LEAF BLIGHT OF BANANA AND ITS CONTROL

By

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THESIS

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DECLARATION

I hereby declare that this thesis entitled "LEAF BLIGHT OF BANANA AND ITS CONTROL" 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.

Place: Vellayani,

Date : 2-8-93

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CERTIFICATE

Certified that this thesis entitled "LEAF BLIGHT OF BANANA AND ITS CONTROL" is a record of research work done independently by Kum.K.V. SAJI under my guidance and supervision and that it has not previously formed the basis for the award of any degree, diploma, fellowship or associateship to her.

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INTRODUCTION

INTRODUCTION

Banana (*Musa paradisiaca L.*) one of the oldest fruits known to mankind is grown through out the warm, moist regions of South East Asia. In India banana is one of the most important fruits occupying about 20 per cent of total area under fruits. In Kerala banana is cultivated through out the state in an area of over 22,000 ha with an average production of 301521 t. Cultivation of banana is mainly done in the paddy fields as an intercrop in the coconut gardens and also in the homesteads.

Banana being an important fruit crop it is widely disseminated in the markets of different parts of the world or even the planting materials are carried to different regions. Thus the chances of occurrence of the disease is also distributed.

Leaf diseases are of major concern especially in banana crop, as the number of leaves produced in a plant directly/indirectly contribute to the number of bunches as well as the bunch weight. Even though a number of leaf spot diseases have been reported from different parts of India, no effort has been made so far in Kerala to study the various aspects of the pathogen(s) involved in the incidence of leaf blight of

banana and methods of control of this disease. Leaf blight is a very severe disease in banana especially in the lowlying areas which causes extensive damage to the foliage which ultimately reflect on the yield of the crop. Most of the banana varieties cultivated are prone to this disease. Detailed information on the season of occurrence of the disease, symptomatology, resistance/susceptibility of different varieties etc. are necessary for formulating suitable strategy for any plant disease management. Considering these aspects, the following items of work have been under taken in the present study-

Survey on the occurrence and severity of leaf blight of banana in different localities in Thiruvananthapuram district, study the symptomatology of the disease on popular varieties of banana, isolation, purification and maintenance of the pathogen(s), characterisation of the pathogen(s), laboratory evaluation of fungicides against the pathogen(s), testing the efficacy of the promising fungicides under field condition and scoring the disease intensity in the promising varieties of banana.

REVIEW OF LITERATURE

2. REVIEW OF LITERATURE

Various leaf spot diseases of banana caused by Cercospora musae Zimm., Deightoniella torulosum (Syd. and Ashby) Ellis., Cordana musae (Zimm.) Von Hohnel., Helminthosporium gibberosporium Curzi. and Septoria keralensis Elizabeth (Elizabeth, 1964) have been reported from Kerala. Combined infections of Cordana musae (Zimm.) Von Hohnel and Helminthosporium gibberosporium Curzi. have also been reported by various workers. The present study covered some of the leaf blighting fungi in banana. A brief review on some of the fungi causing leaf blight in banana is given below.

2.1 Fungal Pathogens of Banana

2.1.1 Colletotrichum sp

In 1912, Sydow and Sydow recorded Gloeosporiun chioneum Syd. from the flaccid or drying leaves of Musa sp in Congo. The fungus produced erumpent acervuli which are ochraceous white coloured initially and white coloured later, irregularly and scantly distributed over the leaf surface. The conidia are abundant, hyaline, cylindrical or slightly curved, obtuse at both ends and measure 12-16x3-4 micron in size. Petch (1917) isolated a species of

Glomerella, described as G. musarum from the leaves of plantains affected by Cordana musae. The fungus produced perithecia which are superficial or erumpent, black coloured, ovate, shortly rostrate, membraneous and measure 100-120 micron in dia. Asci which are clavate, 50-60 micron in size and eight spored are produced inside the perithecia. Spores are hyaline, continuous, cymbiform, straight or curved with obtuse ends and 14-18 x 3.5-4 micron in size. No paraphyses was present inside the perithecia. Ocfemia (1924) and Agati (1925) recorded Gloeosporium musarum Cke. and Massee., from the leaves of abaca seedlings in the Philippines. The symptoms are characterised as dark brown or purplish circular to oval spots, particularly during the wet season. The disease is generally outgrown by vigorous seedlings and is of minor economic importance only. The spots vary from 0.5-2mm in width, may coalesce and form irregular brown or purple lesions. On the leaves of older plants, the diseased areas are fewer in number but of larger size, 10-15 x 5-10cm. Occasionally they may extend from midrib to margin. Strains of Gloeosporium musarum Cke. and Massee. occur in association with the Cercospora leaf spot in Fiji was reported by Campbell in 1925.

Davis (1931) observed that Glomerella cingulata, the ascomycetous stage of Gloeosporium

musarum can also attack a wide host range including banana. Simmonds (1933) reported that Colletotrichum acervuli may occasionally appear on old leaf spot. Wardlaw (1934) isolated strains of Glomerella cingulata from the leaf spot of Cavendish banana variety in Trinidad. Wardlaw (1934) reported Gloeosporium musarum from the speckled leaf tissues of banana. In Brazil, Deslandes (1937) has reported several fungi associated with leaf spots viz., Gloeosporium musarum, Deightoniella torulosa and Nigrospora sp. A new leaf spot of banana incited by Colletotrichum psidii Curz. was reported from Poona in 1966. Plata et al. (1974) gave an account of the morphology of the Colletotrichum musae (Berk. and Br.) Arx. obtained from different Musa spp. Colonies are often with abundant white aerial mycelium becoming grey with age, with abundant cinnamon conidial masses. Sclerotia and setae are absent. Conidia are straight, cylindrical, obtuse at the apices, 12-17 x 4.5-5.5 micron in size. Appressoria are common, medium to dark brown, irregular in shape, often with large or deep lobes, 9-13 x 9-11.5 micron in size.

2.1.2 Guignardia musae

The fungus, Guignardia musae Racib. was reported for the first time from the living leaves of

Musa sapientum L. by Srivastava (1975). The fungus incited minute, rusty, dark brown, circular - oval blisters on the lamina (0.5-1.0 mm) in dia. Sections of the infected leaves through the blisters show the mycelium as endophytic, branched, septate and intracellular. Hyphae profusely ramifying in the intercellular spaces also. Conidia are roughly spherical (10-15 micron in dia) thick walled and produced in chains. The fungus with its perithecial state was recorded on the living leaves of Musa paradisiaca L. for the first time from Java. Srivastava (1981) reported Guignardia musae on Musa paradisiaca from Jaunpur.

Chuang (1981) isolated Phyllosticta musarum, the causal agent of banana freckle from the surface sterilized banana leaves showing new freckle spots in Taiwan. Fungus produced two types of isolate when plated on V-8 juice agar medium. In the first type, fungus produced blackish minute colonies designated as M-type was isolated. Another fungus which produced relatively large blackish green colonies designated as L-type was isolated. M-type isolate produced black pycnidia which contained hyaline, obovoid conidia (10-22 x 6-13 micron) with a mucilaginous envelope and appendage. L-type of isolate produced black pycnidia

which contained hyaline, obovoid conidia (6-12 x 6-8 micron) with a gelatinous envelope and an apical appendage. Chuang also studied the perfect stage of the fungus. L-type isolate produced black ascocarps which contained hyaline, fusiform to ellipsoidal ascospores (10-14x6-7 micron) with an apical gelatinous plug at each end. The morphological characters of the L-type isolate fit the descriptions of those Musa isolate studied by Punithalingam (1974). He considered those isolate to be Phyllosticta musarum with the ascogenous state Guignardia musae Raciborski. Jone and Alcorn (1982) recorded Guignardia musae causing freckle disease of banana from Far North Queensland. It was observed that the disease was more widespread on the islands in the Torres strait and on the east coast of Cape York Peninsula.

2.1.3 Khuskia oryzae

Wardlaw (1934) reported the association of Nigrospora oryzae (B. and Br.) Petch., the conidial stage of Khuskia oryzae from the banana leaf spot incited by Cordana musae. It was in the same year Wardlaw isolated Nigrospora oryzae from the speckled leaf tissue of banana. Association of the fungus Nigrospora oryzae with Deightoniella leaf spot of banana has also been reported. The fungus occurs

frequently on diseased or unhealthy banana leaves. On discoloured chlorotic patches it forms a slight surface mycelium from which hyphae pass into the leaf tissue through the stomata. The conidia are smoky brown or shiny jet black, round or oval, measuring 13.0 - 15.5 x 10-13 micron. They are borne singly on short inflated stalks and are abundantly produced on leaf surface. The fungus is very common on banana leaves.

2.1.4 Pestalotiopsis versicolor

Om Prakash and Singh (1973) observed a new leaf spot of banana caused by Pestalotiopsis versicolor in Cavendish banana variety Robusta. The initial symptoms of the disease is characterised by numerous pinhead like spots on the leaf surface. Spots are circular in the beginning but at maturity they become elongated. There is general yellowing of the infected leaves. The spots are dull white in the centre and enclosed by reddish brown or brick coloured necrotic zones, 0.5 - 2mm in dia, later coalesced and formed oval, elongate or irregular big spots. They are irregularly distributed on leaf and can sometimes be seen on the midrib and petiole also, but comparatively smaller in size than on leaves. Consequent to infection the whole leaf turns yellowish brown and start drying. On PDA, fungus produced conidia, which

are five celled, clavate or elliptic, fusiform, 20-22.5x7-8.5 micron in dia; intermediate cells coloured, 13-18.2 micron long; upper two coloured cells fuliginous, opaque, swollen; lowest coloured cells olivaceous, constricted at the dividing septa; apical cells are hyaline, short, conic, cylindric, consisting of three divergent setulae, 7.5 - 20.5 micron long; basal cells hyaline and short, obtuse or conoid, pedicels upto 5 micron.

2.1.5 Phaeoseptoria sp

Reghunath (1963) described a new leat spot of banana incited by Phaeoseptoria sp from Kerala. Symptoms are exhibited mainly on mature leaves as numerous oblong to elliposoid necrotic areas of 1.5 x 0.7 cm having white centre and dark brown margins. Later these spots coalesced, forming irregular necrotic patches. Usually young and growing leaves are unaffected.

2.2 Characterisation of pathogen(s)

2.2.1 Utilisation of different carbon and nitrogen sources

Misra and Thakur (1965) studied the nutritional requirement of the fungus Gloeosporium

musarum. Among the nine carbon sources tested, lactose, arabinose and galactose were found to be best for the maximum growth of the fungus under laboratory condition. Lactose also supported the maximum sporulation. Lactose at 2.5-3 per cent concentration gave the best growth and sporulation of the fungus. Among the nitrogenous sources used, peptone, urea and calcium nitrate supported maximum growth. Sporulation and length of conidia were maximum in peptone. Nitrogen at 0.15 per cent level proved best for maximum growth, sporulation and length of conidia.

Greene and Morales (1967) observed that Gloeosporium musarum (Colletotrichum musae) causing anthracnose of banana grew well on all carbon sources used except galactose. The best growth of the fungus was obtained with maltose followed by DIFCO starch, fructose, glucose, banana starch, sucrose and galactose.

Nutritional requirements of the fungus Nigrospora (Khuskia) oryzae was studied by Rawla and Tandon (1976). He reported that the fungus gave its best growth with maltose; good with dextrose, sucrose and mannose; fairly good with cellulose, soluble starch and fructose; poor with mannitol and lactose. No growth of the fungus was observed with sorbose, n-butyl

alcohol, stearic, oxalic, lactic, succinic, citric and propionic acids. The optimum growth of the fungus was obtained after seven days of incubation with maltose, eleven days with dextrose, fructose, mannose, lactose, soluble starch, cellulose, mannitol and thirteen days with sucrose.

2.2.2 Toxin Production

The production of toxin as metabolic byproducts in culture filtrates of plant pathogenic fungi and their principles of action in disease development was studied by Meehan and Murphy (1946). Production of toxic metabolites of species of Colletotrichum was reported by various workers. Lin (1948) reported the production of a powerful toxic substance by Glomerella cingulata. Production of toxin by tobacco anthracnose organism (Colletotrichum nicotianae) was observed by Wolf and Flowers (1957). They obtained anthracnose symptoms on the leaves and petioles with the sterile filtrates of cultures of the organism thus revealing the secretion of a toxic metabolite into the growing medium. The production, physiological activity and chemical nature of colletotin, a toxin produced by C. fuscum was described by Goodman (1960). Hen (1968) isolated a toxic

metabolite from the culture filtrates of C. musae. Lycomarasmic acid production by C. gloeosporioides was reported by Ballio et al (1969). Detailed studies on the toxin produced by C. capsici associated with turmeric leaf spot disease was done by Nair (1972).

2.2.2.1 Effect of substrates on toxin production

The effect of various media supporting toxin production was studied by various workers. It was Berry and Futrell (1961) who showed that varying degrees of pathogenicity of a fungus results in the production of phytotoxic metabolites on different composition of the medium. Production of toxin by Colletotrichum gloeosporioides causing citrus die back occured in Richard's solution after 22 days of growth (Sharma and Sharma, 1969). Nandi and Santra (1974) had tested the differential phytotoxicity of metabolic byproducts of Nigrospora oryzae in different nitrogen sources on some varieties of Oryza sativa. It was observed that culture media containing asparagine, tryptophan or tyrosine produced significant amount of toxic byproducts in the culture filtrate. In the case of inorganic sources, production of such toxic byproducts seem to be much lower than those exhibited by the organic sources.

2.2.2.2 Bioassay technique

Phytotoxic behaviour of metabolic byproducts of Nigrospora oryzae (Berk. and Br.) Petch on some varieties of Oryza sativa was tested by Santra (1969). It was observed that there is considerable inhibition of growth in roots and shoots when treated with metabolic solution in comparison to the control.

Moreover, in 1:10 dilution greater inhibition occurs than in 1:100 dilution. Nair and Ramakrishnan (1973) studied the production of toxic metabolites by Colletotrichum capsici and its role in leaf spot disease of turmeric. The concentrated toxic metabolites from the mycelium (endo toxin) and that from culture filtrate (exotoxin) were bio assayed on turmeric leaves. In treatments with endotoxin as well as exotoxin, visible signs of necrosis were noted within 4h.

2.3 Chemical Control

2.3.1 Laboratory Evaluation

Kothari and Bhatnagar (1966) reported complete inhibition of spore germination of Colletotrichum capsici with ferbam even at the lowest concentration tried (2 ppm). Fytolan and Dithane Z-78

showed complete inhibition of spore germination at 64 and 128 ppm respectively. Chin (1967) observed that Dithane M-45 inhibited growth of Colletotrichum musae. Good control of the fungus Gloeosporium musarum (Colletotrichum musae) was obtained with F 1991 (benomyl), which suppressed the mycelial growth but did not entirely inhibit spore germination. This was found out by Ogawa et al. (1968). Beaudoin et al. (1969) reported that thiabendazole powder suspended in water at 300-400 ppm controlled Gloeosporium musarum, if applied within three days of inoculation. Beccart et al. (1969) showed the inhibition of growth of Colletotrichum musae with benomyl at 500 ppm and thiabendazole at 450 ppm. Shilling ford (1970) found out that moderate control of Gloeosporium musarum causing banana fruit rot was achieved by Dithane M-45 at 2000 ppm. Rippon (1972) reported that best control of Gloeosporium musarum on inoculated banana hands was given by post harvest application of benomyl at 100ppm.

2.3.2 Field evaluation

Control of various leafspot diseases of banana has been attempted by several workers. Carpenter (1918) obtained good control of freckle disease of banana with Bordeaux mixture. Calpouzos et

al. (1959) reported the action of petroleum and silicone oil sprays in the control of sigatoka leaf spot of banana caused by Mycosphaerella musicola. Kapoor and Tandon (1968) observed that Deightoniella torulosa (syd.) Ell. infection in banana could be controlled by Dithane Z-78 and Bordeaux mixture (4:4:50) when repeated applications were made. Cercospora leaf spot, freckle leaf spot and Cordana leaf spot of banana could be controlled by spraying mancozeb and maneb. (Pont, 1970). Brodrick and Kuhne (1971) had used a mixture of mancozeb and white oil for the control of sigatoka disease of banana. Suharban and Paily (1977) observed that one per cent power oil was effective in controlling leaf spot disease of banana.

Cercospora musae infections of young banana leaves could be prevented by spraying the leaves with maneb or zineb at 2 Kg/ha or copper based fungicides at 3 Kg/ha (Anon, 1977). Fungicides alone and mixed with mineral oil for the control of sigatoka leaf spot of banana has been reported by Perez vicente (1978). Fitzpatrick (1980) reported that black leaf streak of banana could be controlled by aerial spraying with mancozeb and or benomyl. Effective control of black sigatoka disease of banana could be achieved with Dithane M-45 in an oil

water emulsion at 2 Kg/ha, 75 per cent Daconil at 1.5, 1.75 and 2 Kg/ha in water or Bravo 6 F at 2 and 2.51 in water or Dithane M-22 in oil water emulsion (Chuang, 1981). A field trial conducted at Banana Research Station, Kannara, Kerala Agricultural University indicated that Dithane M-45 at 0.2 per cent was found to be significantly superior to others in reducing the per cent of leaf infection (Anon, 1982). Chuang et al. (1984) reported that Dithane M-45 in oil water emulsion is more tenacious during rainfall, more inhibitory to conidial germination and gave higher average bunch weight of banana than Dithane M-45 in water only. Eswaramurthy et al. (1988) obtained good control of sigatoka leaf spot of banana with Bavistin at 60-70mg. A field trial conducted at Banana Research station, Kannara, Kerala Agricultural University, proved that sigatoka leaf spot of banana could be effectively controlled by spraying Difolatan (0.3 per cent) or Bordeaux mixture (1 per cent) (Anon, 1985).

2.4. Varietal resistance.

According to Cheeseman and Wardlaw (1937) and Wardlaw (1939) the banana variety monthan (Musa sapidisiaca Jacab.) is susceptible to leaf spot diseases, while another species of Musa, M. balbisiana Colla. is resistant to leaf spot. Simmonds (1939)

observed that initial microscopic spots taken four to eight weeks to develop and hence the youngest leaves of even a heavily infected plant are apparently healthy. The same was also reported by Klein (1959). Nair (1966) reported that three fungi viz., Cordana musae, Septoria keralensis and Macrophoma musae causing leaf spot diseases in banana under Vellayani conditions. With the introduction of Gros Michel and Cavendish varieties, the incidence of sigatoka leaf spot diseases caused by Mycosphaerella musicola was found to be prevalent in Kerala. In all these diseases the youngest leaves were apparently healthy while old ones were infected.

A study conducted by Meredith and Lawrence (1970), found out that varieties belonging to the commonly important triploid group (AAA) were all very susceptible to leaf spot diseases of banana whereas AAB and ABB groups included some moderately susceptible cultivars. The diploids were either very or moderately susceptible. It was also found out that most of the important varieties like Giant and Dwarf Cavendish, Robusta, Valery and Gros Michel were highly susceptible to M.. fijiensis. Ic-2, Silk, the Hawaian bananas and 3 AAB groups were moderatly susceptible and Saba and an unidentified diploid AA were slightly susceptible.

A study on the clonal reaction of banana varieties to leaf spot diseases of banana was conducted by Gopimony (1977). It was observed that most of the varieties belonging to ABB group were either tolerant or highly tolerant. Popular varieties of Kerala like Chemkadali, Ambalakadali, Palayamkodan, Poomkalli, Monthan, Neypoovan and Njalipoovan were found to be tolerent to leaf spot diseases of banana caused by Cordana musae, Septoria keralensis and Macrophoma musae when compared to the introduced varieties like Gros Michel and Robusta.

It was observed by Laville (1983) that genome ems to confer reduced susceptibility to Cercospora disease of banana. Pearson et al. (1983) reported that among the 35 clones showed low levels of susceptibility to black sigatoka, 15 of these are having AA genome. Foure (1987) found out that varieties with genomic combination ABB and AA were more resistant to black leaf streak than in AAA groups.

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1 Survey on the occurrence and severity of leaf blight of banana in different localities in Thiruvananthapuram district.

A survey was conducted in order to find out the occurrence and severity of the various leaf blighting organism(s) in banana in different localities in Thiruvananthapuram district. For this, the three agricultural sub divisions of Thiruvananthapuram district, viz., Nedumangad, Neyyattinkara and Attingal were selected, and the survey was conducted in the banana growing areas of these sub divisions. This was conducted during 1990 to 1992. The survey was carried out on the popular varieties of each locality. The variety, age of the plant, season, type of leaf blight and the various fungi obtained during the survey were recorded. In each field twenty plants were selected randomly. Leaves in each plant were graded by numbering from one, the youngest unfurled leaf consecutively downwards to the oldest upright leaf.

During the survey it was found that the varieties such as Nendran, Monthan, Red banana, Palayamkodan and Rasakadali were severely affected by the leaf blighting organisms irrespective of the locality, followed by

Mysore annan. Among the organisms obtained, leaf blighting disease(s) caused by Colletotrichum musae, Guignardia musae, Khuskia oryzae, Nodulisporium gregarium and Phaeoseptoria sp. were found most destructive in most of the varieties. Hence detailed studies were carried out on these organisms, in different varieties.

The disease intensity was measured by using the following disease grades (Plate 1).

Grade	Disease intensity (per cent)	Description
0	0	No spots
1	5 - 10	2-10 spots
2	10 - 25	10 or more spots
3	26 - 50	Half of the leaf area infected
4	51 - 75	1/2 - 3/4 of the leaf area affected
5	76 - 100	Almost complete infection of leaf

Disease index was calculated using the formula,

$$\text{Disease index (DI)} = \frac{\text{sum of grades of each leaf} \times 100}{\text{Total number of leaves assessed} \times \text{maximum score (5)}}$$

Plate 1. Score chart for Leaf Blight of Banana.

Grade	Description
0	No spots
1	2-10 spots
2	10 or more spots
3	Half of the leaf area infected
4	1/2 - 3/4 of the leaf area affected.
5.	Almost complete infection of leaf.

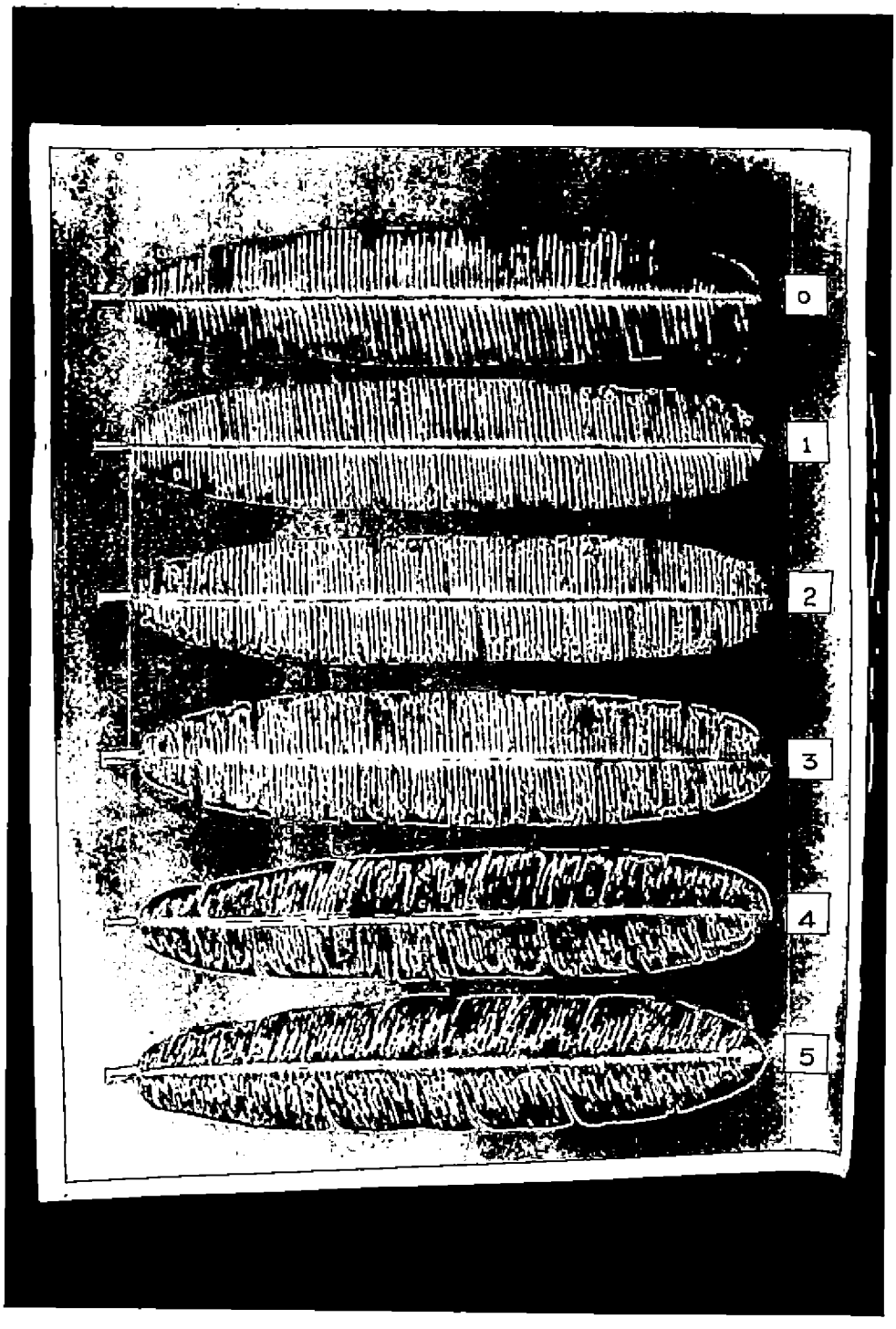


Plate 1

3.2 Isolation of the pathogen(s)

Banana leaves showing initial symptoms of leaf blight were collected from different banana growing localities in each sub divisions and used for the isolation of the pathogens. The infected leaves were washed in tap water, cut into small bits and surface sterilized with 0.1 per cent mercuric chloride solution for one min and washed in two to three changes of sterilized distilled water. These bits were then placed in sterilized petri dishes containing potato dextrose agar (PDA) medium. The dishes were then incubated at room temperature (28^{o}C). After two to three days, when the growth of the fungus was visible, mycelial bits were transferred aseptically to PDA slants.

3.3 Symptomatology of the disease on popular varieties of banana.

Symptoms of the disease was studied by observing naturally infected plants and also following the course of development of the disease under artificial inoculation. Inoculations were made individually and also by combining different organisms on the leaves of young plants and covering it with polythene bag to maintain humidity. Course of

of commencement of symptom development, variation in symptom development and pattern of development were noted in each case.

3.4 Purification and maintenance of the cultures

Fungal cultures were purified by single conidium isolation method. A dilute suspension of about 20 spores per microscopic field was prepared. This suspension was mixed with ten ml plain agar (2 per cent) medium and allowed to solidify. Then the dishes were kept upside down on the stage of the microscope and observed under low power objective. With the help of a needle, the single spores were placed aseptically on PDA. Growth was observed daily and the purified cultures were maintained on PDA slants, at room temperature (28^{o}C) by periodical subculturing and also under refrigerated condition.

3.5 Morphology of the fungi

A detailed study on the morphological characters of the isolated fungi such as nature of mycelium, growth of fungal colony, nature and formation of conidia and their measurements were made.

The colony characters of the fungi were studied by growing them in petri dishes on PDA and incubating at laboratory condition (28^{o}C). After seven days of growth, slide cultures were prepared for the study of ontogeny of conidia following the method described by Riddel (1950).

For confirmation of the isolated organism(s) the cultures were sent to International Mycological Institute (IMI), Kew, Surrey, England and their results were noted. The systematic position of the isolated organism(s) were noted.

3.6 Pathogenicity tests

Pathogenicity of the fungi was tested by artificially inoculating detached young banana leaves with seven day old cultures of the fungi and also in four to five month old plants in the field. For this study the variety Nendran was used. The leaves were surface sterilized with mercuric chloride solution and repeatedly washed with sterile water. Inoculations were done with and without making injury by applying mycelial bits. Controls were also maintained. The leaves were covered with polythene bags to maintain humidity.

Inoculated plants were observed for symptom development and observations were noted.

3.7 Characterisation of the pathogens

3.7.1 Growth and sporulation of the pathogens on different culture media

3.7.1.1 Solid media

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

1. Potato dextrose agar (PDA)
2. Czapek (Dox) agar
3. Richard's agar
4. Banana leaf extract agar
5. Carrot leaf extract agar

The composition of the media used a
under Appendix-I.

The media were prepared and steri
autoclaving at 1.05 kg/cm^2 for 15 min. It wa
and poured into sterilized dishes at the rate of 15 ml in each dish and allowed to solidify. Circular mycelial discs of five mm dia cut out by means of a sterile cork borer from the outer edge of a seven day old PDA culture of the fungi were placed in the centre of each dish. The plates were incubated ar room

temperature (28°C). Observations were recorded when full growth of the fungus was obtained in any one of the medium tested. The observations were analysed statistically and is presented.

3.7.1.2. Liquid Media

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

1. Potato dextrose broth
2. Czapek (Dox) medium
3. Richard's medium
4. Banana leaf extract
5. Carrot leaf extract

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

The media were prepared and poured into 250 ml conical flasks at the rate of 50 ml and sterilised by autoclaving at 1.05 kg/cm^2 for 15 min. Each flask was inoculated with circular discs of five mm dia cut out from the edge of seven day old culture plates by means of sterile cork borer. The flasks were incubated at room temperature (28°C). After 15 days of incubation, the culture was filtered through previously weighed Whatman No.1 filter paper and the dry weight of

the mycelium was determined when two consecutive weights were obtained. For each treatment three replications were maintained. Observations were recorded and analysed statistically.

3.7.2. Utilisation of different carbon sources on the growth of the pathogens

The best liquid medium for each fungus was selected and this was used as the base medium for this study. Various carbon compounds such as dextrose, inositol, maltose and starch were substituted for sucrose in the basal medium so as to give an equivalent amount in the each case. Controls were kept without adding any carbon source.

50ml medium was dispensed into 250 ml conical flasks and autoclaved at 1.05 kg/cm^2 for 15 min. The medium was inoculated with culture discs cut out from an actively growing seven day old culture and incubated at room temperature. The mycelial growth was harvested after 15 days and the dry weight was recorded in each case. Three replications were maintained for each treatment. Observations were recorded and analysed statistically.

3.7.3. Utilisation of different nitrogen sources on the growth of the pathogens

The effect of both organic and inorganic forms of nitrogen on the growth of the fungus was studied. They were substituted for the nitrogen source in the basal medium. The concentration of different nitrogen compounds were so adjusted as to contain an equivalent amount that is present in the basal medium. Inorganic sources such as sodium nitrite, sodium nitrate and potassium nitrate and organic sources such as asparagine and glutamine were tried. Controls were kept without adding any nitrogen source.

50 ml of the medium was taken in 250 ml. conical flask and sterilised at 1.05 kg/cm^2 for 15 min. They were inoculated with mycelial discs cut out from seven day old fungal cultures and incubated at room temperature. After 15 days of growth, mycelial growth was filtered, dried and dry weight was recorded. Three replications were maintained for each treatment. Observations were recorded and analysed statistically.

3.7.4 Effect of various media on toxin production

An attempt has been made to assess the capacity of the pathogens in the production of toxic metabolites if any, in the culture medium. For this the following liquid media were used.

1. Potato dextrose broth
2. Czapek (Dox) medium
3. Richard's medium
4. Host extract dextrose medium
5. Carrot extract medium

Composition of the media are given in Appendix-I.

Each medium was prepared and taken in 250 ml conical flasks at the rate of 50 ml and sterilised by autoclaving at 1.05 kg/cm^2 for 15 min. The medium was inoculated with 5 mm dia mycelial discs cut out from seven day old cultures. The flasks were incubated at room temperature. After 15 days of incubation, the cultures were filtered through Whatman No.1 filter paper. The comparative toxic activity of the filterate was studied by the following bioassay method.

Banana leaves detached from the young plants were inoculated with culture filterate. The leaves were incubated and observed for lesion development. Leaves treated with sterile water, served as control. Observations were recorded and expressed in four grades, according to necrotic area produced in each leaf.

3.7.5. Host range studies of the pathogens

Host range of the pathogens studied by inoculating the following plants as these plants were found very common in banana growing areas. Inoculations were done both on detached leaves and also on standing crops with culture bits with and without injury. The inoculated plants were covered with polythene bags for maintaining humidity and the symptom development was observed and recorded by comparing with that in the control plants without any culture bits.

Plants used for host range study

Common name	Botanical name
1) Tapioca	*Manihot esculenta* Crantz.
2) Nutmeg	*Myristica fragrans* Houtt.
3) Clerodendron	*Clerodendron infortunatum* Gaertn.
4) Cocoa	*Theobroma cacao* L.
5) Colocasia	*Colocasia esculenta* (L.) Schott.
6) Clove	*Eugenia caryophyllata* Thumb.

3.8 Laboratory evaluation of fungicides against the pathogens

The following fungicides at three different concentrations each as shown below were used for the laboratory evaluation against the pathogens.

Sl. No.	Trade name	Generic name	Chemical name	Concentration (ppm)
1.	--	Bordeaux mixture	Copper sulphate - lime mixture	2500, 5,000, 10,000
2.	Foltaf	Captafol	Cis-N-(1,1,2,2, tetra chloroethyl thio)-4- cyclo hexene 1,2-dicarbo-ximide.	1000, 2000, 3000
3.	Bavistin	Carbendazim	Methyl-2-benzimida-zole carbamate.	250, 500, 1000.
4.	Dithane M-45	Mancozeb	Manganese ethylene bisdithio carbamate and zinc ions.	1000, 2000, 3000.
5.	Calixin	Tridemorph	2,6 - dimethyl-4-tridecyl morpholine	250, 500 1000.

Inhibition of growth of the fungus was studied by poisoned food technique (Zentmyer, 1955).

The required quantity of each of the fungicides was weighed out and added to 50 ml of sterilised PDA 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 with mycelial discs of five mm dia. cut out from an actively growing colony of the fungi. Controls consisting of PDA without any fungicides, were also inoculated. The dishes were then

incubated at room temperature (28°C) and observed daily for the growth of the fungi. Observations were taken when growth was completely covered in the control plates, and recorded. For each treatment three replications were maintained. Per cent inhibition of growth of different fungi 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.

Recorded observations were analysed statistically and presented.

3.9 Field evaluation of fungicides

Among the five organisms viz, *Colletotrichum musae*, *Guignardia musae*, *Khuskia oryzae*, *Nodulisporium gregarium* and *Phaeoseptoria sp*, causing leaf blighting diseases in banana, the most frequently occuring blighting was the one caused by *Colletotrichum musae*. Hence a field trial was conducted to assess the efficacy of the fungicides in the management of the blighting caused by *C. musae*.

The trial was conducted in a farmer's banana field at Balaramapuram under Neyyatinkara sub division.

The three fungicides viz., Bordeaux mixture, Dithane M-45 and Mineral oil at the recommended concentration (1, 0.2 and 1 per cent respectively) were used in the field.

Plants were selected randomly from an existing plantation. There were seven replications for each treatment. The banana variety used was Nendran. The plants were given two sprayings with the fungicides first on the 90th day of planting and the second two weeks after the first spraying. Pre and post treatment observations on disease intensity by each fungus was recorded. The disease scoring was done using the score chart mentioned under item No: 3.1 and the Disease Index was calculated using the formula,

$$\text{Disease Index (DI)} = \frac{\text{Sum of grades of each leaf} \times 100}{\text{Total number of leaves assessed} \times \text{Maximum score (5}}$$

Disease Index obtained was tabulated and analysed statistically.

3.10 Scoring the leaf blight intensity in promising varieties of banana.

The intensity of leaf blighting in banana caused by the common leaf blighting organism(s) was carried out. The disease scoring was done on the following banana varieties under natural conditions

viz., Nendran, Red banana, Palayamkodan and Rasakadali. The experiment was conducted in a banana field at College of Agriculture, Vellayani. For each variety, there were ten replications. The observations on disease scoring was done from the second month of planting. Three observations were recorded for each variety at an interval of one month. The disease index (DI) in each case was calculated using the method mentioned under item No.3.1.

RESULTS

4. RESULTS

4.1. Survey on the occurrence and severity of leaf blight of banana in different localities in Thiruvananthapuram district.

The results of the survey conducted at the three agricultural sub divisions of Thiruvananthapuram district are presented in the tables 1,2 and 3.

The common varieties cultivated in the three agricultural sub divisions include Nendran, Red banana, Palayamkodan, Rasakadali, and Mysore annan. It was found that the varieties such as Nendran, Red banana, Palayamkodan and Rasakadali were severly affected by the leaf blighting organism(s) followed by Mysore annan. During the survey it was observed that old leaves showed severe leaf blighting than young ones. Disease intensity was high in the case of four to five month old plants. Occurrence and spread of the disease were more during rainy season. Among the three sub divisions, disease intensity was more severe in Nedumangad, in the variety Nendran.

4.2. Isolation of the patnogen(s)

The causal organism(s) isolated from the samples collected from the infected leaf tissue include

Table 1. Details of survey conducted at Nedumangad Agricultural Sub Division

Sl.No.	Fungi Isolated	Varieties	Season	Symptoms
1.	Colletotrichum musae (Berk. and M.A.Curtis) Von Arx. and Curvularia sp.	a) Red banana	April 1991	Isolated from Vattappara and Chellamcode areas. Symptoms characterised as black coloured lesions, enlarged and became oval to irregular shaped with grey centre, black margin without yellow halo. In advanced stages, spots coalesced to form patches. Clear spots also appeared on the lower side of the leaf.
		b) Mysore annan	April 1991	Same as above
2.	Guignardia musae Racib.	a) Nendran	August 1991	Isolated from Chellamcode area. Symptoms characterised by small dark brown to black streaks parallel to veins. The streaks expands laterally to form elliptical spots. In advanced stages, centre of the spots dries out becoming light grey, but a narrow dark brown to black border exist. Yellow halo present around these spots. Coalescing of these spots lead to scorching of leaves. petiole collapses and leaves hang down from the pseudostem.

b) Red banana	August 1991	Isolated from Chellamcode area. Symptoms are characterised as small dark brown streaks, enlarged and became broader, than the above case. The spots became 'eye' shaped with grey centre, dark brown margin and a yellow halo. Coalescing of these spots lead to entire blighting of leaf lamina.
c) Poovan	September 1991	Isolated from Peringamala area. Characterised by small dark brown to black streak parallel to veins. The streaks expand laterally to form elliptical spots. In advanced stages, centre of the spots dries out becoming light grey, but a narrow dark brown to black border exist. A yellow halo was present around these spots, Coalescing of these spots lead to scorching of leaves. Petiole collapses and leaves hangdown from the pseudostem.
d) Vella-palayamkodan	September 1991	Same as above

3.	Khuskia oryzae H.J. Hudson	Robusta	September 1991	Symptoms started as small dark brown coloured streaks along the veins through out the leaf lamina, in chains. Later these streaks elongated and became linear spots with grey centre, dark brown to black margin and yellow halo around. These spots coalesced to form pale brown coloured patches from tip inwards.
4.	Nodulisporium gregarium (Berk and M.A.Curtis) J.A. Meyer	Red banana	September 1991	Isolated from Peringamala area. Characterised as dark brown to black lesions from mid rib or base of the leaf lamina. Coalescing of these lesions lead to the formation of large patches surrounded by yellow halo. Sometimes spots became oval shaped with grey centre and dark brown margin.

Table 2. Details of survey conducted at Neyyattinkara Agricultural Sub Division

Sl.No.	Fungi Isolated	Varieties	Season	Symptoms
1.	Colletotrichum musae	a) Nendran	April 1991	Isolated from Vellayani area. Symptoms are characterised as oval shaped spots along the veins through out the lamina. Spots are having dark brown centre, indefinite margin and yellow halo around. Later the centre dries out becoming grey coloured and dark brown margin. In advanced stages, spots coalesced to form patches of blighted area.
		b) Red banana	April 1991	Same as above
2.	Guignardia musae Racib.	a) Red banana	June 1991	Isolated from Vellayani area. Symptoms are characterised as small dark brown streaks, enlarged and became broader. The spot become 'eye' shaped with grey centre, dark brown margin and a yellow halo. Coalescing of these spots lead to entire blighting of leaf lamina.

3. <u>Pestalotiopsis versicolor</u> (Speg.) Stey.	a) Nendran	August 1991	Isolated from Balaramapuram area. Symptoms started as minute circular dark brown spots surrounded by a yellow halo. Later turned into elongated forms. On maturity these spots became dull white at the centre surrounded by dark brown margin and yellow halo and became oval, elongate or irregular shaped. Coalescing of such spots resulted in the formation of large patches from midrib to margin.
4. <u>Phaeoseptoria sp.</u>	Rasakadali	May 1991	Isolated from Vellayani area. Symptoms started as grey coloured patches from the margins of the leaf lamina having a dark brown margin and yellow halo. On maturity these patches covered the entire area. Small black coloured dots appeared on the blighted area were the pycnidia of the fungus.

Table 3. Details of survey conducted at Attingal Agricultural Sub Division

Sl.No.	Fungi Isolated	Variety	Season	Symptoms
1.	Colletotrichum musae (Berk. and M.A.Curtis) Von Arx.	a) Nendran	September 1991	Isolated from Chirayinkil area. Symptoms are characterised as oval shaped spots along the veins throughout the lamina. Spots are having dark brown centre and indefinite margin and yellow halo around. Later the centre dries out becoming grey coloured and dark brown margin. In advanced stages, spots coalesced to form patches of blighted area.
		b) Palayamkodan	September 1991	Isolated from Chirayinkil area. Symptoms same as above.
2.	Pestalotiopsis versicolor (Speg.) Stey.	a) Nendran	August 1991	Isolated from Kilimanoor area. Symptoms started as minute circular dark brown spots surrounded by an a yellow halo. Later turned into elongate forms. On maturity, these spots became dull white at the centre surrounded by dark brown margin and yellow halo, oval, elongate or irregular shaped. Coalescing of such spots resulted in the formation of large patches from midrib to margin.
		b) Rasakadali	September 1991	Isolated from Kilimanoor area. Same as above.

Colletotrichum musae (Berk. and M.A Curtis) Von Arx., Curvularia sp., Guignardia musae Racib., Khuskia oryzae H.J.Hudson., Nodulisporium gregarium (Berk. and M.A. Curtis) J.A Meyer., Pestalotiopsis versicolor (Speg.) Stey. and Phaeoseptoria sp. These organisms were brought to pure culture and maintained on potato dextrose agar medium.

4.3. Symptomatology of the disease on popular varieties of banana

Pattern of symptom development varied with the variety, age of the plant and season. Same pathogen caused different symptoms in different varieties. Mixed infections were also observed in some varieties.

Colletotrichum musae and Curvularia sp. combinedly caused leaf blighting symptoms in red banana and Mysore annan varieties of banana. This type of combined infection could be collected from Vattappara and Chellamcode areas of Nedumangad sub-division. The symptoms are characterised by black coloured lesions initially and later enlarged and became oval to irregular in shape with grey centre and black margin (plate 2). In advanced stages, these spots coalesced to form large patches. The same type of symptoms were noticed on the lower side of the leaves also.

Plate 2. Leaf blight caused by the mixed infection of *C. musae* and *Curvularia sp.*

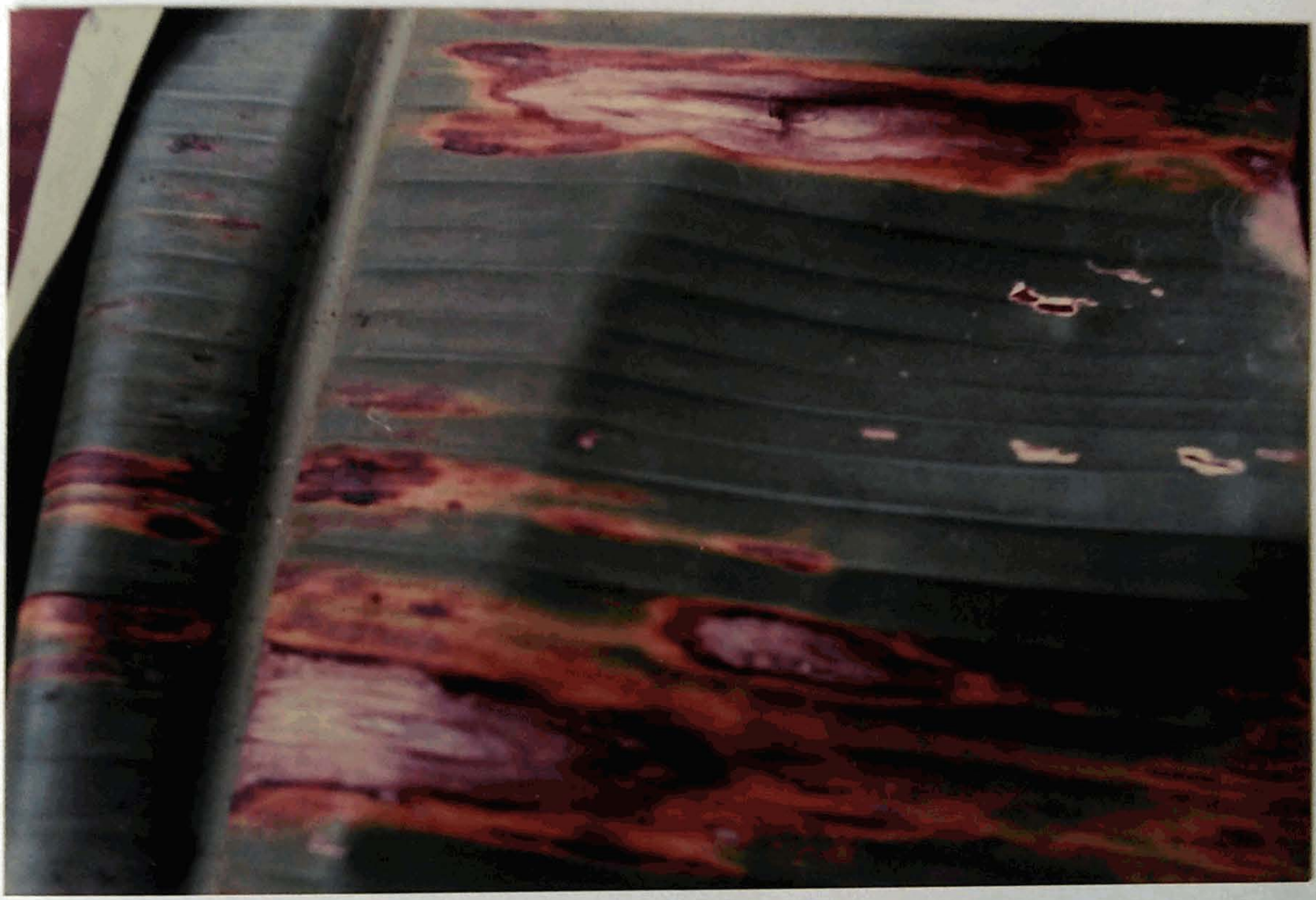


Plate 2

Nendran and Palayamkodan varieties were infected by Colletotrichum musae. The symptoms produced in both the varieties are more or less same with small oval shaped spots appearing along the veins. Spots having dark brown centre and indefinite margins developed initially. Later these spots became grey coloured at the centre surrounded by dark brown margin and yellow halo (plate 3). Severe cases of infection lead to entire blighting of the leaf lamina.

The fungus Guignardia musae Racib. caused leaf blighting in Nendran and Red banana. These type of symptoms could be collected from Nedumangad and Neyyattinkara sub-divisions. In Nendran, symptoms are characterised by small dark brown to black streaks parallel to the veins. The streaks expand laterally to form elliptical spots. In advanced stages, centre of the spots dried out becoming light grey, but a narrow dark brown to black border existed (plate 4). Yellow halo was present around these spots. Coalescing of these spots lead to scorching of leaves. Petiole collapse and leaves hang down from the pseudostem.

In red banana, symptoms produced by Guignardia musae vary with respect to the shape of the spot, even though the initial symptoms appeared to be similar. Here, the spots are comparatively broader

Plate 3. Leaf Blight caused by *C. musae*

Plate 4. Leaf Blight caused by *G. musae*

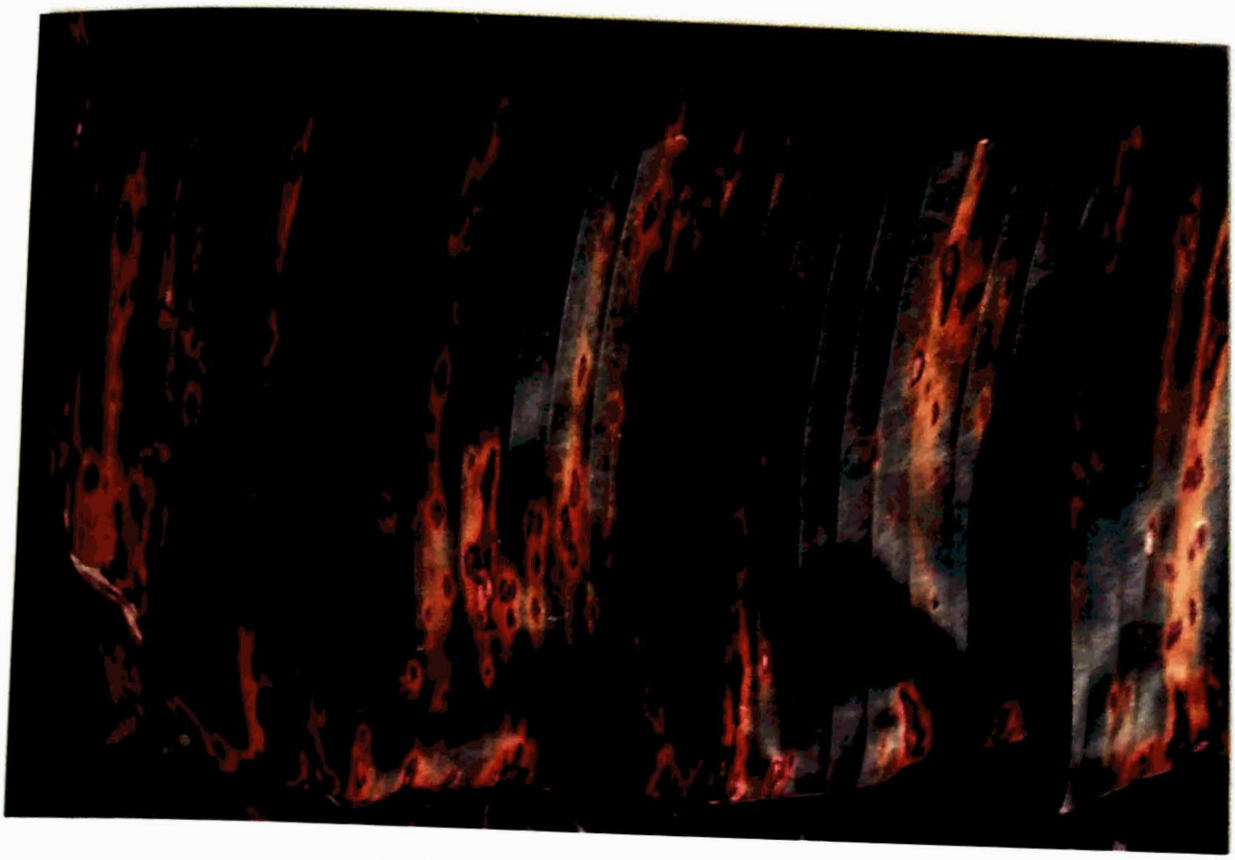


Plate 3

Plate 4

than the above case with a characteristic 'eye' shape, a grey centre, dark brown margin and a yellow halo. The same fungus also caused blighting in Poovan and Vellapalayamkodan varieties collected from Peringamala farm in Nedumangad sub-division.

The fungus Khuskia oryzae could be obtained from the samples collected from Peringamala farm in Nedumangad sub-division in the variety, Robusta. The symptoms are characterised by small dark brown coloured streaks along the veins through out the leaf lamina in chains. Later these streaks elongated and became linear spots with ashy grey centre, dark brown to black margin and yellow halo around (plate 5). These spots coalesced to form pale brown coloured patches leading to blighting of the leaves from tip onwards.

Symptoms on the variety red banana incited by the fungus Nodulisporium gregarium, showing leaf blighting were collected from Peringamala area under Nedumangad sub division. The symptoms initiated as dark brown to black lesions from midrib or base of the leaf lamina (plate 6). Coalescing of these lesions lead to the formation of large patches surrounded by a yellow halo. Sometimes the spots became oval in shape, with grey centre and dark brown margin.

Plate 5. Leaf Blight caused by *K. oryzae*

Plate 6. Leaf Blight caused by *N. gregarium*

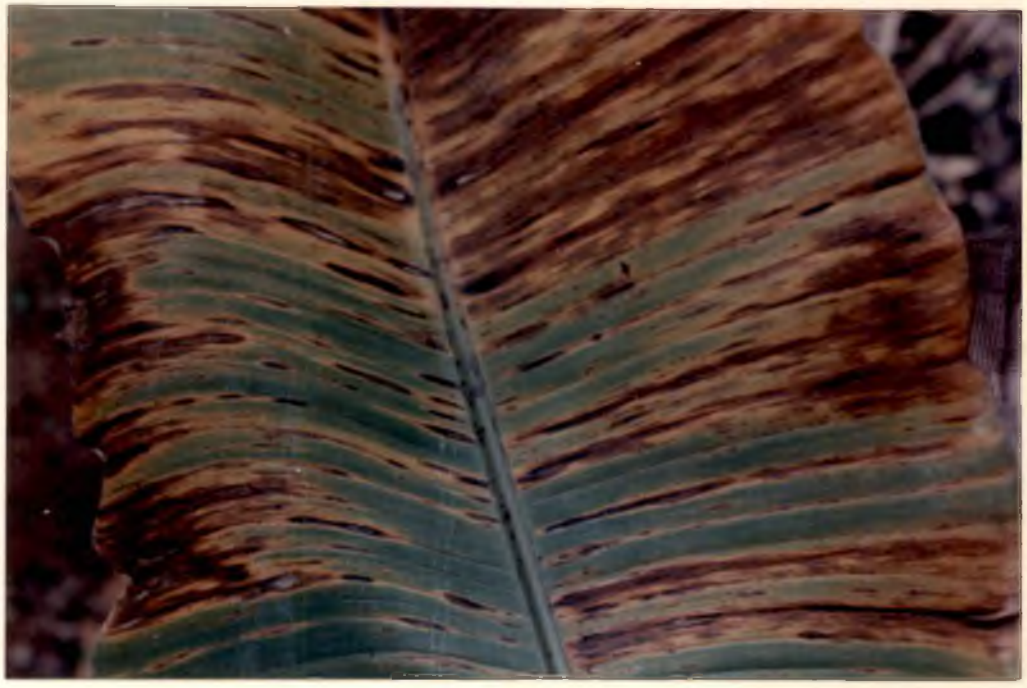


Plate 5

plate 6

The varieties such as Nendran and Rasakadali showed another type of leaf blighting incited by Pestalotiopsis versicolor. These symptoms could be observed from samples collected from Neyyattinkara and Attingal sub divisions. In both the varieties symptoms are characterised by minute circular dark brown spots surrounded by a yellow halo. Later these spots turned into elongated lesions. On maturity, these spots/lesions became oval, elongate or irregular shaped with dull white centre surrounded by dark brown margin and yellow halo around (plate 7). Coalescing of such spots resulted in the formation of large patches from midrib to margin.

Yet another type of leaf blighting was observed in Rasakadali collected from Vellayani area of Neyyattinkara sub division incited by Phaeoseptoria sp. Symptoms are characterised as grey coloured patches from the margins of the leaf lamina having dark brown border and yellow halo. On maturity these patches covered the entire lamina. Small black coloured dots appeared on the blighted area representing the fruiting bodies of the fungus (plate 8).

4.4 Purification and maintenance of the cultures

Fungal cultures purified by single condium isolation were maintained on PDA slants, at room temperature and also under refrigerated condition.

Plate 7. Leaf Blight caused by *P. versicolor*

Plate 8. Leaf Blight caused by *Phaeoseptoria sp.*

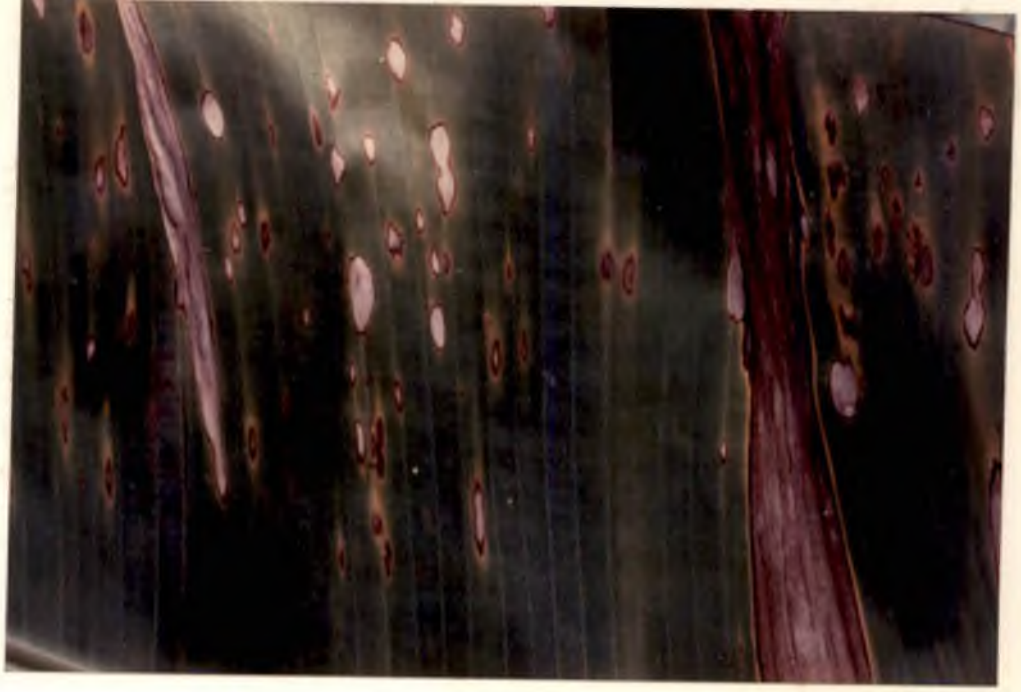


Plate 7

Plate 8

4.5 Morphology of the pathogens

4.5.1 *Colletotrichum musae* (Berk. and M.A. Curtis) Von Arx. (IMI type culture No.351354)

Colonies are formed with abundant aerial mycelium, becoming grey with age, with profuse orange coloured conidial masses. Microscopic observation of the culture revealed the presence of black coloured, branched, septate mycelium. Sclerotia and setae were absent. Conidia are formed in dark brown coloured acervuli. Conidia are somewhat cylindrical or oval in shape with constricted centre, rounded ends and nonseptate with 7-14 x 2-6 micron in size (plate 9). Based on the above mentioned characters, the fungus was identified as *Colletotrichum musae* (Berk. and M.A. Curtis) Von Arx., coming under the class Deuteromycetes and order Melanconiales, of the family Melanconiaceae.

4.5.2 *Curvularia sp.*

The colour of the colony on PDA was creamy white and velvetty. Mycelium is septate, profusely branched and light brown in colour with coloured and unbranched conidiophores. Conidia are boat shaped, generally three celled but rarely having four cells, (plate 10). Middle cell coloured and broader than the

Plate 9. Conidia of *C. musae* (X 200)

Plate 10. Conidia of *Curvularia sp.* (X 200)

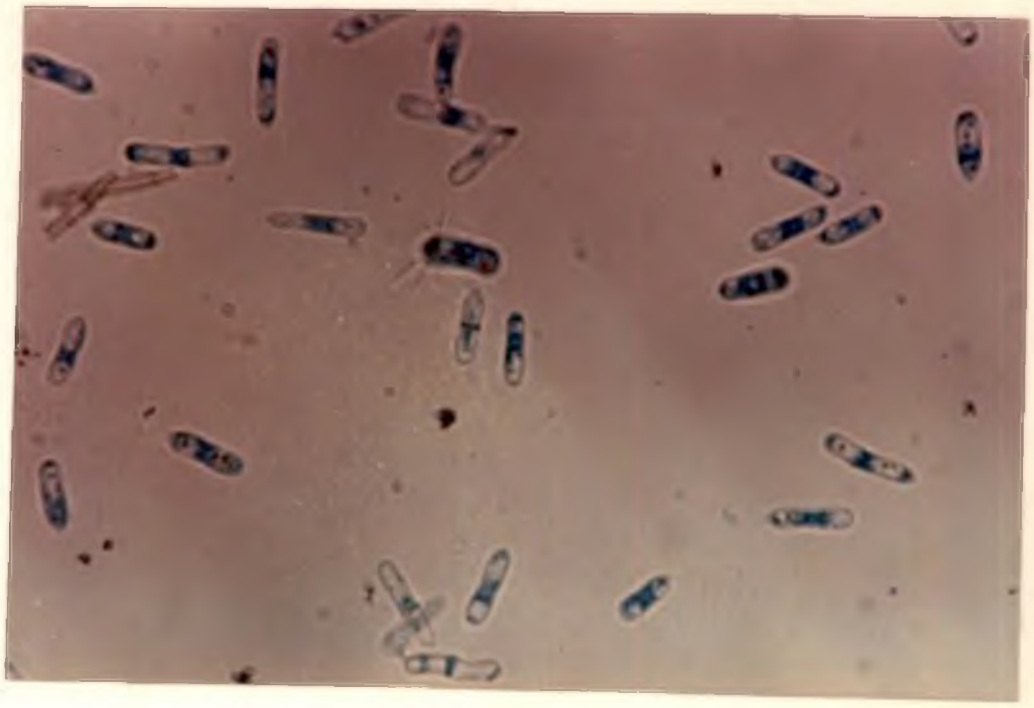


Plate 9

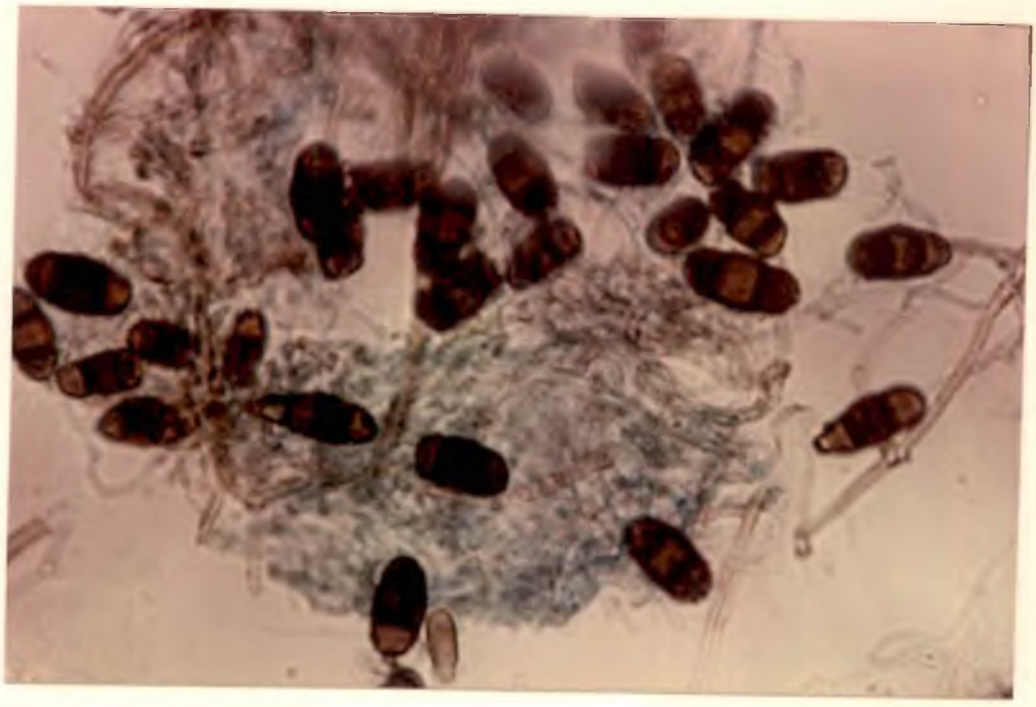


Plate 10

other two cells. End cells are pale coloured. The basal cell having a scar indicating the point of attachment to the conidiophore. The spores measured 15-20 x 4.75-7.5 micron in size. Based on these characters, the organism was identified as *Curvularia sp.* coming under the class Deuteromycetes and order Moniliales of the family Dematiaceae.

4.5.3 *Guignardia musae* Racib. (IMI type culture No.351351)

Colonies are slow growing and black coloured. Margins smooth or lobed. Microscopic observations revealed the presence of dark brown, septate and branched mycelium. Chlamydospores are present. Pycnidia brown to black solitary or in groups. Conidia are hyaline, roughly spherical with gelatinous envelope and 10-15 micron in dia and formed inside the fruiting structures. Pycnidia are often intermixed with black coloured ascocarp; asci subclavate to cylindrical and eight spored. Ascospores are hyaline, unicelluar, with obtuse ends and 10-14 x 6-7 micron in size (Fig. 1). Based on these characters, the organism was identified as *Guignardia musae* Racib., belonging to the class Ascomycetes and order Sphaeriales of the family Polystigmataceae.

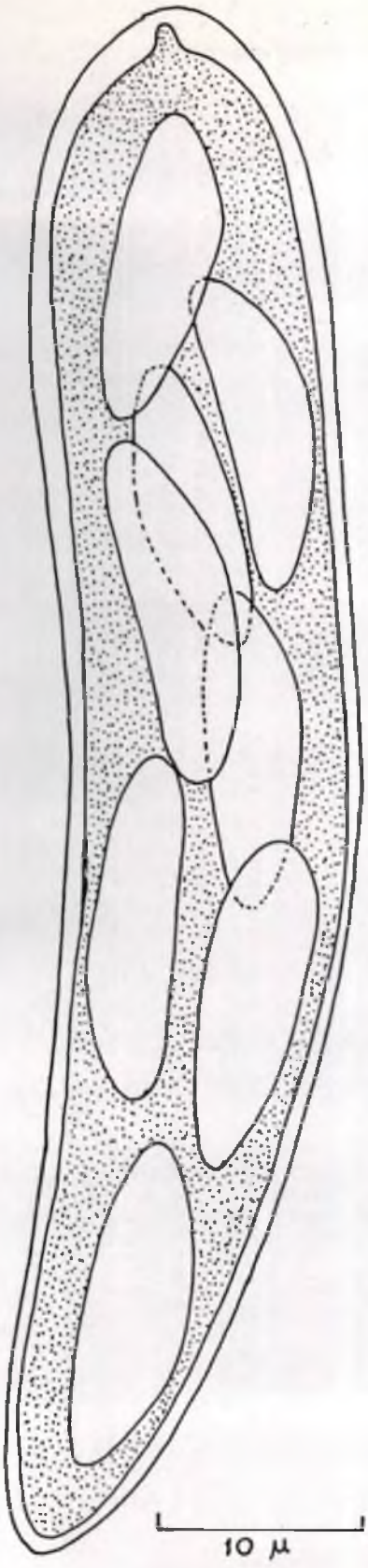


FIGURE. 1.

ASCUS AND ASCOCARP OF *Guignardia musae*

At first colonies are white in colour with small shining black conidia, later became brown or black (Fig.2). Conidia are brown coloured, oval in shape measuring 13-15.5 x 10-13 micron in size, borne singly on short inflated stalks. Based on these characters, the organism was identified as Khuskia oryzae H.J. Hudson., the perfect state of Nigrospora oryzae (Berk. and Br.) Petch. belonging to the class Deuteromycetes and order Moniliales of the family Dematiaceae.

4.5.5 Nodulisporium gregarium (Berk. and M.A. Curtis) J.A. Meyer. (IMI type culture No.351355)

Organism exhibited reddish brown colonies gradually became black coloured. Hyphae are dark brown, septate and branched. Conidiophores erect, pale olivaceous brown, septate, racemosely branched, branches arise immediately below the septa, shorter than parent branches. Conidia produced on sporogenous cells which are wide towards the base and tapering above terminating into some what flattened apex with many denticles (Fig. 3). Conidia are produced singly

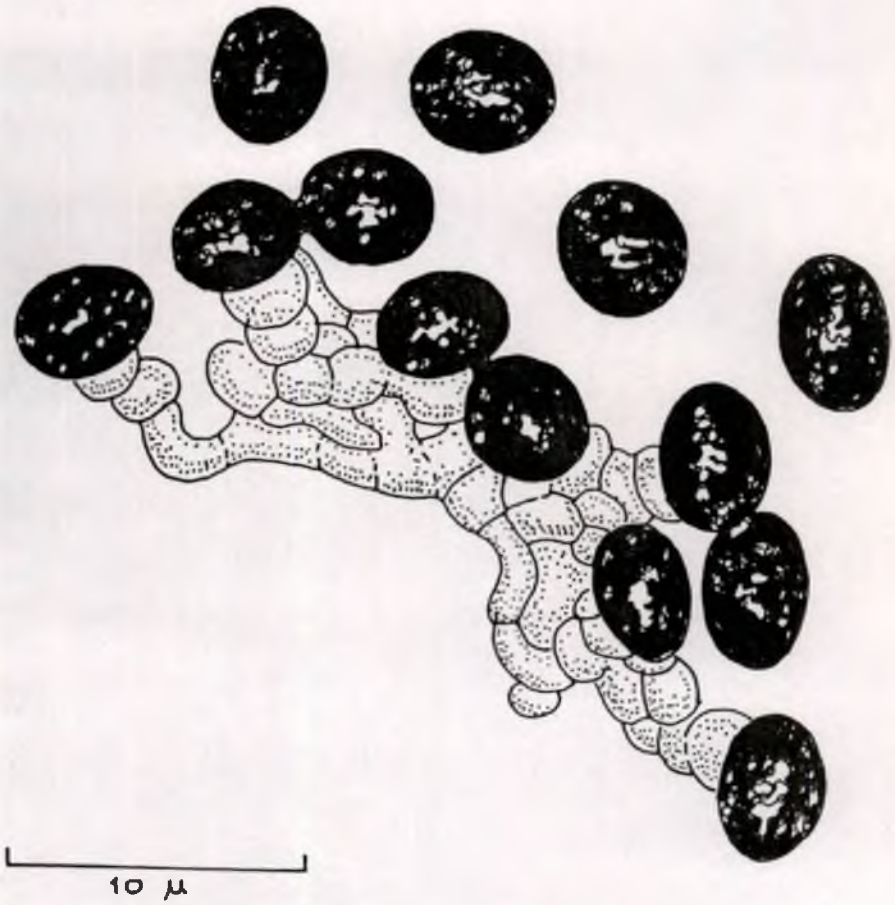


FIGURE. 2.

CONIDIA OF *Khuskia oryzae*

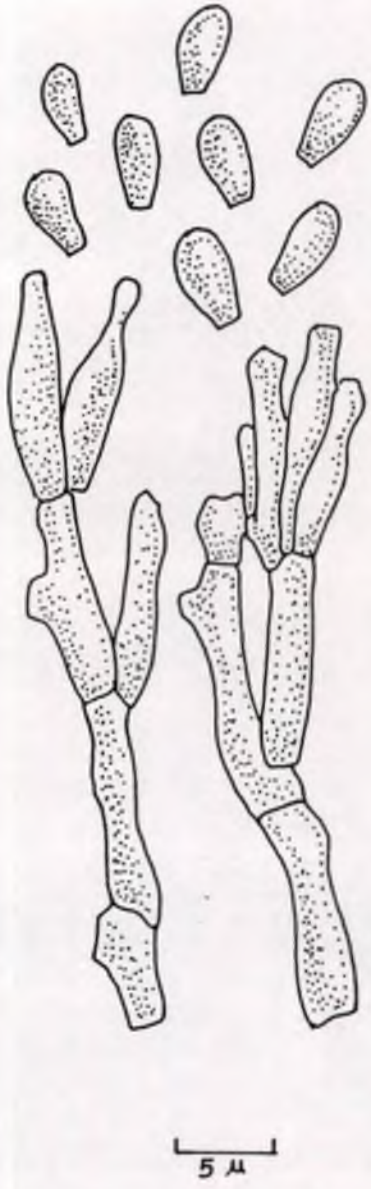


FIGURE. 3.

CONIDIA OF *Nodulisporium gregarium*.

on the denticles globose, subhyaline, apiculate at the base, 3.7 to 5.4 micron in dia. Based on these characters, the organism was identified as Nodulisporium gregarium (Berk. and M.A. Curtis) J.A. Meyer. coming under the class Deuteromycetes and order Moniliales and family Dematiacease.

4.5.6 Pestalotiopsis versicolor (Speg.) Stey.

(IMI type culture No. 351353)

White cottony growth of the mycelium and black coloured fruiting bodies appeared on the mycelial mat after four to five days. Conidia are five celled clavate or elliptic, 18-22.5 x 6-8 micron in dia; intermediate cells coloured 13-17 micron long; upper two coloured cells fuliginous, opaque, swollen; lowest coloured cells olivaceous, constricted at the dividing septa; apical cells are hyaline, short, conic, cylindric consisting of three divergent setulae 7.5-20 micron long; basal cells hyaline and short, pedicels upto 5 micron long. Based on these characters the organism was identified as Pestalotiopsis versicolor (Speg.) Stey. belonging to the class Deuteromycetes and order Melanconiales of the family Melanconiaceae.

4.5.7 Phaeoseptoria sp. (IMI type culture No.351348)

Colonies which are white at first became dark coloured later, with black coloured round structures appearing on the mycelial mat. Pycnidia are embedded on the leaf tissue, subglobose to globose, narrowly ostiolate with membraneous pseudoparenchymatous wall (Plate 11). It produced yellowish brown conidia which are cylindrical, straight to fusiform with rounded base and septate. Based on these characters the organism was identified as Phaeoseptoria sp. belonging to the class Coelomycetes and order Sphaerosidales.

4.6 **Pathogenicity tests**

Artificial inoculation studies conducted revealed that all the fungi could infect the leaves more easily on young ones than on old ones. Similarly the injured leaves showed the symptoms of disease development earlier than the uninjured leaves. However, both the leaves showed the blighting symptoms at a later stage, and they are more or less the same. Successful infection could be obtained when the leaves were inoculated with culture bits of the respective fungi. Even though initial symptoms developed after four to five days of inoculation, it took eight to ten days to develop typical symptoms of blighting in both the cases. The symptoms produced by each pathogen are described below.

Plate 11. pycnidia of *Phaeoseptoria sp.* (X 200)

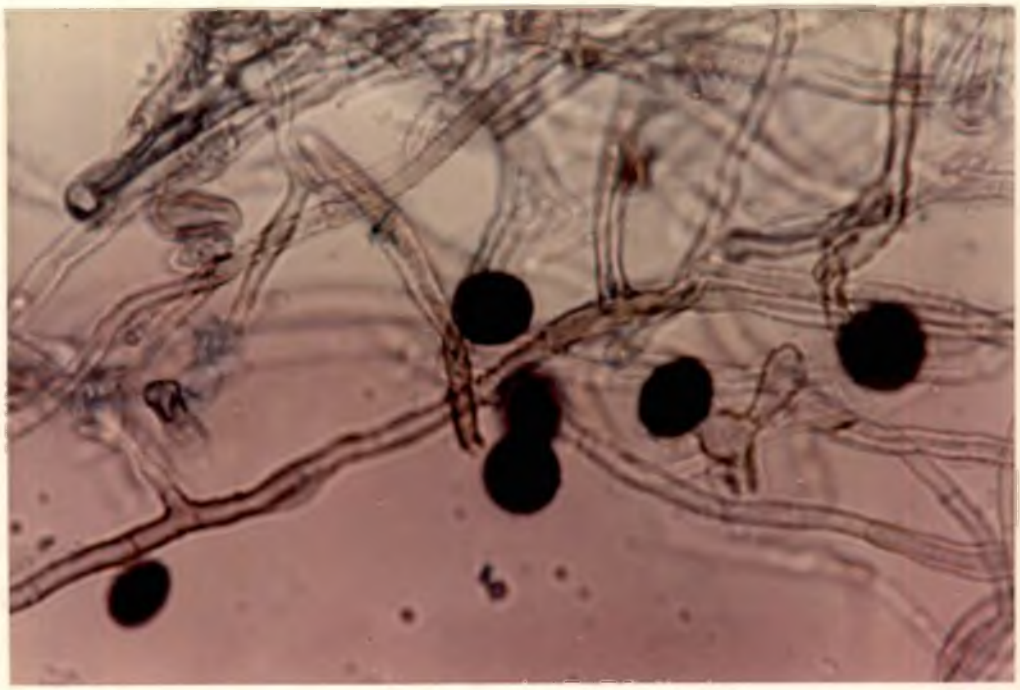


Plate 11

4.6.1 Colletotrichum musae

Symptom development was very slow in the case of C. musae by culture bit inoculation as well as by spore suspension spraying. Symptoms started as small dark brown spots, enlarged upto one mm dia.

4.6.2 Curvularia sp

Symptoms developed as dark brown coloured lesions, which enlarged to form patches.

4.6.3 Guignardia musae

Symptoms started four to five days of inoculation as pale brown to dark brown spots. Gradually the spots enlarged and became oval in shape. A yellow halo could be seen around these spots in due course.

4.6.4 Khuskia oryzae

Symptoms started as small dark brown to black coloured lesions. Gradually enlarged and coalesced to form dark brown patches. Symptoms started only after four to five days of inoculation.

4.6.5 Nodulisporium gregarium

Symptoms started five to six days after inoculation as small reddish brown coloured dots.

These dots enlarged in size and coalesced to form long lesions, having dark brown centre, pale brown margin and a yellow halo. These lesions extended upto the margin.

4.6.6 Pestalotiopsis versicolor

Small oval shaped spots developed having grey coloured centre, pale brown diffused margin and yellow halo. Symptoms started on the third day of inoculation.

4.6.7 Phaeoseptoria sp.

Symptoms developed as small black coloured dots later enlarged with papery white centre and brown margin. Black coloured fruiting bodies could be observed on the surface of these spots. Coalescing of spots lead to formation of some what larger lesions.

4.7 Characterisation of the Pathogens

4.7.1 Growth and sporulation of the pathogens on different culture media

4.7.1.1 Solid media

4.7.1.1.1 Colletotrichum musae

The effect of the different solid media on the growth and sporulation of the pathogen was studied.

The mean radial growth and cultural characters of the organism in different media are presented in the table 4 (Fig. 4). The results of the study revealed that Richard's agar was the best medium for the growth of the fungus followed by banana leaf extract agar and potato dextrose agar. Good sporulation was also obtained in Czapek (Dox) agar.

Statistical analysis of the data revealed that Richard's agar is significantly superior (9.33) to all other media used. There was no significant difference exist between banana leaf extract agar and potato dextrose agar. The same was the case with potato dextrose and Czapek (Dox) agar.

4.7.1.1.2 Guignardia musae

The mean radial growth and growth characters of the organism is presented in the table 5 (Fig.5). The results of the study revealed that Richard's agar was the best medium for the growth of the fungus followed by Czapek (Dox) agar and potato dextrose agar. The growth was very poor in carrot leaf extract agar. Good sporulation was observed in all the media except carrot leaf extract agar.

Statistical analysis of the data revealed that Richard's agar is significantly superior (8.49) to

Table 4. Growth and sporulation of Colletotrichum musae on different solid media

Sl. No.	Medium	* Mean colony dia in mm.	Colony characters
1.	Potato dextrose agar	81.00 (8.99)	White fluffy growth of the mycelium was observed on the second day of inoculation later became black in colour. Orange coloured conidial masses observed on the mycelial mat.
2.	Czapek (Dox) agar	80.00 (8.94)	Dirty white mycelial growth from the second day of inoculation. Later became dark coloured. Orange coloured conidial masses appeared on the mycelial mat after one week.
3.	Richard's agar	87.00 (9.33)	Dirty white coloured mycelial growth from the second day of inoculation, became dark coloured later. Rapid growth and good sporulation was observed.
4.	Banana leaf extract agar	82.00 (9.06)	Started as white coloured growth, became dark coloured later. Orange coloured conidial masses appeared on the mycelial mat.
5.	Carrot leaf extract agar	45.50 (6.74)	Scanty growth of dirty white mycelial growth was observed. No sporulation was observed even after two weeks of inoculation.

CD = 0.09
(0.05)

* Average of 3 replications.
values in parenthesis indicate transformed values.

Fig. 4. Growth of *Collectotrichum musae* on different solid media

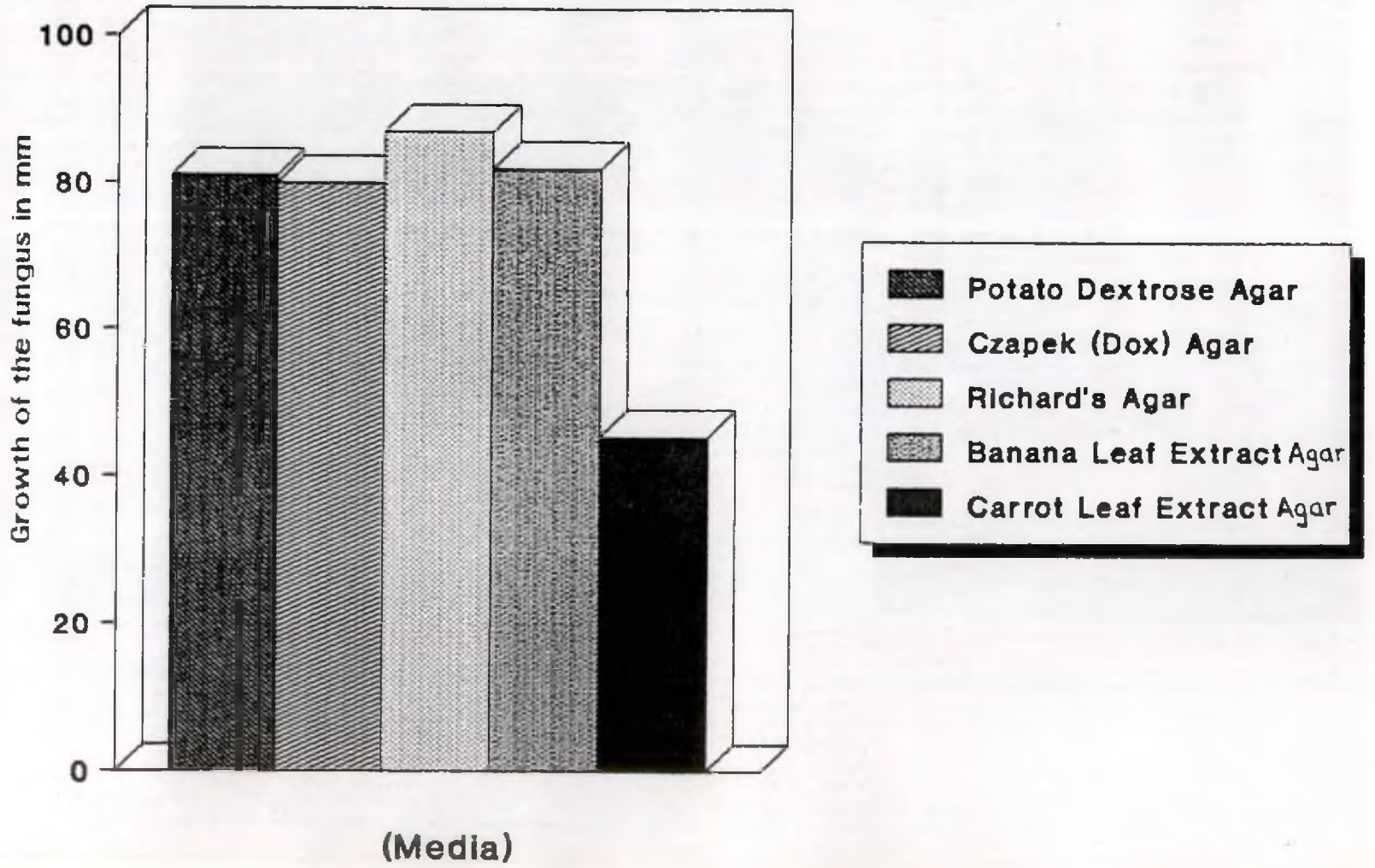


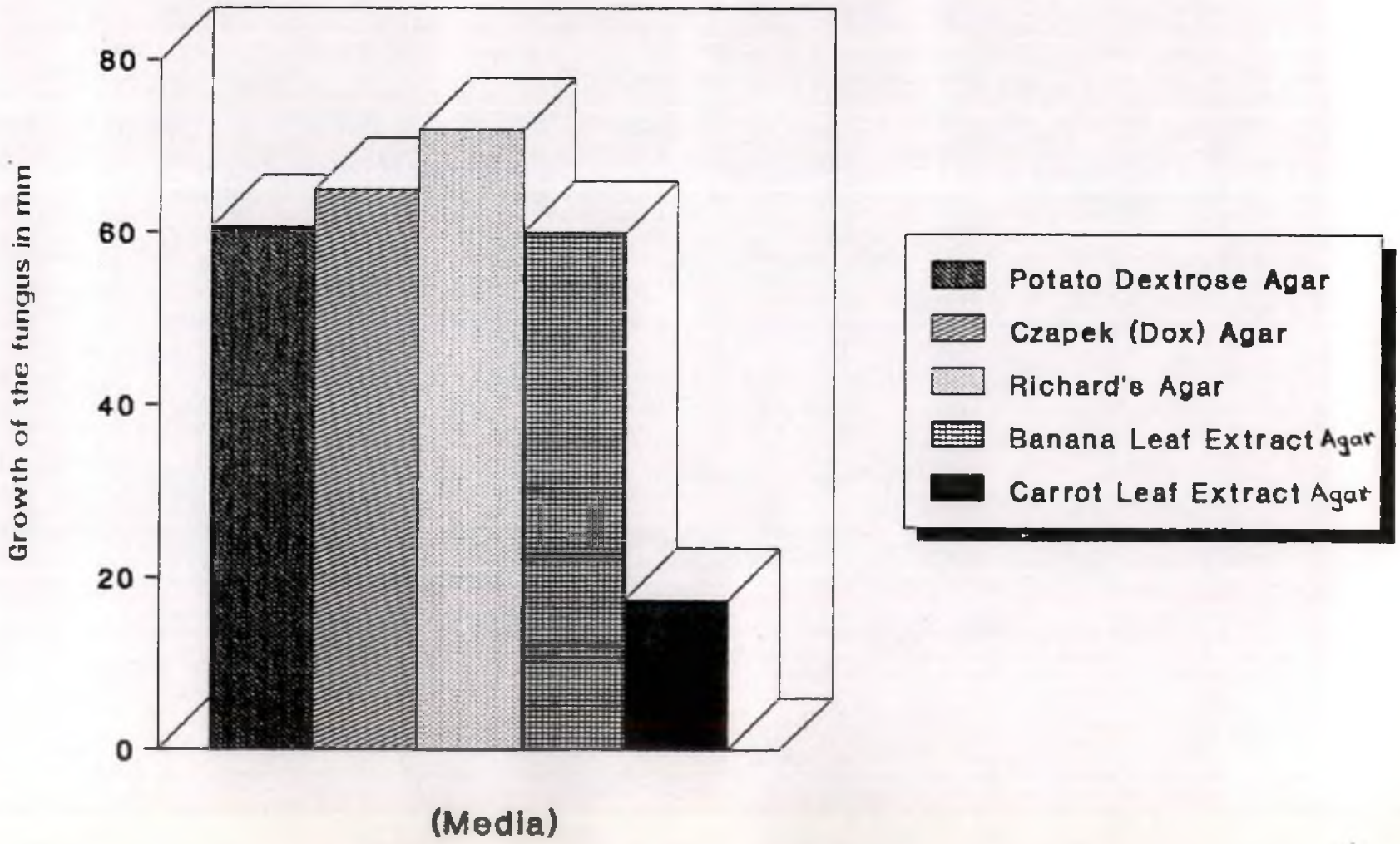
Table 5. Growth and sporulation of Guignardia musae on different solid media

Sl. No.	Medium	*Mean colony dia in mm	Colony characters
1.	Potato dextrose agar	60.66 (7.79)	Growth started on the third day of inoculation as black coloured and puckered mycelial growth. Margins became lobed later. Good sporulation.
2.	Czapek (Dox) agar	65.00 (8.06)	Black coloured mycelial growth. Good sporulation was observed
3.	Richard's agar	72.00 (8.49)	Black coloured mycelial growth which was puckered. Good sporulation was observed.
4.	Banana leaf extract agar	60.00 (7.75)	Ashy to black mycelial growth. Abundant sporulation was observed.
5.	Carrot leaf extract agar	17.50 (4.18)	Ashy black coloured growth. Slow growing. No sporulation.

CD = 0.27
(0.05)

*Average of 3 replications
values in parenthesis indicate transformed values.

Fig. 5. Growth of *Guignardia musae* on different solid media



all other media used. Potato dextrose agar and Czapek (Dox) agar are equally effective. No significant difference exist between potato dextrose agar and banana leaf extract agar.

4.7.1.1.3 Khuskia oryzae

The mean radial growth and growth characters of the organism in different media are presented in table 6 (Fig. 6). The results of the study showed that potato dextrose agar was the best medium followed by Richard's agar and Czapek (Dox) medium. Good sporulation was observed in potato dextrose agar.

Statistical analysis of the data revealed that potato dextrose agar is found significant (9.27) to all other media used. No significant difference exist between Richard's agar and Czapek (Dox) agar.

4.7.1.1.4 Nodulisporium gregarium

The mean radial growth and growth characters of the organism in different media are presented in the table 7 (Fig. 7). The results of the study showed that potato dextrose agar was the best medium for growth of the fungus followed by banana leaf extract agar, Richard's agar and Czapek (Dox) agar. Growth was comparatively poor in carrot leaf extract agar. Good sporulation was obtained in potato dextrose agar.

Table 6. Growth and sporulation of Khuskia oryzae on different solid media

Sl. No.	Medium	* Mean colony dia in mm	Colony characters
1.	Potato dextrose agar	86.00 (9.27)	Growth started as dirty white coloured mycelium gradually turn into pinkish tinge. Good sporulation was obtained.
2.	Czapek (Dox) agar	77.00 (8.77)	Growth started as dirty white coloured colonies, became dark ash coloured and white centre.
3.	Richard's agar	78.00 (8.83)	Growth started as cottony white mycelia which became intermixed with cream colour. Centre of the colony was found dark in colour.
4.	Banana leaf extract agar	60.50 (7.77)	Dirty white mycelial growth. No sporulation.
5.	Carrot leaf extract agar	67.50 (8.16)	Dirty white mycelial growth. No sporulation

CD = 0.17
(0.05)

* Average of 3 replications
Values in parenthesis indicate transformed values.

Fig. 6. Growth of *Khuskia oryzae* on different solid media

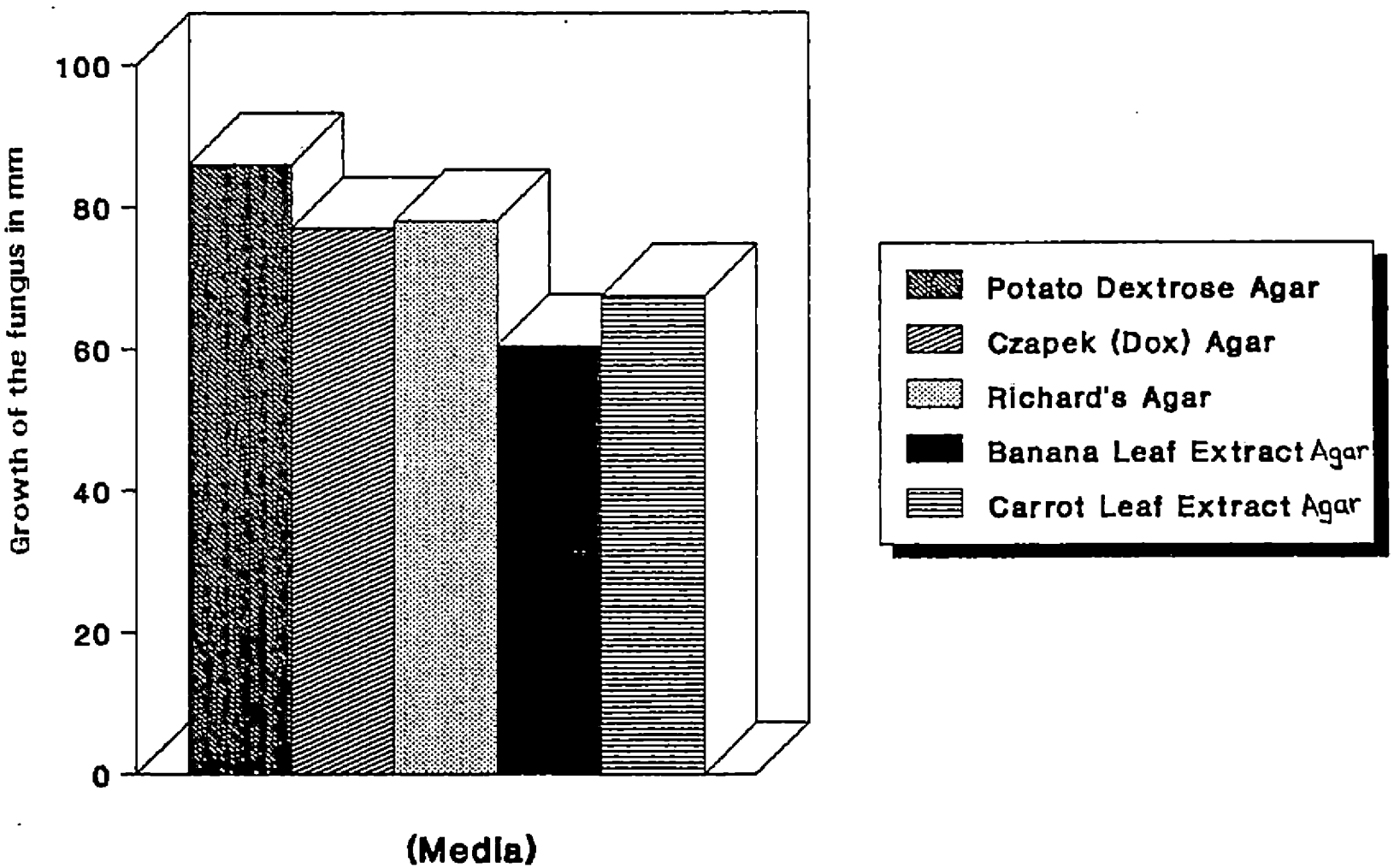


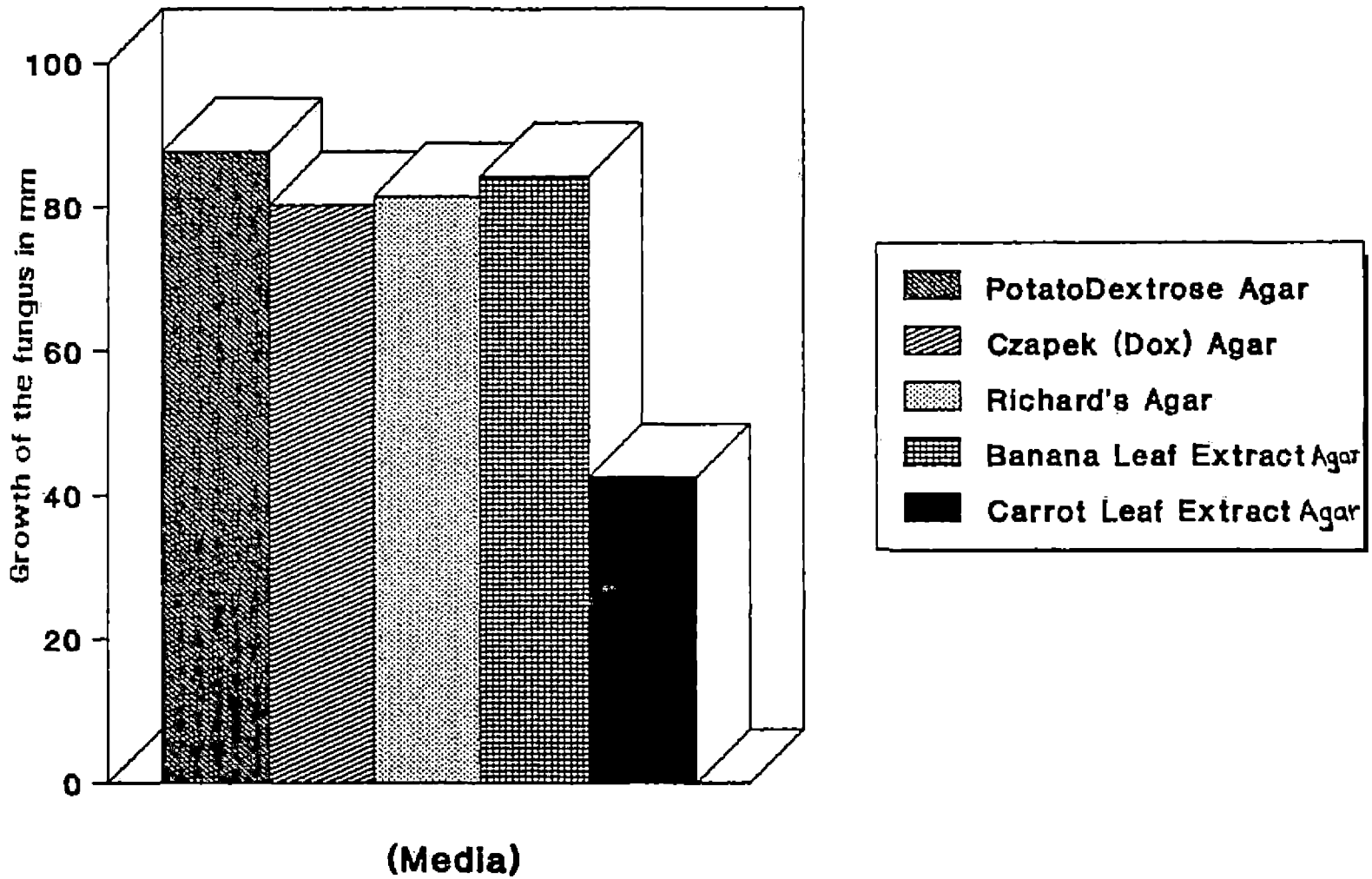
Table 7. Growth and sporulation of *Nodulisporium gregarium* on different solid media

Sl. No.	Medium	*Mean colony dia in mm	Colony characters
1.	Potato dextrose agar	87.75 (9.37)	Dirty white coloured mycelial growth became yellowish brown after three to four days. Good sporulation was observed.
2.	Czapek (Dox) agar	80.33 (8.96)	Dirty white coloured mycelial growth later yellowish brown in colour, Fair sporulation.
3.	Richard's agar	81.50 (9.03)	Dirty white coloured mycelial growth became yellowish brown later. Meagre sporulation.
4.	Banana leaf extract agar	84.25 (9.18)	Dirty white to pale brown coloured mycelial growth. No sporulation was observed.
5.	Carrot leaf extract agar	42.50 (6.52)	Scanty mycelial growth. No sporulation.

CD = 0.06 (0.05)

* Average of 3 replications.
Values in parenthesis indicate transformed values.

Fig. 7. Growth of *Nodulisporium gregarium* on different solid media



Statistical analysis of the data revealed that potato dextrose agar is significantly superior (9.37) to all other media. Carrot leaf extract is found to be a poor medium (6.52) for the growth of the fungus.

4.7.1.1.5 Phaeoseptoria sp

The mean radial growth and growth characters of the organism in different media are presented in the table 8 (Fig. 8).

The results of the study showed that potato dextrose agar was the best medium for growth of the fungus followed by Czapek (Dox) agar and carrot leaf extract agar. Sporulation was abundant on potato dextrose agar.

Analysis of the data revealed that potato dextrose agar was statistically significant (9.29) to all other media except Czapek (Dox) agar. No significant difference existed between carrot leaf extract agar and banana leaf extract agar. The same was the case with potato dextrose agar and Czapek (Dox) agar.

4.7.1.2. Liquid media:

Mean dry weight of the mycelium obtained in different media for five leaf blighting pathogens are presented in the table 9 (Fig. 9).

Table 8. Growth and sporulation of *Phaeoseptoria sp.* on different solid media

Sl. No.	Medium	* Mean colony dia in mm.	Colony characters
1.	Potato dextrose agar	86.33 (9.29)	Growth started as ashy white and later became dark coloured. Whitish tinge appeared on the centre. Good sporulation.
2.	Czapek (Dox) agar	73.00 (8.54)	Dirty white mycelial growth, became dark coloured later. Good sporulation.
3.	Richard's agar	26.66 (5.14)	Slow growing. Started as dirty white mycelial growth. Meagre sporulation.
4.	Banana leaf extract agar	49.66 (7.02)	Growth started as dirty white coloured mycelium became dark coloured later. Scanty growth of mycelium.
5.	Carrot leaf extract agar	58.33 (7.64)	Growth started as dirty white mycelial growth, dark coloured later. No sporulation.

CD = 0.84 (0.05)

* Average of 3 replications
Values in Parenthesis indicate transformed values.

Fig. 8. Growth of *Phaeoseptoria sp.* on different solid media

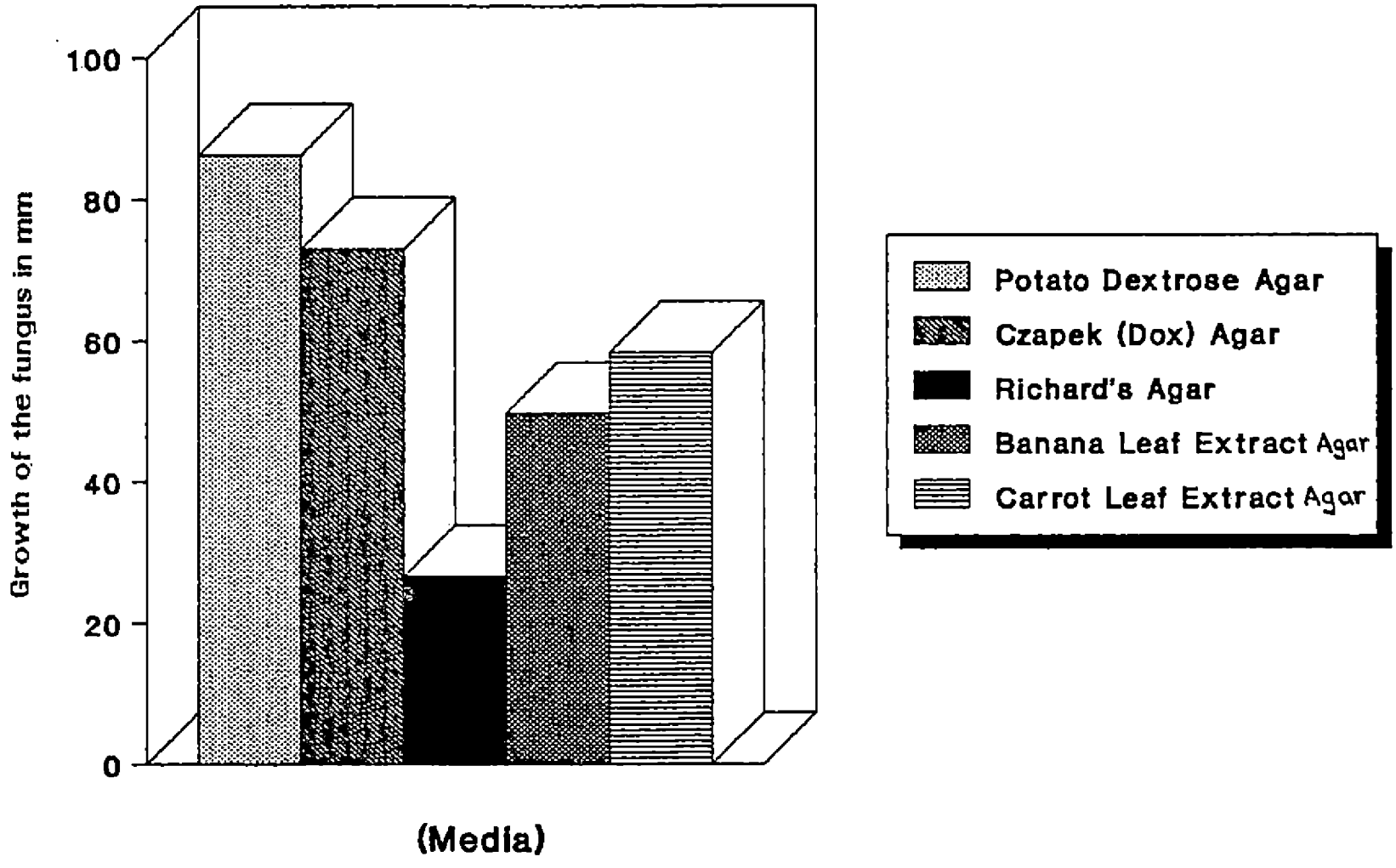
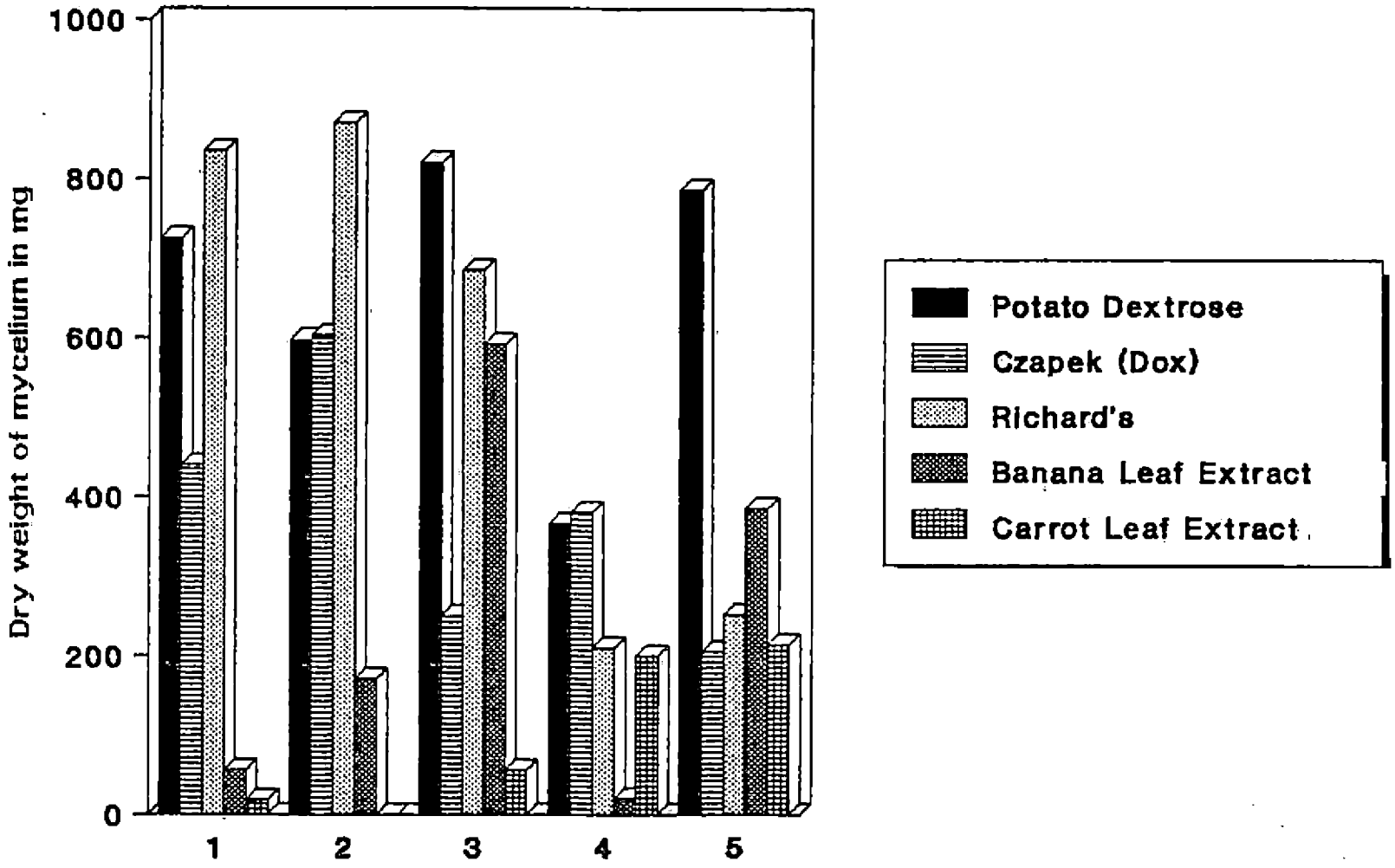


Table 9. Growth and sporulation of five leaf blighting pathogens on different liquid media

Pathogen	Liquid media					
	Potato dextrose	Czapek (Dox)	Richard's	Banana leaf extract	Carrot leaf extract	CD (0.05)
1. Colletotrichum musae	*726.00 (2.87)	440.00 (2.65)	836.00 (2.93)	56.00 (1.82)	18.00 (1.45)	0.01
2. Guignardia musae	598.00 (2.78)	604.00 (2.79)	871.00 (2.95)	171.00 (2.26)	0.00 (1.00)	0.002
3. Khuskia oryzae	821.00 (2.92)	251.00 (2.42)	686.00 (2.81)	592.50 (2.78)	56.25 (1.82)	0.002
4. Nodulisporium gregarium	366.00 (2.58)	380.00 (2.59)	210.00 (2.34)	20.00 (1.48)	200.00 (2.32)	0.01
5. Phaeoseptoria sp	786.00 (2.90)	205.00 (2.33)	252.00 (2.42)	386.00 (2.59)	215.00 2.35)	0.003

* Mean dry weight of mycelium (Average of three replications)
Values in parenthesis indicate transformed values.

Fig. 9. Growth of five leaf blighting pathogens on different liquid media



4.7.1.2.1 Colletotrichum musae

Maximum dry weight was obtained in Richard's solution followed by potato dextrose broth and Czapek (Dox) medium. Growth was very poor in banana leaf extract and carrot leaf extract. Good sporulation was obtained in potato dextrose broth, Czapek (Dox) medium and Richard's solution.

Statistical analysis of the data revealed that Richard's solution is significantly superior (2.93) to all other media used.

4.7.1.2.2 Guignardia musae

Maximum dry weight was obtained in Richard's broth followed by Czapek (Dox) medium. No growth was obtained in carrot leaf extract. Good sporulation was also obtained on potato dextrose agar and banana leaf extract agar.

Statistical analysis of the data revealed that Richard's broth is significantly superior (2.95) to all other media used.

4.7.1.2.3 Khuskia oryzae

Results of the laboratary study indicated that potato dextrose broth was the best medium for the

growth of the fungus followed by Richard's solution. Growth was very poor in carrot leaf extract.

Statistical analysis of the data showed that potato dextrose broth is significantly superior (2.92) to all other media used.

4.7.1.2.4 Nodulisporium gregarium

Results of the study showed that Czapek (Dox) medium was the best medium for the growth and sporulation of N. gregarium followed by potato dextrose broth. Poor growth was obtained in banana leaf extract.

Statistical analysis of the data revealed that potato dextrose broth and Czapek (Dox) medium are on par, followed by Richard's broth and carrot leaf extract.

4.7.1.2.5 Phaeoseptoria sp.

Maximum dry weight was obtained in potato dextrose broth followed by banana leaf extract. Growth was poor in carrot leaf extract and Czapek (Dox) medium. Good sporulation was obtained in all media except carrot leaf extract.

Analysis of the data showed that potato dextrose broth is statistically significant (2.90) to all other media used followed by banana leaf extract.

4.7.2. Utilisation of different carbon sources on the growth of the pathogen(s)

Mean dry weight of the mycelium obtained in five carbon sources for five leaf blighting pathogens in banana are presented in the table 10 (Fig. 10).

4.7.2.1. Colletotrichum musae

Results of the study showed that, maximum growth was obtained in maltose followed by sucrose and starch. Least growth was obtained in inositol.

Statistical analysis of the data revealed that maltose was significantly superior (3.13) to all other sources used.

4.7.2.2 Guignardia musae

Results showed that, maximum growth was obtained in starch followed by maltose, dextrose and inositol. Sucrose was found to be a poor carbon source for the growth of G. musae.

Statistical analysis of the data revealed that starch was significantly superior (2.91) to all other carbon sources used followed by maltose (2.86).

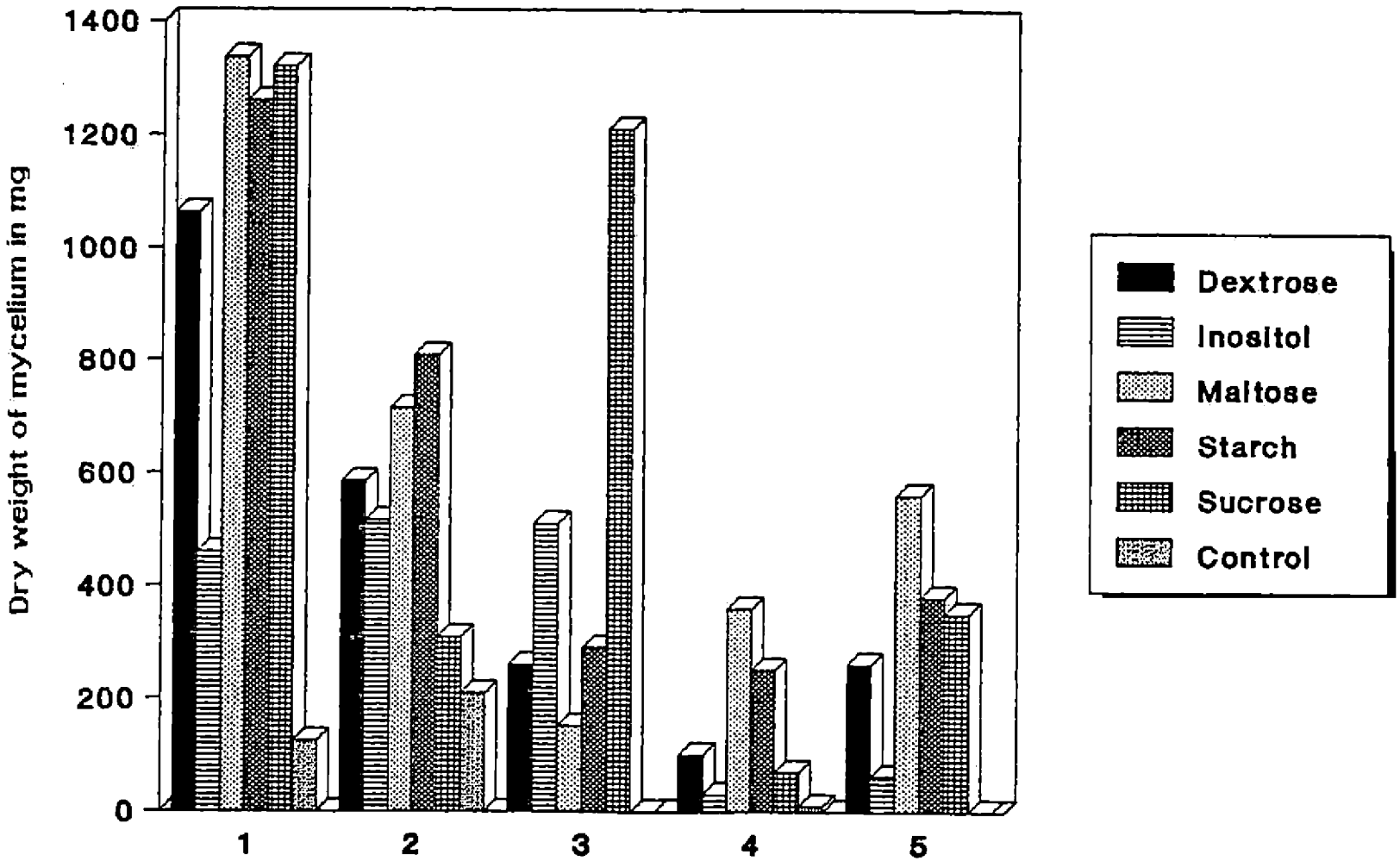
Table 10. Growth of five leaf blighting pathogens on different carbon sources

Pathogens	Carbon sources						
	Dextrose	Inositol	Maltose	Starch	Sucrose	Control	CD (0.05)
1. *Colletotrichum musae*	*1062.00 (3.03)	461.00 (2.67)	1338.00 (3.13)	1261.00 (3.10)	1322.00 (3.12)	125.00 (2.13)	0.002
2. *Guignardia musae*	586.60 (2.78)	516.67 (2.72)	716.07 (2.86)	810.00 (2.91)	310.00 (2.51)	210.00 (2.34)	0.002
3. *Khuskia oryzae*	261.10 (2.43)	512.11 (2.72)	152.11 (2.21)	291.10 (2.44)	1211.00 (3.09)	0.00 (1.00)	0.04
4. *Nodulisporium gregarium*	101.33 (2.01)	30.66 (1.49)	360.12 (2.55)	251.67 (2.41)	70.11 (1.85)	10.13 (1.01)	0.001
5. *Phaeoseptoria sp.*	261.00 (2.43)	62.00 (1.86)	561.00 (2.76)	381.00 (2.59)	352.00 (2.56)	0.00 (1.00)	0.004

* Mean dry weight of the mycelium in mg.

Figures in parenthesis indicate transformed values.

Fig. 10. Growth of five leaf blighting pathogens on different carbon sources



4.7.2.3 Khuskia oryzae

From the table it is clear that sucrose was the best carbon source for the growth of the fungus followed by inositol, starch and dextrose.

Statistical analysis of the data revealed that sucrose was significantly superior (3.09) to all other media used. No significant difference existed between starch and dextrose. Least growth was obtained in maltose (2.21)

4.7.2.4 Nodulisporium gregarium

Results of the study showed that best carbon source for the growth of the fungus was maltose followed by starch, dextrose and sucrose. Inositol was found to be the least carbon source for the growth of Nodulisporium gregarium.

Statistical analysis of the data revealed that maltose was significantly superior (2.55) to all other sources tried, followed by starch (2.41).

4.7.2.5 Phaeoseptoria sp.

Among the five carbon sources used, maltose was found to be the best one for the growth of Phaeoseptoria sp. followed by starch, sucrose and dextrose. Very poor growth was obtained in inositol.

Statistical analysis of the data revealed that maltose is significantly superior (2.76) to all other media used.

4.7.3 Utilisation of different nitrogen sources on the growth of the pathogens

Mean dry weight of the mycelium obtained in the five nitrogen sources for five leaf blighting fungi are presented in the table 11 (Fig. 11).

4.7.3.1 Colletotrichum musae

Results of the study showed that best nitrogen source for the growth of the fungus was sodium nitrate followed by asparagine and potassium nitrate. No growth was obtained in sodium nitrite.

Statistical analysis of the data revealed that sodium nitrate was significantly superior (3.28) to all other nitrogen sources used, followed by asparagine (3.26) and potassium nitrate (3.09).

4.7.3.2 Guignardia musae

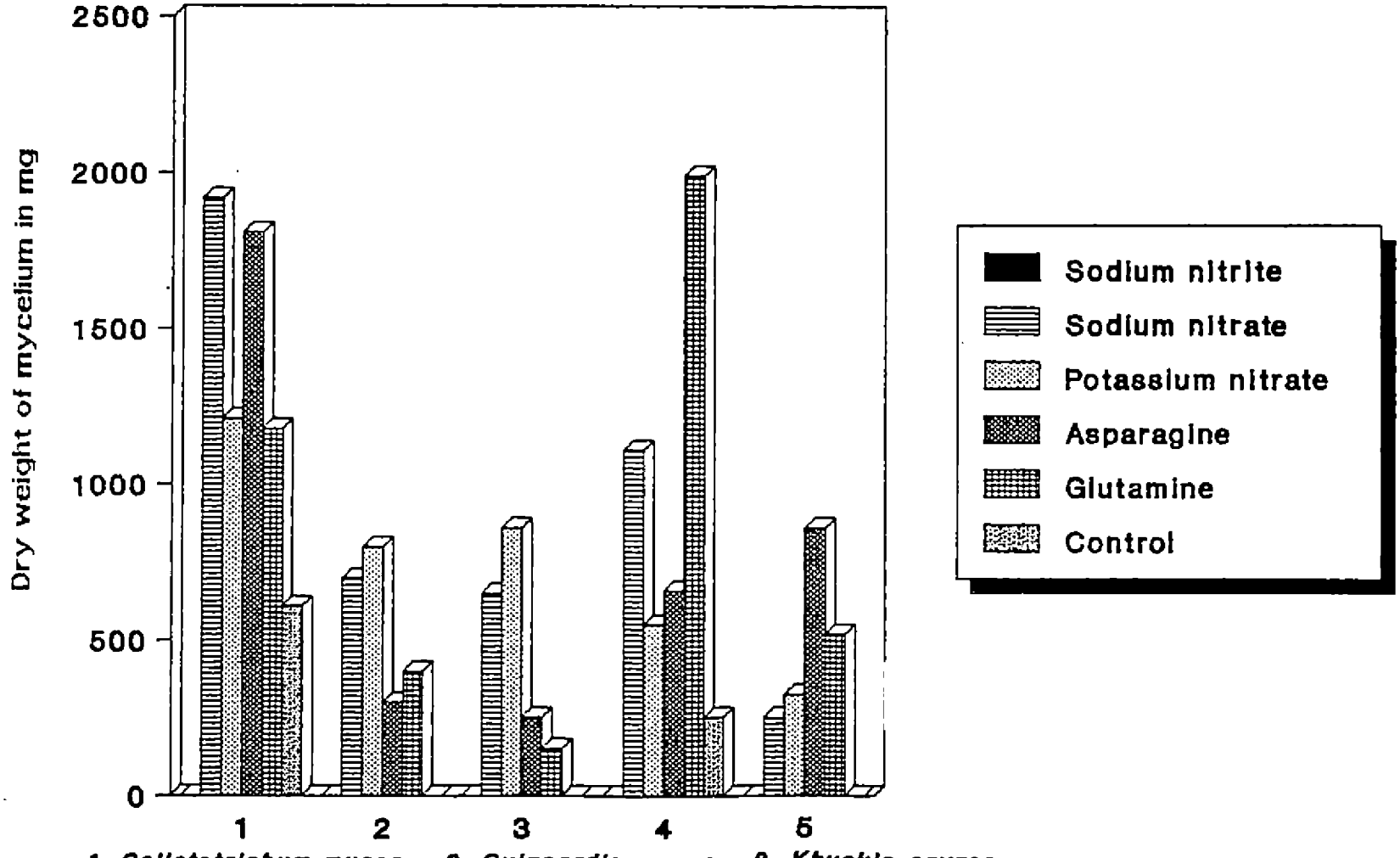
From the table it is clear that the best nitrogen source for the growth of the fungus was potassium nitrate followed by sodium nitrate and glutamine. No growth was obtained in sodium nitrite.

Table 11. Growth of five leaf blighting pathogens on different nitrogen sources

Pathogens	Sodium nitrite	Sodium nitrate	Potassium nitrate	Asparagine	Glutamine	Control
Colletotrichum musae	*0.00 (1.00)	1991.00 (3.28)	1210.00 (3.09)	1810.00 (3.26)	1180.00 (3.08)	610.00 (2.79)
Guignardia musae	0.00 (1.00)	700.00 (2.85)	800.00 (2.91)	300.00 (2.49)	400.00 (2.61)	0.00 (1.00)
Khuskia oryzae	0.00 (1.00)	652.12 (2.82)	862.10 (2.94)	252.00 (2.42)	151.90 (2.21)	0.00 (1.00)
Nodulisporium gregarium	0.00 (1.00)	1110.00 (3.05)	550.00 (2.75)	660.12 (2.83)	1990.11 (3.30)	252.23 (2.42)
Phaeoseptoria sp.	0.00 (1.00)	252.12 (2.42)	326.13 (2.53)	862.10 (2.94)	521.00 (2.73)	0.00 (1.00)

* Mean dry weight of mycelium in mg.
Figures in parentheses indicate transformed values.

Fig. 11. Growth of five leaf blighting pathogens on different nitrogen sources



Statistical analysis of the data revealed that potassium nitrate was significantly superior (2.91) to all other sources tried. Sodium nitrite and control were statistically on par.

4.7.3.3 Khuskia oryzae

From the study it is noticed that best nitrogen source for the growth of K. oryzae was potassium nitrate followed by sodium nitrate. No growth was obtained in sodium nitrite.

Statistical analysis of the data revealed that potassium nitrate was significantly superior (2.94) to all other sources tried. Sodium nitrite and control were on par.

4.7.3.4. Nodulisporium gregarium

Best nitrogen source for the growth of the fungus was found to be glutamine followed by sodium nitrate and asparagine. No growth was observed in sodium nitrite.

Statistical analysis of the data revealed that glutamine was significantly superior (3.30) to all other sources tried in the present study.

4.7.3.5 Phaeoseptoria sp.

From the results it is clear that asparagine was the best nitrogen source for the growth of the fungus followed by glutamine and potassium nitrate. No growth was observed in sodium nitrite.

Statistical analysis of the data revealed that asparagine was significantly superior (2.94) to all other sources tried followed by glutamine (2.73). Sodium nitrite and control were satistically on par.

4.7.4 Effect of various media on toxin production. Bioassay using host leaf

Effect of various media on the toxin production by the five leaf blighting pathogens are presented in the table 12.

4.7.4.1 Colletotrichum musae

From the results, it could be noticed that maximum area of necrosis was produced in potato dextrose broth and Richard's solution. No lesion was formed in the case of carrot leaf extract even after ten days of incubation.

Table 12. Effect of various media on toxin production by the five leaf blighting pathogens

Pathogens	Potato dextrose			Czapek (Dox)			Richard's			Host leaf dextrose			Carrot leaf extract			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	R_1	R_2	R_3	R_1	R_2	R_3
1. *Colletotrichum musae*	3	2	3	2	2	1	3	2	3	2	2	1	0	0	0	0	0	0
2. *Guignardia musae*	1	1	1	2	2	2	3	3	3	2	3	2	2	1	1	0	0	0
3. *Khuskia oryzae*	3	2	3	2	2	2	3	3	2	2	2	1	0	0	1	0	0	0
4. *Nodulisporium gregarium*	4	4	4	4	3	4	4	4	3	3	3	2	2	2	2	0	0	0
5. *Phaeoseptoria sp.*	1	2	1	1	1	1	3	3	2	2	2	1	1	1	2	0	0	0

0 - No lesions
1 - Lesion length less than 1 cm
2 - Lesion length between 1-2 cm
3 - Lesion length between 2-3 cm
4 - Lesion length more than 3 cm

4.7.4.2 Guignardia musae

Results showed that maximum area of necrosis was obtained in the case of Richard's solution followed by host leaf extract dextrose medium and Czapek (Dox) broth. Minimum necrotic area was obtained in the case of potato dextrose broth.

4.7.4.3 Khuskia oryzae

From the table it is clear that maximum area of necrosis was produced in potato dextrose broth and Richard's solution followed by Czapek (Dox) and host leaf extract dextrose medium. Least area of necrosis was obtained in carrot leaf extract.

4.7.4.4. Nodulisporium gregarium

From the results it could be observed that maximum area of lesion was developed in the case of potato dextrose broth followed by Czapek (Dox) medium and Richard's broths. Least area of necrosis was obtained in carrot leaf extract.

4.7.4.5 Phaeoseptoria sp.

Results of the study revealed that maximum area of necrosis was produced in the case of Richard's

solution followed by host extract dextrose, potato dextrose and carrot leaf extracts. Least area of necrosis was obtained in Czapek (Dox) medium.

4.7.5 Host range of the pathogens

Inoculation of six plants with culture bits of test fungi revealed that some of them were infected and others do not develop any symptoms. Plants showed symptoms after four to five days of inoculation. It was noticed that wounds are prerequisite for the successful development of symptoms. Intensity of infection varied with age of the plant. Injured young leaves were found to be more susceptible than uninjured ones.

4.7.5.1 Colletotrichum musae

a) Clove

Here the symptoms developed as reddish brown coloured spots, later enlarged and coalesced to form large patches. The centre of the spots became grey in colour in due course.

b) Cocoa

Symptoms started on the third day of inoculation as dark brown coloured specks which later

enlarged into large lesions. Centre became grey coloured with dark brown margins. The leaves showed symptoms of wilting.

c) **Colocasia**

Symptoms started as pale brown coloured specks. Later these specks enlarged and are irregular in shape with definite margin. A yellow discolouration appeared around these spots. Entire leaf showed yellowing. Symptoms were more pronounced on cut leaves than in standing crop.

d) **Nutmeg**

Symptoms started from the third day of inoculation as dark brown coloured patches, later enlarged and became greyish brown in colour. These spots attained a dia of one cm. Spots are having diffused margin. Symptoms on cut twigs and standing crop were more or less same.

No symptom development was observed in clerodendron and tapioca.

4.7.5.2 Guignardia musae

a) **Clove**

Symptoms started as small black coloured spots. Leaves showed symptoms of wilting.

b) Cocoa

Symptoms developed as dark brown spots. Entire leaf showed a yellow discolouration. These dark brown spots gradually covered the entire area of leaf lamina.

c) Colocasia

The entire leaves showed yellowing and wilting within four to five days.

d) Nutmeg

Symptoms started after five days of inoculation as pale brown spots, enlarged with indefinite margin. Centre of the lesion became pale brown in colour with dark margins and without any specific yellow halo.

No symptom development was observed in clerodendron and tapioca.

4.7.5.3 Khuskia oryzae

a) Clove

Symptoms started as dark brown coloured spots gradually became pale brown in colour.

b) Colocasia

Symptoms started as dark brown spots. Later enlarged having definite margins. Entire leaf showed yellowing within a few days.

c) Nutmeg

Symptoms developed as reddish brown coloured patches. Leaves showed wilting within four to five days.

No symptom development was observed in clerodendron, cocoa and tapioca.

4.7.5.4 Nodulisporium gregarium

a) Colocasia

Symptoms started from the fourth day of inoculation as dark brown to black spots. These spots enlarged and attained a dia of 2 mm. These spots does not have a definite margin. Later showed symptoms of yellowing.

b) Nutmeg

Symptoms started as black spots, later enlarged with grey centre and reddish brown margin. Each spot had definite margins. No yellow discolouration could be observed.

c) Tapioca

Symptoms developed as dark brown small lesions. Leaves showed yellowing at a later stage.

No symptoms could be seen in clerodendron, clove and cocoa.

4.7.5.5 Phaeoseptoria sp.

a) Clove

Symptoms started as dark brown spots. Symptoms developed more on detached leaves than on standing crop. Later these spots enlarged and extended to the whole leaf.

b) Cocoa

Symptoms started as pale brown specks, later developed into lesions having reddish brown coloured lesions spreading to entire leaf.

c) Nutmeg

Symptoms developed on detached leaves and on standing crops from the fourth day of inoculation. Symptoms started as reddish brown small lesions which gradually leads to wilting of the entire lamina. Symptoms were more pronounced on young leaves than on old ones.

No symptom development could be observed in clerodendron, colocasia and tapioca.

4.8 Laboratory evaluation of fungicides against the pathogens - Poisoned Food Technique (Solid medium)

The results of the effect of various fungicides on the radial growth of the five pathogens causing leaf blighting in banana are presented in table 13.

4.8.1 Colletotrichum musae

Of the five fungicides tested, each at three concentrations complete inhibition of the fungus could not be obtained with any of the fungicides. Calixin at 1000 ppm was significantly superior to all other fungicides tested followed by Bavistin at 1000 ppm, Calixin at 500 ppm and 250 ppm. Least effective fungicide among the five was found to be Bordeaux mixture (Fig. 12).

4.8.2 Guignardia musae

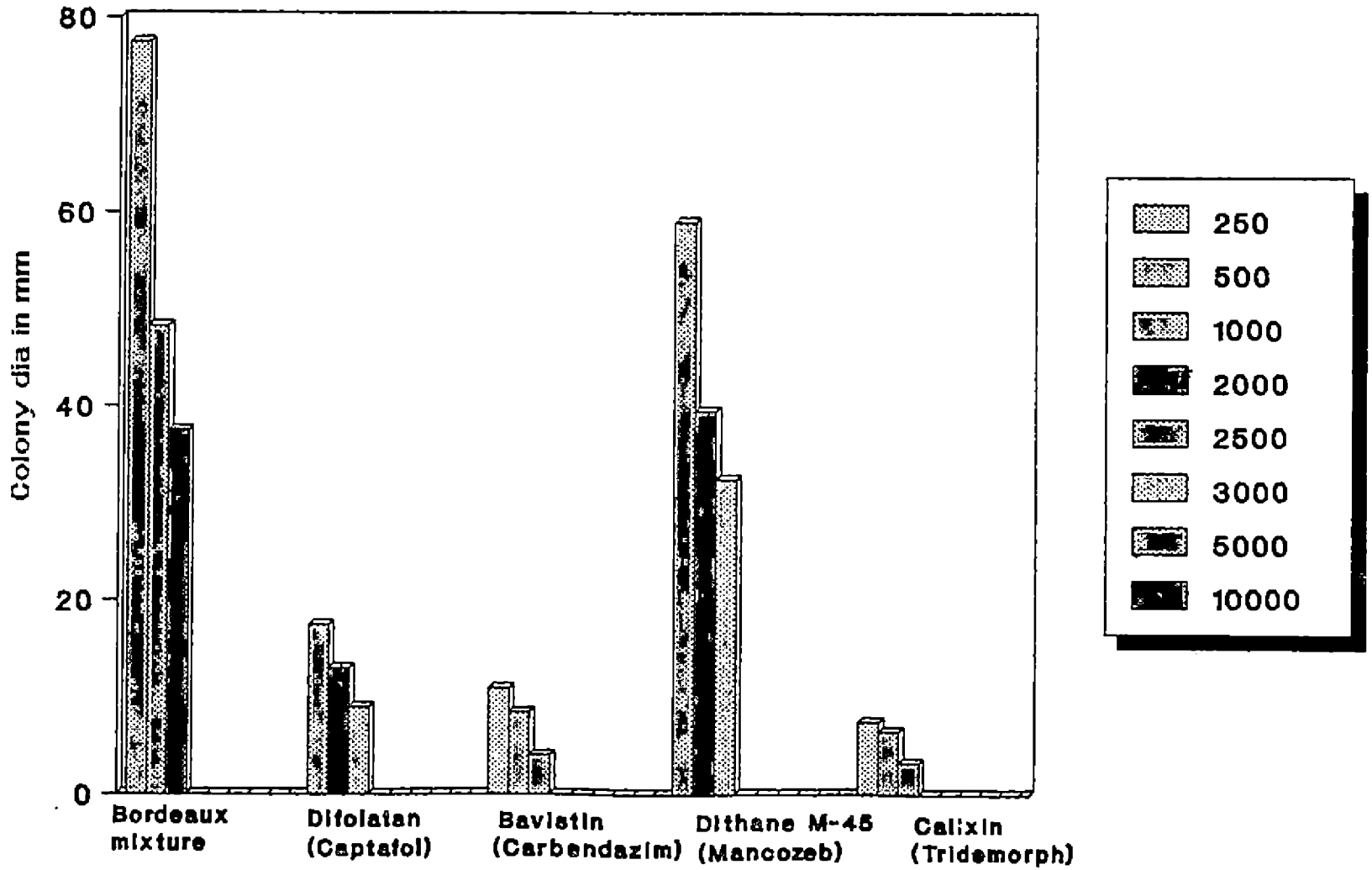
Complete inhibition of the fungus could not be achieved with any of the fungicides tested. Dithane M-45 at 3000 ppm was found to be significantly

Table 13. Laboratory evaluation of fungicides against leaf blighting Pathogens in Banana

Pathogens	Fungicides															
	Bordeaux Mixture			Difolatan (Captafol)			Bavistin (Carbendazim)			Dithane M-45 (Mancozeb)			Calixin (Tridemorph)			CD (0.05)
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	
1. Colletotrichum musae	2500	77.49 (8.86)	13.90	1000	17.49 (4.30)	80.57	250	10.99 (3.46)	87.79	1000	59.00 (7.75)	34.44	250	7.49 (2.92)	91.68	CD_1 = 0.04
	5000	48.29 (7.02)	46.34	2000	13.00 (3.74)	85.56	500	8.49 (3.08)	90.57	2000	39.50 (6.36)	56.11	500	6.49 (2.74)	92.79	CD_2 = 0.03
	10000	37.49 (6.20)	58.34	3000	9.00 (3.16)	90.00	1000	4.00 (2.24)	95.56	3000	32.50 (5.79)	63.89	1000	3.25 (2.06)	96.39	
2. Guignardia musae	2500	26.99 (5.29)	70.01	1000	12.25 (3.63)	86.39	250	12.49 (3.67)	86.12	1000	14.50 (3.94)	83.89	250	11.5 (3.54)	87.22	
	5000	23.99 (4.99)	73.34	2000	11.49 (3.54)	87.23	500	9.00 (3.16)	90.00	2000	13.50 (3.81)	85.00	500	8.29 (3.05)	90.79	CD_1 = 0.04
	10000	12.25 (3.63)	86.39	3000	8.00 (3.00)	91.11	1000	8.00 (3.00)	91.11	3000	5.00 (2.45)	94.44	1000	7.49 (2.92)	91.68	CD_2 = 0.03
3. Khuskia oryzae	2500	70.83 (8.48)	21.30	1000	8.17 (3.03)	90.92	250	4.50 (2.35)	95.00	1000	0.00 (1.00)	100	250	0.00 (1.00)	100	
	5000	68.00 (8.31)	24.44	2000	7.16 (2.86)	92.04	500	3.08 (2.02)	96.58	2000	0.00 (1.00)	100	500	0.00 (1.00)	100	CD_1 = 0.03
	10000	62.00 (7.94)	31.11	3000	5.16 (2.48)	94.27	1000	1.23 (1.49)	98.63	3000	0.00 (1.00)	100	1000	0.00 (1.00)	100	CD_2 = 0.02
4. Nodulisporium gregarium	2500	85.49 (9.30)	5.01	1000	1.50 (1.58)	98.33	250	0.00 (1.00)	100	1000	0.00 (1.00)	100	250	0.00 (1.00)	100	
	5000	74.99 (8.72)	16.68	2000	0.99 (1.41)	98.90	500	0.00 (1.00)	100	2000	0.00 (1.00)	100	500	0.00 (1.00)	100	CD_1 = 0.02
	10000	60.00 (7.81)	33.33	3000	0.49 (1.22)	99.46	1000	0.00 (1.00)	100	3000	0.00 (1.00)	100	1000	0.00 (1.00)	100	CD_2 = 0.02
5. Phaeoseptoria sp.	2500	84.99 (9.27)	5.57	1000	15.33 (4.04)	82.97	250	89.49 (9.51)	0.57	1000	0.00 (1.00)	100	250	0.00 (1.00)	100	
	5000	83.99 (9.22)	6.67	2000	14.17 (3.89)	84.26	500	84.99 (9.27)	5.57	2000	0.00 (1.00)	100	500	0.00 (1.00)	100	CD_1 = 0.04
	10000	82.99 (9.17)	7.79	3000	8.66 (3.11)	90.38	1000	79.99 (8.99)	11.12	3000	0.00 (1.00)	100	1000	0.00 (1.00)	100	CD_2 = 0.03

A - Concentration of fungicides in ppm.
B - Mean colony dia in mm (Values in parenthesis indicate transformed values)
C - Per cent inhibition over control.
CD_1 - Between fungicides
CD_2 - Between concentration.

Fig. 12. Laboratory evaluation of fungicides against *C. musae*



superior to all other fungicides tested followed by Calixin at 1000 ppm. No significant difference existed between Difolatan at 3000 ppm and Bavistin at 1000 ppm and statistically they are on par. The same was the case with Calixin at 250 ppm and Difolatan at 1000 ppm, Bordeaux mixture at 10,000 ppm and Difolatan at 1000 ppm (Fig. 13).

4.8.3 Khuskia oryzae

Complete inhibition of the fungus could be obtained on potato dextrose agar medium containing Dithane M-45 and Calixin at all the tested concentrations. They were found to be significantly superior to all other fungicides tested; followed by Bavistin at all concentrations tried (1000, 500 and 250 ppm) (Fig. 14).

4.8.4 Nodulisporium gregarium

Of the five fungicides tested, complete inhibition of growth of fungus was obtained with Bavistin, Dithane M-45 and Calixin at three tested concentrations. They were found to be significantly superior to all other fungicides tested; followed by Difolatan (3000, 2000 and 1000 ppm) (Fig. 15).

Fig. 13. Laboratory evaluation of fungicides against *G. musae*

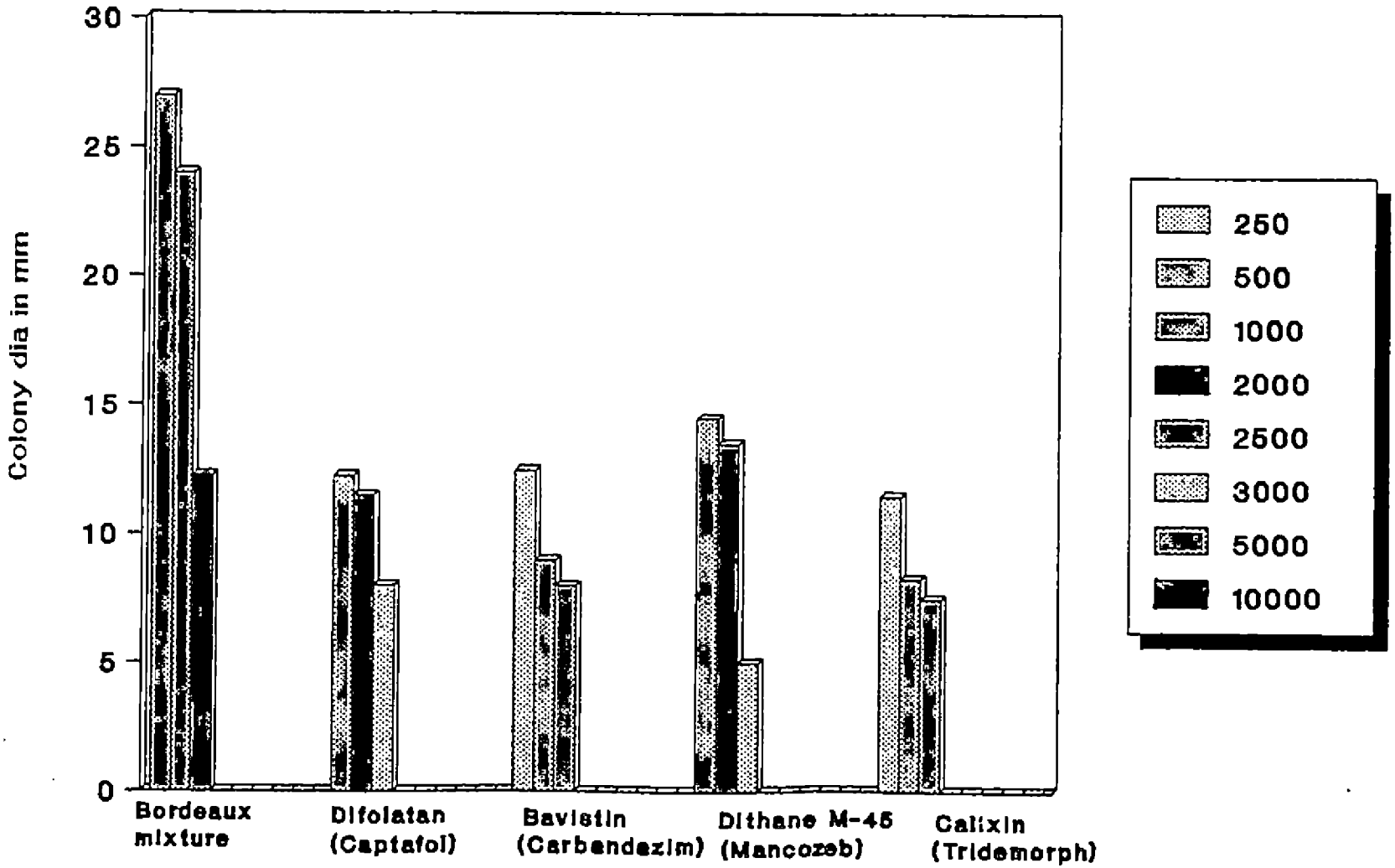


Fig. 14. Laboratory evaluation of fungicides against *K. oryzae*

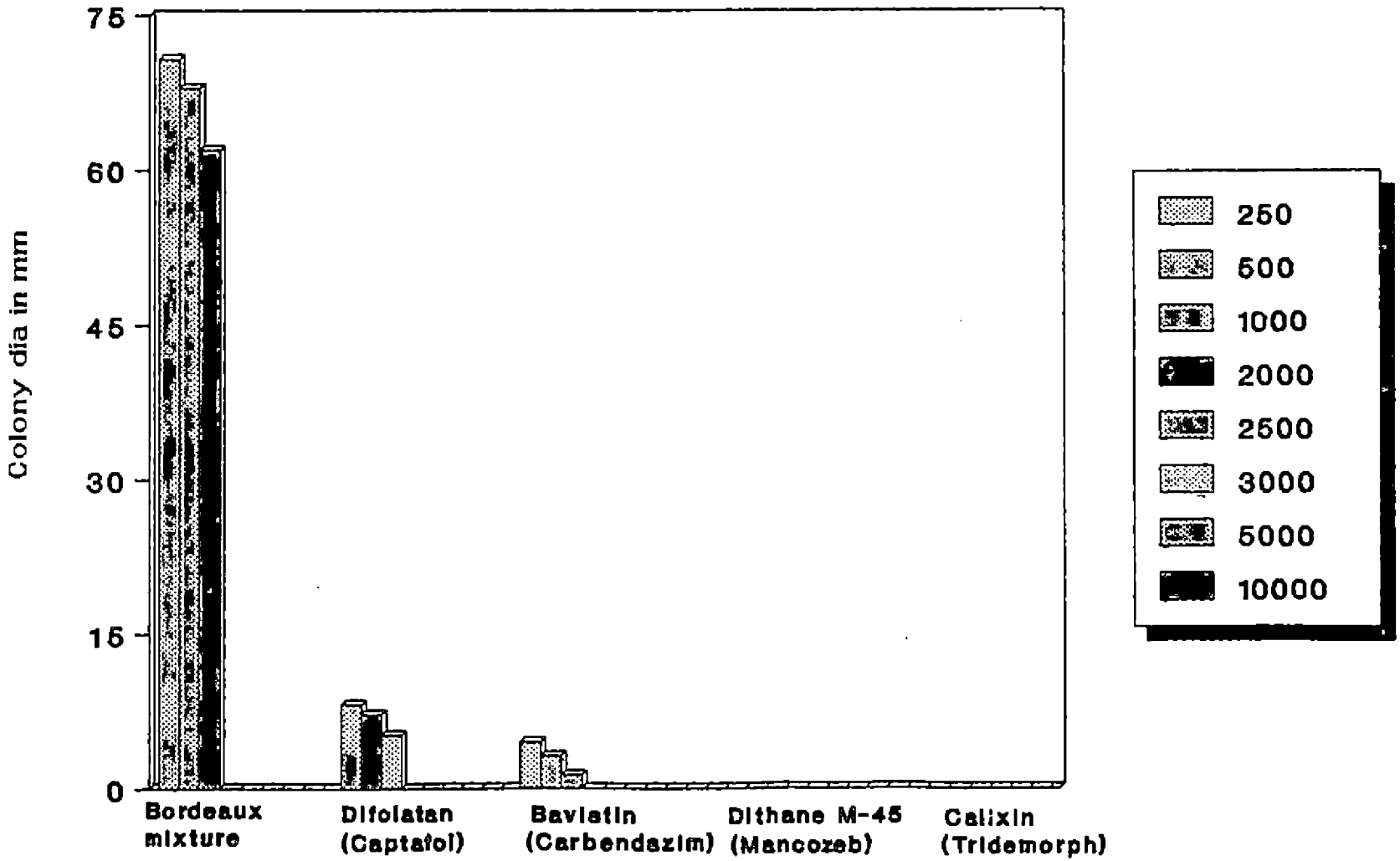
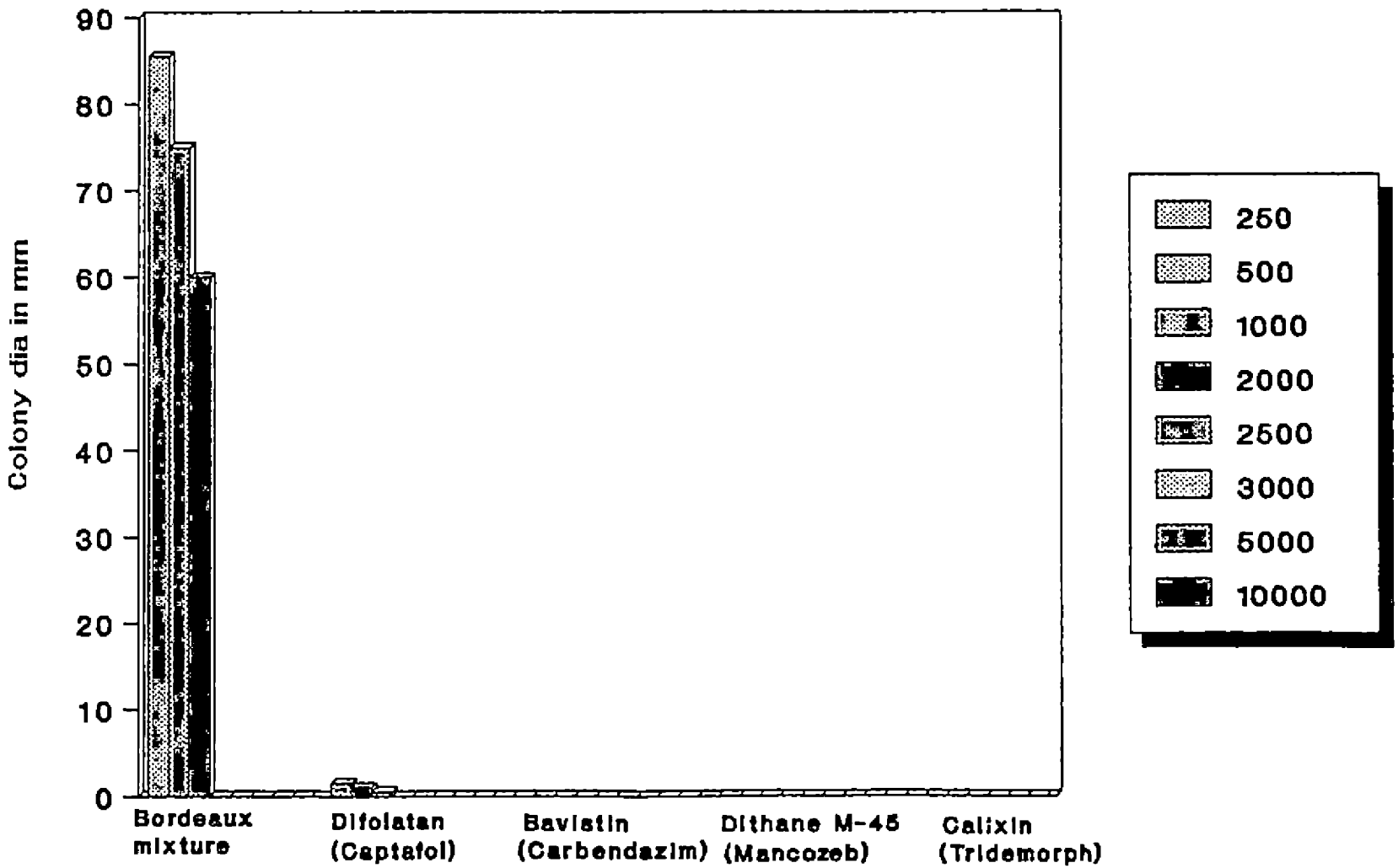


Fig. 15. Laboratory evaluation of fungicides against *N. gregarium*



4.8.5 *Phaeoseptoria sp.*

Complete inhibition of growth of the fungus was caused by Dithane M-45 and Calixin at all the three concentrations and were found to be significantly superior to all other fungicides tested, followed by Difolatan at all the three concentrations (1000, 2000, and 3000 ppm). Bavistin was found to be least effective fungicide in this case (Fig. 16).

4.9 **Field evaluation of fungicides**

Pre and post treatment disease indices obtained were tabulated, statistically analysed and presented in table 14.

The efficacy of three fungicides, viz., Bordeaux mixture, Dithane M-45 and Mineral oil at the field dose each was tested against the important leaf blighting organism, *Colletotrichum musae* in banana in the field.

From the table it is clear that after the first application of Mineral oil (1%) the disease index has been reduced considerably with a reduction in the disease index of 13.14, followed by Dithane M-45 (9.87) and Bordeaux mixture (4.0).

Fig. 16. Laboratory evaluation of fungicides against *Phaeoseptoria* sp.

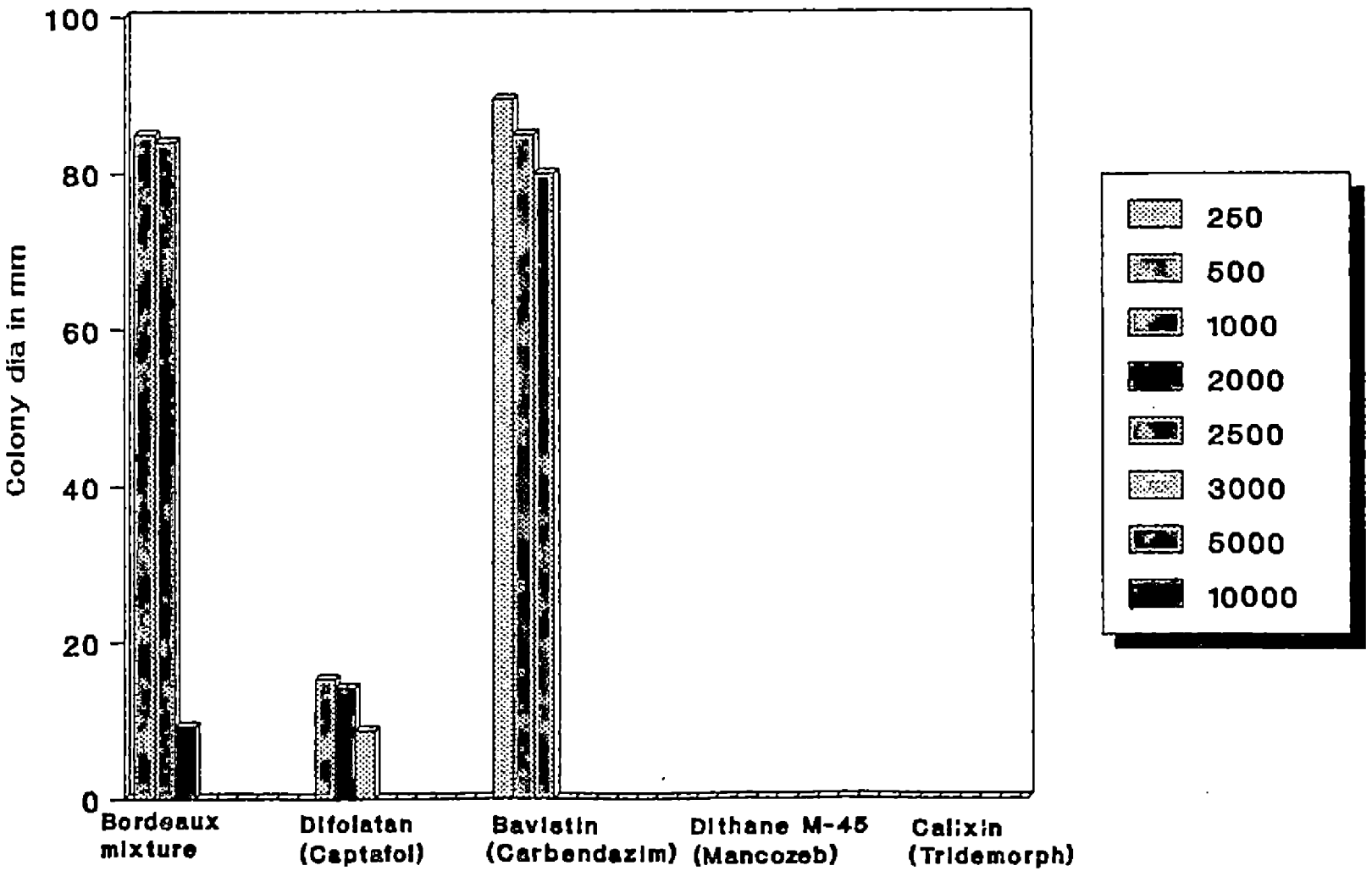


Table 14. Observation on the intensity of leaf blighting in banana

Treatments	Pretreatment observations R_1	R_2	R_3	R_4	R_5	R_6	R_7	Marginal means CD = 3.53 (0.05)
T_1	50	52	52	52	52	42	52	50.28
T_2	50	51.2	53.4	50	52	50	50	50.94
T_3	50	52	52	52	52	42	52	50.28
Post treatment observation (10 days after first spraying)								CD=3.05 (0.05)
T_1	48	48	46	40	48	48	46	46.28
T_2	40	36	38	45.5	44	42	42	41.07
T_3	40	38	36	38	36	36	36	37.14
Post treatment observation (10 days after second spraying)								CD=2.92 (0.05)
T_1	40	38	36	38	36	36	36	37.14
T_2	32.8	26	26	27.2	26	24	24	26.57
T_3	29	27.2	32.8	32.8	34.6	34.6	34.6	32.23

T_1 - Bordeaux mixture at 1 per cent concentration
T_2 - Dithane M-45 at 0.2 per cent concentration
T_3 - Mineral oil at 1 per cent concentration.

But after the second application of fungicides, maximum reduction in disease index from the pre treatment values was obtained with Dithane M-45 (24.37) followed by mineral oil (18.05) and Bordeaux mixture (13.14).

Even though mineral oil was found more effective in reducing the disease index after the first application of fungicides, the difference in disease index obtained with Dithane M-45 at the second spraying was significantly superior to mineral oil and Bordeaux mixture ie., Dithane M-45 significantly reduced the disease than mineral oil and Bordeaux mixture after the second application.

4.10 Scoring the leaf blight intensity in promising varieties of banana

Leaf blight intensity caused by the common pathogens in banana was scored using four cultivars of banana. The disease index for each variety was calculated at an interval of one month (table 15).

Results showed that minimum disease index values (2.61, 3.71 and 3.40) were obtained in the variety Rasakadali at all the three stages of observation, indicating that the variety Rasakadali is

Table 15. Marginal mean values of Disease index

Variety	Disease index at first observation	Disease index at second observation	Disease index at third observation
T_1	13.53 (3.68)	21.47 (4.63)	26.12 (5.11)
T_2	8.03 (2.83)	18.15 (4.26)	24.37 (4.94)
T_3	10.65 (3.26)	13.75 (3.71)	18.77 (4.33)
T_4	6.79 (2.61)	13.74 (3.71)	11.57 (3.40)
	CD = 0.43 (0.05)	CD = 0.36 (0.05)	CD = 0.44 (0.05)

T_1 = Nendran

T_2 = Red Banana

T_3 = Palayamkodan

T_4 = Rasakadali

moderately resistant/tolerant to leaf blighting compared to the other varieties used.

In the case of Palayamkodan, the disease index values were 3.71 and 4.33 at second and third stages of observations respectively which was lower than that obtained for Red banana (4.26 and 4.94). This indicate that the variety Palayamkodan is next to Rasakadali in resistance/tolerance, against the leaf blight pathogens.

The disease index values for Red banana at all the three stages of observation was lower (2.83, 4.26 and 4.94) than that of Nendran (3.68, 4.63 and 5.11) and these values were found statistically significant. This indicate that the variety Nendran is comparatively susceptible to leaf blight disease compared to the other three. The next susceptible variety to the leaf blight is Red banana followed by Palayamkodan and Rasakadali.

DISCUSSION

5. DISCUSSION

Banana crop is found affected by a number of leaf blighting organisms in the southern parts of Kerala. Severe infections resulted in the complete drying up of the leaves. During the survey, in the present study, seven fungal pathogens were found to be causing leaf blighting in banana. They were isolated, identified and described. They were the following:-

1. Colletotrichum musae (Berk. and M.A. Curits) Von Arx.
2. Curvularia sp
3. Guignardia musae Racib.
4. Khuskia oryzae H.J. Hudson
5. Nodulisporium gregarium (Berk. and M.A. Curtis) J.A.Meyer.
6. Pestalotiopsis versicolor (Speg.) Stey.
7. Phaeoseptoria sp.

Among these seven leaf blighting organisms, those caused by C. musae, G. musae, K. oryzae, N gregarium and Phaeoseptoria sp were more severe when compared to the other two.

Mixed infections caused by C. musae and Curvularia sp could be noticed in the variety Red

banana and Mysore annan in areas coming under Nedumangad agricultural subdivision. Leaf blighting intensity in banana was found severe in the above subdivision. This may be attributed to the condusive conditions prevailing there. Most of the banana plantations are in the paddy fields.

Varietal susceptibility is noticed in the three agricultural subdivisions. The variety Nendran is more susceptible to leaf blighting organisms followed by Red banana, Palayamkodan, Rasakadali and Mysore annan. Varietal susceptibility is attributed to biochemical characteristics of leaves. Presence of amino acids, carbohydrates, organic acids and phenol contents in banana varieties showed variations to fungal infection. (Reghunathan et al., 1968).

Old leaves were more prone to leaf blighting than young ones. This may be due to the retarded nutritional status and larger exposure leading to increased trauma of the older leaves. The presence of certain inhibitory substances in the younger leaves which are absent in older leaves, may also be responsible for such a response. Reghunathan et al. (1968) reported that the content of aminoacids like histidine and tryptophan in banana leaves decreased during maturity. Both glutamic acid and cystine

declined in the old tissues, while their concentration was high in young tissues. The similar situation was reported by Rangaswami and Natarajan (1965). Klein (1959) noted that the youngest leaves of actively growing plants were free from diseases. Echandi and Fernandez (1962) found that young branches of coffee plants by virtue of their higher content of phenols and chlorogenic acid were resistant to canker caused by Ceratocystis fimbriata.

Variations in symptom development was noticed in different varieties by the same fungus as noticed in Nendran and Redbanana caused by G. musae.

The morphological characters and pathogenicity tests of all the seven isolated cultures were studied and described. Detailed studies were carried out on the five leaf blighting organisms, viz., C. musae, G. musae, K. oryzae, N. gregarium and Phaeoseptoria sp.

Studies conducted on the growth and sporulation of the fungi in different solid media are presented in the table 4-8. Growth and sporulation of C. musae was best in Richard's agar followed by banana leaf extract agar. In the case of G.musae also maximum growth and sporulation was in Richard's agar followed

by Czapek (Dox) agar. Potato dextrose agar was found to be the best medium for the growth and sporulation of K.oryzae, N.gregarium and Phaeoseptoria sp. The second best medium for the growth of K.oryzae, N.gregarium and Phaeoseptoria sp. was found to be Richard's agar, banana leaf extract agar and Czapek (Dox) agar respectively.

Studies conducted on the growth and sporulation of the five fungi on different liquid media showed that, for different organisms the media also varies. In the case of C.musae and G.musae, Richard's solution is found the best medium for the growth and sporulation, whereas potato dextrose broth for K.oryzae and Phaeoseptoria sp and Czapek (Dox) for N.gregarium. This indicates that media has profound influence on the growth and sporulation of the leaf blighting fungi. Best growth of Colletotrichum gloeosporioides was reported in Richard's broth (Santhakumari, 1980). PDA supported for the best growth in both solid and liquid media for Alternaria porri affecting onion plants. (Sitarama Raju and Mehta, 1982).

Utilisation of different carbon sources on the growth of the five fungi conducted revealed that, the best source varies with the pathogen. Maltose was found to be best carbon source in terms of maximum dry

matter production in C. musae, N. gregarium and Phaeoseptoria sp. Greene and Morales (1967) reported that Gloeosporium musarum grew well on maltose and sucrose. For G. musae the best carbon source was found to be starch and for K.oryzae, it was sucrose. The optimum dry weight of mycelium in the case of K. oryzae after 13 days of growth was obtained in sucrose (Rawla and Tandon, 1976). The same observations were recorded by Lilly and Barnett (1953) with many fungi.

Studies conducted on the growth of the fungi using various nitrogen sources indicated varying results. Sodium nitrate supported maximum growth and dry matter production with the fungus C. musae, whereas G. musae and K. oryzae yielded maximum dry matter when potassium nitrate was added in the medium. Potassium nitrate supported maximum growth in the case of Alternaria porri affecting onion (Sitarama Raju and Mehta, 1982). Sodium nitrate was reported as best nitrogen source for the growth of Colletotrichum gloeosporioides (Santhakumari 1980). Nair (1972) reported ammonium nitrate and sodium nitrate as the nitrogen sources which supported best growth of Colletotrichum capsici. Maximum growth and dry matter production in the case of N. gregarium was obtained with glutamine and asparagine to that of Phaeoseptoria sp. Dayal and Ram (1968) reported the best nitrogen

source for the growth of Cercospora jasminicola Muller and Chupp as asparagine.

Toxin production capacity of five leaf blighting pathogens in banana was tested on different media. From this study it was found that media have considerable influence on the production of toxins. Potato dextrose broth and Richard's solution were found to be best for maximum toxin production by C.musae and K.oryzae, whereas G.musae and Phaeoseptoria sp produced maximum toxin only in Richard's solution. Sharma and Sharma (1969) reported Richard's liquid medium as the best medium for the production of toxin by Colletotrichum gloeosporioides causing citrus dieback. Chauhan (1961) and Chauhan (1964) also found Richard's solution as the best medium for the toxin production by Fusarium orthoceros var. ciceri and Ascochyta rabiei. The toxic effect of the culture filtrate, bioassayed against clove leaves revealed that maximum lesion length was in Richard's culture filtrate. (Sulochana, 1980). Nandi and Santra (1974) observed that culture media containing asparagine, tryptophan and tyrosine produced significant amount of toxic byproduct by Nigrospora oryzae (conidial state of K. oryzae). Nodulisporium gregarium produced maximum toxin in potato dextrose broth.

Host range studies of the pathogens conducted indicated that among the six plants used viz; clove, cocoa, colocasia, nutmeg, tapioca and clerodendron, none of the pathogens could infect clerodendron after fifteen days of incubation, even in the cut twigs. For different fungi, the time taken for sympotom expression in different hosts varied. In all the host plants injury was found to be a pre-requisite for the production of symptoms.

C. musae and G. musae could infect clove, cocoa, colocasia and nutmeg. Clove, colocasia and nutmeg were found to be the host plants for K. oryzae. N. gregarium produced symptoms in colocasia, nutmeg and tapioca and Phaeoseptoria sp in clove, cocoa and nutmeg.

Clerodendron was not affected by any of the fungi. The resistance/tolerant nature of clerodendron can be attributed to the presence of an alkaloid viz., clerodendrin in this plant. (Wada and Munakata, 1968). The fact that all the five fungi had a wide host range and ı cause: infection to many important crop plants is of great concern.

Results of the laboratory evaluation of fungicides indicated that there was complete inhibition of K. oryzae and Phaeoseptoria sp. with Dithane M-45 (1000, 2000 and 3000 ppm) and Calixin (250, 500 and

1000 ppm) in all the three test concentrations; whereas in the case of N.gregarium complete inhibition was obtained with Dithane M-45, Calixin & Bavistin (250,500 and 1000 ppm). Complete inhibition of Leveillula taurica with Dithane M-45 (200 micro gram/ml) was reported (Srivastava and Rajkumari Rawat, 1982). Complete inhibition of Bipolaris hawaiiensis could be achieved by Dithane M-45 at 1000 ppm (Thomas john, 1989). Complete inhibition of growth of Diplodia natalensis could be obtained with Dithane M-45 at 3000 ppm. (Rajagopalan and Wilson, 1972). Vijayan (1978) obtained complete inhibition of radial growth of Botryodiplodia theobromae with Bavistin 250 ppm and Dithane M-45 at 1000 ppm.

Dithane M-45 (3000 ppm) was able to inhibit the growth of G. musae under laboratory conditions, followed by Calixin (1000 ppm). Calixin and Bavistin at 1000 ppm each were able to inhibit the growth of C. musae. Cox (1956) reported that Mancozeb at 0.5 per cent was effective in inhibiting the growth of Helminthosporium maydis. Naseema (1981) reported that radial growth of Botryodiplodia theobromae could be inhibited by Dithane M-45 at 1000 ppm. Bavistin and Dithane M-45 have been reported to be effective in checking the growth of many fungi in nutrient medium.

(Zachos et al., 1963; Sen and Kapoor, 1975; Kataria and Grover, 1977).

Three fungicides viz., Bordeaux mixture, Dithane M-45 and mineral oil each at field recommendation were tested against the leaf blighting pathogen in banana in a field experiment. The studies conducted indicated that there was considerable reduction in the disease index with one per cent mineral oil after the first round of spraying. Suharban and Paily (1977) conducted a field trial on fungicidal control in banana revealed that one per cent power oil was effective in controlling the leaf spot diseases of banana. The same results were reported by different workers (Rhodes, 1960; Houghton, 1965 and Kranz, 1965).

Medium viscosity refined oils are used for the control of plant disease, along with finely divided copper fungicides, on the principle that being less volatile than water, smaller droplets and lower volumes could be used. (Guyot, 1953). Oil possibly inhibit the fungus inside the banana leaf at some stage after stomatal penetration and before symptom appearance. (Calpouzos et al., 1959). Under field conditions oil spray reduced germination, germ tube growth and appressoria formation of Mycosphaerella musicola for

two days to two weeks. Spores were inhibited only on the leaves to which oil was applied. It is remarkable that the application of oil upto two weeks before and after infection reduces the number of spots and retards their development. Oil greatly inhibits disease progress after host penetration; but leaf spot is still able to build up on sprayed plants, when abundant inoculam is present and weather conditions are suitable. Behaviour of the pathogen on sprayed susceptible plant is similar to that on partially resistant plant. (Stover and Dickson, 1968).

Fungicide alone and mixed with mineral oil for the control of sigatoka leaf spot of banana has been reported by Perez (1978) and West (1983).

But in the present study, after the second application of fungicides, ie. two weeks after the first spraying, Dithane M-45 significantly reduced the disease than mineral oil and Bordeaux mixture. Maximum reduction in the disease index was obtained with Dithane M-45 after the second application of fungicides followed by mineral oil. Pont (1970) obtained control of Cercospora leaf spot, freckle leaf spot and Cordana leaf spot of banana by spraying with mancozeb and maneb. A field trial conducted at Banana Research station,Kannara indicated that Dithane M-45 at 0.2 per

cent concentration was found to be best for reducing the percentage of leaf infection. Mancozeb was more effective than benomyl, and mineral oil was ineffective when single leaf tests were conducted against banana speckle disease caused by Phyllosticta musarum. Under field conditions maneb and mancozeb in oil-water emulsion or in oil only and Bravo 6F (Chlorothalonil) and Daconil in water only gave good control. Benomyl in oil was ineffective (Chuang, 1983). In comparative trials, Dithane M-45 (mancozeb) WP (in oil-water emulsion) and Dithane M-45F (in water only) both controlled Mycosphaerella fijiensis var. difformis, but the latter formulation is effective in inhibiting the conidial gemmination. (Chuang et al., 1984). Moderate control of Gloeosporium musarum (C.musae) causing fruit rot in banana could be achieved by Dithane M-45 and maneb at 2000 ppm. (Shilling ford, 1970).

In the present investigation, even though mineral oil was found effective in reducing the disease considerably after the first spraying of fungicides, maximum disease control after the second spraying of fungicide was obtained with Dithane M-45. Hence Dithane M-45 (0.2%) is the best fungicide among the three fungicides used in the present study for the control of leaf blight disease in banana (table No.14).

Studies conducted on scoring of leaf blight intensity in the four promising varieties of banana revealed that the variety Rasakadali was moderately resistant/tolerant to leaf blighting followed by Palayamkodan and Red banana. Nendran was found to be the least resistant/tolerant variety to leaf blighting fungi. Gopimony (1977) reported that most of the Kerala varieties like Chemkadali, Palayamkodan, Monthan, Neypoovan and Njalipoovan (Rasakadali) were found to be tolerent to leaf spot diseases when compared to the introduced varities like Gros Michel and Robusta.

SUMMARY

6. SUMMARY

A survey was conducted to study the occurrence and severity of leaf blight disease in banana in the three agricultural sub divisions of Thiruvananthapuram district. Seven fungal pathogens causing leaf blighting in banana were described here. They were Colletotrichum musae (Berk and M.A. Curtis) Von Arx., Curvularia sp., Guignardia musae Racib., Khuskia oryzae H. J. Hudson., Nodulisporium gregarium (Berk. and M.A. Curtis) J.A. Meyer., Pestalotiopsis versicolor (Speg.) Stey. and Phaeoseptoria sp. Mixed infections caused by Colletotrichum musae and Curvularia sp was reported in Red banana and Mysore annan varieties of banana from Nedumangad agricultural sub division.

The leaf blight caused by Curvularia sp, Khuskia oryzae and Nodulisporium gregarium are new reports from India.

From the results of the survey it was revealed that the banana variety Nendran was severly affected by leaf blight disease followed by Red banana, Palayamkodan and Rasakadali whereas Mysore annan is less susceptible to the disease. Old banana leaves were severly affected by the leaf blighting organisms

than the young ones. Disease intensity was high when the plant attained four to five month old. Occurrence and severity of the disease was more during rainy season. Among the three agricultural sub divisions surveyed, leaf blighting was severe in Nedumangad subdivision.

Detailed studies were conducted on the five leaf blighting organisms viz., C. musae, G.musae, K. oryzae, N. gregarium and Phaeoseptoria sp since severe infections could be noticed by these five fungi.

Studies conducted on the growth and sporulation of the fungi in different media (both solid and liquid) revealed that C. musae and G. musae grew best on Richard's medium whereas K. oryzae and Phaeoseptoria sp utilised potato dextrose medium extensively for their growth. In the case of N. gregarium potato dextrose agar was the best solid medium and Czapek (Dox) broth was the best liquid medium for its growth and sporulation.

Utilisation of different carbon sources on the growth of fungi indicated that maltose was found to be the best carbon source for the growth of C. musae, N. gregarium and Phaeoseptoria sp. G. musae and K.oryzae utilised maximum starch and sucrose for their

growth and sporulation respectively indicating these as the best carbon sources.

Effect of different nitrogen sources on the growth of fungi revealed that C. musae yielded maximum mycelial weight with sodium nitrate, G.musae and K. oryzae with potassium nitrate, N gregarium with glutamine and Phaeoseptoria sp with asparagine.

Toxin production capacity of the fungi in different media tested indicated that potato dextrose broth and Richard's solution as the best medium for the maximum production of toxin by C.musae and K. oryzae,G. musae and Phaeoseptoria sp produced maximum lesion length in Richard's solution. In the case of N. gregarium maximum lesion length was produced when potato dextrose broth was used as the medium.

Host range studies of the pathogens conducted revealed that clerodendron was a non host plant for all the five pathogens under study. Cocoa, clove, colocasia and nutmeg were found to be the host plants for C. musae and G. musae, whereas Phaeoseptoria sp could infect the above host plants except colocasia. Clove, colocasia and nutmeg were found to be the host plants for K. oryzae and for N. gregarium colocasia, nutmeg and tapioca.

Results of the laboratory evaluation of fungicides against five pathogens indicated that there was complete inhibition of K. oryzae and Phaeoseptoria sp with Dithane M-45 and Calixin at all the tested concentrations whereas in the case of N. gregarium, complete inhibition was obtained with Bavistin also, apart from the above two. Dithane M-45 at 3000 ppm was found to be best fungicide for the inhibition of G. musae whereas Bavistin and Calixin at 1000 ppm each were able to inhibit C. musae effectively under laboratory condition.

Field evaluation of three fungicides conducted revealed that Dithane M-45 was the best and could give satisfactory control for the leaf blight disease in banana.

Among the four varieties viz., Nendran, Palayamkodan, Rasakadali and Redbanana screened against the leaf blight disease in banana, the variety Rasakadali was found moderately resistant/tolerant than the other three varieties. The variety Nendran was found most susceptible to the leaf blight pathogen(s).

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* Originals not seen.

APPENDIX

APPENDIX - I

I. Solid Media

Potato dextrose agar medium

Peeled and sliced potato	-	200g
Dextrose	-	20g
Agar agar	-	20g
Distilled water	-	1000 ml
pH	-	6.0 to 6.5

Czapek (Dox) agar medium

$Mg\ So_4 . 7H_2O$	-	0.50g
KH_2PO_4	-	1.00g
KCl	-	0.50g
$FeSo_4$	-	0.01g
$NaNo_3$	-	2.00g
Sucrose	-	30.00g
Agar agar	-	20.00g
Distilled water	-	1000 ml.
pH	-	6.5

Richard's agar medium

KNo_3	-	10.00g
KH_2PO_4	-	5.00g
$MgSo_4 . 7H_2O$	-	2.50g
$FeCl_2$	-	0.02g
Sucrose	-	50.00g

Agar agar	-	20.00g
Distilled water	-	1000ml
pH	-	6.6 - 7.2

Host leaf extract agar

Banana leaves	-	200g
Agar agar	-	20g
Distilled water	-	1000 ml.

Carrot leaf extract agar

Carrot leaves	-	200g
Agar agar	-	20g
Distilled water	-	1000 ml.

Host extract dextrose medium

Banana leaves	-	200g
Dextrose	-	20g
Distilled water	-	1000ml.

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

LEAF BLIGHT OF BANANA AND ITS CONTROL

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ABSTRACT

Survey conducted to study the fungal pathogens causing leaf blight disease in banana in the three agricultural subdivisions of Thiruvananthapuram district, yielded seven fungal pathogens viz., Colletotrichum musae, Curvularia sp, Guignardia musae, Khuskia oryzae, Nodulisporium gregarium, Pestalotiopsis versicolor and Phaeoseptoria sp. Among these, Curvularia sp, Khuskia oryzae and Nodulisporium gregarium are new reports.

Morphological characters and pathogenicity tests of all the seven isolated cultures were studied and described. Detailed studies were conducted on five fungal pathogens viz., C. musae, G. musae, K. oryzae, N. gregarium and Phaeoseptoria sp., since severe infections could be noticed by these fungi.

Studies conducted on the growth and sporulation of pathogens on different media indicated that Richard's medium was the best for C. musae and G. musae, potato dextrose medium for K. oryzae and Phaeoseptoria sp. In the case of N. gregarium, potato dextrose agar and Czapek (Dox) broth were found to be the best solid and liquid media respectively for its growth.

Best growth of C. musae, N. gregarium and Phaeoseptoria sp was obtained with maltose as the carbon source, whereas G. musae and K. oryzae utilized maximum starch and sucrose for their growth. Maximum mycelial weight of C. musae was obtained with sodium nitrate as nitrogen source, G. musae and K. oryzae with potassium nitrate, N. gregarium with glutamine and Phaeoseptoria sp with asparagine.

Potato dextrose broth and Richard's solution were found to be best media for the toxin production by C. musae and K. oryzae. G. musae and Phaeoseptoria sp. produced maximum toxin in Richard's solution and N. gregarium in potato dextrose broth.

Host range studies of the pathogen(s) conducted indicated that they can infect a number of economically important plants like, clove, cocoa, colocasia, nutmeg and tapioca, but none of the pathogens could infect clerodendron.

Dithane M-45, Calixin and Bavistin were able to inhibit the growth of the leaf blighting pathogens under laboratory conditions and under field conditions Dithane M-45 (0.2%) was found to be the best.

Varietal screening trials showed that variety Rasakadali was moderately resistant/tolerant among the four popular varieties screened, whereas Nendran was the most susceptible variety.