	-	-	-	-
	2000	-	-	-	-	-	-	-	-	-	-
	3000	-	-	-	-	-	-	-	-	-	-

m - Minute h - Hour

\- - No growth
\+ - 2 to 3 hyphae protruding out upto 1 cm diameter
++ - Hyphal growth upto 1 - 2 cm diameter
+++ - Hyphal growth between 2 - 3 cm diameter
++++ - Hyphal growth between 3 - 4 cm diameter
+++++ - Hyphal growth between 4 - 5 cm diameter

immersion of the culture discs in the fungicidal solution whereas Dithane M-45 inhibited after 24 hours immersion of the culture discs in the fungicidal solution.

Rovral was able to inhibit the growth of the fungus at 2000 ppm when the culture discs were immersed in the fungicidal solution for a period of 24 hours.

Difolatan inhibited the growth of the fungus at 3000 ppm after 24 hours immersion of the culture discs in the fungicidal solution, and Fytolan could not inhibit even at 3000 ppm.

Daconil-2787 was able to inhibit the growth of the fungus at 3000 ppm when the culture discs were immersed for 24 hours in the fungicidal solution.

DISCUSSION

DISCUSSION

The fungus, Cylindrocladium quinqueseptatum Boedijn et Reitsma, was found to cause leaf blight disease of clove, at Patton, Trivandrum District, Kerala. Seedlings were found to be more susceptible than mature plants. Besides leaf blighting, it also caused twig drying of the infected plants.

Sloof (1941), Reitsma and Sloof (1950), Boedijn and Reitsma (1950) and Sarma and Nambiar (1978) have reported the leaf diseases of clove plants caused by C. quinqueseptatum. Wilson et al. (1977) reported a mixed infection of clove by C. quinqueseptatum and Colletotrichum capsici causing leaf blight of clove in South India.

The fungus was isolated from naturally infected clove leaves and brought into pure culture on PDA and Czapeks' agar media. Morphological characters of the fungus was studied in detail and it was compared with those reported by Boedijn and Reitsma (1950), Peerally (1972), Sobers et al. (1975) and Sarma and Nambiar (1978). The detailed morphological studies carried out in the present investigation has showed that most of the measurements were in agreement with those reported by Peerally (1972). Production of tertiary sterigmata was observed very frequently in the present study while Sarma and Nambiar (1978) recorded production of the tertiary sterigmata as a rare phenomenon.

Artificial inoculations conducted revealed that the fungus was able to infect clove leaves of all stages of maturity, infection was severe especially at younger stages. Injury of the host tissue was found to be a pre-requisite for the successful infection by the fungus. However, it is noticed that detached young clove leaves, when inoculated with mycelial bit as well as by spraying with spore suspension got infected within 8 to 10 days even without injury. Sarma and Nambiar (1978) described the symptoms developed on artificially inoculated Eugenia jambolana, Pimenta dioica, Eucalyptus grandis, E. maculata and E. globulus.

Host range studies indicated that the fungus could infect a large number of plants. In this study new additional hosts such as tapioca, cashew, citrus, guava, cherry, nutmeg, clerodendron, synedrella, lucas, croton, ageratum, sida, euphorbia and sapota were recorded for the organism. C. quinqueseptatum could infect Anona, Camellia sinensis, Eucalyptus, Eugenia caryophyllata and Hevea (Peerally, 1972). The organism is also known to infect a variety of host plants, viz., Eugenia jambolana, Pimenta dioica, Eucalyptus grandis, E. maculata and E. globulus (Sarma and Nambiar, 1978). The fact that this fungus has a wide host range and it causes infection to many important crop plants is of great concern in the cultivation of these crops. Further the wide host range of this fungus has epidemiological significance and this organism can be a potential pathogen for many of the important crop plants in future.

The fungus was able to grow well on a number of culture media. Good sporulation was obtained on potato dextrose agar, Coon's agar and Czapeks' agar. It grew well on a number of liquid culture media. Maximum dry weight of the mycelium was obtained on potato dextrose broth followed by Czapeks' and Fries' broth. Sarma and Nambiar (1978) reported good growth of the fungus on potato sucrose agar (PSA).

Studies conducted on the growth of the fungus on solid medium incorporated with different oils indicated that among the five different oils used, maximum growth was obtained on gingelly oil amended medium, which was followed by coconut and clove oils. Studies with liquid medium amended with oils also showed similar results.

Growth of the fungus at pH 9 was found to be significantly superior. Maximum dry weight of the mycelium was obtained at this pH.

The toxic effect of the culture filtrate bioassayed against the host leaf revealed that maximum lesion length produced with Richards' and Czapeks' culture filtrate after 72 hours incubation. Sharma and Sharma (1969) reported Richards' liquid medium as the best medium for the production of toxin. Exotoxin production was found to be more than endotoxin. The exotoxin production of Colletotrichum capsici is found to be lesser than endotoxin (Nair and Ramakrishnan, 1973). The culture filtrate of C. gloeosporioides showed toxicity to Citrus limettoides, C. aurantifolia, C. aurantium,

C. limon, C. maxima, C. reticulata, C. medica, C. sinensis var. mosambi, C. sinensis var. nairang, Carissa carandas and Cicer arietinum. The toxicity was expressed in the form of dropping down of the leaves, complete wilting of twigs, defoliation and change in the colour of leaves and twigs (Sharma and Sharma, 1969). As the incubation period increased, there was a proportionate increase in the area of lesion.

The effect of heat on the toxin produced by the fungus was studied. The culture filtrate which was boiled for 15 minutes and that autoclaved at 1.05 kg/cm^2 for 15 minutes produced lesions of 4 to 6 cm length whereas the untreated filtrate produced lesions of 6 cm length. The mycelial extract of the fungus also produced lesions on treated leaves but the effect was more with the culture filtrate. These studies indicate that the toxic principle produced by the fungus is thermostable.

The undiluted and unsterilized culture filtrate produced the maximum lesion length after 48 hours. The diluted unsterilized xculture filtrate also produced lesion but the effect was less than the undiluted ones. The results reveal that the toxin can withstand a dilution of 1:4 without loosing its effect. The effect of dilution of culture filtrate on toxicity of Colletotrichum gloeosporioides showed that toxicity decreased with the dilution. The filtrate retained its activity upto 10 times dilution though it was decreased to a great extent with dilution (Sharma and Sharma, 1969).

Toxic effect of the culture filtrate translocated on the cut twigs of plants showed wilt like symptoms followed by defoliation. Leaves lost their turgidity and curled downwards. Defoliation was found to be more in the case of clove followed by cashew and tapioca. Toxic effect of the culture filtrate of C. quinqueseptatum, the causal organism of seedling blight of Eucalyptus hybrid, was demonstrated by Anahosur et al. (1976). Katznelson and Richardson (1948) reported the toxic effect of Cylindrocladium sp. on complete wilting of strawberry seedlings. Marginal necrosis of leaves, leaf spots and wilting were the characteristic effects observed by them. The culture filtrate of Colletotrichum gloeosporioides was found to be toxic to Citrus spp. (Sharma and Sharma, 1969).

The spore germination of Colletotrichum gloeosporioides and Curvularia sp. were inhibited by the culture filtrate. Eighty nine per cent inhibition was obtained in the case of C. gloeosporioides, and sixty one per cent with Curvularia sp. Toxin produced by Helminthosporium sativum could inhibit the spore germination of Sclerotinia fructicola (Ludwig, 1955). Alternaric acid produced by Alternaria solani prevented the spore germination of Myrothecium verrucaria, at 1 per cent concentration (Brian et al., 1949). Phomic acid produced by Phoma medicaginis inhibited the spore germination of Penicillium notatum, Fusarium culmorum and Aspergillus niger at 50 ppm concentration (Lakshmanan and Vanterpool, 1967). Dwivedi and Singh (1971) reported inhibitory effect of metabolites produced by Botryodiplodia theobromae on the occurrence of

microorganisms in the rhizosphere of Andrographis paniculata. Rajagopalan (1971) noted inhibition of the spore germination of Gloeosporium psidii and Pestalotia psidii by the culture filtrates of Diplodia natalensis, causing fruit rot of guava. Sharma and Sharma (1969) reported that the culture filtrate of Colletotrichum gloeosporioides was toxic to the spore germination of C. gloeosporioides itself. Species of Diplodia are known to produce metabolites toxic to the germination of maize, cucumber and snakegourd seeds (Orshanskaya, 1960; Rajagopalan, 1971).

Effect of culture filtrate and mycelial extract on clove leaves of different stages of maturity indicate that both preparations are toxic and produced lesions on leaves of different ages. But the culture filtrate induced larger lesions than the mycelial extract in all types of leaves with injury. Activity of the toxin produced by Colletotrichum fuscum is measured by intensity of symptoms (Goodman, 1960).

The studies on the production of toxic metabolites by Cylindrocladium quinqueseptatum and its role in the pathogenesis of leaf spot of clove, gave positive results and these results are suggestive of the role of a toxin(s) produced by the organism in the pathogenesis. However, more detailed studies are necessary to establish the exact chemical nature of toxic metabolites and its role in pathogenesis.

Results of the laboratory evaluation of fungicides indicated that the growth of the fungus was completely inhibited by Bavistin at 250 ppm, 500 ppm and 1000 ppm; Dithane M-45 at 1000 ppm, 2000 ppm and 3000 ppm; Mildothane at 500 ppm, 1000 ppm and 2000 ppm and Thiride at 1000 ppm, 2000 ppm and 3000 ppm, when tested by the 'poisoned food technique' using Czapeks' agar as basal medium. Bavistin and Dithane M-45 have been reported to be effective in checking the growth of many fungi in nutrient media (Zachos et al., 1963; Sen and Kapoor, 1975; Kataria and Grover, 1977). In fungicide incorporated liquid medium, growth of the fungus was completely inhibited with Bavistin, Difolatan, Dithane M-45 and Mildothane at all concentrations, whereas Daconil-2787. Fytolan, Rovral and Thiride did not completely inhibit the growth of the fungus even at the maximum concentration tested. However, Rovral was reported as an effective fungicide for the control of Botrytis cineria causing grey rot of grapes (Boley et al., 1976; Schuepp, 1977). Except Difolatan and Thiride, all the fungicides showed similar effects, both on solid and in liquid medium. Difolatan is known to be effective for the control of boll rot of cotton caused by Diplodia gossypine (Chen and Yu, 1967) and panel necrosis of rubber caused by Botryodiplodia theobromae (Anon, 1971).

When the efficacy of fungicides was tested by the agar culture disc immersion method, it was noticed that,

Bavistin and Thiride were able to inhibit the growth of the fungus at the minimum concentration (250 ppm of Bavistin and 1000 ppm of Thiride) when the culture discs were tested for the viability of the fungus after immersion for 15 minutes in fungicidal solutions. Mildothane and Dithane M-45 inhibited the growth of the fungus at the middle concentration (1000 ppm and 2000 ppm respectively), when the culture discs were tested for the viability of the fungus after immersion for one hour in fungicidal solution. Rovral was able to inhibit the growth after 2 hours immersion of the culture discs in the fungicidal solution at 1000 and 2000 ppm concentrations.

Fytolan and Difolatan were able to inhibit the growth of the fungus at the maximum concentration (both at 3000 ppm) only after 24 hours immersion in the fungicidal solution. Daconil-2787 was able to inhibit the growth of the fungus at 2000 and 3000 ppm concentrations when the discs were immersed for 24 hours in the fungicidal solution.

Observations taken after 48 hours indicated that minimum concentrations of Bavistin and Thiride (250 ppm and 1000 ppm respectively) were enough to inhibit the growth of the fungus by immersing the culture discs for 15 minutes. Mildothane and Dithane M-45 inhibited the growth of the fungus at the maximum concentration tested with slight difference. Mildothane inhibited the growth after 2 hours immersion of the culture discs in the fungicidal solution, whereas

Dithane M-45 inhibited after 24 hours immersion of the disc in the fungicidal solution. Rovral was able to inhibit the growth of the fungus at 2000 ppm when the culture discs were immersed in the fungicidal solution for a period of twenty four hours. Difolatan inhibited the growth of the fungus at 3000 ppm after 24 hours immersion of the culture discs in the fungicidal solution. But Fytolan could not inhibit even at 3000 ppm.

Daconil-2787 was able to inhibit the growth of the fungus at 3000 ppm when the culture discs were immersed for 24 hours in the fungicidal solution.

In the experiments conducted to study the inhibition of spore germination of the fungus on glass slides, it was noticed that there was complete inhibition of germination of spores of the fungus at all concentrations of the eight fungicides when observed 24 hours after treatment. Daconil-2787, Dithane M-45, Fytolan and Thiride were able to cause 94, 97, 94 and 95 per cent inhibition respectively upto 12th day at the maximum concentration tested (3000 ppm). But at the middle concentration (2000 ppm) tested, only Dithane M-45 and Thiride could cause 90 per cent inhibition upto 12th day, and at the minimum concentration (1000 ppm), none of these could cause ninety per cent inhibition. Mildothane was able to cause 95, 95 and 98 per cent inhibition at 1000, 2000 and 3000 ppm respectively upto 3rd day; but at maximum concentration this effect was retained upto 6th day of observation and thereafter decreased.

Kovral could cause more than 90 per cent inhibition upto 6th day at all concentrations tested. None of the fungicides could effect complete inhibition upto the 12th day of observation. However on the 12th day, Dithane M-45 exhibited maximum per cent inhibition at the maximum concentration followed by Thiride and Daconil-2787. Laboratory evaluation of fungicides against C. quinqueseptatum has been reported by Anahosur et al. (1977). They studied the efficacy of 10 fungicides at three concentrations by the poisoned food technique. Out of the 10 fungicides tested, Bavistin and Thiram at all concentrations and Hexaferb at 0.3 per cent concentration completely inhibited the growth of the fungus, whereas Calixin and Dithane Z-78 showed least inhibition of growth at all concentrations.

Of the eight fungicides tested for the in vitro control of the fungus (C. quinqueseptatum). Bavistin, Dithane M-45, Mildothane, Thiride were found to be equally effective in all the 3 concentrations by the poisoned food technique. However more detailed in vivo studies are required before any one of them can be recommended for the field control of the disease.

SUMMARY

SUMMARY

Cylindrocladium quinqueseptatum Boedijn et. Reitsma causing leaf blight disease of clove at Pattom, Trivandrum District, Kerala was isolated and brought into pure culture. Artificial inoculations revealed that the fungus was pathogenic to clove leaves of all stages of maturity.

Host range studies of the pathogen indicated that the fungus could infect a large number of plants including cashew, tapioca, citrus, guava, cherry, eucalyptus, nutmeg, lucas, croton, sida, anona. Morphological characters of the organism agreed with those reported by other investigators.

Cultural studies indicated that the fungus could grow well on potato dextrose agar, Coon's agar and Czapeks' agar. Abundant growth and sporulation was obtained on potato dextrose agar, Coon's agar and Czapeks' agar. It could also grew well on a number of liquid culture media. Maximum dry weight of the mycelium was obtained on potato dextrose broth followed by Czapeks' and Fries'.

Growth of the fungus on media incorporated with different oils indicated that the fungus could grow well on media amended with gingelly oil. Regarding its pH requirements pH 9 was found to be best for growth and sporulation.

The toxic effect bioassayed against the host leaf revealed that maximum area of symptom expression was obtained with culture filtrate of the organisms grown in Richards'

and Czapeks' medium. Exotoxin production was found to be more than endotoxin.

Heating effect on the culture filtrate showed that the untreated culture filtrate produced more lesioned areas than those boiled for 15 minutes and autoclaved. Studies on the effect of dilution of culture filtrate revealed that undiluted unsterilized culture filtrate produced more necrotic areas than diluted unsterilized one.

Toxic effect of the culture filtrate translocated on cut clove twigs showed more wilting symptoms than other twigs treated. The culture filtrate of the fungus inhibited the germination of spores of Colletotrichum gloeosporioides and Curvularia sp. Activity of the culture filtrate on different stages of maturity of the clove leaves showed that it could produce lesions on all stages of growth of the leaves.

Laboratory evaluation of fungicides:- Regarding the tests conducted to study the inhibition of spore germination of the fungus on glass slides, it was noticed that there was complete inhibition of germination of spores of the fungus, with all the eight fungicides when tested 24 hrs after treatment in all the concentrations. Dithane M-45 and Thiride could cause ninety per cent inhibition upto twelth day at the middle concentration tested (2000 ppm).

Rovral could cause more than ninety per cent inhibition upto sixth day at all concentrations tested. None of the fungicides could effect complete inhibition upto twenth day of observation.

Inhibition of growth of the fungus on solid Czapeks' medium incorporated with different fungicides by poisoned food technique, revealed that; Bavistin, Dithane M-45, Mildothane and Thiride could completely inhibit the growth of the fungus at all the concentrations tested. In fungicide incorporated liquid medium, growth of the fungus was completely inhibited with Bavistin, Difolatan, Dithane M-45 and Mildothane at all concentrations tested.

When the efficacy of fungicides was tested by the agar culture disc immersion method, it was noticed that Bavistin and Thiride were able to inhibit the growth of the fungus at the minimum concentration (250 ppm of Bavistin and 1000 ppm of Thiride), when the culture discs were tested for the viability of the fungus after immersion for 15 minutes in fungicidal solution. Whereas Mildothane and Dithane M-45 inhibited the growth of the fungus at the middle concentration (1000 ppm and 2000 ppm respectively) tested, when the culture discs were tested for the viability of the fungus after immersion for one hour in fungicide solution.

Rovral was able to inhibit the growth after 2 hours immersion of the culture discs in the fungicidal solution at 1000 and 2000 ppm concentration.

Fytolan and Difolatan were able to inhibit the growth of the fungus at the maximum concentration (both at 3000 ppm) only after 24 hours immersion in the fungicidal solution.

Daconil-2787 was able to inhibit the growth of the fungus at 2000 and 3000 ppm concentrations when the discs were immersed 24 hours in the fungicidal solution.

Observations taken after 48 hours, indicated that 250 ppm Bavistin and 1000 ppm Thiride were enough to inhibit the growth of the fungus by immersing the culture discs for 15 minutes in the fungicidal solutions.

Mildothane and Dithane M-45 inhibited the growth of the fungus at the maximum concentration tested.

Rovral was able to inhibit the growth of the fungus at 2000 ppm when the culture discs were immersed in the fungicidal solution for a period of 24 hours.

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*Originals not seen

APPENDICES

APPENDIX I

Potato dextrose agar medium

Pealed and sliced potato	-	200 g
Dextrose	-	20.0 g
Agar agar	-	20.0 g
Distilled water	-	1000 ml

Czapeks' agar medium

$MgSO_4.7\ H_2O$	-	0.50 g
NH_2PO_4	-	1.00 g
KCl	-	0.50 g
$FeSO_4$	-	0.01 g
$Na\ NO_3$	-	2.00 g
Sucrose	-	30.00 g
Agar agar	-	20.00 g
Distilled water	-	1000 ml

Sabouraud's medium

Glucose	-	40.00 g
Peptone	-	10.00 g
Agar agar	-	20.00 g
Distilled water	-	1000 ml

Oat meal agar

Rolled oats	-	40.00 g
Agar agar	-	20.00 g
Distilled water	-	1000 ml

Coon's agar

$MgSO_4.7\ H_2O$	-	1.23 g
Sucrose	-	7.20 g
Dextrose	-	3.60 g
KNO_3	-	2.20 g
Agar agar	-	20.00 g
Pot.acid phosphate	-	2.72 g
Distilled water	-	1000 ml

Host extract agar

Clove leaves	-	200.00 g
Agar agar	-	20.00 g
Distilled water	-	1000 ml

Host extract dextrose agar

Clove leaves	-	200.00 g
Dextrose	-	20.00 g
Agar agar	-	20.00 g
Distilled water	-	1000 ml

Richards' agar

KNO_3	-	10.00 g
KH_2PO_4	-	5.00 g
$MgSO_4.7\ H_2O$	-	2.50 g
$FeCl_3$	-	0.02 g
Sucrose	-	50.00 g
Agar agar	-	20.00 g
Distilled water	-	1000 ml

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

Fries' medium (Pringle and Scheffer, 1963)

$(NH_4)_2NO_3$	-	1.00 g
KH_2PO_4	-	1.00 g
$MgSO_4$	-	0.50 g
NaCl	-	100 .00 mg
$CaCl_2$	-	130.00 mg
Sucrose	-	30.00 g

Appendix I continued

$MnSO_4 \cdot 7\ H_2O$	-	10.00 g
$FeSO_4$	-	20.00 mg
Boric acid	-	1.00 mg
$CuSO_4$	-	0.10 mg
$ZnSO_4$	-	0.01 mg
Distilled water	-	1000 ml

For the preparation of liquid medium agar agar was not added.

APPENDIX II

Analysis of variance table
(Growth of the fungus on different solid media)

Source	S.S.	df	M.S.	F
Treatment	141.376	7	20.197	35.67
Error	9.060	16	0.566	

C.D. for comparison = 13.03

APPENDIX III

Analysis of variance table
(Growth of the fungus on different liquid media)

Source	S.S.	df	M.S.	F
Total	480311.70	19		
Treatment	388237.20	4	97059.30	15.81
Error	92074.50	15	6138.30	Sig

C.D. = 117.98

APPENDIX IV

Analysis of variance table
(Effect of pH on the radial growth of the fungus)

Source	S.S.	df	M.S.	F
Total	0.0420	20		
Treatment	1.0831	6	0.1805	
Error	1.0411	14	0.0743	2.43

C.D. = 0.47

APPENDIX V

Analysis of variance table
(Growth of the fungus on media incorporated with different oils - on solid media)

Source	S.S.	df	M.S.	F
Total	22957.68	23		
Treatment	21076.77	5	4215.35	40.34 Sig
Error	1880.91	18	104.50	

C.D. = 15.18

APPENDIX VI

Analysis of variance table

(Growth of the fungus on media incorporated with different oils - on liquid media)

Source	S.S.	df	M.S.	F
Total	471728.50	23		
Treatment	434084	5	86816.80	
Error	37644.50	18	2091.36	41.51

C.D. = 67.91

APPENDIX VII

Analysis of variance table

(Effect of different fungicides on the growth of *Cylindrocladium quinqueseptatum* on solid medium)

Source	S.S.	df	M.S.	F
Treatment	4020.5	24	16.752	37.878
Error	22113.0	50	442.260	

C.D. for comparison between fungicides = 6.3
C.D. for comparison between levels = 10.9

APPENDIX VIII

Analysis of variance table

(Effect of fungicides on the growth of the fungus on liquid medium)

Source	S.S.	df	M.S.	F
Total	2397831.00			
Treatment	2038653.73	24	84943.00	11.83
Error	359177.61	50	7183.55	

C.D. = 139.09

STUDIES ON THE LEAF BLIGHT DISEASE OF CLOVE CAUSED BY *Cylindrocladium sp.*

By

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ABSTRACT OF A THESIS

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ABSTRACT

Leaf blight disease of clove caused by Cylindrocladium quinqueseptatum Boedijn et Reitsma was investigated. The fungus infected clove leaves at all stages of maturity, but the seedlings were found to be more susceptible to the disease than mature plants. Injury of the host tissue was found to be a pre-requisite for successful infection by the fungus.

The organism infected a wide variety of plants including some of the weed plants on artificial inoculation.

Good growth and sporulation of the fungus was obtained on potato dextrose agar followed by Coon's agar and Czapeks' agar. In liquid media, maximum dry weight of the mycelium was obtained on potato dextrose broth, followed by Czapeks' broth.

Maximum growth of the fungus was obtained on medium amended with gingelly oil followed by coconut and clove oils. In liquid media, maximum dry weight of the mycelium was obtained in the medium amended with gingelly oil followed by coconut and clove oils. Optimum pH range for the growth of the fungus was found to be 7 to 9.

Richards' broth was found to be the best medium for the production of toxin followed by Czapeks' and Fries' media. Exotoxin production was found to be more than endotoxin. The toxic metabolite is found to be thermostable. Diluting the culture filtrate to 4 times its volume showed a reduction in the toxic effect. However, the treatments did not completely eliminated the toxic effect of the preparation.

The toxic effect of the culture filtrate translocated by

defoliation on the cut twigs of plants. Culture filtrate of the fungus inhibited the spore germination of Colletotrichum gloeosporioides and Curvularia sp.

The culture filtrate as well as the mycelial extract produced lesions on clove leaves of different maturity, with pronounced effect on tender leaves.

Spore germination of the fungus could be completely inhibited with all the eight fungicides in all concentrations on the first day of observation. Daconil-2787, Dithane M-45, Fytolan and Thiride were able to cause 94, 97, 94 and 95 per cent inhibition of spore germination respectively upto 12th day at maximum concentration tested (3000 ppm).

Growth of the fungus was completely inhibited with Bavistin 250, 500 and 1000 ppm, Dithane M-45 1000, 2000 and 3000 ppm; Mildothane 500, 1000 and 2000 ppm and Thiride 1000, 2000 and 3000 ppm when tested in Czapeks' agar medium. In Czapeks' solution Bavistin, Difolatan, Dithane M-45 and Mildothane at all concentrations tested, there was complete inhibition of growth of the fungus.

Bavistin at 250 ppm and Thiride at 1000 ppm were able to inhibit the growth of the fungus by 15 minutes immersion, when the culture discs were tested for the viability of the fungus. Mildothan and Dithane M-45 inhibited the growth of the fungus at 1000 and 2000 ppm respectively, when the culture discs were tested for the viabilit of the fungus after immersion for one hour in fungicidal solution. Fytolan and Difolatan were able to inhibit the growth of the fungus at the maximum concentration (both at 3000 ppm), only after 24 hours immersion in the fungicidal solution.