ETIOLOGY AND CONTROL OF SEEDLING BLIGHT OF COCOA

By

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THESIS

Submitted in partial fulfilment of the
requirement for the degree

Master of Science in Agriculture

Faculty of Agriculture
Kerala Agricultural University

Department of Plant Pathology
COLLEGE OF HORTICULTURE
Vellanikkara - Thrissur

1995

DECLARATION

I hereby declare that the thesis entitled **"ETIOLOGY AND CONTROL OF SEEDLING BLIGHT OF COCOA"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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We, the undersigned members of the Advisory Committee of **Mr. E. Edwin Prem**, a candidate for the degree of Master of Science in Agriculture with major in Plant Pathology, agree that thesis entitled **"ETIOLOGY AND CONTROL OF SEEDLING BLIGHT OF COCOA"** may be submitted by Mr. E. Edwin Prem, in partial fulfilment of the requirement for the degree.

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ACKNOWLEDGEMENTS

It is with great pleasure that I express my deep sense of gratitude and indebtedness to Dr. Koshy Abraham, Associate Professor, Department of Plant Pathology for his constant encouragement and valuable guidance. As the chairman of the advisory committee he has given me the maximum help and encouragement by taking keen and lively interest throughout the course of investigation and preparation of this manuscript.

I am really grateful to Prof. C.K. Ramakrishnan, Professor and Head, Department of Plant Pathology (Retd.), Dr. James Mathew, Professor and Head, Department of Plant Pathology and Dr. A. Sukumara Varma, Associate Professor, Plant Pathology for having served as members of advisory committee, and for their valuable suggestions and help rendered in the preparation and presentation of this thesis.

I am grateful to Dr. R. Vikraman Nair, Professor, CCRP, College of Horticulture as a member of my advisory committee and for providing all the facilities in the field for the successful completion of this work.

I wish to express my sincere thanks to all the staff members of the Department of Plant Pathology for the timely

and whole hearted co-operation, assistance they always accorded me during the course of investigation.

I am indebted to Dr. N.K. Vijayakumar, Associate Professor, College of Forestry, Vellanikkara for his help in taking the photographs.

I am extremely grateful to Dr. Chandramohanan, Senior Scientist, CPCRI, Regional Station, Vital for his valuable help in taking photomicrographs.

I am very much thankful to Prof. P.V. Prabaharan, Professor and Head, Department of Agricultural Statistics and Smt. V.J. Joicy of the Department of Agricultural statistics for their good help in the statistical analysis of the data.

It is my bounden duty to register the deep sense of gratitude and sincere thanks to my classmates cum friends for their co-operation rendered then and there.

The help and co-operation rendered by Mr.S. Jothimani, Mr. K. Angaiah and Mr. Ashok B. Divatar is gratefully acknowledged.

I express my thanks to the workers of the CCRP project for their co-operation in carrying out the field works.

I am lovingly thank my parents and sisters whose inspiration, timely persuasion, good wishes and prayers were very vital for successful completion of this work.

The grant of Junior Fellowship by the Kerala Agricultural University is gratefully acknowledged.

I thank Shri. O.K. Ravindran, C/o. Peagles, Mannuthy for neat and prompt typing of this document.

Above all, I bow my head before God Almighty whose blessings enabled me to undertake this venture successfully.

E. EDWIN PREM

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Introduction

INTRODUCTION

Cocoa (Theobroma cacao L.) ranks third as a beverage crop in the world preceded by tea and coffee. It belongs to the family Sterculiaceae, a group of small trees, native of the rain forests of Tropical America. Cocoa is extensively cultivated in Ghana, Nigeria, Brazil, Ivory Cost, Cameroon, Ecuador, Papua New Guinea, Mexico, Costa Rica, Philippines, Malaysia and Indonesia. In India, the commercial cultivation of cocoa was started in early 1960's. Kerala is the principal cocoa growing state of India accounting for about 80 per cent of the area under cultivation followed by Karnataka and Tamil Nadu. The area under cocoa in Kerala is about 10,000 hectares with an annual production of 5,400 tonnes.

Cocoa is prone to the attack of many diseases. Among the various diseases, seedling blight is the most serious one in the nursery. At times, this disease inflicts heavy crop losses and was first reported from Nigeria (Chant, 1957). Though there was no authentic report on the occurrence of seedling blight from India, the prevalence of the disease is noticed in most of the cocoa nurseries. However, there was a report of a seedling die back of cocoa caused by Phytophthora palmivora from India (Chandramohanan, 1979, 1983).

Phytophthora diseases act as one of the important constraints in cocoa cultivation. Many species of Phytophthora are known to infect cocoa plant. Among the different species, P. palmivora is the most important one. This cosmopolitan fungus is very active in the warm tropical regions receiving high amount of rainfall. A number of diseases like pod rot, canker, chupon blight and seedling blight are known to be caused by P. palmivora.

In seedlings, Phytophthora infection results in leaf blight, stem infection, die back and death, posing serious threat to the production of quality planting materials. Since this disease causes heavy damage to seedlings and budded plants in nurseries, a better understanding of the disease is essential for proper monitoring and control of the disease. However, no systematic attempt has been made to study the various aspects of seedling blight of cocoa in India. In view of the serious nature of the disease, the present investigation was carried out with the following objectives.

1. Isolation of the causal organism from different parts of the plant and testing its pathogenicity.

2. Identification of the causal organism based on morphological characters.

3. Critical study of the symptomatology of the disease.

4. Screening of cocoa types for host resistance against seedling blight.

5. Studies on the influence of age of seedlings and budded plants on the incidence and severity of the disease.

6. *In vitro* and *in vivo* screening of fungicides/antibiotics for the control of the disease

Review of Literature

REVIEW OF LITERATURE

The occurrence of seedling blight of cocoa was first recorded by Chant (1957) from Nigeria. Subsequently, the disease was reported from Malaysia (Chee, 1969a), Ghana (Asare-Nyako *et al.*, 1972) and Papua New Guinea (Mc Gregor, 1984). In India, Chandramohanan (1979; 1983) observed the occurrence of a seedling die-back of cocoa from Karnataka and Kerala State.

Chant (1957) reported more than 70 per cent mortality of cocoa seedlings due to *Phytophthora palmivora* under high humid conditions. *P. palmivora* caused occasional serious loss of cocoa planting material in the nurseries (Mc Gregor, 1984).

Mc Laughlin (1950) observed that the die back of cocoa shoot was due to *P. palmivora* rather than to *Colletotrichum* sp. or *Diplodia* sp. Over watering under heavy shade and use of heavy soils have been attributed for the outbreaks of cocoa seedling infection (Chant and Hall, 1969). Chee (1969a) reported an outbreak of cocoa seedling die back in Perak, Malaysia caused by *P. palmivora*. Williams (1969) noticed persistent occurrence of die-back of cocoa in the Tawar residency of Sabah and he reported that it was due to improper land preparation and lack of soil and seedling protection.

Fireman and Vernon (1970) reported that P. palmivora isolates from black pod, canker and green shoots were equally pathogenic both to pods and seedling stems. Asare-Nyako et al. (1972) reported the incidence of seedling blight, on seedlings planted directly on harrowed seed beds or in plastic potting bags.

According to Manco (1974) the blight of shoots, chupons, cuttings, grafts and seedlings of cocoa was caused by P. palmivora. P. palmivora caused canker on mature cocoa trees and nursery seedlings (Campelo et al., 1984). Keane (1974) and Chandramohanan (1979) observed that the infection of P. palmivora was more on the seedlings of less than a month old. Lawrence et al. (1982) found that P. palmivora was consistently more virulent than P. capsici on attached and detached cocoa pods, germinated seeds and seedlings. Chandramohanan (1983) reported that the die-back of cocoa seedlings was frequently observed during rainy season in the nurseries of Kerala, Karnataka and Tamil Nadu. Holderness (1992) reported that infection of unhardened seedling leaves occurred primarily through rain (or watering) splash from the soil and the inoculum of seedling blight was introduced into the nursery via, contaminated soil or debris or splash from adjacent mature cocoa.

observed the leaf spot in cocoa nurseries caused by Colletotrichum gloeosporioides from Costa Rica. Infection of cocoa seedlings with C. crassipes was reported by Ram et al. (1973). Chandramohanan and Kaveriappa (1983) found that leaf blight and shot hole symptoms caused by C. gloeosporioides occurred on plants of all ages including seedlings. Chandramohanan et al. (1987) observed that C. gloeosporioides incidence was highest on 18-day-old cocoa seedlings. Infection of cocoa seedlings and young flushes by Moniliophthora roreri has been demonstrated under green house condition (Evans, 1981). Gonzalez et al. (1990) reported a scion die-back in graft propagated cocoa from Pureto Rico. They observed the association of Botrydiplodia theobromae and Fusarium latartium with the disease.

2.1 Causal organism

In all cocoa growing countries of the world, black pod, stem canker, chupon blight and seedling blight of cocoa caused by Phytophthora spp. are economically important. Among the various Phytophthora diseases, seedling blight caused by P. palmivora was one of the important diseases in the nursery (Chant, 1957; Chee, 1969a; Asare-Nyako et al., 1972; Manco, 1974 and Chandramohanan, 1983).

Carruthers (1898) was the first to record the fungus associated with black pod and identified as Peronospora corda in Ceylon. Massee (1899) after examining infected pod from Trininad, identified the fungus as Phytophthora omnivora de Bary. Faber (1909) studied the fungus associated with the black pod disease in Cameroon and noted its difference from P. omnivora. He retained only the generic name (Phytophthora sp.) to this fungus. Based on Faber's work, Maublanc (1909) described the Phytophthora infecting cocoa as a new species and identified it as P. faberi Maubl. Coleman (1910) described the fungus associated with disease in Ceylon as P. theobromae Coleman. Rosenbaum (1917) received a culture of Phytophthora from J.B. Rorer which he identified as P. faberi. Reinking (1919) reported that the fungus causing coconut (Cocos nucifera L.) bud rot was identical with P. faberi. He also noted that P. faberi isolated from cocoa caused bud rot of coconut. After a careful examination of Reinking's figures and description of P. faberi, Butler (1925) confirmed that this fungus was morphologically identical with Phytophthora palmivora (Butl.) Butl., which was reported as the causal organism of bud rot of palmyra palm (Borassus flabellifer L.) (Butler, 1907, 1919). He described the fungus having intercellular mycelium, only haustoria passing into the cells, hyphae large, often irregularly swollen, upto 7 µm in diameter, ramifying between the cells of parenchyma, sporangia

inverted pear shaped or more rarely round, and always terminal, which germinate readily in water giving rise to rather large zoospores. The sporangia measure 50 x 35 µm on an average (extreme 38-72 x 33-42 µm).

Ashby (1929) described P. palmivora as having sporangia which, when mature fall away from the sporangiophores by natural absession with a short, often stout and more or less occuluded pedicel, mostly 2-4 µm long and not exceeding 6 µm in length. Tucker (1931) recognised two groups of P. palmivora isolates and referred as 'typical' and 'atypical' forms which were distinguishable on the basis of sporangial morphology. The average length/breadth (L/B) ratio of 'typical' form was 1.5 to 2.1 and that of 'atypical' was 1.3 to 1.6. Waterhouse (1963) constructed a key to the species of Phytophthora considering the characters of sporangial apex, occurrence of oospores and antheridial relationships, and classified the then known species into six groups and placed P. palmivora in group I. In a further paper, based on the sporangial morphology she stated that P. palmivora existed in two morphological forms (MF) namely MF_1 (typical form) and MF_2 (atypical form) (Waterhouse, 1974). She also found A_1 and A_2 compatibility types in each of the morphological forms. These groups were identified based on sporangial morphology. Sporangia of MF_1 were ellipsoidal or

ovoid and having round base with short stalk (2-10 μm in length) and L/B ratio usually more than 1.4, whereas the sporangia of the MF_2 were shorter, obpyriform and ovoid with an average L/B ratio of 1.3 to 1.4. Sansome et al. (1975) demonstrated chromosome differences among the isolates from cocoa in West Africa.

During the Rothamsted cocoa Phtophthora workshop in 1976, it was agreed that there were at least three and possibly more distinct morphological forms within P. palmivora. These forms were temporarily designated as MF_1, MF_2, MF_3 and MF_4, 'other types' and 'pepper forms' (Griffin, 1977). Differences in cultural characteristics, sporangial morphology, pedicel length and chromosome number formed the basis of grouping the isolates into morphological forms. The important characteristics of these four morphological forms are given below.

MF_1: Cultures on carrot agar medium was stellate, striate (smooth-combed) with sharply well defined edge and aerial mycelium usually sparse. Sporangia with round base, shed with a short, broad and occluded pedicel (<5 μm in length), predominantly A_2 compatibility, small chromosomes (n = 9-12). L/B ratio of sporangia 1.2 to 1.8.

MF_2: They have stellate or striate pattern of colony growth. Sporangia similar to MF_1, sporangia with round base and decidious with a short occluded pedicel (5 μm in length).

MF_3: Cultures on carrot agar with plentiful cotton wool like aerial mycelium over the entire colony, edge was more diffused and less regular than MF_1. Sporangia had rounded base, decidious with thin stalks (10-15 μm in length). Large chromosome (n = 5-6). Predominantly A_1 compatibility. L/B ratio of sporangia 1.2 to 1.6.

MF_4: Cultures on carrot agar mostly petaloid with a moderate amount of aerial mycelium, rarely stellate radiating pattern. Elongated sporangia, its base usually tapered towards the stalk giving a 'sloping shoulders' appearance, shed with thin long stalks (>15 μm in length). Chromosome not determined. Both A_1 and A_2 compatibility types were present. L/B ratio of sporangia 1.9 some upto 2.3.

Based on the pedicel length, Zentmyer et al. (1977) and Kaosiri et al. (1978) classified the cocoa isolates of Phytophthora into four groups.

Group I : Short and thick sporangial stalks; average length <5 μm.

Group II : Thin sporangial stalks intermediate in length (average 5-15 µm).

Group III : Unusual and characteristically long stalks with an average length of >15 µm.

Group IV : Non-caducous sporangia

The first three groups corresponds to MF_1, MF_3 and MF_4 of the cocoa isolates of P. palmivora (Griffin, 1977). The fourth group with non-caducous sporangia were found to be P. citrophthora (Smith & Smith) Leonian. (Campelo and Luz, 1981; Kellam and Zentmyer, 1986).

The sporangial stalk length as a valuable taxonomic criterion for identifying the isolates in the P. palmivora complex of cocoa was reported by Waterhouse (1974), Zentmyer et al. (1977), Kaosiri et al. (1978) and Al-Hedaithy and Tsao (1979).

Size, shape and length to breadth ratio of sporangia were frequently considered as important characteristics in identifying Phytophthora species (Rosenbaum, 1917; Tucker, 1931; Leonian, 1934; Waterhouse, 1963; Newhook et al., 1978; Ho, 1981; Waterhouse et al., 1983 and Stamps et al., 1990).

Sporangial ontogeny was considered as important taxonomic criterion in distinguishing Phytophthora species (Tsao and Tummakutte, 1977; Alizadeh and Tsao, 1985 and Zentmyer, 1988). The formation of sporangia of P. palmivora and P. megakarya of cocoa has been reported as typical sympodial (Waterhouse, 1963, 1974; Idosu and Zentmyer, 1978; Brasier and Griffin, 1979; Zentmyer, 1988). Idosu and Zentmyer (1978) and Zentmyer (1988) reported the umbellate type of sporangial formation in P. capsici.

Chandramohanan et al. (1979) reported that the sporangia of P. palmivora causing twig die-back and chupon blight were ellipsoidal or ovoid, papillate and caducous. The L/B ratio was 1.3 to 2.2 usually 1.6. The stalks of the sporangia were short and thick, which closely resembled the group I of the morphological groups (Griffin, 1977). The chlamydospores were produced in short lateral branches, intercalary and mostly spherical in shape.

Based on the detailed studies on the morphological and physiological characteristics of 950 isolates of Phytophthora collected from all the major cocoa growing areas of the world, Brasier and Griffin (1979) reported that majority of the isolates of P. palmivora belonged to either one of the three main groups viz., MF_1 ('S'-type), MF_3 ('L'-type) and MF_4 and they were of the opinion that the three forms should be

designated as separate species. Therefore, MF_1 ('S'-type) and MF_2 forms were redesignated as P. palmivora, whereas MF_3 ('L'-type) was described as a new species P. megakarya Brasier and Griffin. They also noted that Phytophthora isolates from pepper closely resembled with P. palmivora MF_4 in sporangial shape, pedicel type and oogonial morphology and therefore suggested both pepper and MF_4 form as one and the same species. Later, it was renamed as P. capsici Leonian. (Kaosiri et al., 1978; Zentmyer et al., 1981 and Waller, 1981).

Das (1986) observed that, the Phytophthora sp. infecting arecanut, rubber, cocoa, coconut and cardamom had close similarity with black pepper isolates of P. palmivora in their morphological characters. He also noted that fungus produced ovoid to lamoniform sporangia having round base with L/B ratio ranging from 1.07 to 1.67 and pedicel length 2.00 to 6.00 µm. Fagan (1988) studied six cocoa isolates of Phytophthora palmivora from Jamaica and found the mean sporangial size as 48 x 27.7 µm and L/B ratio varying from 1.52 to 1.8.

2.2 Growth on different media

Turner (1969) reported that P. palmivora grew rapidly on oat meal agar medium at 25 to 28°C. Weststeijn and Okafor

(1971) found better growth and sporulation of P. palmivora on cassava dextrose agar. According to Waterhouse (1974), the amount of aerial mycelium varied with the medium and to a certain extent with the isolate, ranging from sparse (corn meal agar) to fluffy (potato dextrose agar). Abundant sporangia were observed in corn meal agar, lima bean and oat meal agar. Many workers had the opinion that carrot agar medium was the best for studying colony morphology (Sansome et al., 1975; Brasier and Griffin, 1979; Idosu and Zentmyer, 1978; Kellam and Zentmyer, 1986).

P. palmivora produced abundant sporangia and chlamydospores on potato dextrose agar and oat meal agar (Chandramohanan et al., 1979). Brasier and Griffin (1979) observed the growth of P. palmivora MF_1 on carrot agar. According to them, the aerial mycelium was sparse except in the centre, produced stellate and striate pattern with defined edge. Al-Hedaithy and Tsao (1979) studied three Phytophthora isolates including P. palmivora on carrot agar, oat meal agar and V_8-$CaCO_3$ and found that different media did not or slightly affected the pedicel length. Pimental et al. (1981) studied three Phytophthora isolates of cocoa and found no difference in the production of zoosporangia at 21, 24 and 27°C while carrot agar and pea culture media gave better results than potato dextrose agar. Alizadeh and Tsao (1985)

found that on carrot agar plates sporulation was enhanced in continuous light. Das (1986) studied the growth of P. palmivora on potato dextrose agar, oat meal agar, corn meal agar and carrot agar. He observed the maximum sporangial production in carrot agar medium.

2.3 Symptomatology

The major symptoms produced by Phytophthora palmivora on cocoa plant include seedling blight, trunk canker, die-back of twigs, blight and necrosis of leaf and petiole resulting in leaf fall and rotting of fruits (Gregory, 1974).

The symptomatology of seedling blight of cocoa was described by some workers. Chant (1957) reported the infection of cocoa seedlings by P. palmivora. The first symptom noted was a brown discolouration of leaves at the growing point of the seedlings which led to infection of the cotyledons and eventual collapse of the stem. According to Keane (1974), the leaves, petioles and shoot tips of cocoa seedlings rapidly withered, turning black and died. Within few days the rot extended to the lower stem and cotyledons. P. palmivora was observed on the surface of infected seedlings.

Manco (1974) described the symptoms occurring on the shoots, cuttings, grafts and seedlings due to P. palmivora.

He observed that symptom varied according to site of occurrence (leaves, stem or petioles) and with age of the tissue. Accordingly, on young succulent leaves, initial symptom appeared within 24 h after inoculation as little blackened spots. The blackened spots grew very rapidly when conditions were humid. The veins turned dark and when the fungus reached the secondary or main veins the necrosis spread through them, forming a 'V' and turning brown. On unhardened stem tissues the lesion appeared as a dark spot. High humidity always increased the growth of the spot, led to die-back. The lesion advanced to the petiole of leaves causing necrosis. When the petiole was infected, the darkening progressed first towards the midrip and then through the stem. Die-back was always a symptom of infection on stem or petiole. The symptom on mature leaves initially developed as dark spots between the veins, spreading to the main vein and then to the petiole. The leaves became yellow with necrotic spots and they dropped. During the arrival of dry condition the growth of the fungus stopped on leaves and the necrotic tissues became light brown and a marked chlorosis appeared surrounding the necrosis.

Chandramohanan (1979, 1983) reported the symptomatology of seedling die-back of cocoa caused by P. palmivora. He observed that, infection started from the tip of the stem and

spread downwards as dark brown to black water soaked linear lesions. The lesion extended to the leaves through the petioles resulting in wilting and subsequent defoliation of the seedlings. The infection also initiated from the collar region, cotyledonary stalks or leaves as dark brown to black discolouration. In all the cases, infection spread to the entire stem causing wilting, defoliation and finally death of the seedlings.

Studies on the symptomatology of seedling blight of cocoa conducted at the Cadbury KAU Co-operative Cocoa Research Project revealed that the disease initiated on young leaves and stem as water soaked lesions, later turned to black or dark brown areas and finally resulted in the blightening of leaves and stem. In severe cases of infection, defoliation and death of seedlings occurred. In young budded plants stem infection occurred at any portion of the stem. Infection at the base of the budded plants resulted in their death (KAU, 1993).

2.4 Host range

Narasimhan (1927) found that the leaves of Colocasia antiquorm Schott., Santalum album L., Mangifera indica L. and Hevea brasilliensis muell. Arg. were infected by P. palmivora. Ashby (1929) described P. palmivora as an omnivorous tropical

fungal pathogen of world wide distribution attacking a wide range of cultivated plants. Muller (1936) reported that the pepper Phytophthora was capable of infecting papaw, cocoa, rubber, caster, egg plant, tobacco and Colocasia antiquorm. He also found that the cocoa isolate of P. palmivora did not infect black pepper. Bobr-Tylingo (1954) listed the susceptible plants and stated that P. palmivora principally attacks cocoa, hevea rubber, palms and various citrus fruits in addition to 56 species which are less severely affected. Infection of Bougainvillea spectabilis by P. palmivora was reported by Ramakrishnan and Seethalakshmi (1956). Hickman (1958) found that the fungus parasitizes plants of 51 genera in 21 families of flowering plants. Pieris (1962) reported that P. palmivora from cocoa could infect coconut but coconut strain infected cocoa pods only with difficulty. Holliday and Mowat (1963) observed P. palmivora from cocoa did not infect leaves of pepper. Pieris (1963) showed that the cocoa strain could infect hevea rubber and vice versa. Waterhouse (1963) reported that the genus Phytophthora contains 39 species which affect a range of annual and perennial plants, shrubs and herbaceous flowering plants of many families. Chee (1969b) listed 138 plants as hosts of P. palmivora. Of which, 78 are economically important and rest were ornamental, shade or hedge plants. Umbala and Rama Rao (1972) reported a non-oospore forming isolate of P. palmivora as the causal agent of

leaf blight of Colocasia esculenta (Linn.) Schott. from Hyderabad. P. palmivora cause blight on plants like rubber, papaya, bougainvillea, fig, sunflower, avacado, citrus and coconut (Manco, 1974).

Chee (1974) listed the economically important plants attacked by P. palmivora. It included the plants grown as fruits and nuts, vegetables, oil crops, other plantation crops and a variety of ornamental shade and hedge plants. Chandramohanan et al. (1979) found that the P. palmivora isolate from cocoa did not infect areca palm fruits. Das (1986) found that cross inoculation test with cocoa isolate was successful on arecanut, rubber, coconut, and cardamom.

2.5 Screening for host resistance

Asare-Nyako and Amponsah (1973) described the glasshouse method for screening the cocoa seedlings against black pod. In this method, pre-germinated seeds were inoculated with zoospore suspension of the fungus. Seedling mortality after eight weeks of inoculation was related with the susceptibility of the parents to black pod in the field. Evaluation of methods for assessing resistance of cocoa cultivars and hybrids to P. palmivora was suggested by Lawrence (1978). Result indicated that, out of 51 cultivars screened, nine cultivars such as EET 59, EET 376, pound 7,

UF 713, UF 715, SCA 6, SCA 12, Catongo and Diamantes 800 showed a promising degree of resistance. Results of the laboratory inoculation trials conducted by Sri-Sukamato and Mawardi (1986) indicated that, the cocoa types DRC 16, SCA-6, SCA-12 and ICS-6 were highly resistant to back pod pathogen P. palmivora. Pinto et al. (1989) screened cocoa hybrid progenitors resistant to Phytophthora spp. They crossed the clones BE 5, CAS 1, EEG 8,9 and 65, EET 103, ICS 84 and 95, MA 15, SGU 3 and SPA 17 with the clone SIC 802 as a common progenitor during the seedling stage and observed that the progenies of the clones BE 5, EEG 8, 9 and MA 15 showed the highest level of resistance, while ICS 84, 95 and CAS 1 the highest level of susceptibility.

Preliminary screening on the budded plants of 10 high-yielding cocoa types at Cadbury KAU Co-operative Cocoa Research Project, College of Horticulture showed that the cocoa type GVI-10 recorded the lowest percentage of mortality followed by types S-45.5 and S-40.7. The highest percentage of mortality were observed in the types S-39.9 and S-31.11 (KAU, 1993).

2.6 Disease control with chemicals

2.6.1 In vitro studies

Okaisabor (1970) found low concentrations of Copper

oxide, Dexon, Plantvax and Tillet were highly effective against black pod pathogen P. palmivora in agar culture and in soil in the lab. Chandramohanan et al. (1979) found that the fungicides Captafol (Difolatan 80 WP) at 0.2 per cent and Copperoxy chloride (Cupramar), Guazatire (Panoctine) and Fenfuram (Panovam) each at 0.3 per cent concentration inhibited the lesion development on detached cocoa pods. Kueh (1980) observed the reduction of sporulation of P. palmivora in in vitro by Metalaxyl, Dowco 444 and Previcur-N (Propanocarb). Effect of Copper oxychloride in inhibiting the growth of P. palmivora under in vitro condition was reported by Figueiredo and Lellis (1981). Tey and Wood (1984) tested the in vitro effect of eight fungicides against P. palmivora and found Cycloheximide and Mancozeb were highly toxic at low concentrations. Campelo et al. (1984) reported the in vitro effect of Ridomil and found that the mycelial growth of P. palmivora was completely inhibited at 50 ppm. Reddy and Chandramohanan (1984) tabulated the relative efficacy of 24 fungicides against black pod pathogen P. palmivora. They observed that the fungicides Bordeaux mixture (0.75%), Fytolan and Thiram (each at 1%), Dithane-M.45 (0.4%), Difolatan (0.3%) and Captan (0.5%) completely inhibited the growth of the fungus in glucose nitrate agar medium. Raghu and Chandramohanan (1993) assessed the in vitro sensitivity of certain fungicides on P. palmivora and found that Ridomil-MZ

at 0.2 and 0.3 per cent, Fytolan, Blitox, Foltaf, Dithane M.45 and Captaf each at 0.3 per cent concentrations were fungicidal. Ridomil-MZ, Foltaf and Captaf each at 0.1, 0.2 and 0.3 per cent concentration inhibited P. palmivora infection on detached cocoa pods completely even seven days after inoculation.

Not much work has been conducted on the in vitro effect of antibiotics against Phytophthora spp. However, Voros (1963) reported that the growth of P. infestans was strongly inhibited by 25 ppm Streptomycin and almost completely by 200 ppm. Ersek et al. (1972) observed the in vitro inhibitory effect of Chloramphenicol to P. infestans causing late blight of potato. In vitro effect of Aureofungin against P. parasitica var. piperina was reported by Chaurasia et al. (1973). Complete inhibition of the growth of black pod pathogen P. palmivora by Aureofungin at 200 ppm was noticed by Reddy and Chandramohanan (1984).

2.6.2 In vivo studies

Field control of seedling blight of cocoa caused by P. palmivora was studied by few workers. Newhall and Diaz (1966) compared Malachite green with seven other fungicides on cocoa seedlings. Bordeaux mixture proved best and Malachite green the poorest. Diaz and Newhall (1966) compared visual

field scoring with three methods of laboratory scoring of cocoa seedlings for evaluating effectiveness of four fungicides in the control of Phytophthora leaf blight. They found that Difolatan and Copper Sandoz gave the best control of the leaf blight disease. Muller and Njomou (1970) reported that Difolatan and Copper oxychloride gave good control of Phytophthora leaf blight. According to Asare-Nyako (1972), Kocide at 0.91 kg/45 l of water gave control of pre- and post-emergence death of cocoa seedlings. Chandramohanan (1983) reported that drenching the seedlings with Bordeaux mixture or any of the Copper oxychloride just before the onset of monsoon and thereafter at frequent intervals resulted in good control of seedling die-back caused by P. palmivora. Mc Gregor (1984) observed the effectiveness of Metalaxyl both as foliar spray and as a seed treatment for the control of Phytophthora seedling blight. Chan and Lim (1987) obtained good control of P. palmivora with Ridomil MZ as protective and curative foliar sprays and as soil drench on cocoa and durian seedlings. Anderson and Guest (1990) reported that Potassium phosphonate (0.4%) sprays controlled seedling blight of cocoa caused by P. palmivora. A preliminary study conducted at Cadbury KAU Co-operative Cocoa Research Project, College of Horticulture, indicated that Potassium phosphonate was effective than Indofil-M.45 in checking the severity of seedling blight of cocoa (KAU, 1993).

Many reports on the control of other Phythphthora diseases of cocoa are available. Successful control of cocoa Phytophthora pod rot disease using Bordeaux mixture was reported by Faber (1907), Bowman (1952), Siller (1954), Swarbrick (1965) and Gorenz (1972) and Copper oxychloride by Holliday (1954, 1960); Liabeuf (1964) and Braudeau and Muller (1971). Effect of Kocide against black pod of cocoa caused by P. palmivora was reported by Jones (1971); Rocha et al. (1973) and Asare-Nyako (1972). Turner (1974) found that treatment with 2% Difolatan 4F was effective against cocoa canker caused by P. palmivora. Chandramohanan et al. (1979) reported that P. palmivora is the most destructive of all the fungal pathogens attacking cocoa in India and require regular prophylactic control measures. Kueh (1980) observed that the pod infection by P. palmivora (MF_1) was reduced by Etridiazole and Metalaxyl. Mc Gregor (1983, 1984) proved Metalaxyl as the most effective systemic fungicide so far tested against P. palmivora on cocoa. Reddy and Chandramohanan (1984) conducted a field evaluation of five fungicides and indicated that all the fungicides reduced black pod incidence but the copper based fungicides performed best. Ventilborgh (1987) reported the effectiveness of copper contact fungicides and Ridomil plus-72 against P. palmivora causing black pod of cocoa. In vivo effectiveness of Potassium phosphonate in the control of black pod and canker of cocoa caused by

P. palmivora was reported by Anderson and Guest (1990) and Holderness (1992).

Usefulness of antibiotics in controlling some Phytophthora diseases of crop plants have been reported by few workers. Effectiveness of Streptomycin against Oomycetes such as those causing late blight of tomatoes and potatoes was reported by Muller et al. (1954) and Crosse et al. (1960). Somani and Patel (1970) observed the effectiveness of Aureofungin to P. nicotianae var. nicotianae. Treatment of potato tuber slices with Chloramphenicol (50-800 ppm) or Streptomycin (3.125 to 50 ppm) inhibited the growth of P. infestans (Barna et al., 1972). In vivo effectiveness of Chloramphenicol against P. infestans causing late blight of potato was reported by Ersek et al. (1972). Chaurasia et al. (1973) tested the efficacy of five antibiotics and found that Streptocycline and Streptomycin were most effective against P. parasitica var. piperina on leaves of Piper betle L. Vyas and Chaurasia (1978) obtained successful control of leaf-rot and foot-rot of betel vine caused by Phytophthora sp. by dipping the cuttings of betel vine in one per cent solution of Streptomycin sulphate for about 10 minutes before planting.

Materials and Methods

MATERIALS AND METHODS

3.1 Location of the experiment

The field experiments were conducted at the nursery of Cadbury KAU Co-operative Cocoa Research Project, attached to the College of Horticulture and the laboratory experiments were conducted at the Department of Plant Pathology, College of Horticulture, Vellanikkara, Trichur.

3.2 Isolation of the pathogen

The pathogen causing seedling blight disease of cocoa was isolated from the infected leaves and stems showing typical symptoms. The infected portions of leaves and stems with advancing lesion were cut into small bits, surface sterilized with 1:1000 mercuric chloride solution for 60 seconds and washed in three changes of sterile water. The bits were then placed in sterile petri dishes previously poured with potato dextrose agar (PDA). The dishes were incubated at room temperature. After two to three days, when the growth of the fungus was visible, bits of mycelium were transferred to PDA slants. The culture was purified by hyphal tip method. The fungal culture was maintained on PDA and oat meal agar media.

3.3 Pathogenicity

Both zoospore suspension and culture discs of the isolated fungus were used for pathogenicity test. Zoospore suspension was prepared by putting seven day old culture in sterile water for three days. After three days, fungal mycelia were separated by filtering through a fine muslin cloth. Zoospore suspension was obtained from the filtrate (Mammootty, 1978).

The seedlings were sprayed with zoospore suspension and kept inside the polythene bags to provide high humidity. In another set of experiment, culture discs of 8 mm diameter containing mycelia and sporangia of the fungus were placed on the lower surface of the leaves and covered by a disc of moist blotting paper. The inoculated leaves were provided with high humidity. Stem of seedlings and budded plants were also inoculated by placing 8 mm culture discs containing sporangia and mycelia and covered by moist cotton and provided high humidity.

3.4 Morphological characters of the pathogen

Morphological characters of the pathogen were studied by the standard "slide culture" method except for decidious sporangia. Decidious sporangia were obtained and observed by slightly touching the mycelial disc one or two times in a drop

of cotton blue stain. Observations on the morphological characters such as breadth of the hypha, length of the sporangiophore, length and breadth of the decidious sporangium and the length of pedicel were taken using Olympus research microscope and measurements taken after calibrating with micrometer. The mean of 30 observations of each character was taken. Microscopic drawings of the fungus were done using a Leitz research microscope with zoom attachment at maximum possible magnification. Photomicrographs of the pathogen were also taken.

3.5 Growth of the fungus on different media

3.5.1 Growth on solid media

The following media were used for the study.

1. Potato dextrose agar
2. Czapek's agar
3. Oat meal agar
4. Corn meal agar
5. Carrot agar
6. Cocoa pod extract agar
7. Cocoa leaf extract agar
8. Cocoa pod extract dextrose agar

The composition of the media are given in Appendix I. The media were prepared and poured into sterile petri dishes at the rate of 15 ml/plate. Circular discs of 8 mm diameter were cut from the seven day old culture of the fungus by means of a sterile cork borer. The culture discs were placed in the centre of the solid medium. Four replications were maintained for each medium. The dishes were incubated at room temperature. Observations on the growth of the fungus were taken at an interval of 24 h for eight days. Sporangial production on different solid media were also assessed. For this, culture discs of 8 mm diameter were taken from each media using a cork borer. The sporangia were obtained by touching the mycelial surface of the disc two or three times in a drop of cotton blue stain. The sporangial count in 10 observtions under low power objective of the microscope were taken and its average worked out.

3.5.2 Growth on liquid media

The growth of the fungus on different liquid media was studied. The liquid media used were:

1. Potato dextrose broth
2. Czapek's broth
3. Oat meal broth
4. Corn meal broth

5. Carrot broth
6. Cocoa pod extract broth
7. Cocoa leaf extract broth
8. Cocoa pod extract dextrose broth

Hundred milli litre quantities of each medium was taken in 250 ml conical flask and sterilized under 1.1 kg/cm^2 pressure for 20 minutes. The media were inoculated with culture discs of 8 mm diameter from seven day old culture of the fungus. Three replications were maintained for each medium. The inoculated flasks were incubated at room temperature for 10 days. The mycelia were filtered through previously weighed Whatman No.1 filter paper and dried at 50°C till a constant weight was obtained. The dry weight of the mycelium was recorded using electronic balance.

3.6 Symptomatology

Symptomatology of the disease on infected seedlings and budded plants was studied both under natural and artificial conditions. Symptomatology under natural condition was studied by close examination of naturally infected seedlings and budded plants during peak period of disease occurrence in the south-west monsoon. The seedlings and budded plants were artificially inoculated with zoospore suspension and culture discs of the pathogen as described in

the pathogenicity test (vide para 3.3) for studying the symptom development under artificial condition.

3.7 Host range of the pathogen

The following plants were inoculated to determine the host range of the pathogen.

Sl. No.	Common name	Botanical name	Plant parts used for inoculation
1.	Pepper	*Piper nigrum* L.	Leaves
2.	Arecanut	*Areca catechu* L.	Leaves and young nuts
3.	Coconut	*Cocos nucifera* L.	Leaves and young nuts
4.	Rubber	*Hevea brasiliensis* L.	Leaves
5.	Bougainvillea	*Bougainvillea* sp.	Leaves
6.	Colocasia	*Colocasia esculenta* L.	Leaves
7.	Betelvine	*Piper betle* L.	Leaves
8.	Long pepper	*Piper longum* L.	Leaves

Detached leaves of pepper, betel vine, arecanut, coconut, bougainvillea, rubber, colocasia and long pepper and, young nuts of coconut and arecanut were inoculated with 8 mm culture discs of the seven day old culture of the fungus. The leaves of the above plants were given pin pricks before inoculation. The young nuts of coconut and arecanut were

inoculated near the stalk end. All the inoculated materials with sufficient replication were kept under high humid condition at room temperature and were observed for symptom development.

3.8 Effect of age of seedlings on the incidence and severity of the disease

This experiment was carried out during May to August, 1993 to study the influence of age of seedling on the incidence and severity of the disease. The seeds were sown at weekly intervals upto eight weeks in polythene bags of size 20 x 15 cm using standard potting mixture. When the seeds sown in the eighth week produced three leaves; all the seedlings sown at different intervals were artificially inoculated with zoospore suspension of the pathogen. Sufficient humidity was provided after inoculation. Fifteen replications were maintained for each age group. Observations on the disease score were taken at five days interval upto 20 days after inoculation. In order to assess the disease intensity a 0-9 scale was prepared based on the percentage of leaf area infected. For facilitating the computation of defoliation and plant mortality two additional scales were provided and accordingly the following 0-13 scale was devised.

Disease scale	Intensity of infection
0	No infection
1	Less than 12.5 per cent leaf area infected
3	13 to 25 "
5	26 to 50 "
7	51 to 75 "
9	More than 75 "
11	Defoliation
13	Mortality of the plant

3.9 Effect of age of budded plants on the incidence and severity of the disease

It was observed that budded plants were more prone to infection by the pathogen resulting in severe stem and leaf symptoms. Thus, a study was conducted, during May to September, 1993 to find out the susceptible stage of budded plants on the incidence and severity of the disease. For this experiment buds of a single cocoa type were taken and budded at weekly interval upto six weeks on six month old root stocks raised in polythene bags using standard potting mixture. When the budded plants of the last interval (6th week) produced 2-3 leaves, all the budded plants were artificially inoculated

with zoospore suspension of the pathogen. Sufficient humidity was provided after inoculation. Fifteen replications were maintained for each age group. Observations on the disease score were taken (0-13 scale) at five days interval upto 20 days after artificial inoculation. In addition to this, the percentage of stem infection was also recorded.

3.10 Screening of cocoa types for resistance/tolerance against seedling blight

This experiment was conducted using 62 promising cocoa types available at the Cadbury KAU Co-operative Cocoa Research Project, College of Horticulture, Vellanikkara. The experiment was carried out during April to September, 1994. Six month old seedlings were budded with each cocoa type. Twenty replications were maintained. The budded plants were kept in a severely infected cocoa nursery. Observations on bud establishment and percentage mortality of budded plants three months after bud establishment were recorded.

3.11 Chemical control of the disease

The efficacy of fungicides/antibiotics were tested both *in vitro* and *in vivo* for the control of the disease.

3.11.1 *In vitro* evaluation of fungicides and antibiotics against the pathogen

The following fungicides and antibiotics were used for the *in vitro* evaluation.

a. Fungicides

Common name	Active ingredient	Concentration (per cent)		
Indofil M.45	Mancozeb	0.1	0.2	0.3
Foltaf	Captafol	0.1	0.2	0.3
Fytolan	Copper oxychloride	0.1	0.2	0.3
Captaf	Captan	0.1	0.2	0.3
Ridomil MZ	Metalaxyl 8% + Mancozeb 64%	0.1	0.2	0.3
Akomin-20	Potassium phosphonate	0.1	0.2	0.3
Bordeaux mixture	Copper sulphate + lime	0.5	0.75	1.0

b. Antibiotics

Common name	Active ingredient	Concentration (ppm)		
Ambistryn-S	Streptomycin sulphate	100	250	500
Streptocycline	Streptomycin sulphate 90%, and Tetracycline hydrochloride 10%	100	250	500
Terramycin	Oxytetracycline hydrochloride	100	250	500
Chloramphenicol	Chloromycetin	100	250	500
Aureofungin	N-methyl aminoacetophenone and mycosamine	100	250	500
Amoxycillin	Amoxycillin trihydrate	100	250	500

The efficacy of different fungicides and antibiotics on the growth of the fungus was tested by the "poisoned food technique" (Riker and Riker, 1936). The required quantity of fungicides and antibiotics were added to the 100 ml sterilized, molten potato dextrose agar, mixed well and poured into sterilized petri dishes at the rate of 15 ml each. Mycelial discs of 8 mm diameter were cut and placed in the centre of each petri dish containing the poisoned medium. Control consisting of PDA medium alone, inoculated with the fungus was also maintained. Three replications were maintained for each concentration of the chemicals. The inoculated petri dishes were incubated at room temperature and

the observations on the radial growth of fungus were taken when the control dishes showed full growth.

3.11.2 Efficacy of fungicides and antibiotics against *Phytophthora palmivora* on detached cocoa leaves

Based on the *in vitro* screening of fungicides/ antibiotics against seedling blight pathogen, seven fungicides and four antibiotics each at one concentration were selected. The effect of these chemicals against *P. palmivora* was tested on detached cocoa leaves. The details of the chemicals used in the study are as follows:

Treatment	Chemical	Concentration
T_1	Fytolan	0.3%
T_2	Captaf	0.3%
T_3	Foltaf	0.3%
T_4	Indofil M.45	0.3%
T_5	Akomin	0.3%
T_6	Ridomil	0.3%
T_7	Bordeaux mixture	1%
T_8	Streptomycin	500 ppm
T_9	Streptocycline	500 ppm
T_{10}	Terramycin	500 ppm
T_{11}	Chloramphenicol	500 ppm
T_{12}	Control	--

The required concentration of each fungicides and antibiotics were prepared and sprayed on young cocoa seedlings. Two hours after spraying the chemicals, leaves of uniform size were taken and were inoculated with 8 mm mycelial disc of the pathogen after giving pin pricks. The inoculated as well as control leaves were kept in a moist chamber. Four replications were maintained for each chemicals. Observations on the diameter of lesion development were recorded on the third and fifth day after inoculation.

3.11.3 *In vivo* control of the disease

The comparative efficacy of fungicides and antibiotics for the control of seedling blight of cocoa was tested in four different pot culture experiments during June to August, 1994. Forty five days old seedlings were used for the experiments. The following chemicals were used for the study.

Treatment	Chemical	Concentration
T_1	Streptomycin	500 ppm
T_2	Streptocycline	500 ppm
T_3	Terramycin	500 ppm
T_4	Chloramphenicol	500 ppm
T_5	Fytolan	0.3 %

T_6	Indofil M-45	0.3 %
T_7	Captaf	0.3 %
T_8	Akomin	0.3 %
T_9	Foltaf	0.3 %
T_{10}	Bordeaux mixture	1 %
T_{11}	Control	--

3.11.3.1 Effect of fungicides and antibiotics in preventing the natural incidence of the disease

The experiment was laid out in completely randomised design with 11 treatments and 10 replications. The seedlings were raised in disease free areas and treated with the fungicides/antibiotics. The sprayed plants were kept in a severely infected cocoa nursery. Further three more sprayings of fungicides/antibiotics were given at 10 days interval. Observations on the disease score were taken at 10 days interval after each spraying using 0-13 scale.

3.11.3.2 Effect of fungicides and antibiotics on naturally infected cocoa seedlings

Infected cocoa seedlings of same age group were selected and sprayed with fungicides/antibiotics. Four

sprayings were given at 10 days interval. This experiment was conducted under shaded and open conditions with eight and seven replications respectively. Observations on the disease score were taken on the day of each spraying and 10 days after fourth spraying.

3.11.3.3 Effect of fungicides and antibiotics on the severity of the disease on artificially inoculated seedlings

The experiment consisted of 11 treatments and eight replications. Seedlings of the same age group were raised in polythene bags and, were artificially inoculated with zoospore suspension of the pathogen. At the onset of the symptom expression, second day after inoculation the chemicals were sprayed. Three more sprayings were given at an interval of 10 days. Observations on the disease score were taken on the day of each spraying and 10 days after fourth spraying.

3.11.3.4 Effect of fungicides and antibiotics in preventing the incidence and severity on artificially inoculated seedlings

The experiment was laid out in completely randomised design with 11 treatments and eight replications. Seedlings of the same age group were raised and sprayed with fungicides/ antibiotics. Two days after spraying, the seedlings were

inoculated with zoospore suspension of the pathogen. Subsequent three sprayings of fungicides/antibiotics were given at 10 days interval after first spraying. observations on the disease score were taken at 10 days interval after each spraying.

Results

RESULTS

4.1 Isolation and pathogenicity of the fungus

The fungal pathogen causing seedling blight of cocoa was isolated from the infected leaves and stems of seedlings and young budded plants on potato dextrose agar (PDA). The isolated fungus was purified by hyphal tip method and maintained on PDA as well as on oat meal agar medium. Koch's postulates were established with the isolated fungus.

4.2 Morphological characters of the pathogen

The morphological characters of the pathogen causing seedling blight of cocoa were studied in detail and the results are presented in Table 1. On potato dextrose agar and oat meal agar the fungus produced cottony white aerial mycelium, but on carrot agar medium, sparse aerial mycelium was observed. The mycelium branched, hyaline and coenocytic. Somatic hyphae measured 3.5 to 5.3 µm (mean 4.6 µm) in breadth. Sporangiphores arose from the somatic hyphae and their tip become swollen, which developed into sporangia. Sporangiophores indeterminate and measured 49-192.5 µm (mean 83.7 µm) long (Plate 1). Young sporangia more or less spherical with less dense protoplasm. At maturity the protoplasm became dense, granular and differentiated into

Table 1. Morphological characters of Phytophthora palmivora causing seedling blight of cocoa

Characters	Mean*	Range
Breadth of hypha (µm)	4.6	3.5-5.3
Length of sporangiophore (µm)	83.7	49-192.5
Length of sporangium (µm)	49.6	28-63
Breadth of sporangium (µm)	27.7	21-35
L/B ratio of sporangium	1.74	1.3-2.3
Length of pedicel (µm)	4.1	3.5-5.3

* Mean of 30 observations

Plate 1. Mycelium with sporangia of Phytophthora palmivora (260 x)

Plate 2. Mature sporangium of Phytophthora palmivora (930 x)

Plate 1

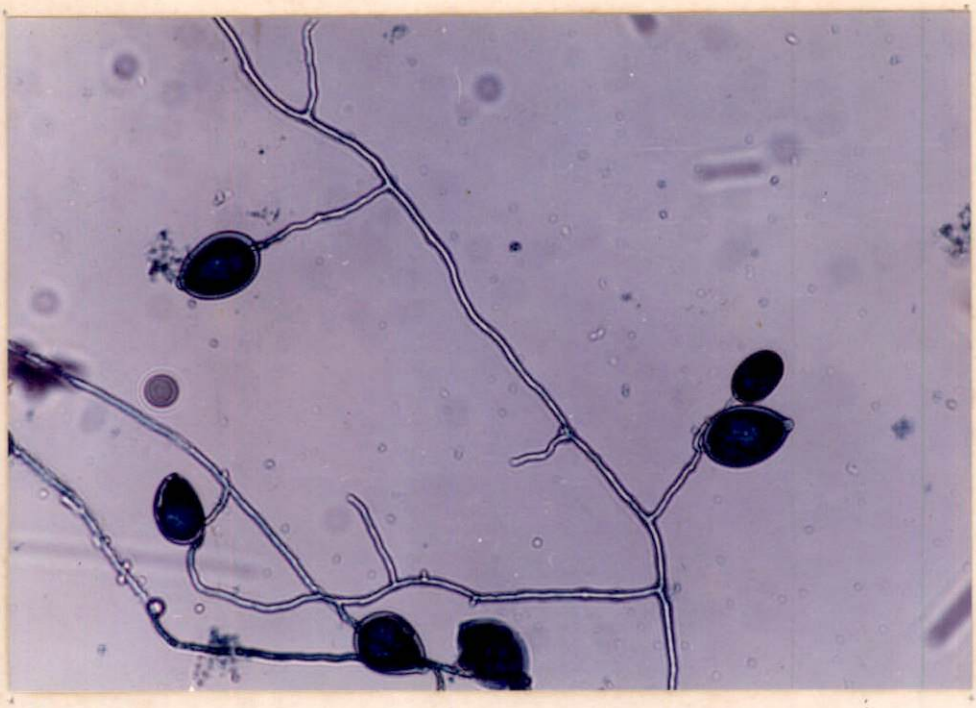


Plate 2

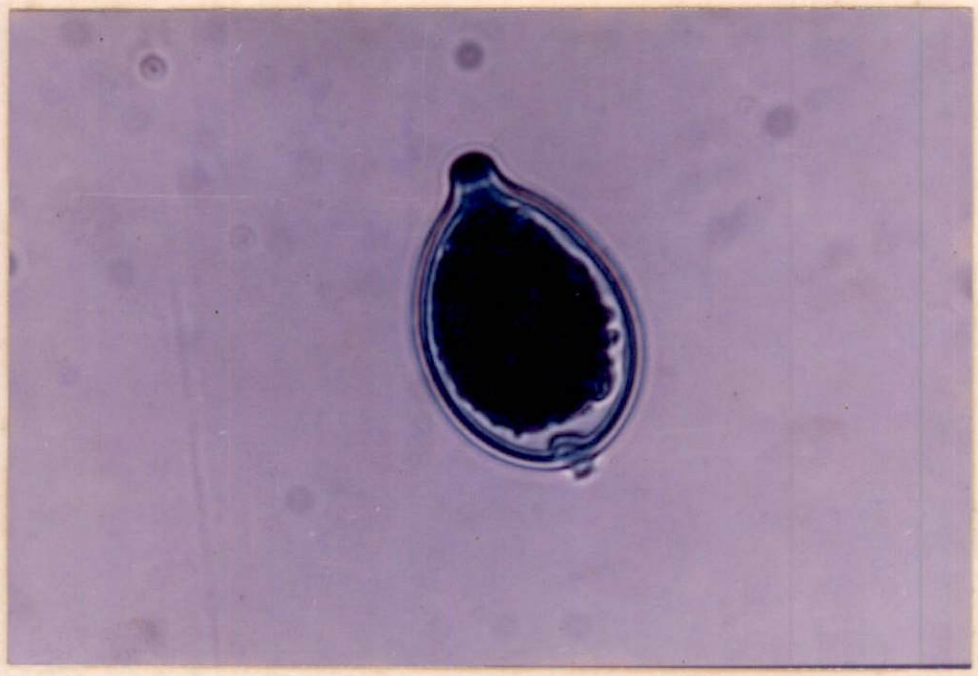


Plate 3. Terminal sporangium of Phytophthora palmivora (850 x)

Plate 4. Sporangial ontogeny of Phytophthora palmivora (260 x)

Plate 3

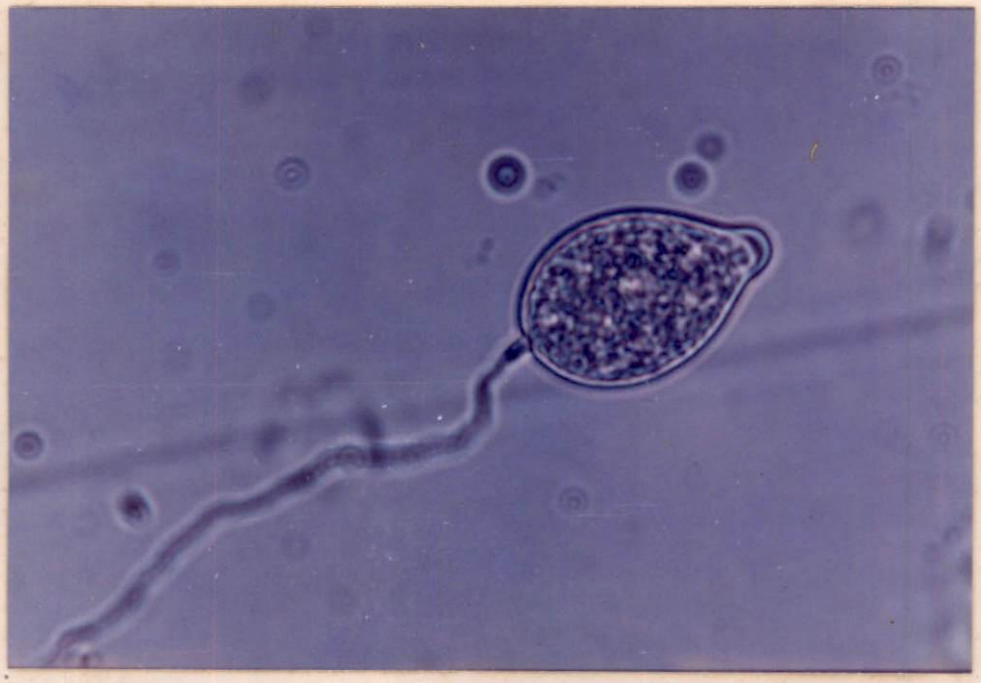
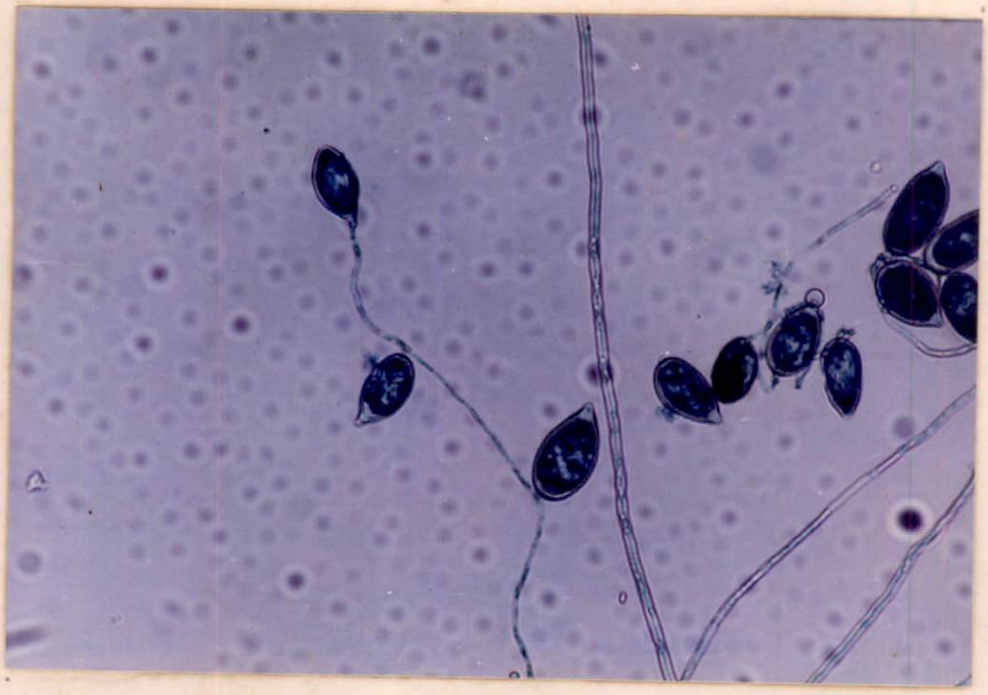


Plate 4



zoospores, within the sporangium itself. The apical portion of the maturing sporangium developed into a papilla and it became well pronounced when fully matured. Fully matured sporangium measured 28-63 x 21-35 µm (mean 49.6 x 27.7 µm) and were near spherical to ovoid with round base (Plate 2 and 3, Fig.1a). The L/B ratio of the sporangium ranged from 1.3 to 2.3 (mean 1.74). The sporangia were borne terminally on the sporangiophores in a simple sympodial fashion and were caducous (Plate 4, 5 and 6; Fig.1b and 2). Decidious sporangia had short and thick stalks which measured 3.5 to 5.3 µm (mean 4.1 µm) long. The fungus produced abundant chlamydospores in old culture of oat meal agar. They were borne intercalerly and more or less spherical. No sexual organs were found to be produced by the fungus in culture media.

4.3 Growth on different solid media

Growth of the fungus on different solid media was studied to find out a suitable medium which produce maximum growth and sporangia and the results are presented in Table 2a and 2b.

1. Potato dextrose agar (PDA)

The fungus initially showed striate growth, and later profuse thick cottony white aerial mycelium was produced.

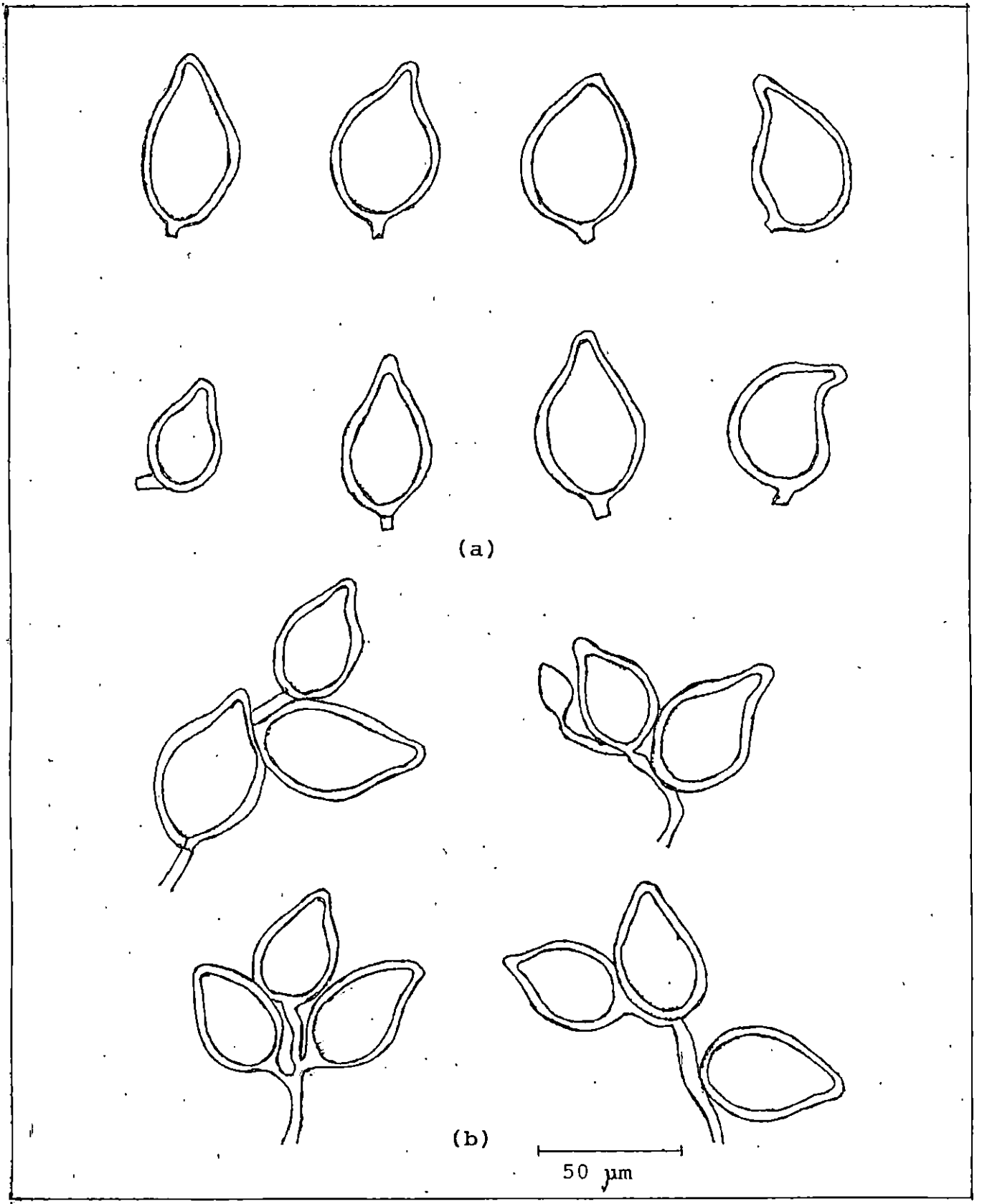


Fig.1 Phytophthora palmivora

a Different shape of mature decidious sporangia

b. Sympodial development of sporangia

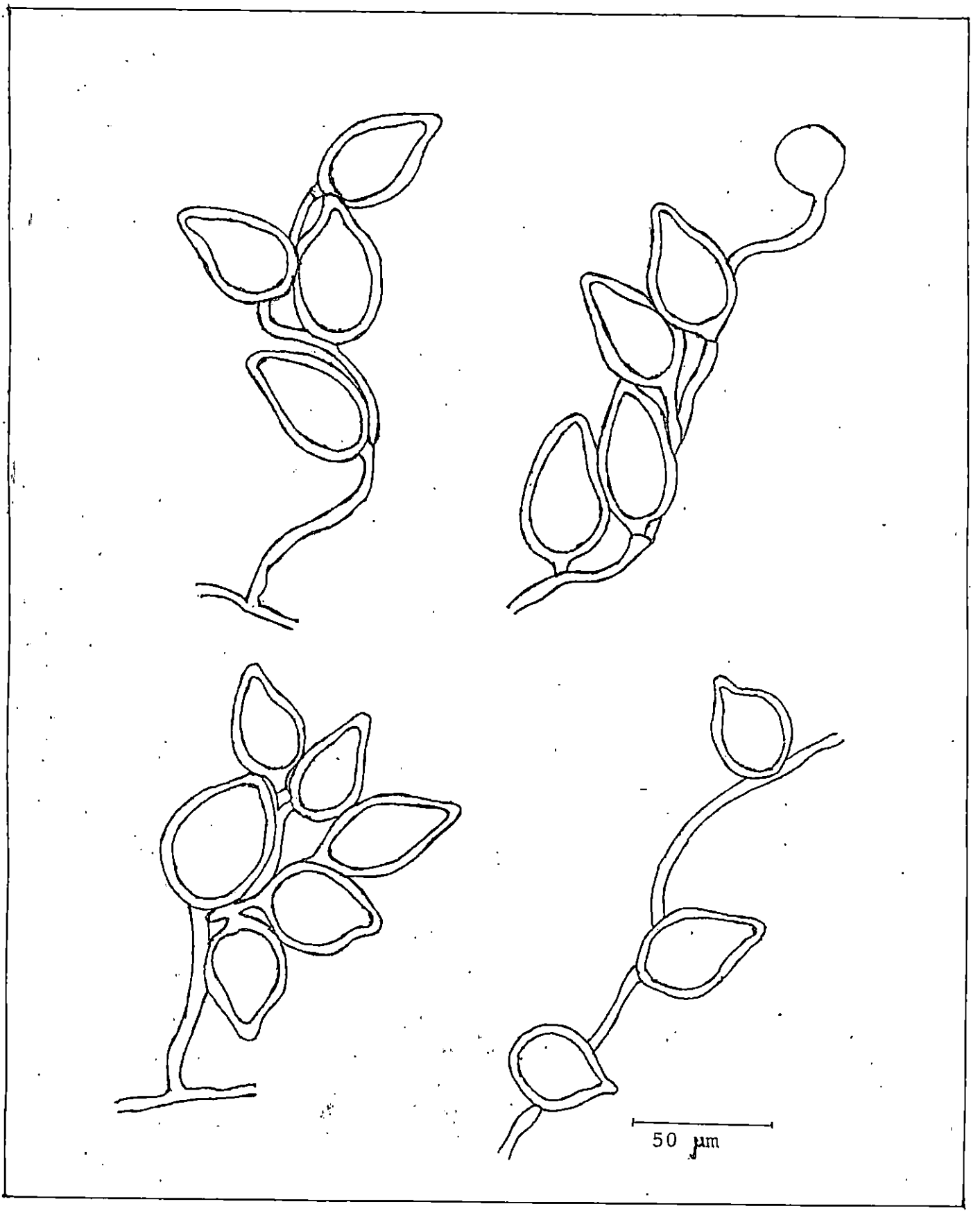


Fig.2 Sporangial ontogeny of Phytophthora palmivora

Plate 5. Close formation of successive sporangia of Phytophthora palmivora (260 x)

Plate 6. Sympodial development of sporangia of Phytophthora palmivora (1060 x)

Plate 5

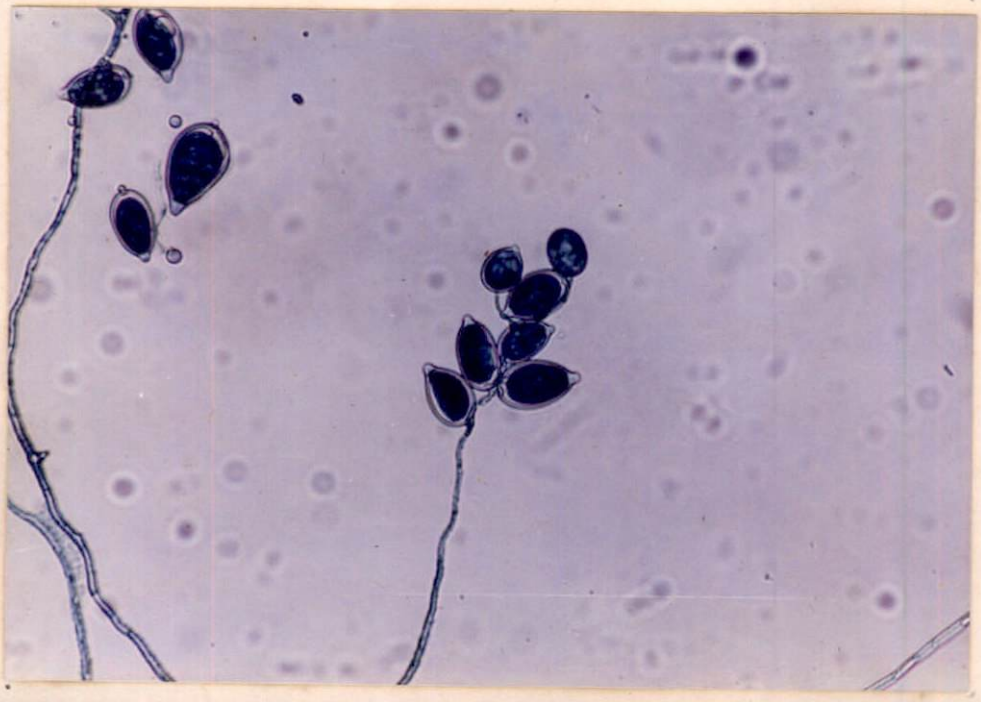


Plate 6

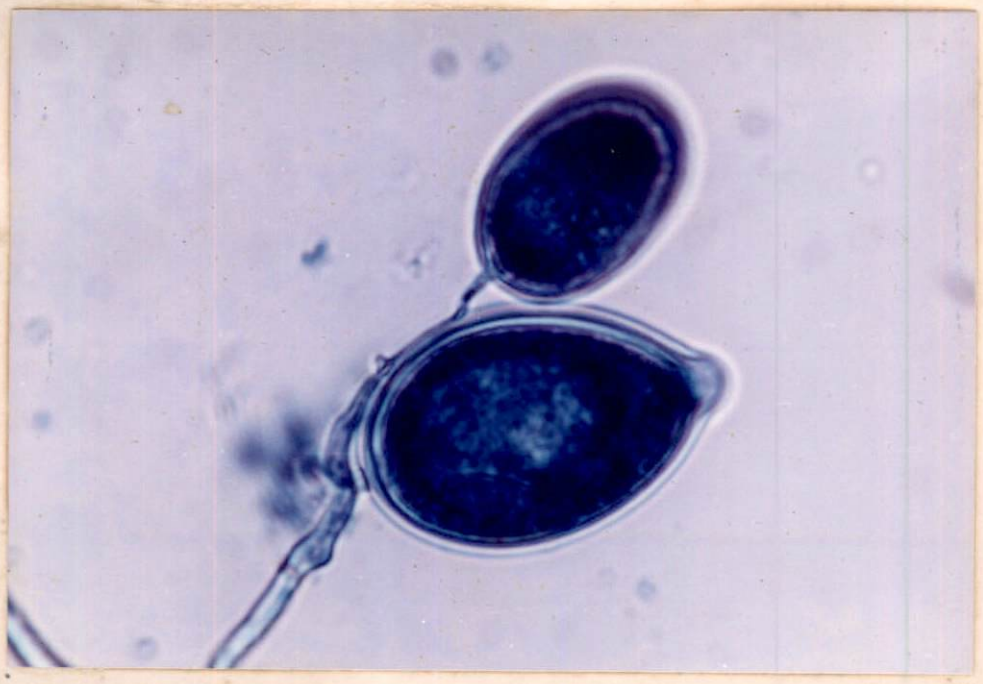


Table 2a. Growth of seedling blight isolate of *Phytophthora palmivora* on different solid media

Sl. No.	Medium	*Colony diameter (cm) after							
		24 h	48 h	72 h	96 h	120 h	144 h	168 h	192 h
1.	Potato dextrose agar	1.1	2.0	2.8	3.9	4.8	5.8	6.6	7.1
2.	Oat meal agar	2.5	5.7	8.5	9.0	9.0	9.0	9.0	9.0
3.	Corn meal agar	2.9	6.0	8.5	9.0	9.0	9.0	9.0	9.0
4.	Carrot agar	1.0	1.9	3.0	4.1	5.3	6.3	7.4	8.3
5.	Czapek's agar	1.1	2.3	3.4	4.4	5.4	6.4	7.3	7.9
6.	Cocoa pod extract agar	1.5	2.9	4.3	5.9	7.5	9.0	9.0	9.0
7.	Cocoa leaf extract agar	1.4	3.1	4.2	5.6	7.1	8.6	9.0	9.0
8.	Cocoa pod extract dextrose agar	1.3	2.7	3.9	5.0	6.4	7.6	8.7	9.0

* Average of four replications

Table 2b. Colony characters and sporangial production of seedling blight isolate of *Phytophthora palmivora* on different solid media

Sl. No.	Medium	Sporangial* production	Colony characters
1.	Potato dextrose agar	++	Aerial mycelium profuse, striate
2.	Oat meal agar	+++	Aerial mycelium profuse
3.	Corn meal agar	+	Aerial mycelium sparse, stellate
4.	Carrot agar	+++	Aerial mycelium sparse except in the centre, striate
5.	Czapek's agar	+	Aerial mycelium sparse except in the centre
6.	Cocoa pod extract agar	+	Aerial mycelium sparse
7.	Cocoa leaf extract agar	+	Aerial mycelium sparse
8.	Cocoa pod extract dextrose agar	+	Aerial mycelium sparse, stellate in the centre

* Average spore count per microscopic field	Grade
Less than 50	+
50 to 100	++
More than 100	+++

Radial growth was slow. Fair sporangial production was observed.

2. Oat meal agar

Initially the fungus grew very fast, sparsely on the surface of medium and completed the growth in a petri dish within 96 h. Later, the fungus produced cottony white aerial mycelium. Sporangia were abundant.

3. Corn meal agar

Rate of growth was similar to oat meal agar medium. Aerial mycelium sparse and stellate. Meagre sporangia were observed.

4. Carrot agar

Similar to PDA radial growth was slow, producing white, striate colony. Aerial mycelium was sparse except in the centre. Abundant sporangia were observed.

5. Czapek's agar

Radial growth of the fungus was irregular. Aerial mycelium white and sparse except in the centre, meagre sporangia were observed.

6. Cocoa pod extract agar

Fungus took 144 h to complete the growth in a petri dish. Aerial mycelium sparse and sporangial production meagre.

7. Cocoa leaf extract agar

Fungus took 168 h to complete the growth in a petri dish. Aerial mycelium sparse and meagre sporangia were observed.

8. Cocoa pod extract dextrose agar

Fungus took 192 h to complete the growth in a petri dish. Colony was white, stellate at the centre. Aerial mycelium sparse and produced meagre sporangia.

4.4 Growth on different liquid media

Growth of the pathogen on different liquid media was studied and results are presented in Table 2c. Analysis of the data revealed that all the liquid media significantly differ from each other in supporting the growth of the pathogen. Among the different media tested, the maximum mycelial dry weight was observed in oat meal broth followed by corn meal broth and these media were significantly superior to all other liquid media tried. Potato dextrose broth was found to be superior in promoting the growth of the pathogen

Table 2c. Growth of seedling blight isolate of Phytophthora palmivora on different liquid media

Sl. No.	Medium	Dry weight of mycelium (mg)
1.	Potato dextrose broth	178.3
2.	Oat meal broth	251.0
3.	Corn meal broth	235.7
4.	Carrot extract	83.3
5.	Czapek's broth	164.7
6.	Cocoa pod extract	62.7
7.	Cocoa leaf extract	55.3
8.	Cocoa pod extract dextrose broth	162.0
	CD (0.05)	17.2

compared to other liquid media except oat meal and corn meal broth. However, it was on par with Czapek's broth and cocoa pod extract dextrose broth. Growth of the fungus on carrot extract broth was superior than cocoa pod extract and cocoa leaf extract. Minimum mycelial weight was observed in cocoa leaf extract broth.

4.5 Symptomatology

Symptomatology of the Phytophthora disease occurring on seedlings and budded plants was studied in detail both under natural and artificial conditions.

4.5.1 Symptoms on leaves

All age group of leaves were susceptible to the pathogen with varying intensities. On young leaves initial symptom appeared as small water soaked lesion on the under surface of leaf lamina (Plate 7). These lesions were either scattered all over the leaves or seen at the distal end and margins of the leaves (Plate 8). Under high humid conditions, the lesion spread very rapidly. As the disease progressed, the watersoaked lesions turned to brown colour. Such lesions coalesced and resulted in leaf blight symptom. Some times yellow halo developed around the blightened area. Later defoliation occurred. In certain cases the lesions were confined in between the veins as circular to irregular with a

Plate 7. Initial water soaked lesions on leaves of cocoa seedling infected with Phytophthora palmivora

Plate 8. Leaf tip infection of cocoa seedling caused by Phytophthora palmivora

Plate 7

Plate 8

clear yellow halo (Plate 9). It was noticed that the spread of the disease was more rapid along with veins as dark brown area (Plate 10). Under wet weather conditions the lesions were uniformly brown in colour but dry spell immediately after wet weather resulted in papery nature of the infected portion of leaves with dark brown margin (Plate 11). During continuous rainy periods the water soaked lesions coalesced and wet rotting developed on young infected leaves (Plate 12).

On mature leaves generally the water soaked lesion appeared along and near the veins later turned to dark brown colour (Plate 13). When the conditions were favourable, such lesions spread and resulted in leaf blight and defoliation. Towards the end of monsoon period, water soaked minute spot appeared all over the leaf lamina and turned into dark brown spots surrounded by chlorotic area (Plate 14). Petiole infection was not common but under prolonged humid conditions, petioles were infected. Dark brown to black lesion developed, as a result the leaves showed yellowing, wilting and drying (Plate 15).

4.5.2 Symptom on stem

4.5.2.1 On seedlings

The infection initiated either from tip of the stem, cotyledonary region, between the cotyledonary and tip of the

Plate 9. Circular to irregular lesion in between the veins of leaves infected with Phytophthora palmivora

Plate 10. Infection along the veins of cocoa leaf infected with Phytophthora palmivora

Plate 9

Plate 10

Plate 11. Papery nature of the blightened portion of leaves due to Phytophthora palmivora

Plate 12. Wet rotting symptom on young leaves caused by Phytophthora palmivora

Plate 11

Plate 12

Plate 13. Infection on mature leaves of cocoa seedlings along the veins due to Phytophthora palmivora

Plate 14. Dark brown small spot with yellow halo on leaves of cocoa seedling infected with Phytophthora palmivora

Plate 13

Plate 14

stem or from the collar region. On succulant immature stem, initial symptoms appeared as water soaked brown lesion later turning to black. Generally the infection started from the tip of the seedlings, spread downward and resulted in defoliation and die-back. Infection near the cotyledonary region spread both upward and downward, resulting in wilting of upper part of the seedlings. Occasionally infection occurred below the cotyledonary region leading to wilting of the plant (Plate 16 and 17).

4.5.2.2 Budded plants

Compared to seedlings, stem of young budded plants were more prone to infection. Here, infection developed on any portion of the stem. The initial symptom appeared as brown water soaked lesion, turning to black (Plate 18). In severe case, the infection spread, covering larger areas resulting in wilt symptoms. Sometimes, leaves of the infected regions fall or remain attached to the plant in a dried up condition. If the infection is near the bud portion, complete death of the plant occurred and the dried up leaves remain attached to the plant (Plate 19). Under high humid conditions white mycelial growth of the pathogen was found on infected portions.

Plate 15. Petiole infection of cocoa seedling caused by Phytophthora palmivora

Plate 16. Cotyledonary infection and tip infection of cocoa seedlings caused by Phytophthora palmivora

Plate 15

Plate 16

Plate 17. Lesion on the stem of cocoa seedling caused by *Phytophthora palmivora*

Plate 18. Stem infection on budded plant of cocoa caused by *Phytophthora palmivora*

Plate 17

Plate 18

Plate 19. Wilting and drying up of budded plant due to Phytophthora palmivora

Plate 20. Water soaked symptoms on the leaves 24 h after artificial inoculation with the zoospore suspension of Phytophthora palmivora

Plate 19

Plate 20

4.5.3 Symptoms under artificial inoculation

4.5.3.1 Symptoms on leaves

Artificial inoculation with zoospore suspension resulted in symptoms similar to that of natural infections on seedlings and budded plants. Initial symptom developed after 24 h of inoculation and full expression of symptoms within 48-72 h. When culture disc of the pathogen was used, water soaked lesion appeared around disc within 24 h and turned dark brown after 48 h. It was noticed that the symptom development was more along the veins with a chlorotic area near by the infected region (Plate 20 and 21).

4.5.3.2 Symptoms on stem

Artificial inoculation using zoospore suspension on seedlings and budded plants showed similar type of symptoms as that of natural infection. Initial symptom developed within 48-72 h after inoculation. The infection was initiated more from the tip of seedling followed by cotyledonary region. On young budded plants the infection developed within 24-48 h and occurred in almost all parts of the stem.

Artificial inoculation using culture discs also showed similar type of symptoms as that of natural incidence both on seedlings as well as on young budded plants. Initial symptom

Plate 21. Symptoms on leaves 48 h after artificial inoculation with the culture disc of Phytophthora palmivora on budded plants

Plate 21

Plate 22. Stem infection of cocoa seedlings 48 h after artificial inoculation with the culture disc of Phytophthora palmivora

Plate 23. Stem infection on the budded plant of cocoa 48 h after artificial inoculation with the culture disc of Phytophthora palmivora

Plate 22

Plate 23

was observed within 24-48 h as light brown water soaked lesion around the culture disc and turned to dark brown within 72 h. On budded plants, the leaves above the artificially inoculated region showed a slight chlorosis (Plate 22 and 23).

4.6 Host range of the pathogen

Studies were carried out to know whether P. palmivora causing cocoa seedling blight of cocoa pathogen would infect other known hosts of Phytophthora spp. The study revealed that out of eight host plants tested, five viz., Piper nigrum L., Cocos nucifera L., Hevea brasilliensis L., Bougainvillea sp. and Colocasia esculenta L. were found to be infected. However, it was found that leaves of Piper betle L., Piper longum L. and leaves and nuts of Areca catechu L. did not take infection.

4.7 Effect of age of seedlings on the incidence and severity of the disease

This experiment was carried out to study the influence of age of seedlings on the incidence and severity of the disease. The different age groups of seedlings were artificially inoculated with zoospore suspension of the pathogen as described in Materials and Methods. Observations on the disease score were recorded at different intervals.

Statistical analysis of the data on the disease score at different intervals showed significant difference among the different age group of seedlings on the incidence of the disease (Table 3). Observations on the disease score five days after inoculation showed that seedlings of 11 days after germination recorded significantly higher disease score than all other age group of seedlings. Plants in age group of 18 days after germination also showed comparatively higher disease score than other age groups except seedlings of 11 days after germination. Minimum disease score was observed on the seedlings of 60 days after germination and was on par with other age group of seedlings except those in 18 and 11 days after germination.

Data on the disease score taken on the 10th and 15th day after inoculation also revealed that seedlings of 11 days after germination had significantly higher disease score than all other age groups. Seedlings of 18 days after germination also showed comparatively higher disease score except seedlings of 11 days after germination but was on par with seedlings of age group 32 and 25 days after germination. Minimum disease score was observed in seedlings of age group 46 days after germination and was on par with other age group of seedlings except plants in 18 and 11 days after germination.

Table 3. Effect of age of cocoa seedling on the incidence and severity of seedling blight

Age of seedling	Disease score			
	5th DAI	10th DAI	15th DAI	20th DAI
60 DAG	0.95 (0.29)	2.24 (0.51)	2.39 (0.53)	2.98 (0.60)
53 DAG	1.40 (0.38)	2.24 (0.51)	2.55 (0.55)	2.89 (0.59)
46 DAG	1.45 (0.39)	1.57 (0.41)	1.95 (0.47)	2.09 (0.49)
39 DAG	1.63 (0.42)	2.09 (0.49)	2.55 (0.55)	3.07 (0.61)
32 DAG	1.75 (0.44)	3.57 (0.66)	4.01 (0.70)	4.50 (0.74)
25 DAG	1.51 (0.40)	2.47 (0.54)	2.89 (0.59)	3.37 (0.64)
18 DAG	2.47 (0.54)	4.37 (0.73)	4.89 (0.77)	5.76 (0.83)
11 DAG	4.62 (0.75)	7.32 (0.92)	8.12 (0.96)	8.33 (0.97
CD (0.05)	0.15	0.16	0.16	0.15

Figures given in parentheses are transformed values (log x+1)

DAG - Days After Germination

DAI - Days After Inoculation

Analysis of data on the disease score on 20th day after inoculation revealed that seedlings of the age group 11 days after germination followed by seedlings of 18 days after germination recorded highest disease score than all other age groups. However, disease score on the seedlings of 18 days after germination was on par with that of the seedlings in age group 32 and 25 days after germination. Minimum disease score was observed in seedlings of 46 days after germination followed by 53 and 60 days after germinated seedlings.

4.8 Effect of age of budded plants on the incidence and severity of the disease

This study was carried out to find the most susceptible stage of the budded plants to infection. The different age groups of budded plants were artificially inoculated. Observation on the disease score, percentage defoliation and percentage stem infection were recorded at different intervals. Analysis of the data on the disease score five days after inoculation revealed no significant difference among the different age group of budded plants on the incidence and severity of the disease (Table 4). However, the minimum disease score was observed on the 66 days old budded plants. Maximum disease score was noticed on 59 days old budded plants.

Table 4. Effect of age of budded plants of cocoa on the incidence and severity of seedling blight

Age of budded plants	Disease score				Per cent stem lesion
	5th DAI	10th DAI	15th DAI	20th DAI	20 DAI
80 DAB	0.20 (0.08)	0.78 (0.25)	1.51 (0.40)	1.57 (0.41)	9.3
73 DAB	0.23 (0.09)	0.91 (0.28)	1.88 (0.46)	1.95 (0.47)	8.2
66 DAB	0.15 (0.06)	0.66 (0.22)	1.29 (0.36)	1.34 (0.37)	8.6
59 DAB	1.04 (0.31)	2.31 (0.52)	5.03 (0.78)	5.03 (0.78)	32.8
52 DAB	0.41 (0.15)	1.09 (0.32)	2.24 (0.51)	2.31 (0.52)	22.1
45 DAB	0.45 (0.16)	2.72 (0.57)	5.61 (0.82)	5.61 (0.82)	25.3
CD (0.05)	NS	0.23	0.23	0.23	-

Figures given in parentheses are transformed values (log x+1)

DAB - Days After Budding

DAI - Days After Inoculation

NS - Not Significant

Statistical analysis of the disease score taken on 10th, 15th and 20th day after inoculation showed significant difference among the different age group of budded plants. In all these three intervals 66 days old budded plants recorded the minimum disease score followed by 80 and 73 days old budded plants, and these were on par with each other. Maximum disease score was observed on 45 days old budded plants followed by 59 days old budded plants and these were on par with each other.

4.8.1 Stem infection

Result on the percentage stem infection on 20th day after inoculation is given in Table 4. The maximum percentage stem infection was observed in 59 days old budded plants followed by those in 45 and 52 days old budded plants. Less than 10 per cent stem infection was observed in 73, 66 and 80 days old budded plants. Among them, plants in 73 days after budding recorded the minimum percentage of stem infection.

4.9 Screening of cocoa types against seedling blight disease

Budded plants of 62 high yielding cocoa types of the Cadbury KAU Co-operative Cocoa Research Project were screened against the seedling blight pathogen to know whether any of them possess resistance/tolerance against the disease. The results are presented in Table 5.

Table 5. Screening of cocoa types against seedling blight disease

Sl. No.	Cocoa type	Parentage	Percentage bud establishment	Percentage mortality three months after bud establishment
1.	M-9.16	Local	80	88.0
2.	M-16.9	Local	50	80.0
3.	M-13.12	Local	70	41.2
4.	GI-4.8	Amazon (s) T 63/071/1272	95	52.6
5.	GI-5.2	Amazon scavina(s) T 12/613/972	50	40.0
6.	GI-5.9	Amazon scavina(s) T 12/613/972	15	66.7
7.	GI-5.14	Amazon scavina(s) T 12/613/972	60	33.3
8.	GI-6.17	Iquitos IMC 60/31	60	66.7
9.	GI-9.2	Amazon T 79/501/1424	75	53.3
10.	GI-10.3	Amazon T 63/967/61	90	27.7
11.	GI-10.8	Amazon T 63/967/61	100	54.2
12.	GI-15.5	Iquitos T 16/613/972	100	25.0
13.	GII-14.3	Local	95	26.3
14.	GII-16.3	Local	100	35.0
15.	GII 19.5	Local	90	38.9

Contd.

Table 5 (Contd.

16.	GII-20.4	Local	75	6.7
17.	GII-23.3	Local	100	50.0
18.	GII-24.4	Local	100	85.0
19.	GIII-1.2	Local	90	66.7
20.	GIII-4.1	Local	95	57.9
21.	GIV-1.2	Local	100	30.0
22.	GIV-4.6	Local	95	5.3
23.	GIV-18.5	Local	90	11.2
24.	GIV-32.5	Local	80	50.0
25.	GIV-35.7	Local	100	10.0
26.	GIV-36.6	Local	100	5.0
27.	GVI-2	C 42 (s)	70	50.0
28.	GVI-7	P_3xP_4 (s)	50	20.0
29.	GVI-9	P_3xP_1 (s)	100	30.0
30.	GVI-10	CF 176 x $T_{19/5}$	65	38.5
31.	GVI-15	NA	95	26.3
32.	GVI-17	NA (s)	95	26.3
33.	GVI-19	W 6/56 (T 63/970) (s)	80	43.8
34.	GVI-22	$P_{12}xP_2$ (s)	45	66.7
35.	GVI-23	P_9 x P_4 (s)	25	5.0
36.	GVI-24	W 5/15 (T 63/884) (s)	100	25.0
37.	GVI-25	T 7/12 (s)	20	25.0
38.	GVI-29	P_6xP_6 (s)	60	16.6

Contd.

Table 5 (Contd.)

39.	GVI-33	Amel x Na 33 (s)	90	38.9
40.	GVI-34	Amel x Na 32 (s)	55	36.4
41.	GVI-35	PA.7 x Na 32 (s)	50	60.0
42.	GVI-44	Landas 357 (s)	50	40.0
43.	GVI-50	ICS 6 (c)	70	35.7
44.	GVI-51	IMC 67 (c)	40	12.5
45.	GVI-54	SIAL 93 (b)	80	68.8
46.	GVI-55	IMC 10 (b)	85	41.2
47.	GVI-56	EET 272 (b)	75	26.7
48.	GVI-59	ICS-6 (b)	90	16.7
49.	GVI-60	Na 33 (b)	70	50.0
50.	GVI-61	C 6 (s)	75	60.0
51.	GVI-64	C 3 (s)	95	57.9
52.	GVI-68	P 7c (b)	20	50.0
53.	S-24.1	Local	80	37.5
54.	S-28.3	Local	100	20.0
55.	S-31.11	Local	95	47.4
56.	S-38.1	Local	90	22.2
57.	S-39.9	Local	100	35.0
58.	S-40.7	Local	90	16.8
59.	S-44.1	Local	100	20.0
60.	S-45.5	Local	90	5.6
61.	S-50.12	Local	90	22.2
62.	S-51.1	Local	75	13.3

Data on the percentage mortality three months after bud establishment showed the cocoa types GIV-36.6, GIV-4.6, GVI-23, S-45.5, GII-20.4, GIV-35.7, GIV-18.5 and GVI-51 recorded less than 12.5 per cent mortality with the minimum in types GIV-36.6, GVI-23, GIV-4.6 and S-45.5. The cocoa types S-51.1, GVI-29, S-40.7, S-44.1, S-28.3, GVI-7, S-50.12, S-38.1, GVI-25, GVI-24 and GI-15.5 recorded the percentage mortality ranging from 13 to 25 per cent. A percentage mortality between 26-50 per cent was observed in 28 cocoa types. However, the types GII-14.3, GVI-15, GVI-17 and GVI-56 recorded the mortality percentage near to 26 per cent. More than 50 per cent mortality was observed in 13 cocoa types. Among them, the types M-16.9, GII-24.4 and M-9.16 showed more than 80 per cent mortality.

4.10 Chemical control of the disease

4.10.1 *In vitro* evaluation of fungicides

Seven fungicides viz., Indofil M-45, Foltaf, Fytolan, Captaf, Akomin and Ridomil each at 0.1, 0.2 and 0.3 per cent concentration and Bordeaux mixture at 0.5, 0.75 and 1 per cent concentration were screened for their inhibitory effect on the growth of *P. palmivora*. Among the seven fungicides tested, Fytolan, Captaf, Bordeaux mixture, Akomin and Ridomil at all different concentrations completely inhibited the growth of

the fungus (Table 6). It was noted that Foltaf at 0.3 per cent completely inhibited the growth of the fungus, while its lower two concentrations showed only 90 per cent inhibition over control. Indofil M-45 showed less efficacy than Foltaf. Indofil M-45 at 0.3 per cent concentration recorded 67.6 per cent efficacy over control in inhibiting the fungus, while its lower two concentrations showed 53.9 and 61.1 per cent inhibition..

4.10.2 *In vitro* evaluation of antibiotics

The *in vitro* inhibitory effect of six antibiotics viz., Streptomycin, Streptocycline, Terramycin, Aureofungin Amoxycillin and Chloramphenicol each at 200, 400 and 500 ppm concentrations were tested against *P. palmivora*. Statistical analysis showed that the antibiotics viz., Streptomycin, Streptocycline, Terramycin and Chloramphenicol at all concentrations were significantly superior to control, Aureofungin and Amoxycillin in inhibiting the growth of the fungus (Table 7). Among them, Chloramphenicol and Terramycin at 400 and 500 ppm completely inhibited the growth of the pathogen. In general, increase in the concentration of antibiotics resulted in more inhibition. Amoxycillin and Aureofungin at all concentrations showed the lowest inhibition on the growth of the fungus.

Table 6. *In vitro* sensitivity of *P. palmivora* to fungicides

Sl. No.	Fungicide	Concentration (per cent)	*Mean colony diameter (cm)	Per cent inhibition over control
1.	Indofil M-45	0.1	4.15	53.9
		0.2	3.5	61.1
		0.3	2.92	67.6
2.	Foltaf	0.1	0.85	90.5
		0.2	0.82	90.9
		0.3	0	100
3.	Fytolan	0.1	0	100
		0.2	0	100
		0.3	0	100
4.	Captaf	0.1	0	100
		0.2	0	100
		0.3	0	100
5.	Bordeaux mixture	0.5	0	100
		0.75	0	100
		1.0	0	100
6.	Akomin	0.1	0	100
		0.2	0	100
		0.3	0	100
7.	Ridomil MZ	0.1	0	100
		0.2	0	100
		0.3	0	100
8.	Control	-	9.0	

* Mean of three replications

Table 7. *In vitro* sensitivity of *P. palmivora* to antibiotics

Sl. No.	Antibiotics	Concentration (ppm)	Mean colony diameter (cm)
1.	Streptomycin	200	1.01
		400	0.95
		500	0.85
2.	Terramycin	200	0.82
		400	0.80
		500	0.80
3.	Streptocycline	200	0.93
		400	0.87
		500	0.83
4.	Amoxycillin	200	8.70
		400	9.00
		500	8.70
5.	Aureofungin	200	8.25
		400	7.90
		500	8.00
6.	Chloramphenicol	200	0.87
		400	0.80
		500	0.80
7.	Control	-	9.00
	CD (0.05)	-	0.48

4.10.3 Efficacy of fungicides and antibiotics against *P. palmivora* on detached cocoa leaves

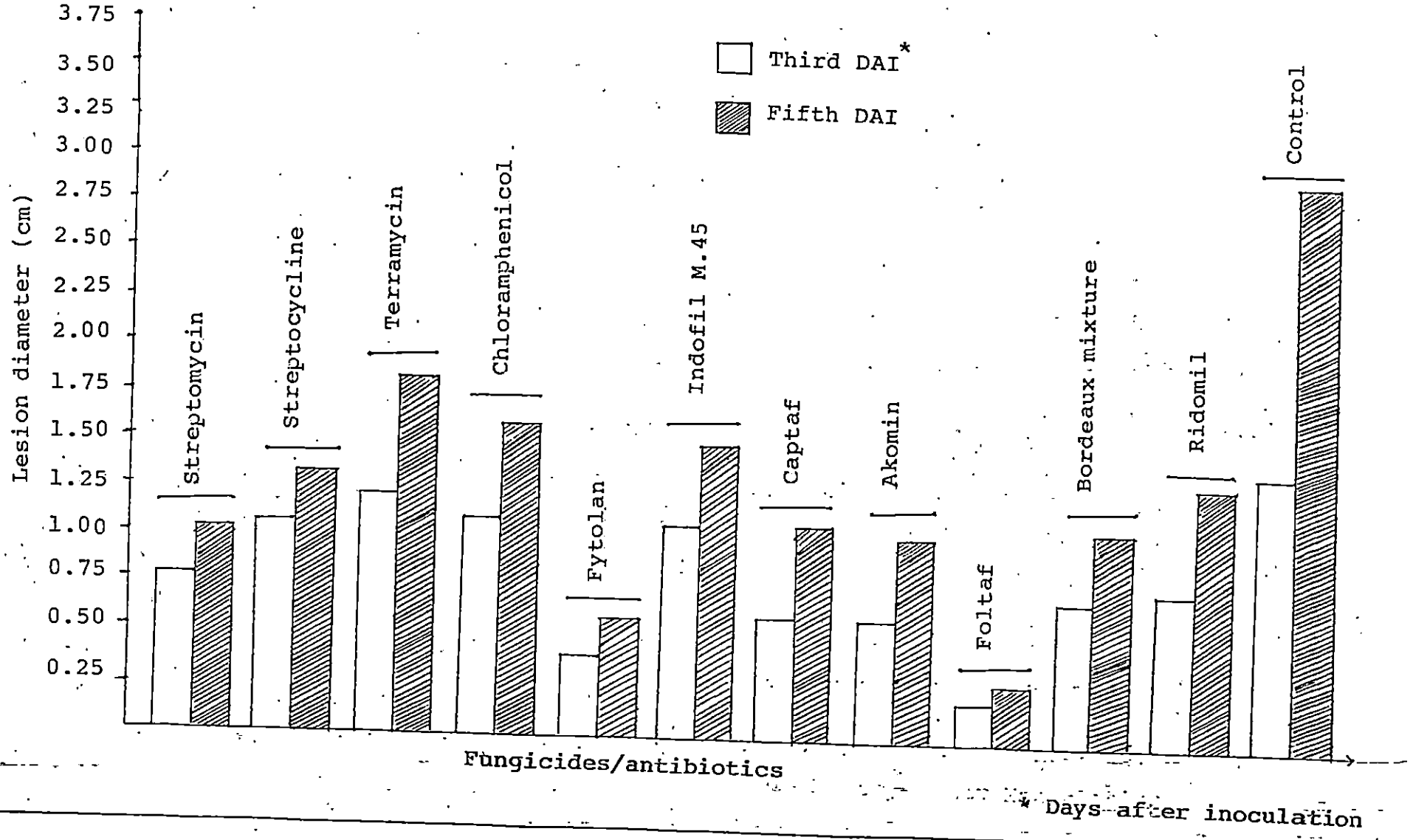
The observation on the lesion diameter recorded on the third and fifth day after inoculation revealed that none of the chemicals completely checked the lesion development (Table 8; Fig.3). But, all the chemicals were significantly superior to control. Observations on the third day after inoculation showed that the fungicides Foltaf followed by Fytolan were significantly superior to all other treatments in preventing the lesion development. Akomin and Captaf were on par but superior to other treatments except Fytolan and Foltaf. Bordeaux mixture and Streptomycin were significantly different from each other in checking the lesion development. Among them, Bordeaux mixture treated leaves showed the minimum lesion length. Among the fungicides/antibiotics treated leaves, the maximum diameter of lesion was observed in Indofil M-45 followed by Terramycin.

Data on the diameter of lesion five days after inoculation revealed that, Foltaf recorded significant effect in checking the lesion development than all other treatments and was on par with Fytolan. However, Fytolan was on par with Streptomycin and Bordeaux mixture and was superior to other treatments. Maximum lesion development was noticed in control.

Table 8. Effect of fungicides and antibiotics against *P. palmivora* on detached cocoa leaves

Sl. No.	Chemical	Concentration	Lesion diameter (cm)	
			Third day after inoculation	Fifth day after inoculation
1.	Streptomycin	500 ppm	0.78	0.97
2.	Streptocycline	500 ppm	0.99	1.29
3.	Terramycin	500 ppm	1.11	1.78
4.	Chloramphenicol	500 ppm	1.03	1.52
5.	Fytolan	0.3 %	0.41	0.66
6.	Indofil M-45	0.3 %	1.13	1.82
7.	Captaf	0.3 %	0.60	1.33
8.	Akomin	0.3 %	0.60	1.40
9.	Foltaf	0.3 %	0.20	0.30
10.	Bordeaux mixture	0.3 %	0.70	1.18
11.	Ridomil	0.3 %	0.81	1.38
12.	Control	-	1.36	3.01
	CD (0.05)		0.07	0.57

Fig.3 Efficacy of fungicides/antibiotics against Phytophthora palmivora on detached cocoa leaves.



Among the fungicides/antibiotics treated leaves, the maximum lesion develoment was noticed in Indofil M-45.

Thus the study revealed that the fungicide Foltaf and Fytolan effectively checked the lesion development in detached leaves inoculated with *P. palmivora*. Maximum lesion was observed in control followed by Indofil M-45.

4.10.4 *In vivo* control of the disease

4.10.4.1 Effect of fungicides and antibiotics in preventing the natural incidence of the disease

The experiment was conducted to study the effect of fungicides and antibiotics in preventing the incidence of the seedling blight of cocoa. The results are presented in Table 9a and 9b. Statistical analysis of the data on the disease score ten days after first spraying revealed no significant difference among the treatments. However, it was noticed that plants in treatment T_{10} (Bordeaux mixture-1%) had the minimum disease score and maximum percentage efficiency over control in preventing the incidence of the disease. Maximum disease score was observed in treatment T_{11} (Control) followed by T_3 (Terramycin-500 ppm) and T_2 (Streptocycline-500 ppm).

Analysis of data on the disease score ten days after second spraying revealed significant difference among the

Table 9a. Effect of fungicides and antibiotics in preventing the natural incidence of seedling blight of cocoa - Disease score

	Treatment	Concentration	Disease score			
			10th day after first spraying	10th day after second spraying	10th day after third spraying	10th day after fourth spraying
T_1	Streptomycin	500 ppm	0.20 (0.08)	0.95 (0.29)	1.34 (0.37)	2.24 (0.51)
T_2	Streptocycline	500 ppm	0.41 (0.15)	1.29 (0.36)	1.82 (0.45)	2.89 (0.59)
T_3	Terramycin	500 ppm	0.45 (0.16)	1.34 (0.37)	1.88 (0.46)	2.80 (0.58)
T_4	Chloramphenicol	500 ppm	0.35 (0.13)	0.86 (0.27)	1.24 (0.35)	1.69 (0.43)
T_5	Fytolan	0.3 %	0.23 (0.09)	0.32 (0.12)	0.58 (0.20)	0.78 (0.25)
T_6	Indofil M-45	0.3 %	0.41 (0.15)	0.55 (0.19)	1.69 (0.43)	1.75 (0.44)
T_7	Captaf	0.3 %	0.12 (0.05)	0.95 (0.29)	1.82 (0.45)	3.07 (0.61)
T_8	Akomin	0.3 %	0.26 (0.10)	0.26 (0.10)	0.35 (0.13)	0.70 (0.23)
T_9	Foltaf	0.3 %	0.15 (0.06)	0.20 (0.08)	0.51 (0.18)	0.66 (0.22)
T_{10}	Bordeaux mixture	0.3 %	0.10 (0.04)	0.41 (0.15)	0.70 (0.23)	0.74 (0.24)
T_{11}	Control	-	0.58 (0.20)	1.45 (0.39)	1.88 (0.46)	3.17 (0.62)
	CD (0.05)		NS	0.17	0.19	0.19

Figures given in parentheses are transformed values (log x+1)

NS - Not Significant

Table 9b. Effect of fungicides and antibiotics in preventing the natural incidence of seedling blight of cocoa - Percentage efficiency over control

Treatment		Concentration	Percentage efficiency of the treatments over control			
			10th day after first spraying	10th day after second spraying	10th day after third spraying	10th day after fourth spraying
T_1	Streptomycin	500 ppm	65.5	34.5	28.7	29.3
T_2	Streptocycline	500 ppm	29.3	11.0	3.2	8.8
T_3	Terramycin	500 ppm	22.4	7.6	0.0	11.7
T_4	Chloramphenicol	500 ppm	39.7	40.7	34.0	46.7
T_5	Fytolan	0.3 %	60.3	77.9	69.1	75.4
T_6	Indofil M-45	0.3 %	29.3	62.1	10.1	44.8
T_7	Captaf	0.3 %	79.3	34.5	3.2	3.2
T_8	Akomin	0.3 %	55.2	82.1	81.4	77.9
T_9	Foltaf	0.3 %	74.1	83.4	72.9	79.2
T_{10}	Bordeaux mixture	1 %	82.8	71.7	62.8	76.7

treatments. It was observed that the treatment T_9 (Foltaf-0.3%), T_8 (Akomin-0.3%), were significantly superior to other treatments except T_5 (Fytolan 0.3%), T_{10} (Bordeaux mixture 1%) and T_6 (Indofil M-45 0.3%). Among them, the plants in treatment T_9 showed the minimum disease score and maximum percentage efficiency over control in preventing the incidence of the disease. Further, the treatment T_5 was significantly superior to the treatments T_7 (Captaf-0.3%), T_2 (Streptocycline 500 ppm), T_3 (Terramycin 500 ppm) and T_{11} (Control). Maximum disease score was observed in control plants and was on par with treatments T_4 (Chloramphenicol 500 ppm), T_1 (Streptomycin 500 ppm), T_7, T_2 and T_3.

On the ten days after third spraying, plants in treatment T_8 (Akomin 0.3%) showed the minimum disease score and was on par with treatments T_9 (Foltaf 0.3%), T_5 (Fytolan 0.3%) and T_{10} (Bordeaux mixture-1%) but it was significantly superior than other chemicals. Maximum disease score was observed on the control plants and it was on par with the treatments T_7 (Captaf 0.3%), T_2 (Streptocycline 500 ppm), T_6 (Indofil M-45 0.3%), T_1 (Streptomycin 500 ppm) and T_4 (Chloramphenicol 500 ppm).

Data on the disease score on the 10th day after fourth spraying revealed that plants in treatment T_9 (Foltaf 0.3%) showed minimum disease score closely followed by T_8 (Akomin 0.3%), T_{10} (Bordeaux mixture 1%) and T_5 (Fytolan 0.3%) and

these were significantly superior than all other treatments. The treatment T_9 recorded the maximum percentage efficiency over control in reducing the incidence. Plants in treatment T_{11} (Control) recorded the maximum disease score, which was on par with treatments T_7 (Captaf 0.3%), T_2 (Streptocycline 500 ppm), T_3 (Terramycin 500 ppm) T_1 (Streptomycin 500 ppm) T_6 (Indofil M-45-0.3%) and T_4 (Chloramphenicol 500 ppm).

4.10.4.2 Effect of fungicides and antibiotics on naturally infected cocoa seedlings

The experiment was carried out to study the effect of fungicides and antibiotics to check the severity of seedling blight disease on naturally infected cocoa seedlings. The experiment was conducted as two sets viz., under open condition and also under shade. A total of four sprayings were given on the naturally infected seedlings at 10 days interval and the observations on the disease score were recorded.

4.10.4.2.1 Seedlings kept under open condition

Analysis of the data on the disease score taken at different intervals revealed no significant difference among the treatments in checking the severity of the disease (Table 10a and 10b). Observation on the disease score 10 days after

Table 10a. Effect of fungicides and antibiotics on naturally infected seedlings (kept under open condition) - Disease score

Treatment		Concentration	Disease score				
			On the day of first spraying	10th day after first spraying	10th day after second spraying	10th day after third spraying	10th day after fourth spraying
T_1	Streptomycin	500 ppm	0.78 (0.25)	0.86 (0.27)	0.95 (0.29)	0.95 (0.29)	1.04 (0.31)
T_2	Streptocycline	500 ppm	1.69 (0.43)	2.24 (0.51)	2.31 (0.52)	2.31 (0.52)	2.31 (0.52)
T_3	Terramycin	500 ppm	1.04 (0.31)	1.19 (0.34)	1.19 (0.34)	1.19 (0.34)	1.40 (0.38)
T_4	Chloramphenicol	500 ppm	1.09 (0.32)	1.57 (0.41)	1.63 (0.42)	1.63 (0.42)	1.63 (0.42)
T_5	Fytolan	0.3 %	0.70 (0.23)	0.78 (0.25)	0.82 (0.26)	0.86 (0.27)	0.86 (0.27)
T_6	Indofil M-45	0.3 %	0.70 (0.23)	1.63 (0.42)	1.88 (0.46)	2.09 (0.49)	2.16 (0.50)
T_7	Captaf	0.3 %	0.82 (0.26)	1.00 (0.30)	1.09 (0.32)	1.14 (0.33)	1.14 (0.33)
T_8	Akomin	0.3 %	0.86 (0.27)	0.86 (0.27)	0.91 (0.28)	0.91 (0.28)	0.95 (0.29)
T_9	Foltaf	0.3 %	0.91 (0.28)	1.09 (0.32)	1.09 (0.32)	1.19 (0.34)	1.24 (0.35)
T_{10}	Bordeaux mixture	0.3 %	1.45 (0.39)	1.63 (0.42)	1.75 (0.44)	1.82 (0.45)	2.16 (0.50)
T_{11}	Control	-	1.51 (0.40)	2.55 (0.55)	2.55 (0.55)	2.55 (0.55)	2.72 (0.57)
CD (0.05)			NS	NS	NS	NS	NS

Figures given in parentheses are transformed values (log x+1)

NS - Not Significant

Table 10b. Effect of fungicides and antibiotics on naturally infected seedlings (kept under open condition) - Percentage efficiency over control

Treatment		Concentration	Percentage efficiency of the treatments over control			
			10th day after first spraying	10th day after second spraying	10th day after third spraying	10th day after fourth spraying
T_1	Streptomycin	500 ppm	66.2	62.7	62.7	61.8
T_2	Streptocycline	500 ppm	12.2	9.4	9.4	1.5
T_3	Terramycin	500 ppm	53.3	53.3	53.3	48.5
T_4	Chloramphenicol	500 ppm	38.4	36.1	36.1	40.1
T_5	Fytolan	0.3 %	69.4	67.8	66.2	68.4
T_6	Indofil M-45	0.3 %	36.1	26.3	18.0	20.6
T_7	Captaf	0.3 %	60.8	57.3	55.3	58.1
T_8	Akomin	0.3 %	66.2	64.3	64.3	65.1
T_9	Foltaf	0.3 %	57.3	57.3	53.3	54.4
T_{10}	Bordeaux mixture	1 %	36.1	31.4	28.6	20.6

first as well as second spraying showed that the plants in treatment T_5 (Fytolan-0.3%) recorded the minimum disease score and maximum percentage efficiency over control followed by treatments T_1 (Streptomycin 500 ppm) and T_8 (Akomin-0.3%). Maximum disease score was observed in treatment T_{11} (Control) followed by plants in T_2 (Streptocycline-500 ppm).

Disease score 10 days after third and fourth spraying revealed that the plants in treatment T_5 (Fytolan-0.3%) recorded the minimum disease score and maximum percentage efficiency over control in checking the severity of the disease followed by T_8 (Akomin-0.3%) and T_1 (Streptomycin 500 ppm). Maximum disease score was observed in control plants. The treatments T_5, T_8, T_1, T_9 and T_7 also recorded comparatively higher percentage efficiency over control.

4.10.4.2.2 Seedlings kept under shade

Statistical analysis of the data on the disease score at different intervals of observations revealed no significant difference among the treatments (Table 11a and 11b). Observation on the disease score 10 days after first spraying showed that seedlings in treatment T_9 (Foltaf-0.3%) showed the minimum disease score followed by T_4 (Chloramphenicol-500 ppm) and T_5 (Fytolan 0.3%). The plants in treatment T_1 (Streptomycin-500 ppm) showed maximum disease score closely

Table 11a. Effect of fungicides and antibiotics on naturally infected seedlings (kept under shade) - Disease score

Treatment		Concentration	Disease score				
			On the day of first spraying	10th day after first spraying	10th day after second spraying	10th day after third spraying	10th day after fourth spraying
T_1	Streptomycin	500 ppm	1.51 (0.40)	2.63 (0.56)	2.63 (0.56)	2.63 (0.56)	2.80 (0.58)
T_2	Streptocycline	500 ppm	1.34 (0.37)	2.55 (0.55)	2.80 (0.58)	2.80 (0.58)	3.17 (0.62)
T_3	Terramycin	500 ppm	1.24 (0.35)	1.57 (0.41)	1.57 (0.41)	2.16 (0.50)	2.55 (0.55)
T_4	Chloramphenicol	500 ppm	1.09 (0.32)	1.34 (0.37)	1.51 (0.40)	2.63 (0.56)	2.72 (0.57)
T_5	Fytolan	0.3 %	1.24 (0.35)	1.57 (0.41)	1.63 (0.42)	2.31 (0.52)	2.31 (0.52)
T_6	Indofil M-45	0.3 %	1.04 (0.31)	2.55 (0.55)	2.89 (0.59)	3.17 (0.62)	3.47 (0.65)
T_7	Captaf	0.3 %	1.24 (0.35)	2.55 (0.55)	2.72 (0.57)	2.98 (0.60)	3.27 (0.63)
T_8	Akomin	0.3 %	1.45 (0.39)	1.82 (0.45)	2.02 (0.48)	2.31 (0.52)	2.31 (0.52)
T_9	Foltaf	0.3 %	1.19 (0.34)	1.19 (0.34)	1.24 (0.35)	1.34 (0.37)	1.40 (0.38)
T_{10}	Bordeaux mixture	0.3 %	1.14 (0.33)	2.24 (0.51)	2.24 (0.51)	2.24 (0.51)	2.24 (0.51)
T_{11}	Control	-	1.51 (0.40)	2.24 (0.51)	2.39 (0.53)	2.72 (0.57)	3.90 (0.69)
	CD (0.05)		NS	NS	NS	NS	NS

Figures given in parentheses are transformed values (log x+1)

NS - Not Significant

Table 11b. Effect of fungicides and antibiotics on naturally infected seedlings (kept under shade) - Percentage efficiency over control

Treatment		Concentration	Percentage efficiency of the treatments over control			
			10th day after first spraying	10th day after second spraying	10th day after third spraying	10th day after fourth spraying
T_1	Streptomycin	500 ppm	-1.7	-1.0	3.3	28.3
T_2	Streptocycline	500 ppm	-1.4	-1.7	-2.9	18.7
T_3	Terramycin	500 ppm	3.0	34.4	21.0	34.6
T_4	Chloramphenicol	500 ppm	4.0	36.8	9.0	30.3
T_5	Fytolan	0.3 %	3.0	31.8	15.1	40.8
T_6	Indofil M-45	0.3 %	-1.4	-20.9	-16.5	11.0
T_7	Captaf	0.3 %	-1.4	-13.8	-9.6	16.1
T_8	Akomin	0.3 %	1.9	15.4	15.1	40.8
T_9	Foltaf	0.3 %	4.7	48.1	50.7	64.1
T_{10}	Bordeaux mixture	1 %	0.0	6.3	17.6	42.6

followed by T_2 (Streptocycline-500 ppm), T_6 (Indofil M-45 0.3%) and T_7 (Captaf 0.3%).

Ten days after second spraying plants in treatment T_9 (Foltaf-0.3%) recorded the minimum disease score and maximum percentage efficiency over control. Maximum disease score was observed in treatment T_6 (Indofil M-45 0.3%) closely followed by T_2 (Streptocycline 500 ppm) and T_7 (Captaf-0.3%).

Data on the disease score 10 days after third spraying showed that plant in T_9 (Foltaf-0.3%) recorded the minimum disease score. This treatment showed the maximum percentage efficiency over control in checking the severity of the disease. Plants in treatment T_6 (Indofil M-45 0.3%) recorded the maximum disease score followed by T_7 (Captaf 0.3%). On the tenth day after fourth spraying, the seedlings in treatment T_9 (Foltaf 0.3%) recorded the minimum disease score and maximum percentage efficiency over control. Maximum disease score was observed in control plants.

4.10.4.3 Effect of fungicides and antibiotics in checking the severity of the disease on artificially inoculated seedlings

The experiment was undertaken to study the effect of fungicides and antibiotics to check the severity of the disease. The seedlings were artificially inoculated with

was taken on the day of each spraying and ten days after fourth spraying.

Analysis of the data on the disease score taken at different intervals showed no significant difference among the treatments except ten days after first spraying (Table 12a and 12b). However, certain chemicals had some effect in checking the severity of the disease. Analysis of the data on the disease score ten days after first spraying revealed that plants in treatment T_8 (Akomin 0.3%) was significantly superior to all other treatments except T_2 (Streptocycline 500 ppm) and T_6 (Indofil M-45 0.3%). Plants sprayed with Akomin 0.3% (T_8) recorded minimum disease score and maximum percentage efficiency over control at all intervals observation. The treatment T_2 (Streptocycline 500 ppm), also showed comparatively higher percentage efficiency over control in checking the severity of the disease than other treatments.

It was observed that the plants in certain treatments showed more disease score than control. Seedlings in treatment T_9 (Foltaf 0.3%) and T_6 (Indofil M-45 0.3%) recorded the maximum disease score 10 days after first and second

Table 12a. Effect of fungicides and antibiotics in checking the severity of the disease on artificially inoculated seedlings - Disease score

	Treatment	Concentration	Disease score				
			On the day of first spraying	10th day after first spraying	10th day after second spraying	10th day after third spraying	10th day after fourth spraying
T_1	Streptomycin	500 ppm	0.91 (0.28)	5.46 (0.81)	7.51 (0.93)	8.77 (0.99)	10.22 (1.05)
T_2	Streptocycline	500 ppm	0.86 (0.27)	4.01 (0.70)	4.89 (0.77)	5.46 (0.81)	5.46 (0.81)
T_3	Terramycin	500 ppm	0.86 (0.27)	7.51 (0.93)	7.71 (0.94)	7.91 (0.95)	8.12 (0.96)
T_4	Chloramphenicol	500 ppm	0.91 (0.28)	8.55 (0.98)	9.00 (1.00)	9.96 (1.04)	10.48 (1.06)
T_5	Fytolan	0.3 %	0.78 (0.25)	7.32 (0.92)	7.71 (0.94)	8.33 (0.97)	8.33 (0.97)
T_6	Indofil M-45	0.3 %	0.78 (0.25)	8.33 (0.97)	9.23 (1.01)	9.23 (1.01)	9.23 (1.01)
T_7	Captaf	0.3 %	0.86 (0.27)	7.51 (0.93)	8.77 (0.99)	9.23 (1.01)	9.47 (1.02)
T_8	Akomin	0.3 %	0.82 (0.26)	3.27 (0.63)	4.25 (0.72)	4.25 (0.72)	4.49 (0.74)
T_9	Foltaf	0.3 %	0.91 (0.28)	8.77 (0.99)	9.00 (1.00)	9.00 (1.00)	9.00 (1.00)
T_{10}	Bordeaux mixture	0.3 %	0.78 (0.25)	8.55 (0.98)	9.23 (1.01)	9.23 (1.01)	9.23 (1.01)
T_{11}	Control	-	0.95 (0.29)	8.33 (0.97)	9.00 (1.00)	9.00 (1.00)	9.96 (1.04)
	CD (0.05)		NS	0.21	NS	NS	NS

Figures given in parentheses are transformed values (log x+1)

NS - Not Significant

Table 12b. Effect of fungicides and antibiotics in checking the severity of the disease on artificially inoculated seedlings - Percentage efficiency over control

Treatment		Concentration	Percentage efficiency of the treatments over control			
			10th day after first spraying	10th day after second spraying	10th day after third spraying	10th day after fourth spraying
T_1	Streptomycin	500 ppm	34.5	24.9	12.3	-2.6
T_2	Streptocycline	500 ppm	51.9	51.1	45.4	45.2
T_3	Terramycin	500 ppm	9.8	22.9	20.9	18.5
T_4	Chloramphenicol	500 ppm	-2.6	0.0	-4.0	-5.2
T_5	Fytolan	0.3 %	12.1	22.9	16.7	16.4
T_6	Indofil M-45	0.3 %	0.0	-2.3	-2.3	1.9
T_7	Captaf	0.3 %	9.8	12.3	-2.3	4.9
T_8	Akomin	0.3 %	60.7	57.5	57.5	54.9
T_9	Foltaf	0.3 %	-5.3	0.0	0.0	3.8
T_{10}	Bordeaux mixture	1 %	-2.6	-2.3	-2.3	2.8

spraying while, Chloramphenicol 500 ppm (T_4) treated plants recorded the maximum during the last two observations.

4.10.4.4 Effect of fungicides and antibiotics in preventing the incidence and severity on artificially inoculated seedlings

The study was carried out to find out the effect of fungicides and antibiotics in preventing the incidence and severity of the disease. Two days after chemical application the seedlings were inoculated with zoospore suspension of the pathogen. Observations on the disease score were taken at ten days intervals after each spraying and the results are presented in Table 13a and 13b.

Analysis of the data on the disease score ten days after first spraying showed that the treatment T_9 (Foltaf-0.3%) was significantly superior to T_6 (Indofil M-45 0.3%) and T_{11} (Control) and was on par with other treatments. Treatment T_9 recorded the minimum disease score and maximum percentage efficiency over control. Seedlings in treatment T_{10} (Bordeaux mixture 1%) was found to be significantly superior than the plants in T_{11} but on par with all other treatments except T_9. It was noticed that the treatments T_9, T_{10} (Bordeaux mixture 1%), T_7 (Captaf 0.3%), and T_1 (Streptomycin 500 ppm), recorded more than 90 per cent efficiency over control in checking the

Table 13a. Effect of fungicides and antibiotics in preventing the incidence and severity on artificially inoculated seedlings - Disease score

Treatment		Concentration	Disease score			
			10th day after first spraying	10th day after second spraying	10th day after third spraying	10th day after fourth spraying
T_1	Streptomycin	500 ppm	0.35 (0.13)	0.35 (0.13)	0.74 (0.24)	0.78 (0.25)
T_2	Streptocycline	500 ppm	0.70 (0.23)	2.09 (0.49)	2.46 (0.54)	2.63 (0.56)
T_3	Terramycin	500 ppm	0.45 (0.16)	0.66 (0.22)	0.78 (0.25)	0.91 (0.28)
T_4	Chloramphenicol	500 ppm	0.41 (0.15)	0.66 (0.22)	0.78 (0.25)	0.78 (0.25)
T_5	Fytolan	0.3 %	0.74 (0.24)	0.91 (0.28)	1.00 (0.30)	1.00 (0.30)
T_6	Indofil M-45	0.3 %	1.24 (0.35)	1.40 (0.38)	1.51 (0.40)	0.75 (0.44)
T_7	Captaf	0.3 %	0.32 (0.12)	1.04 (0.31)	1.34 (0.37)	1.45 (0.39)
T_8	Akomin	0.3 %	0.41 (0.15)	0.45 (0.16)	0.45 (0.16)	0.45 (0.16)
T_9	Foltaf	0.3 %	0.10 (0.04)	0.23 (0.09)	0.51 (0.18)	0.51 (0.18)
T_{10}	Bordeaux mixture	0.3 %	0.26 (0.10)	0.38 (0.14)	0.48 (0.17)	0.55 (0.19)
T_{11}	Control	-	4.01 (0.70)	5.31 (0.80)	5.92 (0.84)	5.92 (0.84)
	CD (0.05)		0.28	0.35	0.32	0.31

Figures given in parentheses are transformed values (log x+1)

Table 13b. Effect of fungicides and antibiotics in preventing the incidence and severity on artificially inoculated seedlings - Percentage efficiency over control

Treatment		Concentration	Percentage efficiency of the treatments over control			
			10th day after first spraying	10th day after second spraying	10th day after third spraying	10th day after fourth spraying
T_1	Streptomycin	500 ppm	91.3	93.4	87.5	86.6
T_2	Streptocycline	500 ppm	82.5	60.6	58.4	55.6
T_3	Terramycin	500 ppm	88.8	87.6	86.6	84.6
T_4	Chloramphenicol	500 ppm	89.8	87.6	86.6	86.6
T_5	Fytolan	0.3 %	81.5	82.7	83.1	83.1
T_6	Indofil M-45	0.3 %	69.1	73.6	74.5	70.4
T_7	Captaf	0.3 %	92.0	80.4	77.4	75.5
T_8	Akomin	0.3 %	89.8	91.5	92.4	92.4
T_9	Foltaf	0.3 %	97.5	95.7	91.4	91.4
T_{10}	Bordeaux mixture	1 %	93.5	92.8	91.9	90.7

severity of the disease. Maximum disease score was observed on control plants.

Observation on the disease score ten days after second spraying revealed that the seedlings in treatment T_9 (Foltaf 0.3%) showed minimum disease score and was significantly superior to treatment T_2 (Streptocycline 500 ppm) and T_{11} (Control), but was on par with all other treatments. The plants in treatment T_9, T_1, T_{10} and T_8 recorded more than 90 per cent efficiency over control in checking the severity of the disease. Maximum disease score was observed in control plants followed by plants in treatment T_2.

Data on the disease score ten days after third spraying showed that the plants in treatment T_8 (Akomin 0.3%) followed by T_{10} (Bordeaux mixture 1%) and T_9 (Foltaf 0.3%) were found to be significantly superior to T_2 (Streptocycline 500 ppm) and T_{11} (Control) and these were on par with other treatments. The treatment T_1 (Streptomycin 500 ppm) was found to be superior to T_{11} and on par with all other treatments except T_9, T_{10} and T_8. Seedlings in treatments T_8, T_{10} and T_9 recorded more than 90 per cent efficiency over control. Maximum disease score was observed in control plants which was on par with the treatment T_2.

Significant difference among the treatment was noticed ten days after fourth spraying. The treatment T_8 (Akomin 0.3%) showed minimum disease score and was significantly superior to the treatments T_2 (Streptocycline 500 ppm) and T_{11} (Control) and was on par with remaining treatments. Application of Akomin 0.3% recorded maximum efficiency over control in checking the disease followed by T_9 (Foltaf 0.3%) and T_{10} (Bordeaux mixture 1%). Maximum disease score was observed in control plants followed by plants in treatment T_2. Thus this study showed that during the first two interval of observation the fungicide Foltaf recorded the minimum disease score while in the last two observations Akomin showed the maximum effect in checking the disease.

Discussion

DISCUSSION

Seedling blight, a serious nursery disease of cocoa, assumes alarming proportions during the monsoon periods resulting in leaf blight, defoliation, stem infection, die-back and finally death of the seedlings. In view of the serious nature of the disease to cocoa planting material, the present investigation was carried out to study the etiology, symptomatology, influence of age of seedlings on disease incidence and also to evolve suitable control measures.

The seedling blight pathogen was isolated from infected leaves and stems of seedlings and budded plants. Isolations from all the infected portions yielded a fungus belonging to Phytophthora sp. and its pathogenicity was established. Occurrence of seedling blight caused by P. palmivora was reported by many workers from various cocoa growing areas of the world (Chant, 1957; Chee, 1969a; Asare-Nyako, 1972; Manco, 1974; Mc Gregor, 1984). There was no report on the occurrence of this disease from India. However, Chandramohanan (1979) reported a seedling die-back due to P. palmivora from Karnataka state of India.

The morphological characters of pathogen causing seedling blight of cocoa were studied, which includes colony

characters and the sporangial characters like sporangial ontogeny, size, shape, L/B ratio and pedicel length.

Many workers considered colony morphology on carrot agar medium in darkness as a reliable character for the separation of Phytophthora spp. of cocoa (Sansome et al., 1975; Griffin, 1977; Brasier and Griffin, 1979; Idosu and Zentmyer, 1978; Kellam and Zentmyer, 1986). The fungus causing seedling blight produced sparse aerial mycelium on carrot agar with striate pattern except in the centre, where cottony white mycelial growth was observed. The mycelium hyaline, coenocytic, measuring 3.5 to 5.3 µm in breadth. Such type of characters were reported by Waterhouse (1974), Griffin (1977), Brasier and Griffin (1979) and Das (1986) while studying the cocoa isolates of P. palmivora. However, stellate pattern of colony observed by these workers have not been observed probably due to some environmental variation.

The young sporangia of the fungus was more or less spherical with less dense protoplasm. On maturity the protoplasm become dense, granular and finally differentiated into zoospores. Similar type of sporangial development of P. palmivora was observed by Zentmyer and Erwin (1970), Christen and Hohl (1972) and Das (1986), while studying the isolates of P. palmivora from cocoa.

Sporangial morphology is considered as an important character for identifying Phytophthora spp. (Rosenbaum, 1917; Tucker, 1931; Leonian, 1934; Waterhouse, 1963; Newhook et al., 1978; Ho, 1981; Waterhouse et al., 1983; Stamps et al., 1990). The mature sporangia of the fungus causing seedling blight of cocoa were spherical to ovoid with round base, papillate and cauducous, measured 49.4 x 27.7 µm with an average L/B ratio ranging from 1.3 to 2.3 (mean 1.74). These observations on the sporangial characters are almost similar to those reported by Griffin (1977), Chandramohanan et al. (1979), Brasier and Griffin (1979), Sreenivasan and Chandramohanan (1984), and Fagan (1988). They reported that the sporangia of P. palmivora causing black pod of cocoa were ellipsoid to ovoid, papillate and cauducous with an L/B ratio ranging from 1.2-1.8 and even upto 2.2.

Another important taxonomic criterion used for distinguishing Phytophthora spp. is the sporangial ontogeny. The fungus causing seedling blight of cocoa produced sporangia terminally on sporangiophorein a typical sympodial fashion (lax or close formation of successive sporangia). Such sympodial formation of sporangia of P. palmivora from cocoa was reported by Waterhouse (1963, 1974), Idosu and Zentmyer (1978), Brasier and Griffin (1979), and Zentmyer (1988). They

also opined that it is a stable diagnostic character of P. palmivora.

Another criterion useful for identification of Phytophthora spp. is the pedicel length. The sporangium of the seedling blight pathogen was caducous with short, thick stalk measuring 3.5 to 5.3 µm (average 4.1 µm) in length. Based on the pedicel length Zentmyer et al. (1977) and Kaosiri et al. (1978) classified Phytophthora isolates of cocoa into four groups viz., Group I (average length <5 µm), Group II (average length 5-15 µm), Group III (average length >15 µm) and Group IV (non-caducous sporangia). The first three groups correspond to P. palmivora, P. megakarya and P. capsici respectively (Kaosiri et al., 1978; Brisier and Griffin, 1979; Waller, 1981; Zentmyer et al., 1981) and Group IV as P. citrophthora (Campelo and Luz, 1981; Kellam and Zentmyer, 1986). Further, it was reported that pedicel length is a stable character under normal conditions (Waterhouse, 1974). In addition, pedicel length is neither affected by age of sporangium bearing culture (Al-Hedaithy and Tsao, 1979) nor was affected by light (Kaosiri et al., 1978). The sporangium of the fungus causing seedling blight of cocoa had short, thick pedicel with average pedicel length of less than 5 um, this corresponds to the original description of Group I of Zentmyer et al. (1977) and Kaosiri et al. (1978) which was

subsequently identified as P. palmivora (Brasier and Griffin, 1979 and Zentmyer et al., 1981).

Based on the colony morphology, sporangial morphology, sporangial ontogeny, L/B ratio and pedicel length the fungus causing seedling blight of cocoa could be identified as Phytophthora palmivora (Butler) Butler. The identity of the fungus was further confirmed from Indian Type Culture Collection, IARI, New Delhi (Acc. No. ITCC. 4527).

The growth of the fungs in different media was studied to select a suitable medium which yields maximum growth and sporangial production. Although media like oat meal and corn meal agar supported maximum growth of the fungus, the sporangial production was maximum in carrot and oatmeal agar. The fungus produced typical striate pattern of growth in carrot agar and potato dextrose agar. The different host extract agar media supported poor growth of the fungus. Usefulness of oatmeal agar for good growth and sporangial production of P. palmivora was reported by Turner (1969), Waterhouse (1974) and Chandramohanan et al. (1979). Typical striate growth of P. palmivora on carrot agar and use of this media for sporangial production was reported by Griffin (1977), Pimental (1981), Alizadeh and Tsao (1985) and Das (1986). Waterhouse (1974) observed abundant sporangia of P. palmivora in corn meal agar. But in the present study corn

meal agar yielded only meagre sporangial production. Hence for maximum growth and sporangial production oat meal agar was the best. Eventhough maximum growth of the fungus was obtained in corn meal agar it was not good for sporangial production. For maximum sporangial production, in addition to oat meal agar, carrot agar can also be utilized.

Among the different liquid media tried, oat meal and corn meal media were found to be superior in supporting the growth of the pathogen, followed by potato dextrose broth. Here also extracts of the different parts of the host in liquid media were ineffective in supporting good growth of the fungus. The sparse growth of the fungus in both solid and liquid host extracts media may be due to the release of certain toxic compounds like phenols.

Symptomatological studies are important in better understanding of the disease. In the present investigation detailed studies were carried out to understand the various types of symptoms produced by the fungus on cocoa seedlings as well as on budded plants both under natural and artificial conditions. It was noticed that on young leaves the disease developed as minute watersoaked lesions, later turned to dark brown in colour. Such lesions coalesced and resulted in blight and defoliation during high humid condition. However, during dry conditions, the initial small watersoaked lesions remained

as such without coalesing and the centre of the lesions turned to dark brown colour with a yellow halo. These symptoms observed on leaves were similar to those already described by Manco (1974) and KAU (1993). Manco (1974) further reported that petiole infection was very common under high humid conditions. However in the present study even during heavy monsoon period, petiole infection was not a common symptom except in stray cases.

Under high humid conditions the spread of the disease was more common along with the veins. It was noticed that the blighted areas of the leaf were papery in nature and in certain case a type of wet rotting symptom was also occurred. These types of symptoms were not observed by earlier workers.

On stems of young seedlings the disease initiated either from the tip or cotyledonary region as water soaked lesions, turned dark brown to black in colour resulting in either die-back or wilt symptom. Such types of symptoms were noticed by Chant (1954), Keane (1974) and Chandramohanan (1979, 1983) while reporting die-back disease of cocoa seedlings.

Stems of young budded plants were found to be more prone to infection than that of seedlings. On budded plants infection occurred at any part of the stem as water soaked

lesions, turning to black. Later they enlarged in size and resulted in defoliation, wilt or die-back depending upon the site of infection. Infection near the bud portion led to wilting and the wilted plants remained as such for longer periods. According to Manco (1974) the symptoms occurring on the shoots, cuttings, grafts and seedlings due to P. palmivora varied according to the site of occurrence and with age of the tissue. Severe infection of budded plants compared to seedlings was also reported (KAU, 1993). It is a fact that shoots arising from buds are more succulent than shoots of seedlings and as such, the budded plants are more prone to infection by the fungus.

According to Campelo et al. (1984) canker symptoms were associated with seedling disease due to Phytophthora sp. But in the present study, canker symptom was not observed either in seedlings or in budded plants.

The symptom produced on artificial inoculation on leaves were similar to that of natural infection. On leaves, first visible symptom developed after 24 h of inoculation and full expression within 48-72 h. This observation was in agreement with that of Manco (1974). According to him seedling blight symptom developed within 24 h of inoculation.

Artificial inoculation on the stems of cocoa seedlings and budded plants produced similar type of symptoms as that of natural infection. The initial symptoms on seedling developed within 48-72 h. Similar result was observed by Manco (1974). However, on succulent budded plants, stem infection developed within 24-48 h.

An important aspect in the continuity of a disease is the host range of the pathogen. Therefore eight commonly known host plants of Phythophthora spp. were tested to know whether cocoa seedling blight pathogen could infect them. It was observed that except Areca catechu L., Piper betle L. and Piper longum L. all other plants tested viz., Piper nigrum L., Cocos nucifera L., Hevea brasiliensis L., Bougainvillea sp. and Colocasia esculenta L. took infection on artificial inoculation.

Wide host range of cultivated plants to P. palmivora was reported by Ashby (1929). Infectivity of P. palmivora on rubber colocasia and coconut was reported by Pieris (1962), Narasimhan (1927), Muller (1936), Bobr-Tylingo (1954) and Das (1986). Infection of coconut caused by cocoa isolate of P. palmivora was observed by Pieris (1962). In the present study also the cocoa seedling blight isolate of P. palmivora infected coconut and colocasia.

Das (1986) also observed that cocoa isolate of P. palmivora caused infection on arecanut, rubber and coconut. It was found that eventhough seedling blight isolate of P. palmivora infected coconut and rubber, it did not take infection in arecanut. Inability of P. palmivora isolate of cocoa to infect arecanut was also reported by Chandramohanan et al. (1979). Contrary to the reports of Muller (1936), Holliday and Mowat (1963) and Das (1986) it was observed that the seedling blight isolate of P. palmivora could cause infection on pepper.

Age of seedling has an important role in the incidence and severity of many seedling diseases. Under natural condition seedling blight disease of cocoa was observed in all stages of the seedlings with varying intensities. The study on the effect of age of seedlings to the disease revealed that the youngest seedlings of 11 days old recorded the maximum disease score followed by 18 days old seedlings. Minimum disease score was observed in 46 days old seedlings followed by 53 and 60 days old. From the study it is evident that the youngest seedlings of age group of less than 18 days after germination was more vulnerable to infection by P. palmivora. Keane (1974) and Chandramohanan (1979) reported that the seedling die-back disease due to P. palmivora was more severe on cocoa seedlings of less than one month old.

It was observed that the budded plants were more prone to seedling blight pathogen P. palmivora, causing severe leaf and stem infections. Thus, an experiment was conducted to find out the most susceptible stage of budded plants on the incidence and severity of the disease. The study revealed that youngest budded plants of 45 days old showed the maximum disease score followed by 59 and 52 days old ones. Minimum disease score was observed in budded plants of 66 days old. It was also found that comparatively younger age group of budded plants viz., 45, 52 and 59 days old budded plants recorded maximum stem infection. Other age groups recorded less than 10 per cent stem infection.

Holderness (1992) observed that the infection of unhardened leaves was primarily through rain splashes from the soil. According to Manco (1974) the infection differ with the site of occurrence (leaves, stem or petioles) or with the age of tissues. In the present study also, it is observed that the youngest budded plants showed the maximum leaf and stem infection. Thus, proving that the young succulent tissues of budded plants were more susceptible to seedling blight pathogen P. palmivora.

The most effective way of controlling a disease is the use of resistant varieties, supplemented with cultural practices and chemical application. An attempt was thus made

to screen 62 cocoa types for host resistance against seedling blight isolate of P. palmivora. Among the 62 cocoa types screened none of them were found to be immune to the disease. However, the cocoa types GIV-36.6, GII-23, GIV-4.5 and S-45.5 showed minimum percentage mortality. Eleven cocoa types showed the mortality ranging from 13 to 25 per cent. More than 50 per cent mortality was observed in 13 cocoa types. Among them, M-16.9, GII-24.4 and M-19.16, GII-24.4 and M-9.16 recorded more than 80 per cent mortality.

Except for a preliminary study, there are no reports on the reaction of cocoa types against seedling blight disease. The studies conducted at Kerala Agricultural University revealed that the cocoa type GVI-10 recorded the lowest percentage of mortality and S-39.9 and S-31.11 recorded the maximum mortality (KAU, 1993). However, in the present investigation the cocoa types GVI-10, S-39.9 and S-31.11 recorded 25 to 50 per cent mortality. Detailed studies on the resistance of cocoa types to black pod causing P. palmivora has been conducted in various countries. They have identified the cocoa types like pond-7, EET-59, UF-713, SCA-6, ICS-6, DRC-16, BE-5, EEG-8 and MA-16 resistant to black pod (Lawrence, 1978; Sri-Sukamato and Mawardi, 1986 and Pinto et al., 1989). One of the resistant variety against cocoa black pod is ICS-6. In the present study also it has been

found that the cocoa type ICS-6 (b) (GVI-59) comparatively showed less mortality against seedling blight disease ranging from 13-25 per cent. Thus it is revealed that certain cocoa types had resistance against P. palmivora causing seedling blight disease of cocoa. Further, detailed studies incorporating more number of cocoa types and their artificial screening with many isolates of the pathogen from different geographical areas are necessary to find out true resistant cocoa types against the disease.

Plant disease control aims at prevention or reduction in the incidence or severity of the disease. Among the various methods of plant disease control, use of chemicals offers comparatively more effectiveness and quick action in prevention or reduction of disease. As seedling blight of cocoa is very serious in nursery during rainy season use of chemicals offer better control of the disease. So in the present study, both in vitro and in vivo effect of fungicides/ antibiotics against P. palmivora were tested.

Among the fungicides tested in in vitro, the fungicides Fytolan, Captaf, Bordeaux mixture, Akomin, Ridomil each at 0.1, 0.2 and 0.3 per cent concentrations and Foltaf at 0.3 per cent concentration completely inhibited the growth of the fungus. However, Indofil M-45 exhibited low efficacy in inhibiting the fungus.

In vitro effect of fungicides like Ridomil, Bordeaux mixture, Captaf, Foltaf, Fytolan and Akomin were studied by many workers (Kueh, 1980; Figueiredo and Lellis, 1981; Reddy and Chandramohanan, 1984; Campelo *et al*., 1984; Anderson, 1989; Raghu and Chandramohanan, 1993). Tey and Wood (1984) observed that Mancozeb was highly toxic at lower concentration against *P. palmivora*. Reddy and Chandramohanan (1984) and Raghu and Chandramohanan (1993) observed complete inhibition of *P. palmivora* on Dithane M-45 treated medium. However, in the present study Indofil M-45 recorded less efficacy in inhibiting the growth of the seedling blight pathogen. Mammootty (1978) also noted similar result. According to him, less growth of the fungus in Dithane M-45 might be due to the degradation of fungicide into non-toxic compounds by the fungus.

The Oomycetes fungi are considered to be sensitive to many antibacterial antibiotics compared to other fungi, thus represent a distinct specialised group of organisms since they possessed the cellulose containing cell wall. Hence the *in vitro* inhibitory effect of antibiotics against *P. palmivora* was assessed to find out a suitable one which could be utilized for the control of the disease. Among the six antibiotics tested, Chloramphenicol and Terramycin each at 400 and 500 ppm completely inhibited the growth of the

pathogen. Streptomycin also showed comparatively better inhibitory effect. Aureofungin and Amoxycillin exhibited minimum inhibition.

Except for a report by Reddy and Chandramohanan (1984) there are no reports on the sensitivity of antibiotics against cocoa isolate of P. palmivora. They observed complete inhibition of the growth of P. palmivora at 200 ppm of Aureofungin. But in the present study Aureofungin exerted less inhibitory effect. This might be due to the variation in the isolate of the pathogen as they were using the cocoa pod rot isolate of the pathogen. Further the inhibitory effect of Aureofungin against P. piperina var. piperina was reported by Chaurasia et al. (1973). However, there were indications regarding the effectiveness of certain antibiotics against other Phytophthora spp. Usefulness of Streptomycin against P. infestans was observed by Voros (1963) and Barna et al. (1972).

It is a well established fact that many chemicals which are promising in inhibiting the growth of the pathogen in in vitro may not be effective on actual host surface. Therefore, a study was carried out to find the effectiveness of seven fungicides and four antibiotics on detached cocoa leaves against P. palmivora. It was found that none of the chemicals were effective in completely checking the lesion

development. However, all the chemicals were found to be significantly superior to control. Among them, Foltaf followed by Fytolan each at 0.3 per cent concentration were significantly superior than other chemicals in checking the lesion development. Indofil M-45 recorded the least effect.

There are no report of testing fungicides/antibiotics on detached cocoa leaves against seedling blight pathogen P. palmivora. According to Gorenz (1974), screening of fungicides on detached cocoa pods or leaves in the laboratory can be done in a short time at much less cost than by large field experiments. The effectiveness of chemicals on detached cocoa pods against P. palmivora causing pod rot disease was tested by a few workers (Chandramohanan et al., 1979 and Raghu and Chandramohanan, 1993). They reported that Difolatan, Ridomil and Captaf completely inhibited the infection on detached cocoa pods. In the present study also on detached leaves Foltaf at 0.3 per cent showed maximum effect in checking the lesion development.

Field control of seedling blight disease of cocoa was studied in four different experiments.

In the first experiment the fungicides/antibiotics were sprayed on healthy seedlings and kept in a severely infected cocoa nursery. The remaining three sprayings were

done at 10 days interval. On the tenth day after first spraying no significant difference was observed between the treatments. However, observations on the disease score 10 days after second, third and fourth sprayings, the fungicides, Akomin, Foltaf, Fytolan and Bordeaux mixture were on par and significantly superior than other fungicides/antibiotics in preventing the incidence of the disease.

Effect of fungicides and antibiotics on the naturally infected seedlings were carried out both under open and shaded conditions. In this experiment, none of the treatments showed significant effect in checking the severity of the disease under both conditions. However, under open condition, the fungicides Fytolan followed by Akomin recorded the minimum disease score and maximum percentage efficiency over control. Under shade, Foltaf showed the minimum disease score and maximum percentage efficiency over control at all intervals of observations.

In the third experiment, the seedlings were artificially inoculated with the pathogen prior to the chemical treatment. Here, Akomin was significantly superior to all other treatments except Streptocycline and Indofil M-45 during the first observation. But, 10 days after second, third and fourth spraying none of the chemicals showed significant effect in checking the severity of the disease. However,

Akomin showed the minimum disease score and maximum percentage efficiency over control than other chemicals.

In the last pot culture experiment, the seedlings were sprayed with chemicals prior to artificial inoculation. In this study, Foltaf was found to be significantly superior to control and Indofil M-45, and control and Streptocycline on tenth day after first and second spraying respectively. On the tenth day after third spraying Akomin followed by Bordeaux mixture and Foltaf were found to be better than Streptocycline and Control. On the last observation Akomin showed the minimum disease score and maximum percentage efficiency over control followed by Foltaf and Bordeaux mixture.

According to Chandramohanan et al. (1979), P. palmivora is the most destructive of all the fungal pathogens attaking cocoa in India and require regular prophylactic sprayings for its management. Results of the in vivo control experiments indicated that, in general the fungicides like Akomin, Foltaf, Fytolan and Bordeaux mixture had promising effect in checking the incidence and severity of seedling blight of cocoa. Effectiveness of Difolatan, Copper oxychloride, and Potassium phosphonate in controlling seedling blight of cocoa was reported from many countries (Diaz and Newhall, 1966; Muller and Njomou, 1979; Anderson and Guest, 1990 and KAU, 1993). Usefulness of Bordeaux mixture in controlling the seedling die

back of cocoa was noticed by Chandramohanan (1983). Though, Oomycetes fungi are reported to be sensitive to antibiotics, it was found that the different antibiotics tried in the present *in vivo* control experiment did not exert much effect in checking the incidence and severity of seedling blight of cocoa. Also, there are no reports of the effect of antibiotics against seedling blight of cocoa. However, the effect of Streptomycin against *P. infestans* and *P. parasitica* was noticed by some workers (Muller *et al.*, 1954; Crosse *et al.*, 1960; Voros, 1963; Ersek *et al.*, 1972 and Chaurasia, 1973).

Hence, the fungicides, Akomin, Foltaf, Fytolan and Bordeaux mixture found promising in checking the incidence and severity of seedling blight of cocoa could be utilised for better management of the disease.

Summary

SUMMARY

Seedling blight of cocoa is a serious nursery disease inflicting heavy crop losses especially during rainy seasons. Thus, studies were undertaken on the causal organism, symptomatology, influence of age of seedlings on the disease incidence and severity, and to evolve a suitable management/ control measures.

The causal organism was isolated from the infected leaves and stem of seedlings and budded plants and Koch's postulates were established. Cultural and morphological characters of the causal organism were studied for its identification. On carrot agar, the fungus produced sparse, striate growth except in the centre. The mature sporangia were near spherical to ovoid with round base, papillate, caducous, measured 49.4 x 27.3 µm with an average L/B ratio of 1.74. Sprangia were borne terminally in a sympodial fashion. Decidious sporangia had short and thick stalk with an average length of 4.1 µm. Based on the cultural and morphological characters, the causal organism of seedling blight of cocoa was identified as Phytophthora palmivora (Butler) Butler.

For maximum growth of the fungus oatmeal and corn meal agar was the best. On carrot and oatmeal agar the fungus produced maximum sporangia. Among the liquid media, oatmeal

and corn meal broth followed by potato dextrose broth were superior in promoting the growth of the fungus.

Naturally infected cocoa seedlings and budded plants showed different types of symptoms on leaves and stems. On leaves, the disease initiated as watersoaked lesions, later turned to dark brown in colour, coalesced and finally resulted in leaf blight and defoliation. On the stem of seedlings the disease initiated either from the tip or cotyledonary region as watersoaked lesions, turned to black in colour, finally resulting in wilt or die back symptom. Stem of young budded plants were more prone to infection than that of seedlings. On the stem of budded plants, the disease initiated at any point of the stem resulting in symptoms as that of seedlings. The symptom produced on artificial inoculation on leaves and stem of seedling and budded plants were similar to that of natural infection.

Host range study revealed that Piper nigrum L., Cocos nucifera L., Hevea brasiliensis L., Bougainvillea sp. and Colocasia esculenta L. were infected by the pathogen. However, Areca catechu L., Piper betle L. and Piper longum L. did not take up infection.

The youngest seedlings of age group less than 18 days after germination were more susceptible to P. palmivora.

Similarly, the youngest budded plants of age group of 45 days after budding showed the maximum infection.

Among the 62 cocoa types screened for host resistant against seedling blight, the cocoa types GIV-36.6 (local), GVI-23 (P9 x P4), GIV-4.6 (local) and S-45.5 (local) recorded the minimum percentage of mortality, while, the maximum was in types M-9.16 (local), M-16.9 (local) and GII-24.4 (local).

The fungicides Fytolan, Captaf, Bordeaux mixture, Akomin and Ridomil each at 0.1, 0.2 and 0.3 per cent concentrations and 0.3 per cent Foltaf completely inhibited the growth of the fungus in in vitro.. Indofil-M.45 exhibited less efficacy of inhibition.

Among the antibiotics tested in in vitro, Chloramphenicol and Terramycin each at 400 and 500 ppm completely inhibited the growth of the fungus. Aureofungin and Amoxycillin exhibited the least inhibitory effect.

On detached cocoa leaves, Foltaf followed by Fytolan each at 0.3 per cent concentration were significantly superior than other fungicides and antibiotics in checking the lesion development.

In vivo control of the seedling blight of cocoa was carried out in four different experiments. In the first

experiment, Akomin, Foltaf, Fytolan and Bordeaux mixture were superior than other fungicides/antibiotics in checking the natural incidence and severity of the disease. None of the fungicides/antibiotics tried were significantly effective in checking the severity of the disease on naturally infected seedling both under open and shaded conditions. However, Fytolan, Akomin and Foltaf showed comparatively better effect against the disease under open and shaded condition respectively. In the third experiment also, none of the fungicides/antibiotics recorded significant effect against the disease in seedlings artificially inoculated prior to the chemical application. But, Akomin exerted better effect in checking the disease. In the fourth experiment, where the fungicides/antibiotics were applied prior to artificial inoculation, Akomin, Foltaf and Bordeaux mixture exerted better effect against the disease in the last two observations.

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* Originals not seen

Appendices

APPENDIX-I

Potato dextrose agar medium

Peeled and sliced potato	- 200 g
Dextrose	- 20 g
Agar agar	- 20 g
Distilled water	- 1000 ml

Czapek's agar medium

Sodium nitrate	- 2 g
Potassium dihydrogen phosphate	- 1 g
Magnesium sulphate	- 0.5 g
Potassium chloride	- 0.5 g
Ferrous sulphate	- 0.01 g
Sucrose	- 30 g
Agar agar	- 20 g
Distilled water	- 1000 ml

Oatmeal agar

Rolled oats	- 50 g
Agar agar	- 20 g
Distilled water	- 1000 ml

Corn meal agar

Maize	- 30 g
Agar agar	- 20 g
Distilled water	- 1000 ml

Carrot agar

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

Cocoa pod extract agar

Cocoa pod	- 200 g
Agar agar	- 20 g
Distilled water	- 1000 ml

Cocoa leaf extract agar

Cocoa leaves	- 200 g
Agar agar	- 20 g
Distilled water	- 1000 ml

Cocoa pod extract dextrose agar

Cocoa pod	- 200 g
Dextrose	- 20 g
Agar agar	- 20 g
Distilled water	- 1000 ml

APPENDIX - II

Analysis of variance table for the growth of seedling blight isolate of *P. palmivora* on different liquid media

Source	df	Mean squares
Treatment	7	17026.7 **
Error	16	104.5
Total	23	

APPENDIX - III

Analysis of variance table for the *in vitro* sensitivity of *P. palmivora* to antibiotics

Source	df	Mean squares
Treatment	18	43.3 **
Error	38	0.084
Total	56	

APPENDIX - IV

Analysis of variance table for the effect of fungicides and antibiotics against *P. palmivora* on detached cocoa leaves

Source	df	Mean squares	
		Third day	Fifth day
Treatment	11	0.418 **	1.559 **
Error	36	0.069	0.158
Total	47		

** Significant at 1 per cent level

APPENDIX - V

Analysis of variance table for the age of seedling on the incidence and severity of seedling blight

Source	df	Mean squares			
		5th DAI	10th DAI	15th DAI	20th DAI
Treatment	7	0.295 **	0.405 **	0.388 **	0.355 **
Error	112	0.045	0.051	0.049	0.047
Total	119				

APPENDIX - VI

Analysis of variance table for the age of budded plants of cocoa on the incidence and severity of the disease

Source	df	Mean squares			
		5th DAI	10th DAI	15th DAI	20th DAI
Treatment	5	0.125 NS	0.331 **	0.565 **	0.570 **
Error	84	0.057	0.101	0.098	0.098
Total	89				

** Significant at 1 per cent level

NS - Not significant

DAI - Days after inoculation

APPENDIX - VII

Analysis of variance table - Effect of fungicides antibiotics in preventing the natural incidence of seedling blight of cocoa

Source	df	Mean squares			
		10 days after 1st spraying	10 days after 2nd spraying	10 days after 3rd spraying	10 days after 4th spraying
Treatment	10	0.029 NS	0.151 *	0.210 **	0.313 **
Error	99	0.023	0.053	0.065	0.065
Total	109				

APPENDIX - VIII

Analysis of variance table - Effect of fungicides antibiotics on naturally infected seedlings (kept under open condition)

Source	df	Mean squares				
		On the day of 1st spraying	10 days after 1st spraying	10 days after 2nd spraying	10 days after 3rd spraying	10 days after 4th spraying
Treatment	10	0.036 NS	0.073 NS	0.072 NS	0.074 NS	0.169 NS
Error	66	0.022	0.041	0.042	0.040	0.093
Total	76					

* Significant at 5 per cent level ** Significant at 1 per cent level NS - Not significant

APPENDIX - IX

Analysis of variance table - Effect of fungicides antibiotics on naturally infected seedlings (kept under shade)

Source	df	Mean squares				
		On the day of 1st spraying	10 days after 1st spraying	10 days after 2nd spraying	10 days after 3rd spraying	10 days after 4th spraying
Treatment	10	0.008 NS	0.053 NS	0.055 NS	0.056 NS	0.056 NS
Error	77	0.009	0.047	0.044	0.052	0.051
Total	87					

APPENDIX - X

Analysis of variance table - Effect of fungicides antibiotics in checking the severity and spread of the disease on artificially inoculated seedlings

Source	df	Mean squares				
		On the day of 1st spraying	10 days after 1st spraying	10 days after 2nd spraying	10 days after 3rd spraying	10 days after 4th spraying
Treatment	10	0.002 NS	0.128 *	0.082 NS	0.080 NS	0.080 NS
Error	77	0.004	0.066	0.064	0.059	0.056
Total	87					

* Significant at 5 per cent level NS - Not significant

APPENDIX - XI

Analysis of variance table - Effect of fungicides antibiotics in preventing the incidence and severity on artificially inoculated seedlings

Source	df	Mean squares			
		10 days after 1st spraying	10 days after 2nd spraying	10 days after 3rd spraying	10 days after 4th spraying
Treatment	10	0.163 **	0.210 **	0.206 **	0.208 **
Error	44	0.049	0.075	0.062	0.058
Total	54				

** Significant at 1 per cent level

ETIOLOGY AND CONTROL OF SEEDLING BLIGHT OF COCOA

By

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ABSTRACT OF A THESIS

Submitted in partial fulfilment of the requirement for the degree

Master of Science in Agriculture

Faculty of Agriculture

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Vellanikkara - Thrissur

1995

ABSTRACT

The seedling blight is a serious nursery disease of cocoa. The fungus causing seedling blight of cocoa was isolated and Koch's postulates were established.

On carrot agar medium, the fungus produced sparse, striate growth. The mature sporangia were near spherical to ovoid with round base, papillate, caducous with an average L/B ratio of 1.74. Sporangia were borne terminally in a sympodial fashion. It had a short and thick stalk with an average length of 4.1 µm. Based on these characters, the pathogen causing seedling blight of cocoa was identified as Phytophthora palmivora (Butler) Butler.

For maximum growth of the fungus, oat meal and corn meal agar were the best. But, for maximum sporangial production, carrot agar and oat meal agar were good. Among the liquid media, oat meal and corn meal broth supported good growth of the fungus.

The disease produced various type of symptoms on leaves and stem of seedlings and budded plants like water soaking, leaf blight, defoliation, black discolouration, cotyledonary infection, wilting and die back. Stem of budded plants were more prone to infection than that of seedlings.

The seedling blight pathogen infected plants such as Piper nigrum L., Cocos nucifera L., Hevea brasiliensis L., Bougainvillea sp. and Colocasia esculenta L. but did not infect Areca catechu L., Piper betle L. and Piper longum L. on artificial inoculation.

The youngest seedlings of age group less than 18 days after germination were more vulnerable to infection. Similarly, the youngest budded plants of age group 45 days after budding showed maximum infection.

Among the 62 cocoa types screened for host resistance the cocoa types GIV-36.6 (local), GVI-23 ($P_9 x P_4$), GIV-4.6 (local) and S-45.5 (local) showed minimum percentage of mortality.

Among the different fungicides/antibiotics screened in in vitro, Fytolan, Captaf, Bordeaux mixture, Akomin and Ridomil at all concentrations and 0.3 per cent Foltaf and, Chloramphenicol and Terramycin each at 400 and 500 ppm completely inhibited the growth of the fungus. Indofil-M.45, Aureofungin and Amoxycillin exhibited less inhibitory effect.

Foltaf, Fytolan and Akomin each at 0.3 per cent concentration showed better effect in checking the lesion development on detached cocoa leaves.

Result of the different in vivo control experiment indicated that, the fungicides like Akomin, Foltaf, Fytolan and Bordeaux mixture had promising effect in checking the incidence and severity of the disease.