SOME ASPECTS OF THE BIOLOGY AND CONTROL OF LEAF AND FRUIT SPOT DISEASE OF *CITRUS* CAUSED BY *PHAEORAMULARIA ANGOLENSIS* KIRK IN KENYA.

BY

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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Dedicated to my parents Mr. and Mrs. Bernard Waswani

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I extend my thanks to my husband Prof. F. D. Juma for financing the project. I am grateful to John Aswani, Mr.Tepeny and Herbert Khayo for their technical assistance, Mrs. Rose Birgen for the word processing service. In a special way I appreciate the sacrifice, patience and understanding from my children throughout this period.

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ABSTRACT

The leaf and fruit spot disease of *Citrus* also referred to as the vitale syndrome, is a new disease to Kenya. The causal agent is identified as *Phaeoramularia angolensis*. The disease has assumed epidemic levels in Western Kenya and it is a matter of time for it to spread to all *Citrus* producing areas in the country. The control of the disease has been difficult and farmers have expressed fear that the fungus may be resistant to fungicides. The isolation of the fungus was reported to be difficult, and in culture the fungus was said to sporulate only in media containing an extract of *Citrus* leaf and fruit peel.

The objectives of this study were to isolate and characterise the causal agent of the leaf and fruit spot disease, determine the most favourable conditions for growth and sporulation of *P. angolensis*, and to carry out *in vitro* tests on the response of the fungus to selected fungicides.

Field surveys have shown that the disease is severe on all species of *Citrus* growing in Trans-Nzoia district. It was found that the disease occurred in epidemics whose severity coincided with heavy rains from August to December. Random sampling indicated that 60 a% of all fallen leaves and 100% of the dropped

[illegible] were affected. On the average, leaf litter of a single tree was found to contain about 39.[illegible] x 10^5 spores.

Morphological characteristics of the fungus from naturally and artificially infected plants, and axenic cultures, showed that this fungus belongs to the genus *Phaeoramularia*. However, from the literature, the distinction between the genera *Phaeoramularia* and *Phaeoisariopsis* is difficult and requires a taxonomic reappraisal.

Isolates of *P. angolensis* were tested for pathogenecity on disease free *Citrus* seedlings. An inoculum from two week old solid cultures produced typical disease symptoms on young *Citrus* plants. Lesions developed slowly, taking almost seven months for definite necrotic lesions to be formed from the time of inoculation.

Growth in liquid media was more than in solid media with a mean difference of 17% ± 8 The Orange leaf extract gave more growth than other media (viz, Tap water, Potato dextrose , Cornmeal, Potato carrot), mean difference 25% ± 15 . Sparse sporulation was observed on all solid media amended with dextrose. No sporulation was observed in media without dextrose, liquid media and in solid cultures more than two weeks [illegible]

Growth occurred between 6-30^{O}C with the optimum at

25 - 26°C. The fungus grew over a range of pH 3 to 8 with maximum growth occurring at initial pH of 5 to 6. The best mycelial growth was obtained in cultures incubated in continuous white light.

In vitro tests with Copper Oxychloride (50%WP), Kocide 101 (Copper Hydroxide, 50%WP) , Antracol (Propineb 70%WP) and Dithane M45 (Mancozeb 80%WP) showed that these fungicides were effective in stopping spore germination. However, Kocide 101 and Copper Oxychloride were not effective against mycelial growth, but Antracol and Dithane M45 were. Their differential effects on mycelial growth was significant $P<0.05$, although the difference between Kocide 101 and Copper Oxychloride, and between Dithane M45 and Antracol were not significant. The LD_{50} for Antracol and Dithane M45 was found to be less than 10ppm and that for Copper Oxychloride and Kocide 101 more than 3000 ppm.

CHAPTER 1

1.0 INTRODUCTION

1.0 Background to the problem

The *Citrus* originated in North-East India, Burma and in the adjoining areas. They spread from India to the Mediterranean regions and the rest of the world (Opeke, 1982). Though grown all over the tropical areas, commercial production of these fruits is concentrated in subtropical countries mainly with mediterranean type of climate. Tropical countries like Kenya produce only a small quantity for the local market (Ticho, 1972).

The genus *Citrus* belongs to the family Rutaceae which contains approximately 150 genera and nearly 2,000 species. *Citrus* contains about twelve species and many hybrids (Perry, 1987). Most of the cultivated species of *citrus* are found in Kenya. These are: *Citrus sinensis* Osbeck (the sweet orange) , *C. limoni* Burm (the lemon), *C. aurantifolia* Swingle (the lime), *C. aurantium* L. (the sour orange) and *C. reticulata* Blanko (the tangerine) (Watt, 1962). *C. sinensis* is the most common and popular species grown in many parts of Kenya. Coast and Rift Valley provinces with over fifteen thousand hectares are the leading producers of *Citrus*. Western, Central, Nyanza, North Eastern and Nairobi provinces have a total of over five thousand hectares

Production and improved quality have, notwithstanding, never been sufficient to meet the local demand. The government in collaboration with FAO made considerable efforts to expand and supply the home market with high grade *Citrus* both for fresh consumption and for processing. Plans were made to increase hectarage by 300 hectares in the Rift Valley and Eastern provinces. Stage two of the plan was to see a further increase by 800 hectares in the same areas. Such an increase in hectarage was expected to produce 30,000 - 35,000 tons of oranges . Statistics show that although there was increase in hectarage, there has been a dramatic drop in the sweet orange production over the period 1988-1989. In North Eastern province, as hectarage increased by 4%, production dropped by 19%. In West Pokot district, hectarage expanded from 97.7 to 106 hectares, but production dropped by 34%. This drop was attributed to diseases and pests. (Ministry of Agriculture Annual Reports, 1988-1991). Production of sweet oranges in the cooler Western and Central provinces has been curtailed mainly by the greening disease caused by a virus. A new leaf and fruit spot disease caused by *Phaeoramularia angolensis* has aggravated this production drop and worse consequences are expected if measures to curb the spread of the disease are not taken soon.

1.2 Literature review

The form genus *Phaeoramularia* belongs to the form family Stilbellaceae of form class Deuteromycetes (Alexopoulous, 1979). It is characterised by effuse, olivaceous brown, often velvety colonies. Mycelia mostly immersed, stroma small. Conidiophores macronematous, mononematous, caespitose, unbranched or loosely branched, straight or flexous, pale olivaceous brown, smooth and cicatrised. Conidiogenous cells polyblastic, intergrated, terminal becoming intercalary or occasionally discrete. Conidia sympodial, in branched or unbranched chains, acropleurogenous, simple, cylindrical with rounded ends, ellipsoidal or broadly fusiform, hyaline or olivaceous brown, smooth with 1,2 or several transverse septa (Ellis, 1971 & 1985).

Species of *Phaeoramularia* are pathogens to a wide range of plant hosts such as those in the families Compositae, Solanaceae, Anacardiaceae, Convolvulaceae, Cucurbitaceae, Euphorbiaceae, Leguminosae, Rubiaceae, Salicaceae and Vitaceae (Ellis, 1971). The form-species *P. angolensis*, the causal agent of the brown leaf and fruit spot disease of *Citrus*, is the only reported species of *Phaeoramularia* on hosts in the family Rutaceae.

The brown leaf and fruit sport disease of *Citrus* has been reported in Nigeria, Zambia, Zimbabwe, Angola,

Yemen (Kirk, 1986). In Nigeria the causal agent of the disease was initially identified as *Septoria citri* which is the causal agent of *Septoria* spot. Later on, it was identified as *Xanthomonas citri* a bacterium known to cause *Citrus* canker. In 1979, the disease became so severe that closer attention was given to its precise identity and etiology. The causal agent was then identified as a species of the genus *Phaeoisariopsis* (Emechebe, 1980). However, Kirk (1986) described a similar fungus as *Phaeoramularia angolensis* citing *Cercospora angolensis* as a synonym. Secondary metabolite studies carried out on *Phaeoramularia angolensis* isolates from Kenya seemed to suggest that the fungus could actually be a species of *Cercospora* (Siboe, 1989).

Phaeoramularia angolensis is reported to cause necrotic lesions of light to dark brown, surrounded by yellow haloes on the leaves. Lesions on the fruits are reported to be brown in colour with a blistered appearance. The centers of the lesions are said to appear dark brown due to production of synnemata, 120-240 um long. Conidiophores arising from a large stroma are described as macronematous measuring upto 240 um long. The conidia are cylindrical, acropleurogenous, catenulate, pale brown to hyaline, 3-4 septate, a few

1.3 Rationale

Emechebe (1980) reported that the isolation and culture of this fungus was difficult, and that the fungus sporulated only on culture media containing an extract of orange leaf and fruit peel. The fruit and leaf spot disease is most prevalent and severe in Trans Nzoia district. Farmers from this district sold no fruits during the 1986-1988 period There was 100% total loss of income. The sudden occurrence and severity of the disease, and poor response to control methods have forced farmers to either uproot or abandon their orchards. All species of Citrus in the area of study were affected, hence there was no direct link on the influence of the origin and genetics of the cultivars and susceptibility to the disease.

Farmers have attempted to control the fungus using Dithane M45 (Mancozeb 70% WP), and copper fungicides viz: Kocide 101 (Cupric hydroxide 50% WP) and Copper Oxychloride (50% WP). Similar fungicides were used in the control of other Citrus leaf sport diseases (Fisher 1961). In spite of the use of these fungicides, *Citrus* orchards have continued to suffer from severe epidemics each year. This disease together with the existing pests

black leaf spotting disease may leave the country without a single orange if urgent action to stop the spread is not taken.

In view of the above problems relating to the control of the disease it was crucial that proper diagnosis, precise identity of the pathogen and *in vitro* tests on the efficacy of a few popular fungicides on the current market be carried out on the fungus. A clear understanding of the enviromental requirements for optimal growth and reproduction of the pathogen would also be important for the disease control.

1.4 Objectives

The objectives of this study were :

1. To isolate and characterise the causal agent of the leaf and fruit spot disease.

2. To determine the most favourable conditions for growth, sporulation and dissemination of *Phaeoramularia angolensis*.

3. To carry out *in vitro* tests on the response of the fungus to selected fungicides.

CHAPTER 2

2.0 MATERIALS AND METHODS

2.1 Disease incidence and diagnosis

2.1.1 Field studies and collection of samples

The study was carried out in Trans Nzoia district where the disease is most prevalent. Eleven randomly selected farms within a radius of 30 kms from Kitale town were visited and disease occurrence and severity was assessed. Farmers were interviewed using a questionnaire (Appendix I).

An orchard (0.25 acres) on a mixed farm of 10 acres, was selected and visited monthly for one year (from Dec 1990 - Dec 1991) to establish the disease cycle. Changes in rainfall and temperature were obtained from the Meteorological department. Disease symptoms on the leaves and fruits were monitored. Photographs of the diseased plants were taken. Leaf litter was sampled to estimate the amount of diseased leaves, fruits and approximate total inoculum in the litter of the orange trees. Random sampling method using a 50cm x 50cm frame quandrant was used (Odum, 1971). Numbers 1 to 10 were written on equal pieces of paper, carefully folded and placed in a small box. After shaking to mix, one number was blindly picked out. The litter of the orange tree in the first

row in a position corresponding to the picked number was sampled first, followed by every fifth tree. The leaves and fruits within the quandrants for each tree were collected and kept in labelled polythene bags. Diseased leaves and fruits picked from the trees were kept in separate bags. All samples were taken to the laboratory.

2.1.2 Inoculum estimation.

The total number of leaves and fruits in the litter of each tree was counted. Infected leaves (leaves with lesions) were separated from non-infected ones (leaves without lesions). The proportion of infected leaves to total leaf fall was calculated. The count was repeated using fruits and the proportion of infected fruits in the litter also calculated.

A total of 50 lesions were identified on ten leaves. Spores from each lesion were carefully scraped into a 10 ml beaker containing 5 mls distilled water. A drop of this spore suspension was placed on each half of the depression points of an improved Neubauer haemocytometer. The number of spores in five squares on each half of the slide selected in an "X" pattern was counted at the low power (10x10) of the microscope. The total number of spores counted multiplied by 1000 gave the number of spores per ml of the spore suspension. An estimated spore count for each lesion was given as the number of spores per ml x 5. An average spore count for 50 lesions was recorded. The average count of lesions on 50 leaves was also calculated. The product of the two averages gave an estimated spore count per leaf.The spore count per leaf multiplied by total infected leaves gave the estimated inoculum in the leaf litter of each tree. To estimate

the leaf area affected, the surface area of each of the 50 leaves was measured by an automatic area-meter ('Delta-T Device'). All lesions on each leaf were carefully cut out with a sharp scalpel. The surface area of the leaves without lesions was measured. The difference between the two measurements gave the area of the lesions on the leaf. The proportion of lesion area to the total leaf area was equivalent to the proportion of the infected leaf surface area. Fruits were cut into halves to observe disease extension into the inner tissues of the fruits.

2.1.3 Isolation and characterization of the fungus

The fungus was isolated from diseased leaves picked from the trees. Lesions on diseased material were examined under a dissecting microscope for fungal fruitification. Conidia were picked from sporulating lesions with a pointed edge of a sterilized Swann Morton No.11 scalpel . They were transferred onto Tap Water Agar (TWA), (Appendix III). TWA was used to suppress the rapid growth of saprophytes. Axenic cultures of the fungus were used for further cultural and pathogenecity tests. Some fungal materials from the lesions on host leaves and fruits were mounted on slides and observed under a calibrated microscope. Morphological characteristics were recorded. Similar data were recorded for *Phaeoisariopsis griseola* Ferraris isolate from the beans as a representative of

Phaeoisariopsis for taxonomic comparisons. Morphological characteristics extracted from literature on the Nigerian isolate of *Phaeoisariopsis* (Emechebe, 1980) and of *Phaeoramularia angolensis* (Kirk,1986) were also recorded.

2.2 Pathogenecity and disease development.

Two week old cultures grown on potato dextrose agar (PDA), (Appendix III) were harvested and marcerated in a waring blender for 30 seconds. Fifteen colonies were harvested to obtain adequate inoculum. Enough sterile distilled water was added to facilitate easy passage of the inoculum through the atomiser. Ten disease-free potted Sweet orange (Washington navel and Valencia varieties, 60 cm tall) seedlings used, were obtained from disease free nurseries in Nairobi. The plants were sprayed with the culture suspension. They were then covered with polythene bags which had also been sprayed with sterile distilled water to ensure high relative humidity. Ten control plants were sprayed with sterile distilled water only. Wounded plants (plants whose leaves were scratched with a scalpel) were also treated as above. The plants were kept under shade and after 72 hours, the polythene bags were removed. They were observed daily for symptoms and disease development (Conway 1976). Plants were not kept in the laboratory because such plants soon defoliated due to

lack of sun light. Monthly means of air temperatures were obtained from the Meteorological department (Appendix IIb). Data on length of incubation period, rate of lesion development, number of lesions and their sizes on a leaf, amount of sporulation, conidial size, colour etc were recorded. Conidiophore size and colour were also observed.

2.3 Cultural studies

2.3.1 Effect of different solid media on growth and sporulation of *P. angolensis*

Four different media were prepared, Potato dextrose (PD), Potato carrot (PC) , Corn meal (CM) and Orange Leaf extract (Ole) (Appendix III). Agar was added to half of each medium prepared, the other half was kept in a separate bottle and used in the experiment (2:3:2) below .

About 30 mls of each medium was poured in 9 cm petri dishes. Uniform amounts of mycelia from ten day old cultures maintained on TWA were used to inoculate the media. Thirty mls of each medium was poured in 9 cm petri dishes. Each medium had six replicates incubated at room temperature (21-24^{o}C) and in ordinary day and night conditions.

After eight weeks, five colonies on each medium were dislodged with a glass rod. They were oven dried

at 60°C until constant weights were obtained. The sixth colony from each medium was used to rate sporulation. Extra cultures on PCA and CMA adjusted with 20 gms dextrose/litre were also incubated and used to rate sporulation. Data on colony, conidia and conidiophore characteristics were recorded.

2.3.2 Effect of liquid media on growth and sporulation

The liquid media prepared as described above (2.3.1) were used i.e (Potato dextrose, Potato carrot, corn meal and orange leaf extract). The pH of each medium was recorded before and after autoclaving as initial pH. Thirty mls of each was aseptically transferred into six replicates of 250 mls Erlenmeyer flasks. The flasks were plugged with sterilized cotton wool and Aluminium foil. The media were then inoculated with culture suspensions. These were prepared from the remains of colonies used in the solid media experiment (2.3.1 above). Sterilized distilled water was added to the colonies. The colonies were gently scraped with a sterilized scalpel. The suspensions of each colony were transferred to a common sterilized 100 ml beaker. With constant stirring, 5 mls of the mycelial suspension was removed by a pipette and added to the liquid media in the flasks. The cultures were incubated at room temperature (21-24°C). Ordinary conditions of day and night were maintained.

After two weeks, the mycelial mats of five cultures

for each medium were filtered through thimble funnels on weighed filter papers. They were rinsed with distilled water and dried at 60°C until constant weights were obtained. The sixth flask of each medium was used to rate sporulation. The dry weights of the fungus and pH of the filtrates were recorded.

2.3.3 The effect of incubation temperature on growth and sporulation

Inoculum preparation and seeding was done in liquid Potato dextrose medium. Nine incubation temperatures used were: 3, 6 , 8 , 15 , 20 , 25, 26, 30 , 32°C and control room temperature (21-24°C). There were six replicates at each temperature. The incubator used was an open water bath (Grant instrument No 91230 A). The cultures were incubated in the water whose temperatures were altered by an inbuilt heating and cooling system. The cultures were exposed to ordinary light conditions of day and night.

After two weeks, dry weights of five replicates at each temperature were determined as in 2.3.2 above. The sixth replicate was used to study colony characteristics and sporulation.

2.3.4 Effect of different light conditions on growth and sporulation

Six 250 mls Erlenmeyer flasks were prepared with liquid Potato dextrose and seeded with inoculum as in

_.3.2. They were incubated under constant illumination provided by one white, red and blue fluorescent lamp (Model TLD 36 W/54) at a time, located at 25 cm above the flasks. The set up was repeated twice for each light condition.

In the same experiment, six inoculated flasks were maintained in continuous darkness by wrapping up the flasks with three layers of black paper and incubating in a dark cupboard. Six other cultures were incubated on the bench exposed to 12/12 hour natural light/dark as control. After two weeks, dry mycelial weights of five replicates from each experimental set up were recorded. Sporulation studies were made from the sixth replicates. The number of conidia was counted under low power microscope (10 x 10).

2.3.5 Effect of pH on growth and sporulation

Liquid Potato dextrose medium at seven different pH values (2.0-8.0) was used. The medium was buffered with 0.1N hydrochloric acid and 1.0N Sodium hydroxide using (Radiometer pH M61) pH meter. The Control was set up in unbuffered medium at pH 5.6. The medium at different pH was autoclaved. On cooling the pH was measured and recorded as initial pH. Thirty millilitres of each was aseptically transferred into six sterilized 250 mls Erlenmeyer flasks, inoculated with 5 mls of mycelial suspension and incubated at room

temperature (21-24^{o}C) for two weeks on laboratory bench. After incubation period, one replicate was used to study sporulation, while the dry weights of the fungus were determined from the remaining five replicates. The pH values of the filtrates were recorded.

2.4 Effect of selected fungicides on growth and sporulation of *P. angolensis*

2.4.1 Growth and sporulation on solid medium.

The following fungicides were used in this study: Kocide 101 (Cupric Hydroxide 50% WP), Copper Oxychloride (50% WP), Dithane M45 (Mancozeb 70% WP) and Antracol (Propineb 80% WP). Serial dilutions were prepared for each fungicide using the method described by the American Phytopathological Society on Standardisation of Fungicidal Tests (1947), in which, 0.5 gm of each fungicide was suspended in 50 ml sterilized distilled water in a 250 ml sterile Erlenmeyer flask (10,000 ppm). After thorough shaking, 5 ml were withdrawn by a sterile transfer pipette and added to 45 ml sterile distilled water in another flask. After proper mixing, 5 ml were withdrawn from this flask and added to another flask containing 45 mls of sterile distilled water. The above dilution continued until the following dilutions were obtained: 10,000, 1,000, 100, 10, 1 and 0.1 ppm. More dilutions were prepared using the standard formula:

$$\frac{X \text{ units of chemical}}{1{,}000{,}000 \text{ml of water}} = \frac{Z \text{ grams of chemical}}{Y \text{ ml of water}}$$

Potato Dextrose Agar was prepared as in (Appendix III) and, 150 mls of medium were transferred into separate flasks equivalent to the number of prepared dilutions of

tre fungicides. Each diluted fungicide was added to the cooled medium before solidifying at the fungicide : medium ratio of 1:5. The medium and fungicide were shaken to allow mixing and 30 mls of the fungicide amended media were poured into 9 cm petridishes. On solidifying, the medium was inoculated with 1 ml mycelial suspension. Petri dishes were shaken to ensure an even spread of the inoculum on the medium. There were five replicates at each dilution. Control replicates were set up using distilled water and were recorded as zero concentration. The cultures were incubated at room temperature (21-24^{o}c) on the bench.

After two weeks, growth of mycelia on the various fungicide dilutions were scored relative to the control. Where no growth occurred, mycelial fragments were subcultured on fungicide-free PDA to determine their viability. Sporulation was also determined in 2.3.4.

2.4.2 Effect of fungicides on growth and sporulation in liquid medium

Liquid Potato dextrose medium was prepared and 25 mls were aseptically transferred into 250 ml sterile Erlenmeyer flasks. Fungicide dilutions were prepared as in 2.4.1 and 5 mls of each dilution was added to the medium in the flask. Each flask was then inoculated with 1 ml of mycelial suspension. Control flasks were

prepared with 5 mls distilled water in place of the fungicides. There were six replicates for each fungicide dilution and control. The cultures were incubated at room temperature (21-24^{o}C) on laboratory bench.

The LD_{50} was measured from mycelial growth since there was no spore germination at all fungicide dilutions. Mycelial dry weights of five cultures were recorded. The sixth replicates were used to study sporulation. Growth inhibition by each dilution was expressed as a percentage of the control dry weight (Carling, 1990). Dose-response curves were constructed for each fungicide from which the LD_{50} (Lethal Dose 50%) were estimated. Semi-log scale was used to give log-transformations of the fungicide concentrations (Lukens 1971).

2.4.3 Effect of fungicides on conidial germination

Serial dilutions of the fungicides were prepared as described earlier . A suspension of conidia of *P. angolensis* was prepared from host leaves. The suspension was adjusted with distilled water and calibrated on an improved Neubauer haemocytometer to give a spore concentration of about 500,000 spores per ml. Juice from fresh orange (Washington navel variety) was filtered and diluted tenfold with distilled water. Equal volumes of spore suspension and orange juice

stimulant were mixed. Two drops of this mixture were mixed with two drops of each fungicide dilution in the slide depression. A control was set up consisting of two drops of the spore suspension and stimulant mixed with two drops of distilled water. The slides were incubated in a moist chamber and daily observations were made as recommended by the Committee on standardisation of fungicidal tests (1947) and Peterson (1941).

2.5 Statistical analysis

The variations in dry weights with respect to temperature, light, PH, fungicides and fungicide dilutions, were analysed using the Analysis of Variance (ANOVA). When the analysis was restricted to two means, the least significance difference test (LSD) was used. The level of significance was set at 5% $P<0.05$ (Armitage 1971).

CHAPTER 3

3.0 RESULTS

3.1 Disease diagnosis and incidence

Results of the field survey showed that the disease was wide spread in Trans Nzoia district (Table 1). All the eleven farms visited were affected. Farmers noticed the disease for the first time in 1983-84, when only a few leaves and fruits were affected leading to scattered premature defoliation and fruit drop. By 1985-87, the disease had assumed epidemic levels, and no oranges were harvested. All *Citrus* species (Viz: lemons, tangerines, sour oranges etc.) grown in this region were equally affected.

Farmers have been spraying with Kocide 101, Copper Oxychloride and Dithane M45. About 90% of the farmers sprayed only when they noticed severe disease symptoms on the crop. Successful control of the disease had been achieved in one farm out of the eleven farms (Table 1). All eleven farmers interviewed applied these fungicides in mixture combinations of white oil and insecticides to minimise costs. Farmers expressed fear that prolonged use of Copper would in the long run cause Copper toxicity in *Citrus* orchards. To avoid this, over 50% of the farmers were spraying lower fungicide rates

Table 1: Disease incidence and control on some farms in Trans Nzoia

Farm Number	Number of Orange trees	Disease state	Fungicides sprayed	Frequency of application	Control	Action
1	100	severe	1+2+3 in A	fortnightly	poor	abandoned
2	300	severe	1+2+3 in A	fortnightly	poor	abandoned
3	60	severe	1+2+3 in A	fortnightly	poor	hopeful
4	>1,000	not severe	1+2+3 in A	weekly	fair	successful
5	500	severe	1+2+3 in A	weekly	poor	hopeful
6	70	severe	1+2+3 in A	weekly	poor	hopeful
7	20	severe	1+2+3 in A	occasionally	poor	uprooting
8	30	severe	1+2+3 in A	fortnightly	poor	abandoned
9	35	severe	1+2+3 in A	occasionally	poor	uprooting
10	80	severe	1+2+3 in A	weekly	poor	hopeful
11	75	severe	1+2+3 in A	fortnightly	poor	abandoned

Key:

1 = Kocide 101

2 = Copper oxychloride

3 = Dithane M45

A = Mixture of white oil and insecticides

than recommended. Hardly any fungicide residue could be seen on the leaves of the oranges on most farms. The application of expensive fungicides without good fruit harvest had disillusioned two of the farmers into uprooting their *Citrus* trees to give way to more profitable cereal crops. Four of the farmers had neglected their orchards which were getting overgrown with weeds. Three of the farmers who were still hopeful, continued to spray the fungicides weekly or fortnightly.

The 0.25 acre orchard used in this study had three rows of 60 orange trees of Washington navel variety. The first litter sampling was carried out in October, the time when the mean rainfall was 105 mm, and temperature means were 11.3^{0}C (minimum) and 26.3^{0}C(maximum) (Appendix IIa). The results showed that disease infection was severe both on the fruits and leaves (Plates 1 a-b). No infection was observed on the stems and branches. There was heavy leaf and fruit fall and no mature fruits were seen on severely infected trees. Litter sampling estimations showed that on the average, 60.8% of the fallen leaves and 100% of the dropped fruits were infected (Table 2). The leaf litter of a single tree haboured an average of 39.4 x 10^{8} spores. A maximum of 89 lesions on a single leaf of 29 cm^{2} was counted, covering 98.19% of the total leaf surface area (Appendix IV) Lesions had an average diameter of 6 mm and an average spore count of

Plate 1a

Plate 1b

1a Leaves of *Citrus sinensis* exhibiting brown spots caused by *Phaeoramularia angolensis*

1b Brown fruit spot disease of *Citrus sinensis* caused by *Phaeoramularia angolensis*

Table 2: Leaf and fruit litter sampling

Tree	Total count				% infected			
	Leaves		Fruits		Leaves		Fruits	
	1s	2s	1s	2s	1s	2s	1s	2s
1	98	10	5	0	55.1	20	100	0
2	77	9	6	0	58.4	11	100	0
3	78	5	4	2	44.9	0	100	100
4	18	11	8	0	50.0	18	100	0
5	36	2	3	0	50.0	0	100	0
6	72	8	1	1	69.4	38	100	100
7	64	15	11	0	34.4	27	100	0
8	70	5	3	0	82.9	40	100	0
9	54	7	4	0	87.0	14	100	0
10	80	8	7	1	86.3	0	100	100
11	98	3	2	0	57.1	0	100	0
12	89	4	3	0	58.2	0	100	0
Mean se	65.5±22	7±4	5±3	0.4±6	60.8±16	14±15	100	25

1s = First sampling
2s = Second sampling

184×10^4. The second sampling carried out in March, the period with 85.1 mm mean rainfall and temperature means of 12.3°C minimum and 27.8°C maximum, showed reduced disease incidence. There was less leaf and fruit fall due to infection . On the average, 14% of fallen leaves and 100% of dropped fruits were infected (Table 2) . Mature fruits were observed on the trees during this season . The new leaves and fruits that were produced during this period were disease free. Scattered lesions were only seen on a few old leaves and fruits. Leaves with an average area of 27 cm^2 had an average of eight lesions. The lesions were small with an average diameter of 2.5 mm, light brown, lacking haloes and with very sparse sporulation. An average of $12x10^4$ conidia per lesion was counted. Partially developed lesions could be seen as tiny dark spots.

The leaf spots observed from sample material were amphigenous, mainly hypophyllous, sporulating on both sides but more from the under side, and 3-8 mm diameter. On the under side of the leaf, lesions appeared uplifted, but remained flat on the adaxial surface. Young lesions were pale brown while mature ones,brown to dark brown. When sporulating heavily, the lesions were brown, surrounded by a dark brown margin and a yellow halo, the center becoming transparent or detached resulting in a shot hole. Foliar symptoms often

coalesced making the leaves appear yellow followed by premature defoliation .

Lesions on young fruits were slightly sunken, circular, brown, surrounded by a ring of raised epicarp protruding above its normal level, and giving the fruit a blistered appearance. When sporulating, lesions became black, thus the young fruit became covered with black blisters each surrounded by a yellow halo. The merging of several haloes made even immature tiny fruits appear yellow ripe followed by abscission . Lesions on older fruits were more flat and often cracked exuding sticky fruit juice. The fungus did not penetrate the rind and caused no necrosis below the skin, although rotting due to secondary infection in cracked fruits was common .

Conidiophores from the mature lesions were dark brown, mononematous, simple, unbranched, straight or geniculate, septate, smooth, 29 - 156 (255.5) µm high (mode 90 µm), 3.3-4.0 µm wide (mode 3.79 µm), with scars 5.5 µm wide, evenly spaced, 10.97 µm apart. Conidiophores were synnematous, in groups of upto 50 and more, emerging from dark brown to black, erumpent stromatic tissues 71-82 µm in diameter. The lower portions of brown to dark brown synnemata consisted of conidiophores joined at their bases to a length 65.7-

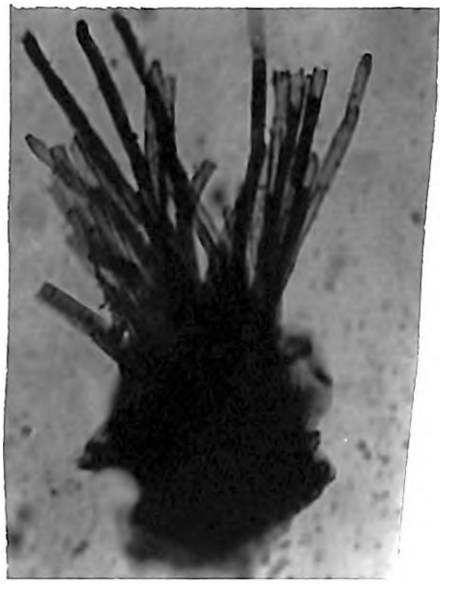


Plate 2. Stroma and conidiophores of *Phaeoramularia angolensis* from inoculated plants.

17.6 µm and 21.9-30.3 µm wide. The overall length of the synnemata 62.1-255.5 µm varied with that of its conidiophores. Conidiogeneous cells were intergrated, terminal, sympodial and cicatrized (Plates 2, Appendix V). Conidia obtained from mature lesions were smooth, light brown to hyaline, cylindrical, straight or slightly curved, solitary, acropleurogenous, often catenulate, (1-), 3-4, (-6) septate, 13.9-79 µm long, (mode 51.1 µm), 3.3-4 µm wide mode (3.65 µm), rounded at the apex and truncated at the base; older conidia often budding daughter conidia (Plate 3). Lesions easily lost their conidia by drop off such that materials kept in the laboratory lost nearly all the conidia after one week of handling.

Conidial measurements of *Phaeoisariopsis griseola* isolated from the bean leaves were 30.4-79.2 µm long 3.6-7.2 µm wide and (1-), 3-4, (-5) septate, (fig 1). Conidia of *P. angolensis* had two modal values of 20-29.9 µm and 50-59.9 µm. *P. griseola* conidial length was unimodal at 50-59.9 µm (Appendix V, fig I)

Conidial germination experiments at different pH and temperature showed that conidia of *P. angolensis* germinated easily in distilled water (pH 5.35) at room temperature (21-24^{o}C) . Spores germinated over temperature range 20-26^{o}C. Below and above this temperature range no spores germinated. High rates of

Plate 3. Conidia of *Phaeoramularia angolensis* from inoculated plants.

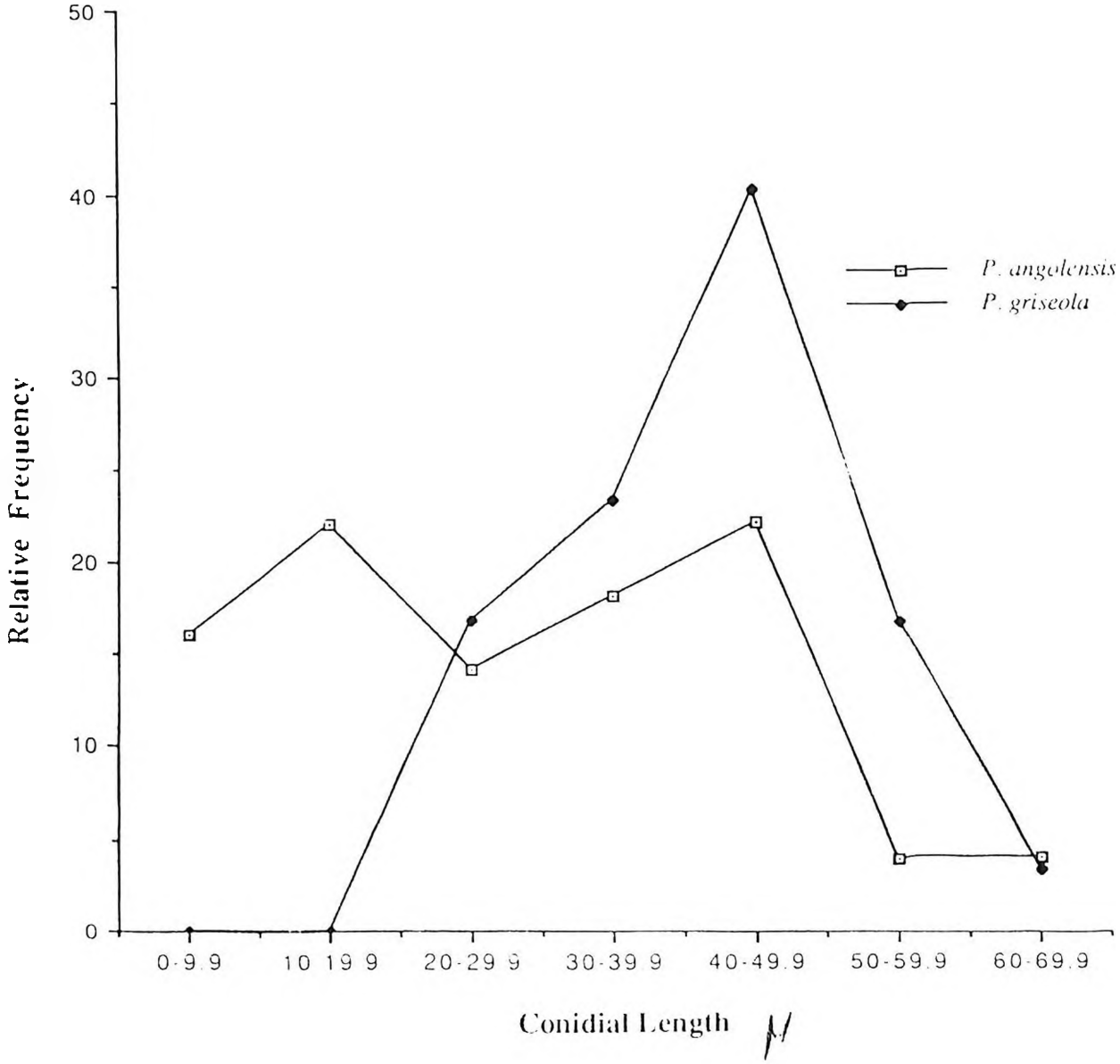


Fig. 1: Comparison of Conidial lengths of *Phaeoramularia angolensis* and *Phaeoisariopsis griseola*

spore germination, of 94 and 93 % were recorded at 25 and 26 ^{o}C respectively. The control at room temperature gave a spore germination of 93 %. The lowest rate of 56 % was observed at 20^{o}C (Appendix VIa). Spores germinated over pH range of 2 - 8, with an optimum of 93 % at pH 4. The lowest rate of 33 % was observed at pH 2. Spore germination in distilled water pH 5.35, gave a rate of 86 % (Appendix VIb).

Spore germ-tubes first appeared from apical and basal cells, but intercalary cells sometimes produced the first germ tubes. Occasionally, more than two germ tubes were produced from a single cell of a conidium (Plate 4).

On the third day following isolation, growth in culture media was only visible under the light microscope. Conidia germinated and formed dark profusely branching mycelia. On the seventh day, growth was observed on top of the medium as a tiny dark dot. This tiny dot of mycelium continued to grow slowly, more vertically than radial, such that radial growth could not be measured. At eight weeks, a colony appearing like a mummified orange fruit was established on the medium . The colonies were solid compact masses easily separated from the medium, dark brown , velvety and immersed (Plate 5). Colonies of *Phaeoisariopsis griseola* isolated from the beans for comparison were

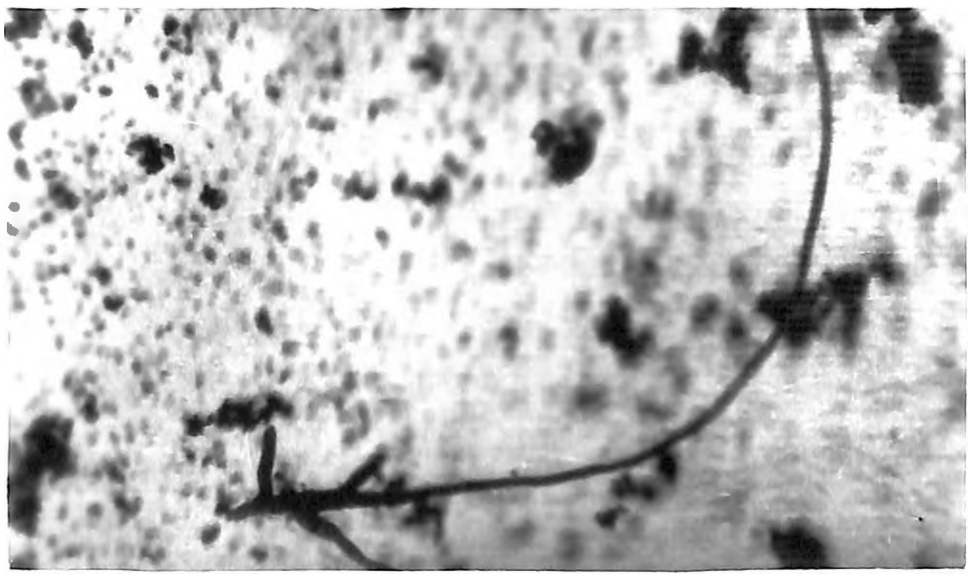


Plate 4. Conidium of *Phaeoramularia angolensis* germinating from terminal and intercalary cells.

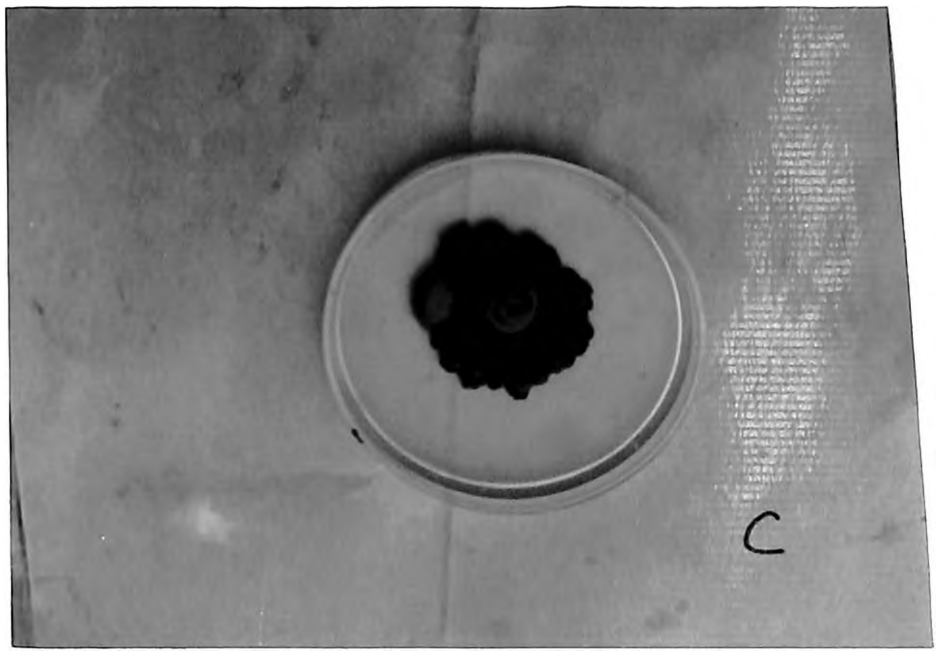


Plate 5. *Phaeoramularia angolensis* colony on orange leaf extract agar. (Four months old).

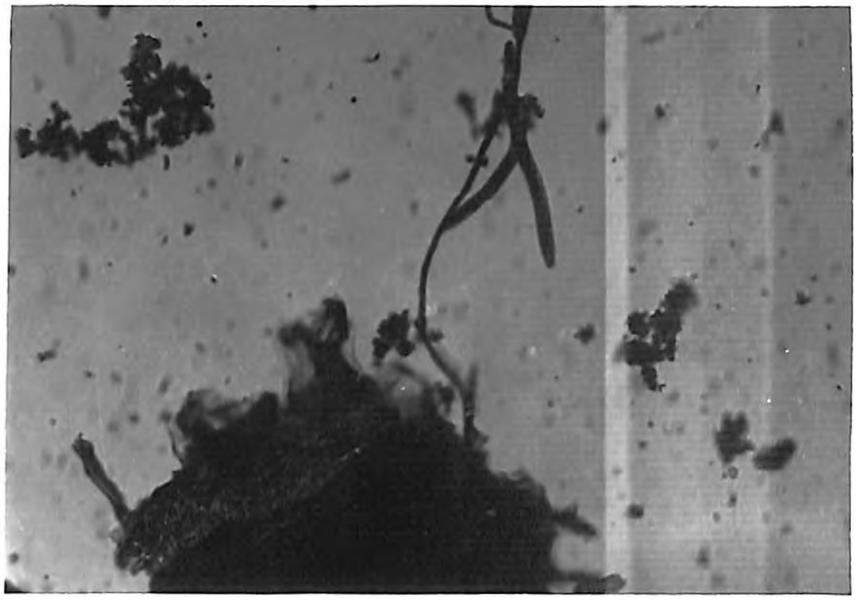
immersed, cottony, or hairy and white grey due to heavy sporulation both in liquid and solid cultures.

Sparse sporulation in cultures of *Phaeoramularia angolensis* was observed in very tiny colonies of upto two weeks old. Cultures of over two weeks had no spores. Subcultures from mature colonies never sporulated. Conidia produced in culture were subhyaline to pale brown, dry, smooth, cylindrical, straight, solitary, acropleurogenous, truncated at the base and rounded at the apex, 9-70 µm long (mode 50 µm) and (3-4) µm wide (mode 3.6 µm), (1-) 3-4 (-6) septate (Plate 6a).

Conidiophores produced in culture were macronematous, straight, smooth, septate, dark, mononematous but synnematous. Conidiophores from an eight day old culture, measured upto 210 µm long forming a huge robust synnema of upto 68.4 µm wide at the base. A big stroma of upto 57 µm wide was also observed. Conidiogeneous cells were intergrated, sympodial and cicatrized (Plate 6b).

Suspensions of two week old cultures of *Phaeoramularia angolensis* sprayed on potted sweet orange seedlings produced typical disease symptoms (

a



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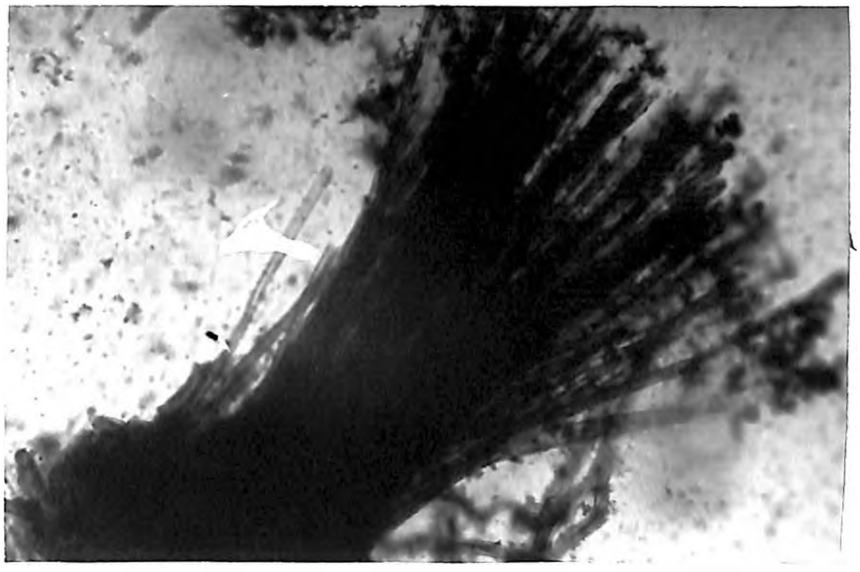


Plate 6.a Conidia of *Phaeoramularia angolensis* from culture

b Stroma and conidiophores of *Phaeoramularia angolensis* from culture

Plate 7a

Plate 7b

7. a *Phaeoramularia angolensis* leaves at an early stage of infection. Note chlorosis and dot-like spot stage.

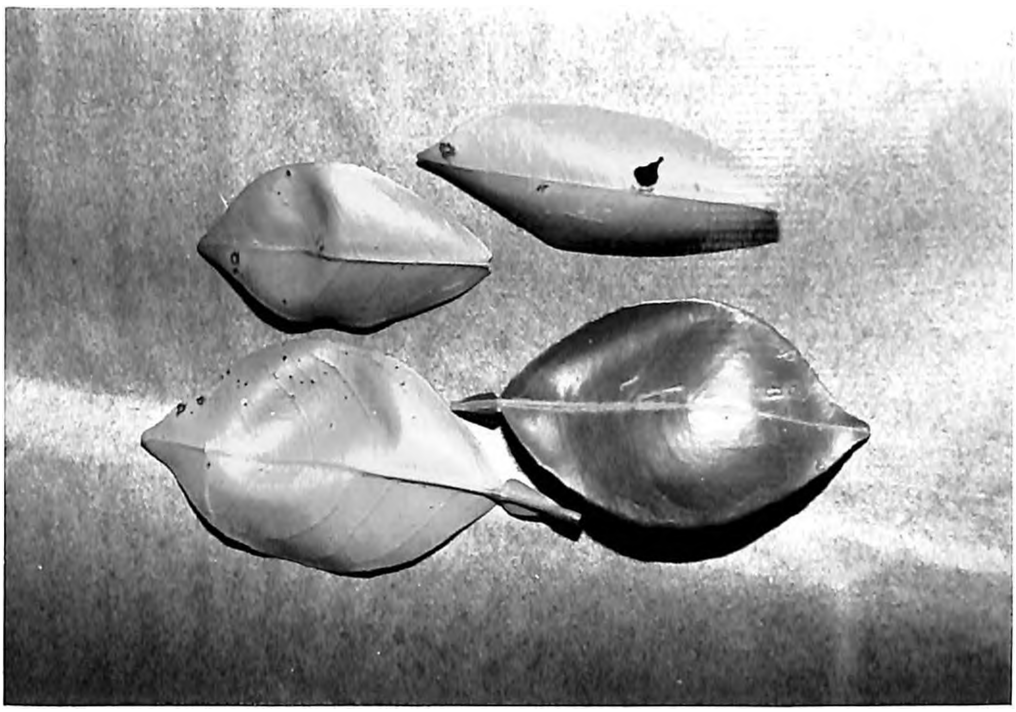
b Typical symptoms of brown spot disease after seven months.

Plate 7a-c). Three months after inoculation, the leaves developed light green to yellow patches becoming more yellow to brown (Plate 7a). Defined tiny spots started appearing in the fifth month. The brown dot-like spots enlarged and by late seventh month, definite necrotic lesions surrounded by narrow yellow haloes had been formed (Plate 7b). The infection of artificially inoculated plants was low, only eleven leaves or less per plant developed lesions. Individual leaves carried a maximum of 15 lesions. Many lesions stopped at the dark, brown dot-like spot development stage (Plate 7c). Mature necrotic lesions were small 2-4 mm diameter, amphigenous, pale brown centres with dark brown margins. Sporulating regions appeared dark brown , scattered in the lesions, both sides of lesions sporulating but more on underside. Conidia, conidiophores , synnemata and stroma isolated from these plants, were as those described earlier. All plants, wounded and unwounded, sprayed with suspensions from mature cultures (three weeks old and more), and cultures maintained on liquid media did not develop disease symptoms.

3.2 CULTURAL STUDIES

3.2.1 Effect of solid medium on growth and sporulation of *P. angolensis*

Data presented in (Appendix VII and fig.2) show



7c. Leaves from artificially infected plants with few developed lesions and more dot-like partially developed lesions.

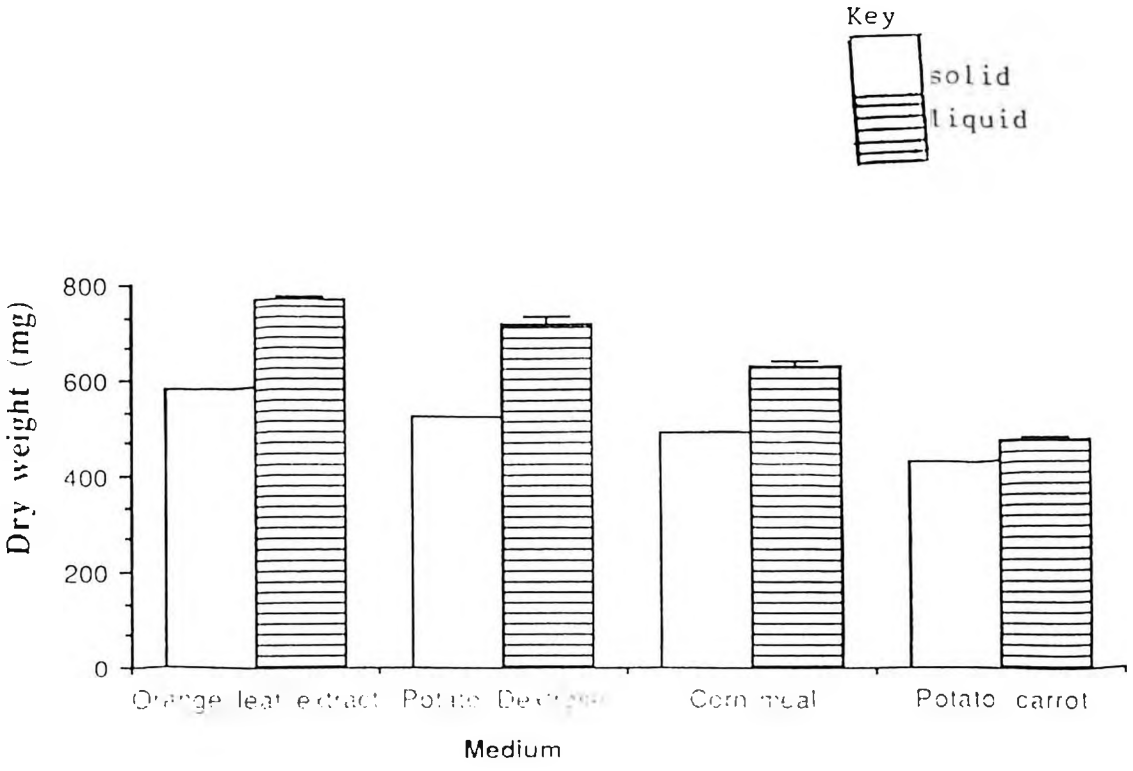


Fig. 2. Effect of medium on growth of *Phaeoramularia angolensis*

that the Orange leaf extract agar with a mean of 582 mgs supported maximum growth of the fungus . Good growth,(mean 519 mgs), was also obtained from Potato dextrose agar . Moderate growth,(mean 488 and 429 mgs), was obtained from Corn meal and Potato carrot agar media respectively. The differences between the means were statistically significant ($F=107.3$, $P<0.05$) . Although sparse sporulation occurred in all the five media with dextrose(initial PH 5.4 - 6.1), spores were only observed at the edges of young colonies that were less than two weeks old. Older colonies with luxuriant mycelial growth, and colonies in media without dextrose never sporulated at all. Subsequent subcultures yielded no spores in all types of media. Conidia, conidiophores, synnemata and stroma described in (3.1) were observed.

3.2.2 Effect of liquid media on growth and sporulation.

Results in (Appendix VII and fig 2) indicate that liquid media supported faster growth of the fungus than solid media. Incubation period for liquid media was two weeks while that of solid media was eight weeks. The orange leaf extract with a mean dry weight of 763 mgs supported maximum growth, followed by Potato dextrose (mean 717 mgs). Good growth with a mean dry weight of 624 mgs was obtained from corn meal, and less growth mean dry weight 468 mgs was obtained on Potato carrot ($F = 234$, $P<0.05$) .

In all liquid media used , pH increased as a result of growth (Appendix VII) and no sporulation was observed in any of the media. In the leaf extract medium, the fungus formed granules adhering onto the sides and bottom of the flasks but in other media, it formed mycelial mats floating over the media (Plate 8).

3.2.3 The effect of incubation temperature on growth and sporulation.

Most growth occured at 20 - 26^{o}C with 25-26^{o}C being the optimum (fig 3). Some growth was obtained at 15^{o}C and at 6^{o}C. No growth was observed at 3 and 32^{o}C. Scanty growth occurred at 30^{o}C (Plates 9). The differences in the means following growth at the various temperatures were statistically significant ($F = 394$, P 0.05). No sporulation was observed at all temperatures (Table 3)

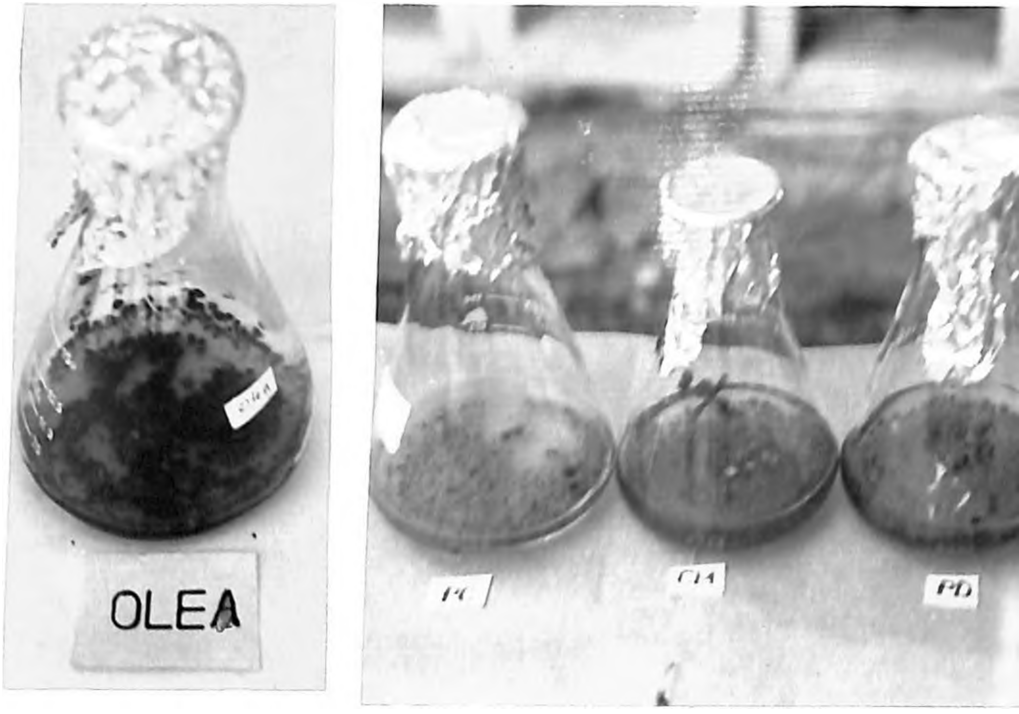


Plate 8. *Phaeoramularia angolensis* in liquid media . Note fungal colonies in lumps adhering onto sides and bottom of flask in the orange leaf extract (OLE) and mycelial mats floating on other media: potato carrot (PC), corn meal (CM) and potato dextrose (PD).

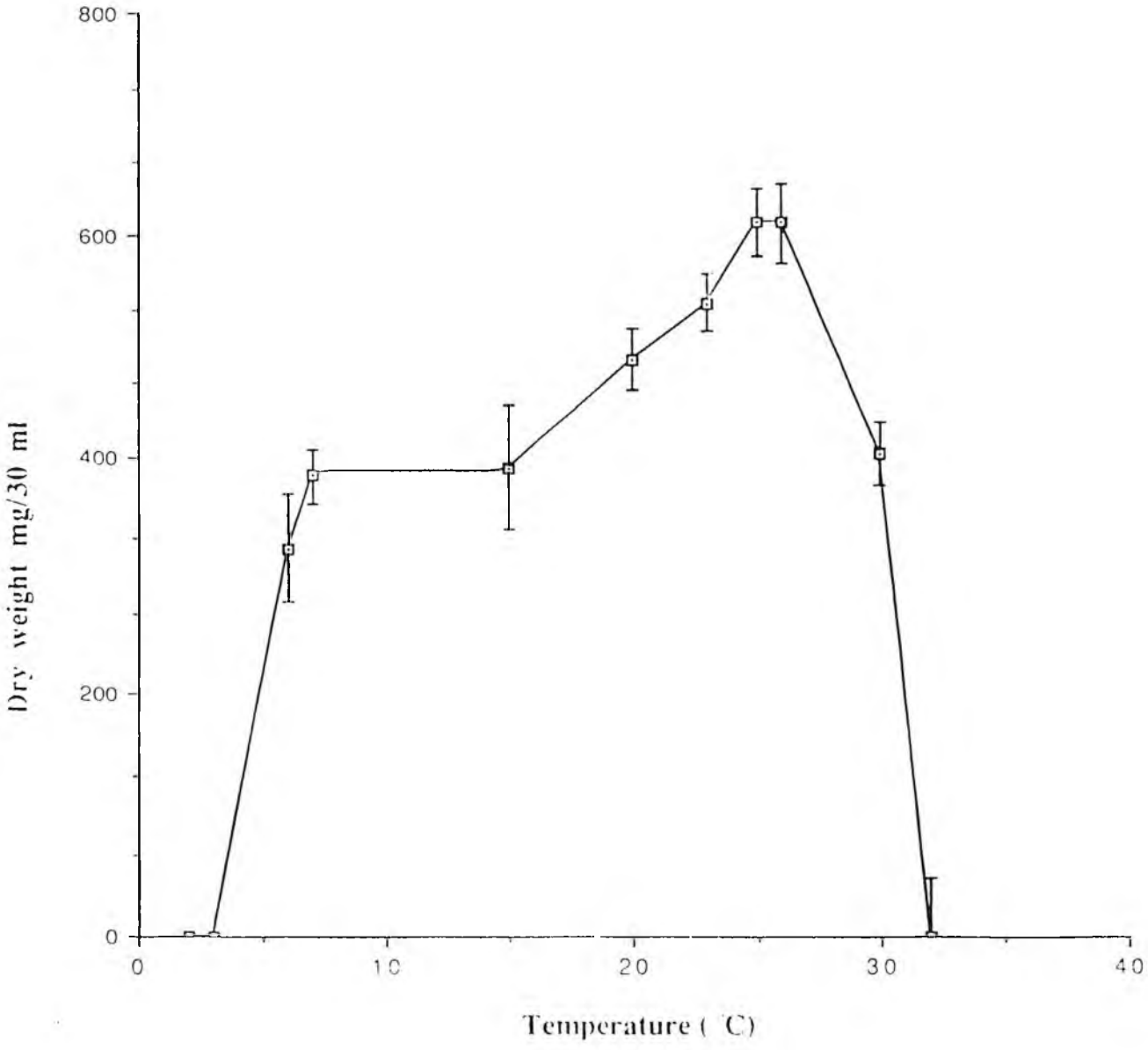


Fig. 3: Effect of temperature on growth of *Phaeocomplex angolensis*

Plate 9: Growth of *Phaeoramularia angolensis* at different temperatures.

a: No growth at 3°c.

b: Little growth at 6°c.

c:Fair growth at 15°c

d: Good growth at 20°c.

e & f Optimal growth at 25 and 26 °c

g:Scanty growth at 30 °c

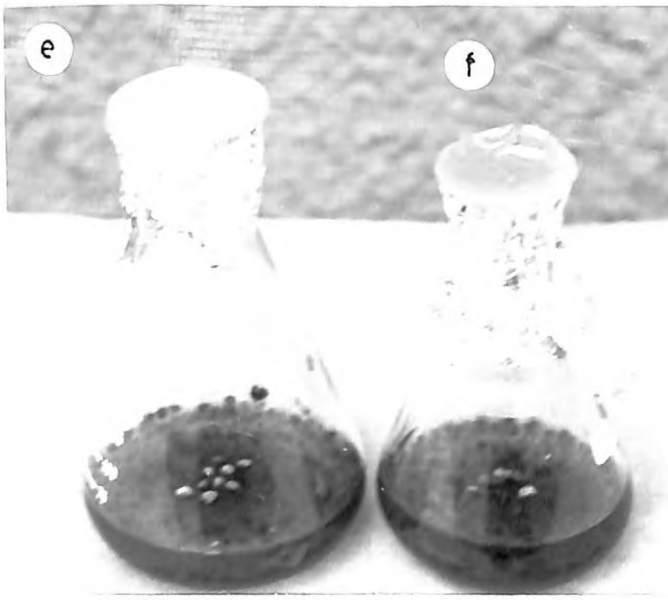
h: No growth at 32°c


a


b


c


d


e
f


g
PYREX
150
125
100
75
150 ml


h

Table 3: Effect of different incubation temperatures on growth and sporulation of *P. angolensis* after 14 days of incubation.

Temperature (°C)	Average dry mycelial wt (mg)*	Sporulation
2	0	Nil
3-5	0	Nil
6	323±10	Nil
6-8	383±15	Nil
15	419±14	Nil
20	512±15	Nil
21-24 (Room)	561±4	Nil
25-26	629±5	Nil
30	432±3	Nil
32	0	Nil

* Each data is an average of five replicates

3.2.4 Effect of different light conditions

All the different light regimes tested supported good mycelial growth (fig.4 and Appendix VIII). Optimum growth (mean 619 mgs) was obtained in cultures under continuous white light. Dry weight of 578 mgs recorded in continuous red light was not significantly different from the control (mean 577 mg). The F ratio however, was significant (F = 16.6, P 0.05). There was no sporulation in any light regime .

3.2.5 Effect of pH on growth and sporulation.

The pathogen grew over a range of pH 3 to 8 (fig.5) with the best growth obtained in media with initial pH of 5 to 6. The growth decreased gradually as pH rose above 6 or fell below 5. However, the decrease in growth at high pH was more gradual as compared to the lower pH levels. No growth occured at pH 2 (Plate 10). The fungus changed the pH of the medium towards neutrality, increasing the level of acidity and lowering the level of alkalinity. No sporulation was observed at all the pH levels (Table 4).
Differences between the means were statistically significant (F = 836, P<0.05).

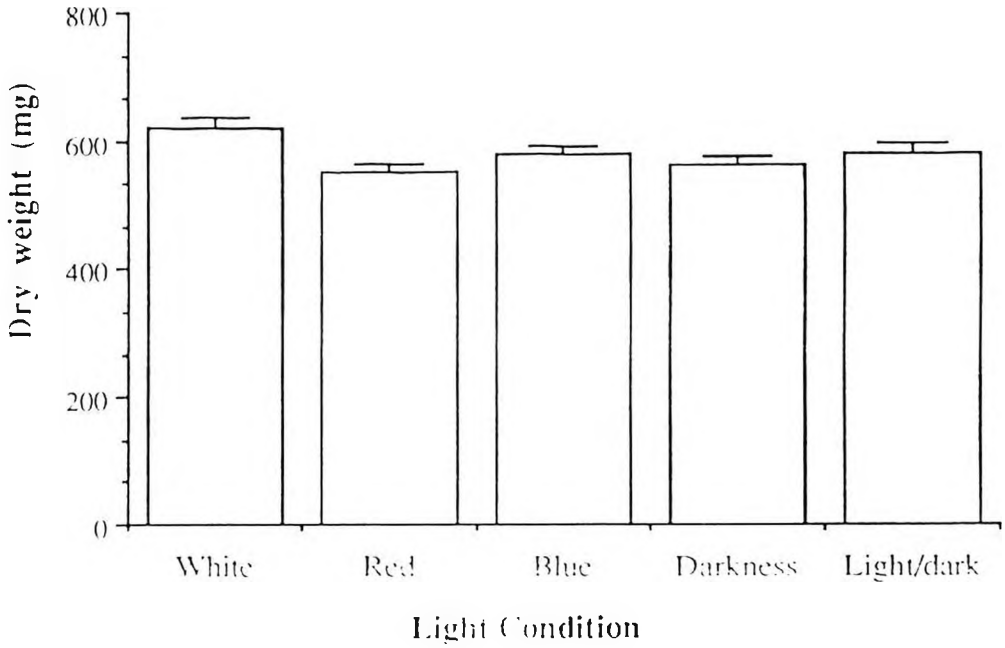


Fig. 4. Effect of light on growth of *Phaeoranularia angolensis*

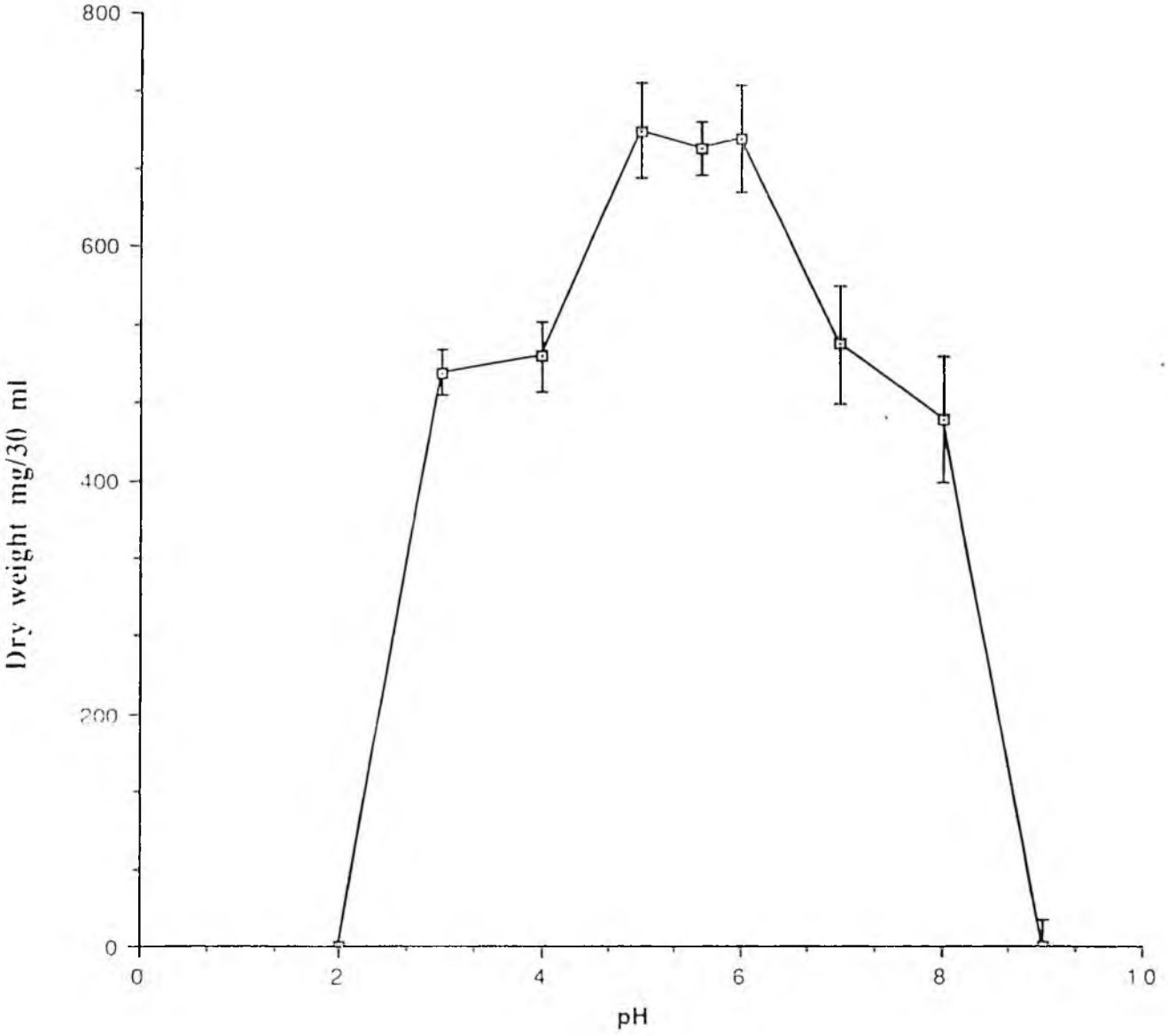


Fig. 5: Effect of pH on growth of *Phaeoramularia angolensis*

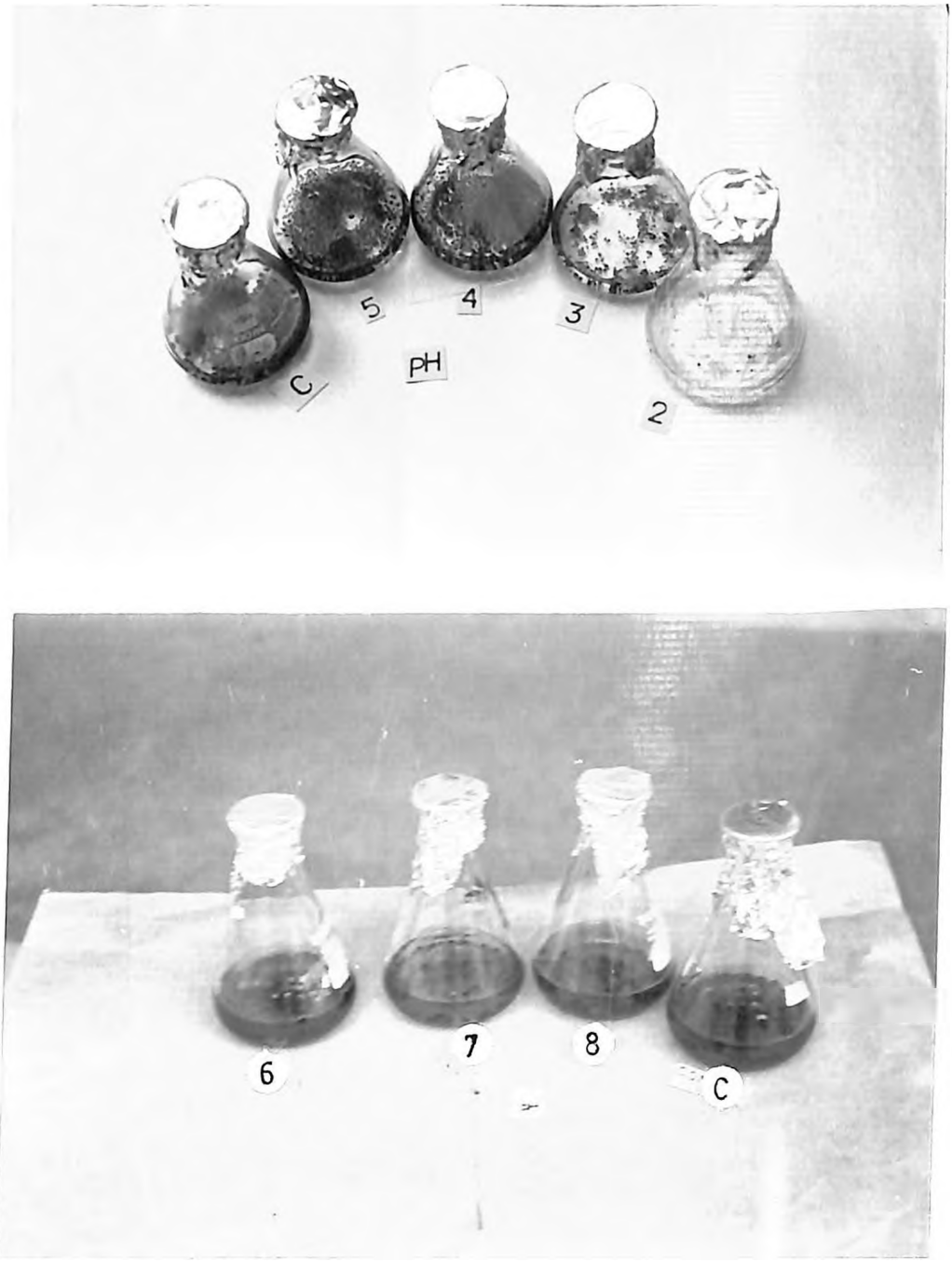


PLate 10. Growth of *Phaeoramularia angolensis* at different pH levels.

Table 4: Effect of different pH levels on growth and sporulation of *P. angolensis* after 14 days of incubation at room temperature (21-24°C).

Initial pH				
Before Autoclaving	After Autoclaving	pH of filtrate	Average dry mycelial weight (mg)*	Sporulation
2	2	2	0	Nil
3	2.9	5.6	490±4	Nil
4	4.09	6.1	503±6	Nil
5	5.1	6.2	697±9	Nil
5.6 (control)	5.8	6.6	682±4	Nil
6	5.9	6.4	690 ±8	Nil
7	7.5	6.9	513±8	Nil
8	8.06	6.8	450±11	Nil
9	9	8.9	0	Nil

* Each data is an average of five replicates

3.3 The effect of selected fungicides on growth, sporulation and conidial germination of *P.angolensis*.

Results from solid media tests (Table 5) show that the fungus did not grow on solid media amended with Dithane M45 and Antracol (Plates 11 a-b). Mycelial fragments subcultured from media treated with fungicides at 10 ppm and below, developed on PDA. Those subcultured from media treated with 100ppm and above shrank and did not grow on PDA. Results from tests using these fungicides in liquid media showed mycelial growth only at fungicide concentrations 1.5ppm and below (Table 6). The recommended dose is 2,500 ppm for both fungicides.

There was growth in solid media amended with Kocide 101 at concentrations 1,000ppm and below (Plates 11c). There was scanty growth at 3,000 and 5,000 ppm. Growth was hardly visible at 6,000 ppm and above, although mycelial fragments sprayed on these media did not shrink and when subcultured on PDA , developed into colonies. Recommended dose for Kocide 101 is 7,500 ppm. In liquid media, there was no mycelial growth at fungicide concentrations 5,000 ppm and above (Table 6).

Copper Oxychloride showed an increase in growth inhibition with increasing fungicide concentration. At 10 ppm there was better growth growth of fungi than

Table 5: Effect of selected fungicides on the growth and sporulation of *P. angolensis* on solid medium after 14 days at room temperature (21-24°C)

	Mycelial growth					
Fungicide concentration (ppm)	Kocide 101	Copper oxychloride	Dithane M45	Antracol	Diazinon + white oil	Diazinon+ white oil + copper oxychloride
10,000	0	0	0	0	0	0
6,000	0	1	0	0	0	1
5,000	1	1	0	0	0	1
3,000	1	2	0	0	0	1
1,000	3	3	0	0	0	2
100	3	3	0	0	0	2
10	3	4	0	0	0	2
*C 0	3	3	3	3	3	3

*C = Control with no fungicide

Key to mycelial growth

0 No visible growth
1 Growth much less than the control
2 Growth slightly less than control
3 Growth equivalent to control
4 Growth almost better than control

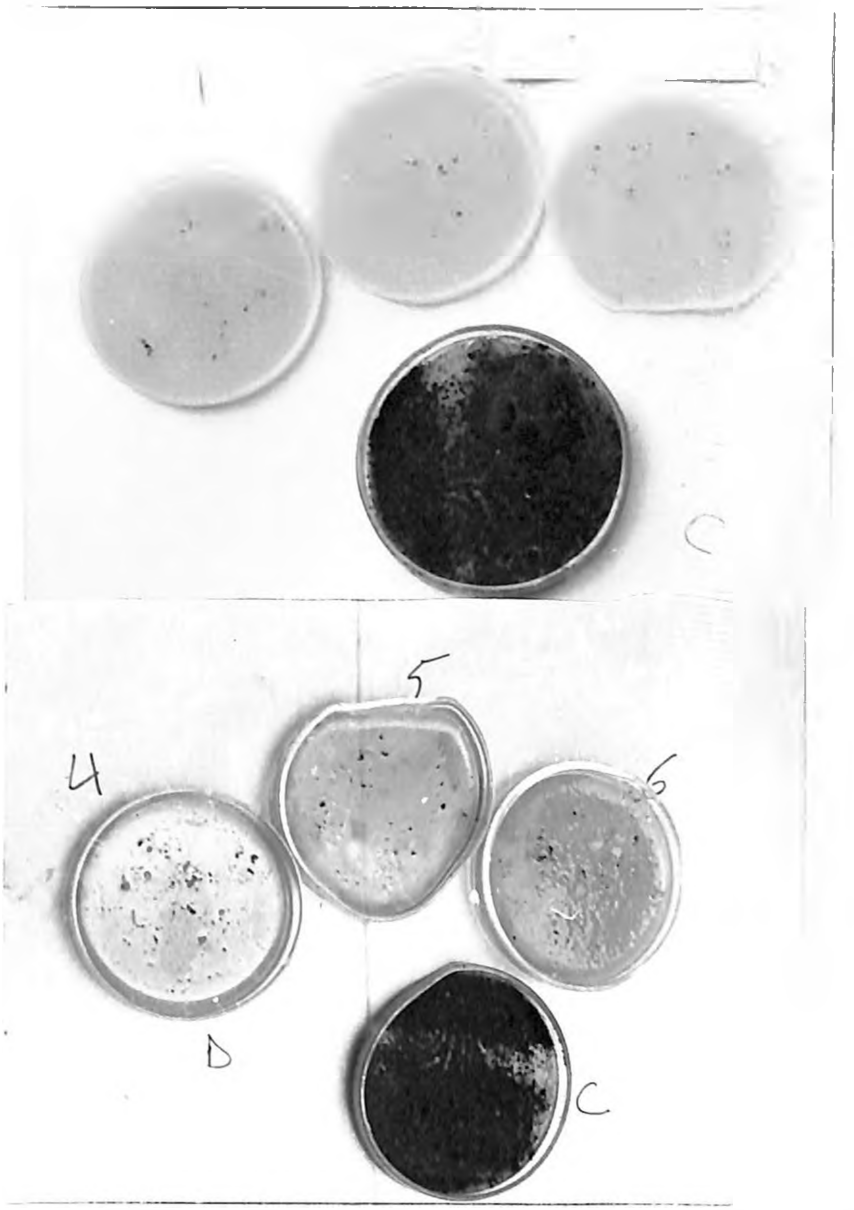


PLate 11a. *Phaeoramularia angolensis* in media ammended with Dithane M45. No growth at all dilutions.

1 =10,000 ppm
2 = 5,000
3 = 2,500 ppm (Recommended dosage)
4 = 3000
5 = 100
6 = 10
C = no fungicide.

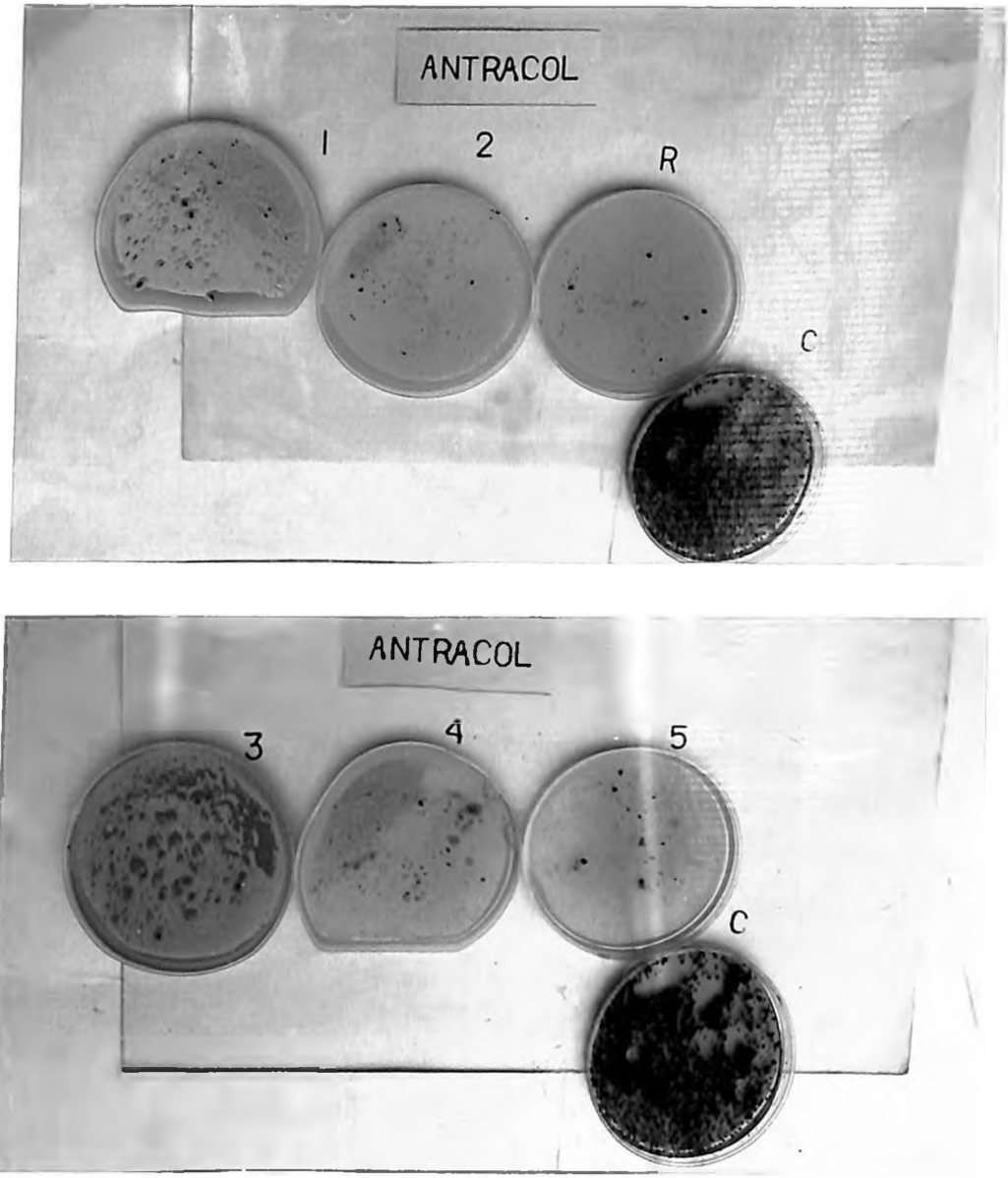


Plate 11b. *Phaeoramularia angolensis* in media ammended with Antracol
No growth at all dilutions.
1 = 10,000 ppm
2 = 5,000
R = 2,500 ppm (Recommended dosage)
3 = 3000
4 = 100
5 =10
C =No fungicide

Table 6: Effect of selected fungicides on growth of *P. angolensis* at temperature (21-24°C) after 14 days of incubation in liquid media.

Fungicide concentration (ppm)	Average dry mycelial weight (mg)* Growth			
	Kocide 101	Copper oxychloride	Antracol	Dithane
0	590±9(0)**	590±9(0)	590±9(0)	590±9(0)
0.1	549±10(7)	589±15(0.2)	342±14(42)	230±15(
0.5	531±15(10)	555±16(6)	254±4(57)	165±6(7
1	507±16(14)	537±11(9)	224±9(62)	165±6(7
1.5	466±14(21)	496±15(16)	206±8(65)	65±4(8
10	460±17(21)	492±13(17)	23±11(96)	0 (10
100	430±12(27)	443±14(25)	0 (100)	
1000	384±10(35)	389±11(34)		
2000	372±8(37)	380±10(36)		
3000	442±9(42)	366±6(28)		
4000	159±5(73)	177±12(70)		
5000	0 (100)	0 (100)		

* Each data is an average of five replicates.
** Numbers in brackets are percentages of inhibited growth

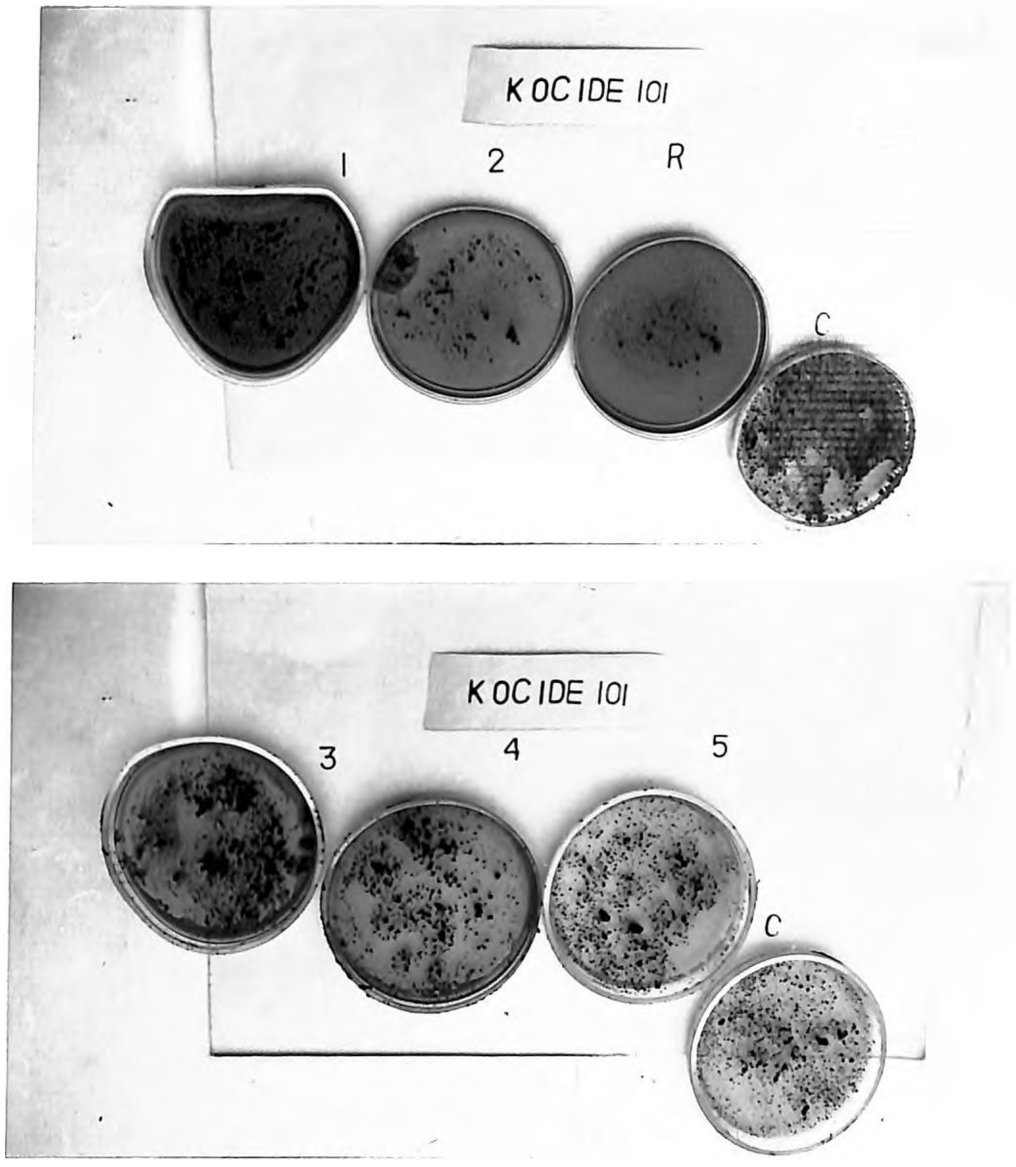


Plate 11c. *Phaeoramularia angolensis* in media ammended with Kocide 101.

1 = 10000 ppm
R = 7,500 ppm (Recommended dosage)
2 = 6500 ppm
3 = 5000 ppm
4 = 3000 ppm
5 = 10 ppm

the control. There was slight growth at 6,000 ppm and at the recommended dose 6,900 ppm, but above this, no growth occured although subcultures on PDA developed into colonies (Plate 11d). In extra experiments carried out with a mixture of white oil and Diazinone insecticide, no growth was observed. When Copper fungicides were added to the white oil mixture, mycelial growth was observed at all concentrations (Plates 11 e-f). In liquid media tests, Copper Oxychloride showed that mycelial inhibition increased with increasing fungicide concentration upto 5,000 ppm where there was total inhibition. No sporulation was observed in any fungicide cultures. The Dose-response curves (fig.6) gave the Lethal Dose (LD_{50}) values as follows: Dithane M45 0.03 ppm, Antracol 0.25 ppm, Kocide 101 3200 ppm and Copper Oxychloride 3600 ppm (Appendix IX). The difference in growth inhibition between the four fungicides was highly significant (F = 53, $P<0.05$). The difference between Kocide 101 and Copper Oxychloride was not significant at all concentrations. The difference between Antracol and Dithane M45 was also not significant. No sporulation was observed in liquid cultures. In all fungicide dilutions, no conidium germinated. Spores incubated in the test dilutions appeared plasmolysed with the cytoplasm shrank away from cell walls particularly those in Dithane M45.

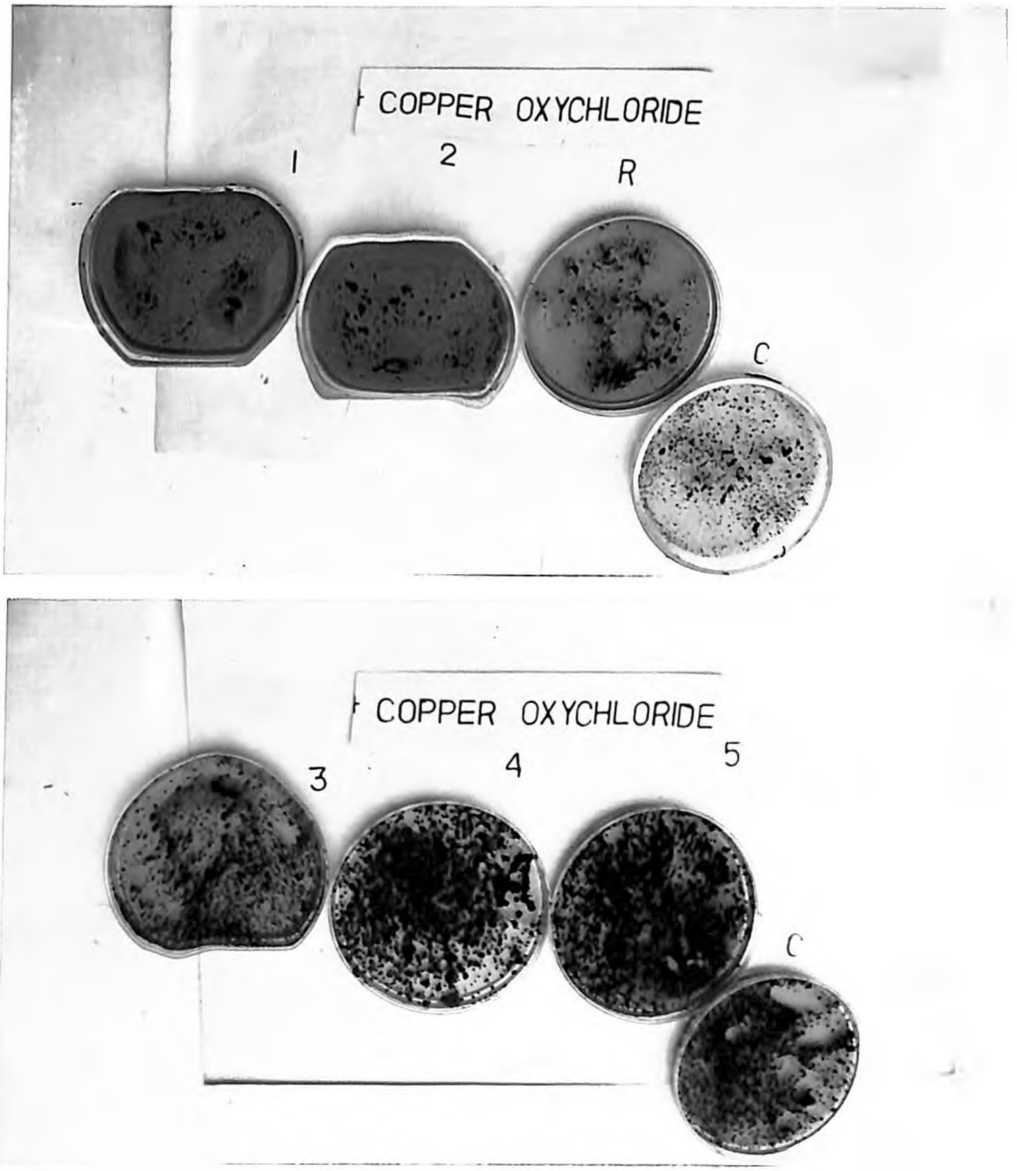


Plate 11d *Phaeoramularia angolensis* in media ammended with Copper Oxychloride

1 = 10000 ppm
R = 6,900 ppm (Reccomended dosage)
2 = 6000 ppm
3 = 5000 ppm
4 = 3000 ppm
5 = 10 ppm

PLate 11e *Phaeoramularia angolensis* in media ammended with Diazinon + white oil. No growth at all dilutions.

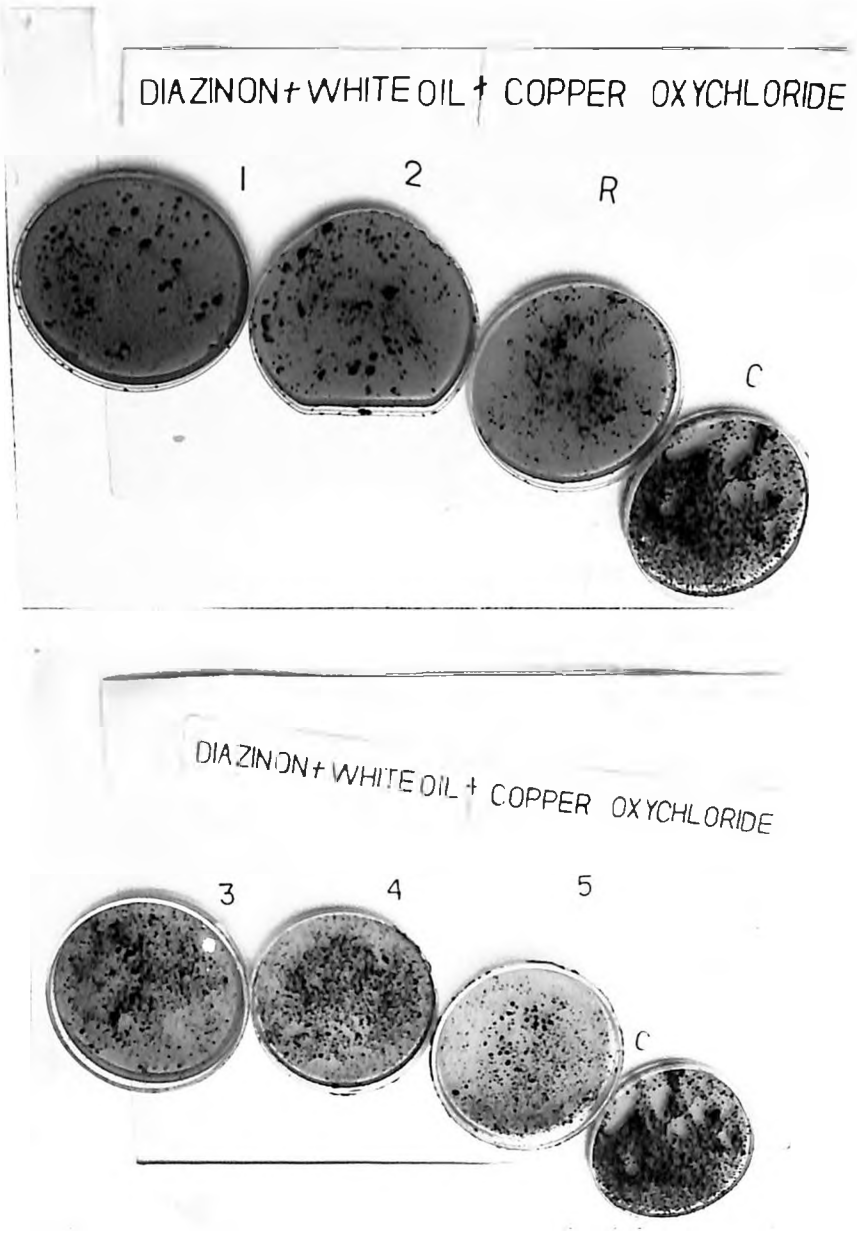


Plate 11f *Phaeoramularia angolensis* in media ammended with Diazinon + white oil + copper oxychloride. Visible growth on all dilutions

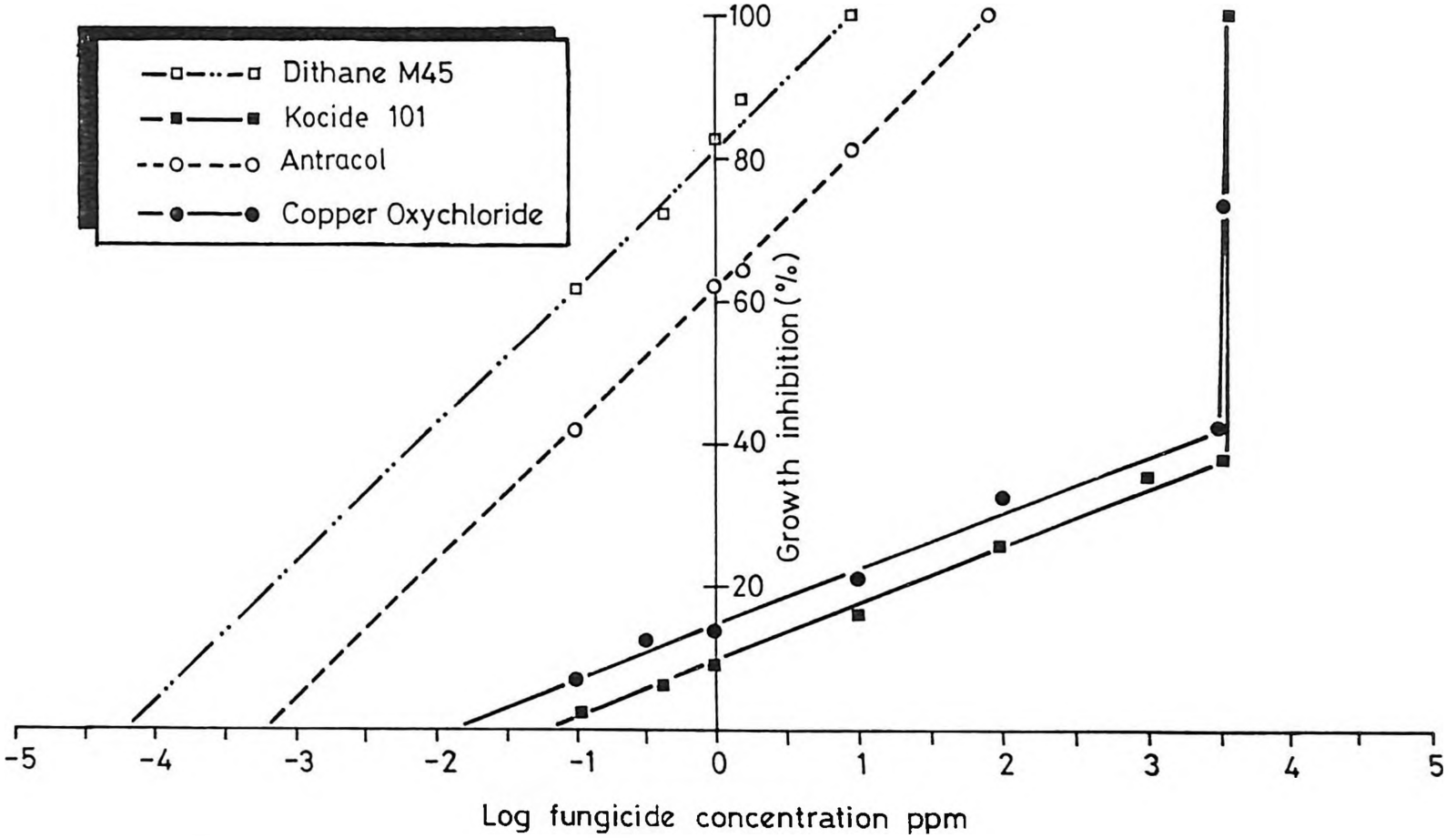


Fig. 6: Log-dose response curves

CHAPTER 4

DISCUSSION AND CONCLUSION

The results presented in this study have shown that the leaf and fruit spot disease of *Citrus* in Kenya is caused by *P. angolensis*. The symptoms and the fungus morphological characters on the artificially infected plants were similar to those from naturally infected plants in the field. The symptomatology and the characteristics of the fungus isolated from Kenyan *Citrus* also agree with the description of the disease attributed to an unknown species of *Phaeoisariopsis* by Emechebe (1980) and to *P. angolensis* Kirk (1986). Therefore , *P. angolensis* described by Kirk (1986), the an unknown species of *Phaeoisariopsis* described by Emechebe (1980), and the causal agent of the leaf and fruit spot disease of *Citrus* in Kenya being described in this study is the same fungus.

P. angolensis. is being reported in Kenya for the first time.This fungus has already been reported in Zambia, Zimbabwe, Angola, Cameroon, Gabon and Uganda. The spartial distribution of the disease in Kenya strongly suggests that the origin of the disease in this country could be Uganda.

There seem to be some taxonomic ambiquity between the genus *Phaeoramularia* and allied genera. *P. angolensis* was originally named *Cercospora angolensis* by

De Carvalho & O. Mendes (Kirk 1986). Emechebe (1980) referred to the same fungus as *Phaeoisariopsis sp.* Kirk (1986) transferred *C. angolensis* to the genus *Phaeoramularia* and named it *P. angolensis*. On the basis of secondary metabolites, Siboe (1989) suggested that *P. angolensis* could be a species in the genus *Cercospora*, somehow agreeing with the name given by De Carvolhos & Mendes.

Comparative measurements of conidial lengths of *P. griseola* as a representative of *Phaeoisariopsis* and *Phaeoramularia angolensis* gave the range of 30-79.9 µm and 10-79.9 µm respectively (Appendix V).This suggests that their conidial lengths overlap. Due to this overlap, conidial length would not be a reliable character in delimiting the form genera *Phaeoramularia* and *Phaeoisariopsis*.

Their colonies on media were different. Colonies of *P. griseola* were cottony, immersed, greyish white sporulating heavily both on solid and liquid media. Colonies of *P. angolensis* were velvety, immersed, dark brown with no sporulation in mature colonies and liquid media. This suggests that the two fungi were different and probably belong to different genera.

Emechebe (1980) argued that *C. angolensis* sporulates on the lower leaf surface only, while his fungus (*Phaeoisariopsis*) sporulated from both surfaces.

Emechebe used this as a distinguishing feature between his fungus and *C. angolensis* which is now known as *P. angolensis* . Findings from this study and from literature (Kirk 1986) show that *P. angolensis* also sporulates from both sides of the lesions though more from the abaxial side. Kirk (1986) used catenulation to transfer this fungus from *Cercospora* to the genus *Phaeoramularia*, and named it *P. angolensis*. Findings from this study were that both conidia of *P.angolensis* and *P.griseola* were occasionally catenulate.

Ferraris (1983) distinquished between the genera *Phaeoramularia* and *Phaeoisariopsis* using insertion of asexual structures. He described the genus *Phaeoisariopsis* as having substomatal stroma and *Phaeoramularia* as having erumpent stroma. Results from this study show that the stroma of both *P.angolensis* and *Phaeosariopsis* are erumpent. Sobers (1968) observed that spore size, septation and even catenulation can be influenced by the environment and hence vary enormously within a genus. Talbot (1971) concluded that morphological characters (viz: conidial length, colour, septation and position of conidia on conidiophores, conidiophore branching and colour) have become the final criteria in delimiting form genera of the Deuteromycetes. Nevertheless, morphological characters do not seem to give clearcut

taxonomic distinction between the genera *Phaeoramularia* and *Phaeoisariopsis*. From the literature (Appendix V), it was not possible to find any distinct morphological differences between the two genera. However, taxonomic data obtained in this study, to some extent, justify the placement of the pathogen in the genus *Phaeoramularia* on the basis of catenulations and growth characteristics. The fungus described by Emechebe also belongs to this genus. Studies using taxonomic criteria other than morphology, are required to differentiate the two genera from each other.

The fungus isolated from lesions of artificially inoculated plants corresponded in all observable characters with the original pathogen from naturally infected *Citrus* plants. This fulfilled Koch's postulate for pathogenicity which stipulates that for a proof of a pathogen, the disease agent should be isolated, grown in culture, be reinfected on host and reisolated from it. Pathogenicity tests characterised by long incubation period, followed by slow disease development, resulting into low infectivity of the test plants may have arisen from a combination of factors. First, cultures used to prepare the inoculum had sparse sporulation hence the spore concentration in the inoculum was low. Wallis (1971) observed that an inoculum of low spore concentration favoured longer incubation periods but did

give a reason for his observation. Probably, the more concentrated the inoculum, the faster the pathogen breaks through the hosts resistance mechanism.

From field and cultural tests, it was estimated that optimum tempratures for fungal growth and spore germination were about 25-26^{o}C (Table 3, and Appendix VI). During the pathogenicity tests, air tempratures around the plants varied between 14-22^{o}C, occasionally falling to 11^{o}C and rising to 25^{o}C (Appendix IIb). Such low temperatures, probably inactivated the fungus enzyme system reducing both the germinability of the conidia and rate of mycelial development. This would also favour long incubation periods. Such long incubation periods would also give the host plant, enough time to organise its defense mechanisms which would arrest much of the disease from developing further, leading to low infectivity and restricted lesion development. This probably explains why most lesions on the artificially infected leaves were small in size and partially developed, stopping at the dot-like spot stage.

Sporulation data revealed that cultures that were more than three weeks old lacked spores, and none were observed in cultures maintained in liquid media. Inoculum prepared from these cultures did not cause disease in artificially inoculated plants. This suggests that, probably this pathogen cannot cause

infection by mycelium alone. Emechebe (1980) also observed similar results, that non-sporulating cultures on PDA did not cause disease in inoculated plants, but did not give a reason for his observation. Probably, mycelium lacks enough energy and enzymes and when deposited on the host surface is incapable of penetrating the host to cause disease.

Observations made on all the eleven farms indicate clearly that the *Citrus* leaf and fruit spot disease has reached severe epidemic levels. The disease control failure could be attributed first, to 90% of the farmers who sprayed only when they noticed severe disease symptoms. This is bad timing. Secondly, severe epidemic conditions exacerbated by the high amounts of inoculum levels from hosts and litter,(39.4×10^8 spores/leaf litter of a single tree) and heavy rains (Appendix IIa). Thirdly, there is increasing awareness among the farmers of the negative side effects of the fungicides being used. Kocide 101 was more popular among the farmers because it was cheaper and more familiar than most of the other fungicides. However, due to suspected phytotoxic effects on *Citrus* fruits, farmers are apprehensive of using the fungicides. As a result, 50% of the farmers were spraying very low dilutions of the fungicides while some were only spraying occasionally. This definetely leads to poor disease control.

Spore counts suggest that the primary source of inoculum of this fungus are the spores, produced in abundance on infected material intact on the plants or in litter and most probably disseminated by wind and water splashing. Litter from a single tree alone was found to produce approximately 39.4×10^8 spores. The severe infection and the resultant epidemic conditions must be an outcome of this high inocculum levels and favourable enviromental conditions that include high rainfall and high optimal temperatures of $25-26^{o}c$ (Appendix IIa).

Evidence accrued from field observations of the disease pattern strongly suggests that the disease epidemics occured in the short rainy season towards the end of the year, particularly from late August. This is because the new flushes of leaves formed during the dry season were disease free just before the rains started but probably with latent infection. Scattered, small non-sporulating lesions were only seen on a few old leaves and fruits. However, three to four weeks after the onset of rains, disease symptoms started to appear on the new leaves. Lesions on the old leaves enlarged and sporulated two to three weeks later. This suggested that infection on the young leaves must have come from the previous seasons lesions. This also suggests that probably the spores of this fungus are able to survive across seasons.

the previous seasons lesions. This also suggests that probably the spores of this fungus are able to survive across seasons.

Comparative studies on growth of the fungus on solid and liquid media indicate that liquid media supported faster growth (Appendix VIII). The fungus showed restricted growth on solid media (plate 5). The growth was more vertical than horizontal on the solid media and appeared to be utilising agar as well. Diffusion of nutrients in solid medium is very slow (Cochrane, 1958). In vertical growth, the fungus was only utilising nutrients from a limited area of the medium. The slow rate of nutrient diffusion would supply essential elements to the colony at a rate too low to support faster growth. If it also utilised agar as a source of its carbon, agar being a complex carbohydrate, the fungus would take longer time breaking it down into utilizable compounds, therefore, could not enhance the growth rate of the fungus. In liquid medium, growth is distributed over a larger surface area and nutrient diffusion is faster hence the fungus is able to grow faster than on solid media.

In both solid and liquid media optimum growth was supported by media with the Orange leaf extract. This being the natural host material, such a medium probably provided the nutritional requirements in

appropriate proportions and form for the fungus. Good growth was also obtained from Potato dextrose medium, whereas reduced growth occurred on corn meal and potato carrot media . Good growth in PDA could be attributed to dextrose which was not in PCA and CMA. Dextrose is chemically a more ready source of energy than the more complex carbohydrates in corn meal and Potato carrot. Potato carrot is generally known to be a poor medium hence it supported minimal growth. Lack of dextrose was also observed to be a limiting factor in sporulation. All the five solid media with dextrose supported sparse sporulation in the first two weeks. When dextrose was added to TWA, PCA and CMA, sparse sporulation occurred as in Orange leaf extract agar and Potato dextrose agar. Probably, dextrose provided the essential energy needed for spore production.

In all cases, spores were only produced at the edges of young cultures of up to two weeks old. Older cultures seemed not to sporulate. Similar observations, without explanation, were made by Kilpatrick and Howard (1956) on some species of *Cercospora*. Generally, fungi are known to produce conidia when the colonies are old and autolyzing. This is contrary to what was observed for *P. angolensis*.

Results on pH tests (Fig.5) indicated that the fungus grew over a wide initial pH range of 3 to 8,

but optimum growth was between pH 5 and 6. Cochrane (1958) also observed that most plant pathogens grew best in media with an initial pH of 5.0 to 6.5. The fungus was able to raise initial acidic pH and lower basic pH. Prithwi (1972) working with some species of *Cercospora* had similar results. During growth, the pH of the medium is affected by the metabolic activities of the fungus. Absorption of anions could raise it, whereas absorption of cations or formation of organic acids could lower it. The pH of the *Citrus* leaf extract was 5.6. This probably explains the *in vitro* preference of the fungus to pH 5-6.

Optimum temperatures for growth of the fungus was observed to be 25-26^{o}C (Fig. 3). This suggests that this fungus is a mesophile. Field studies also revealed that maximum temperatures in Kitale region are in the range 23-28^{o}C (Appendix IIa). This is probably one of the factors that explains why the disease incidence is high in this region, and not found in other *Citrus* growing areas like Coast province or Central province, where temperatures are generally higher or lower respectively, than the observed optimum temperature for the growth of *P. angolensis*. Such optimal temperatures coupled with plenty of rains would enhance severe epidemics in the field. As observed from rainfall statistics (Appendix IIa) , rainfall in Kitale is fairly well distributed with the dry seasons still

receiving some rains.

Barnnet (1950) suggested that the influence of light on fungal growth may be due to the total energy received by the fungus. It was suggested that carbondioxide fixation is increased in light, thus favouring more growth, hence continuous white light seemed to support more growth than other light regimes. Average monthly weather statistics for the year 1990-1991 indicate that Kitale had long periods (over 7 hours on the average) of sun light throughout the year.

Tests with fungicides showed that all the four fungicides inhibited spore germination at all dilutions 0.1 ppm - 10000 ppm (Table 5 and 6). This is in contrast to the situation in the field where the fungus continued to proliferate despite sprays with some fungicides like Kocide 101. Under field conditions, this fungus was observed to produce abundant conidia. Materials brought to the laboratory easily lost conidia by drop off. Conidia dropping off from lesions in the field probably remain suspended in the air supported by air currents. Some conidia get washed down into the litter by rain or are blown to other vegetation in the vicinity. Leaves and fruits dropping off the tree with conidia also increase the inoculum in the litter. Fungicides normally are sprayed on defined trees. Lots of conidia in the air, litter and on other vegetation

escape the sprays. Through wind and water splashing, these conidia serve as inoculum whenever conditions are favourable for infection. Careful spraying to cover the whole plant and other vegetation close by is necessary but expensive therefore not practical. Good sanitation involving burning or burying litter and clearing of all unnecessary vegetation would also improve disease control.

McClellan (1942) observed that toxic materials are least effective at the optimum temperature of the organism concerned and that both above and below this optimum temperature, these materials are more toxic. Kaars (1982) supported this, when he observed that under conditions of high disease pressure like in severe epidemics, most protective fungicides fail to give satisfactory disease control. Optimum growth conditions for this fungus were found to be 25-26^{o}C, with adequate rain, and light. These conditions are optimum in Kitale almost all the year round. This could be another reason to be advanced for the difficulties being experienced in the control of the disease.

During the survey, all *Citrus* species on the orchards were found to be equally susceptible to the disease. According to Bollen (1982) such susceptibility of all species would also enhance severe epidemics. Some farmers had neglected their orchards which were unpruned and getting overgrown by weeds and

only spraying them occasionally. The weeds could act as inoculum spore traps . Lewis (1980) observed that poorly nourished plants have reduced resistance and are more susceptible. He also observed that unpruned trees are more difficult to spray and lead to survival of pathogens in parts where fungicides do not reach. He suggested that there is positive correlation between increase in number of leaves on terminal shoot and increase in disease on the shoots, and that the amount of fungicide spray coverage and deposit decreases with the height of the trees, also dependent upon the spraying equipment. All these factors could be contributing to severe infections in Kitale region. Another limiting factor to the disease control could be incorrect use of fungicides. Farmers complained of the rains washing off the fungicides from the plants. Severe epidemics occur during the rains, when wash off is greatest. On the other hand, farmers who feared phytotoxic effects of Copper fungicides on *Citrus* trees, or found the fungicides to be costly were applying fungicides at lower dilutions than recommended dose rates. Such dilutions would reduce fungicide tenacity.

Dan Neely (1970,1971) observed that in order to protect foliage from pathogenic fungi, fungicides must first be deposited on the leaves surfaces, resist erosion by weathering agents and retain their fungitoxic character through various physical, biological and

chemical reactions. The amount of material remaining on the plant surface to produce protection against plant pathogens depends on the amount of material which sticks to the leaf during spraying and the amount of residue which remain after weathering. This is influenced by the type of foliage to which it is applied. Smooth foliage like those of *Citrus* loose deposits more quickly hence they need to be sprayed more often . Frequent spraying of the *Citrus* is not a common practice to most farmers in Kitale region. Physically, the amount of residual fungicides may be reduced by wind and rain or even undergo photolysis in periods of hot sunny weather. All these weather conditions prevail in Kitale, hence weathering could be a limiting factor in disease control. Farmers should be encouraged to spray at the right time with correct fungicide concentrations to ensure good tenacity. A fungicide formulation with preferably a sticker and spreader to increase foliage retention of fungicide could also be useful.

Dan Neely (1971) observed that if protectants like Copper fungicides are being used, spray timing becomes a critical factor in the management of disease to affect its further development. Most farmers seemed to spray only when the epidemics were observed. This was then too late since protective fungicides were being used. Treatment must begin at bud break or birth of new susceptible individuals. The amount of fungicides

needed for disease control and the number of days between the applications should be timed according to the development, severity of the disease and weather conditions.

Results from this study indicate that effective fungicide doses differed among the tested fungicides. Higher concentrations of Copper fungicides were required to inhibit mycelial growth, as compared to Dithane M45 and Antracol. Mycelial fragments that failed to grow in Copper fungicides developed into colonies when subcultured in potato dextrose agar. This suggested that even at high concentrations, these copper fungicides were only fungistatic. Those fragments subcultured from Dithane M45 and Antracol failed to develop into colonies on PDA. Hence Dithane M45 and Antracol could be fungicidal at concentrations above 10ppm.

Results also indicate that some fungicides like Copper Oxychloride could promote fungal growth instead of being fungitoxic, if applied in doses much below its LD_{50}. Cultures maintained on medium with 10 ppm of fungicide had better growth than the control. Generally *in vitro* growth of the fungus on these levels of Copper fungicides (less than 10 ppm), strongly suggests a potential of the fungus becoming resistant. This calls for close monitoring in the field. However,

this is not conclusive and therefore farmers should be strongly advised to use fungicides in their correct recommended doses. Kocide 101, a Copper fungicide, was found to be popular among the farmers. Farmers contend that it is cheaper and familiar to them. Before the introduction of systemic fungicides, Copper fungicides predominated the fungicide world. If used in correct concentrations, they are known to redistribute well and resist weathering (Barnes, 1976). They reduce inoculum potential by reducing conidial production, their germinability and rate of germ tube elongation (Torgeson, 1969). They are fungistatic through a non-specific denaturation of proteins and enzymes. The fungal spores accumulate free ionic Copper which denature or precipitate the proteins inactivating the enzymes particularly those requiring free sulphlihydryl groups (Buckel, 1983). Though results from cultures show that very low dilutions 0.1 ppm were effective against spore germination, higher Copper concentrations are needed for spore toxicity in the field during heavy rainfalls. However, farmers fear copper fungicide phytotoxic effects on the trees. As a result they may not be complying with this requirement. Chlorosis of brown to purple flecks on the leaves and fruits are the reported Copper phytotoxicity in plants.

Dubin (1974) reported that Copper required a pH 8.5-10 for rapid uptake and that there is reduced uptake

at pH 6.5-7.5. The pH of *Citrus* extract was found to be 5.6. This probably would reduce the uptake of fungicides by the conidia leading to reduced fungicide effectiveness in the field.

From field studies it was learnt that to avoid high input costs, farmers applied fungicides in mixture combinations with oil sprays and insecticides. Although such mixtures are recommended to reduce the risks of resistance development, Togerson (1969) observed that oil reduces Copper redistribution and its toxicity to conidia. It flocculates the Copper into larger less toxic particles. Fisher (1961), however, reported that oil sprays affected stomatal behaviour of *Citrus* affecting its transpiration rate. The fungi thus have difficulty in penetrating the stomata and find the physiology of the plant so altered as to provide an environment unsuitable for their optimum growth. *In vitro*, mycelial growth was inhibited on media with oil alone, but media containing both oil and Copper Oxychloride, supported mycelial growth. The explanation to this is that, the oil reduced the Copper redistribution and toxicity. Therefore farmers should be discouraged from making fungicide mixtures with insecticides that require white oil. Generally, farmers should be advised to make pesticide mixtures recommended by experts only.

Difolatan M.C. inhibited both spore germination and

mycelial growth (Fig 6). Its low LD_{50} of 0.03 ppm indicates that very low doses of the fungicide would be necessary for disease control. Dithane M45 is a fungicide combination of Maneb and Zinc ion with reported good stability, retention and activity. It inhibits energy production by reacting with co-enzyme lipoic acid dehydrogenase. It forms complexes with metal containing enzymes thus inhibiting a large range of enzymes (Lyr & Horstyl, 1987). This explains the high effectiveness of the fungicide. Stefan (1986) reported recurrent effects among leaves treated with mancozeb due to altered intra and interspecific competitions. This can be avoided by applying enough initial doses and then alternating its use with Copper fungicides.

Results show that Antracol is as effective as Dithane M45 (Fig. 6). It had a low LD_{50}, of 0.25 ppm, and inhibited both mycelial growth and spore germination. Antracol is a dithiocarbamate fungicide introduced in 1966 (Martin, 1969) with specific site inhibition at succinic dehydrogenase. Beever (1990) reported that Antracol inhibits mycelial growth more than spore germination. Lyr & Horstyl (1987) observed that Antracol is effective as a protectant, but can also penetrate the host to inhibit post infection or move to untreated portions of the plant for preventive or curative effects. It is reported to have good residual

activit, and its effects on non-target organisms are said to be minimal due to its selective toxicity. It is said to have mild cytokinin effects on a few plants and delay maturity, though yields are increased. Resistance, however, can occur since it is a specific site inhibitor. To delay or prevent development of this resistance, its use can be alternated or combined in mixture with multisite inhibitors such as Dithane M45 or the copper fungicides . Antracol is cheap and can be a substitute for the expensive systemic fungicides on the market.

CONCLUSION

Results from this study indicate that the spore inoculum is available throughout the year. The fruits and leaves of all *Citrus* species at all ages are susceptible to infection by this fungus. The reduction in dependence on fungicides for control of this disease is therefore limited. We can only try to reduce costs by good prediction, careful choice and use of fungicides, and, improved cultural practices e.g. pruning, and destruction of the pathogen through removal and burning of infected material.

Data obtained in this study do not adequately support the hypothesis that the pathogen has developed resistance to the conventional fungicides. Be that as it may, there is an indication that improper use of Copper fungicides could result in disease control failure and consequent resistance. The current poor control of disease could be attributed mainly to:

1. Very high susceptibility of all *Citrus* species to the disease in this region resulting in severe epidemic conditions.
2. Optimal enviromental requirements for growth and reproduction of *P. angolensis*.
3. Poor fungicide applications (viz dose, timing, pesticide combinations etc.) by farmers. This could be improved by good prediction, careful choice and use of fungicides.

4. Poor cultural practices e.g. pruning, abandoned trees, general field sanitation etc. Farmers must be advised to prune, and destroy the pathogen through the removal and burning of infected material.
5. Lack of early diagnosis and precise identity of the pathogen that resulted into epidemic outbreaks.

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Appendix I

Data Sheet

Date --------

Farm No. ..... Name.....

Type ..... (mixed farm etc.)

Citrus acreage or No. of trees .....

Questionnaire

1. When did you notice the infection ?
2. What fungicides do you spray ?
3. How often do you spray ?
4. Why did you choose these fungicides ?
5. What method do you use for spraying ?
6. What crops are affected by this disease ?
7. How much control are you having ?
8. How much fruit do you sell in a year ?
9. What do you plan to do in future ?

Appendix IIa

Average monthly weather statistics

Kitale Oct 1990 - Dec 1991

Month	Rainfall (mm)	Temp ^{o}C		Sunshine hours
		max	min	
Oct	105.9	26.3	11.3	7.9
Nov	73.3	26.2	11.7	7.8
Dec	42.1	26.7	11.8	8.3
Jan	18.7	28.1	10.7	8.4
Feb	47.5	29.1	11.6	7.4
Mar	85.1	27.8	12.3	6.8
April	144.2	23.3	13.7	6.6
May	161.0	25.1	13.1	7.0
June	120.5	22.9	12.9	7.4
July	138	23.5	11.4	6.1
Aug	164	24.7	10.3	6.1
Sept	104	25.1	11.2	7.1
Oct	102	26.4	11.3	7.6
Nov	82.1	26.3	11.7	7.7
Dec	45	26.8	11.9	8.8

Source: Meteorological Department Monthly Reports.

Appendix IIb: Monthly rainfall (mm) and temperature (°C) for Chiromo area of Nairobi (1991).

Month	Temperature (°C) Maximum	Minimum	Rainfall (mm)
January	23.7	12.9	92.9
February	25.7	14.2	139.4
March	24.2	14.6	63.5
April	23.4	14.9	7.8
May	23.6	14.1	7.8
June	22.2	11.5	15.7
July	22.1	13.6	9.7
August	21.2	11.6	163
September	24.6	11.4	247.5
October	25.3	13.8	215.8
November	22.8	14.1	26.8
December	23.1	13.7	52.7

Source: Meteorological department monthly reports

Appendix III

Formulae for media

Tap water Agar (TWA)

(a) Tapwater 1 litre

(b) Agar 15 gms

The tap water and agar were autoclaved for twenty minutes at $121^{o}C$

Potato Dextrose Agar (PDA).

(a)	Potatoes	200 gms
(b)	Dextrose	20 gms
(c)	Agar	15 gms
(d)	Distilled water	1 litre

The potatoes were cleaned with tap water, thinly peeled and cut into thin slices. The slices were rinsed with distilled water and 200g put into a pyrex beaker containing 1 litre of distilled water and boiled until soft. The juice was then filtered through a double layer of muslin cloth. 20g of dextrose and 15g of agar were added and heated till dissolved. The mixture was made up to 1 litre with distilled water and autoclaved for twenty minutes at $121^{o}C$.

Corn Meal Agar (CMA)

(a)	Maize flour	20 gms
(b)	Agar	15 gms
(c)	Distilled water	1 litre

The flour and water were placed in a saucepan and then heated till boiling and stirred continuously for thirty minutes. The juice was filtered through a double layer muslin cloth.

The agar and dextrose were added and heated to dissolve. The mixture was made up to one litre with distilled water and then autoclaved for twenty minutes at 121^{O}C.

Potato Carrot Agar (PCA)

(a)	Potatoes	20 gms
(b)	Carrots	2 gms
(c)	Agar	15 gms
(d)	Distilled water	1 litre

Peeled and washed potatoes and carrots were boiled until soft. The juice was filtered through a double layer muslin cloth. The agar was added and heated to dissolve. The mixture was made up to one litre with distilled water and then autoclaved for twenty minutes at 121^{O}C.

Orange leaf extract agar (OLEA)

(a)	Orange leaves	150 gms
(b)	Dextrose	20 gms
(c)	Agar	20 gms
(d)	Distilled water	1 litre

Fresh sweet orange leaves were weighed, washed with distilled water and then boiled in 1 litre of distilled water for 30 minutes (Emechebe 1980). The decoction was filtered through double layer muslin cloth, the agar and dextrose added and heated to dissolve. The mixture was made up to 1 litre with distilled water and then autoclaved for twenty minutes at 121^{O}C.

Appendix IV Lesion count and estimation of infected leaf surface area.

Leaf area (cm^2)		No. of lesions		% infected surface	
		lesion			
1S	2S	1S	2S	1S	2S
25	4	54	5	99.2	0.2
15	25	11	10	15.1	.7
22	40	23	4	82.2	.1
29	42	89	1	98.2	.1
48	32	62	2	92	.3
40	15	40	3	91.8	.1
43	27	40	1	99.9	.1
45	35	34	11	98.1	.6
46	54	16	14	7.6	.2
60	21	39	7	85.8	.1
19	35	18	6	72.9	.2
33	31	20	13	88.5	.1
22	24	23	1	82.2	.1
28	28	55	7	83.6	.2
36	26	25	4	47.8	.1
26	24	29	3	98.1	.2
34	24	29	9	64.9	.9
28	21	89	1	57.7	.1
22	19	19	4	99.9	.2

Appendix IV Lesion count and estimation of infected leaf surface area.

Leaf area (cm^2)		No. of lesions/lesion		% infected surface	
1S	2S	1S	2S	1S	2S
25	4	54	5	99.2	0.2
15	25	11	10	15.1	.7
22	40	23	4	82.2	.1
29	42	89	1	98.2	.1
48	32	62	2	[illegible]	.3
40	15	40	3	91.8	.1
43	27	40	1	99.9	.1
45	35	34	11	98.1	.6
46	54	16	14	7.6	.2
60	21	39	7	85.8	.1
19	25	18	6	72.9	.2
33	31	20	13	88.5	.1
22	24	23	1	82.2	.1
28	28	55	7	83.6	.2
36	26	25	4	47.8	.1
26	24	29	3	98.1	.2
34	24	29	9	64.9	.9
28	21	89	1	57.7	.1
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43	27	40	1	99.9	.1
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33	31	20	13	88.5	.1
22	24	23	1	82.2	.1
28	28	55	7	83.6	.2
36	26	25	4	47.8	.1
26	24	29	3	98.1	.2
34	24	29	9	64.9	.9
28	21	89	1	57.7	.1
22	19	19	4	99.9	.2

Key to appendix IV

1s = first sampling
2s = second sampling

	1s	2s
Average No. of lesions/leaf =	47	8
Maximum No. of lesions =	89	14

Averages were obtained from 50 leaves

Appendix V

Comparison of conidial lengths of *Phaeoramularia angolensis* and *Phaeoisariopsis griseola*

Conidial length (um)	Relative Frequency	
	P. angolensis	*P. griseola*
0-9.9	0	0
10-19.9	16	0
20-29.9	22	0
30-39.9	14	16.7
40-49.9	18	23.3
50-59.9	22	40
60-69.7	4	16.7
70-79.9	4	3.3

Appendix VIa

Conidial germination at different temperatures and pH levels.

Temp. °C	Total spore count*	No. germinated*
2	12	0(0)
3-5	10	0
6	16	0(0)
6-8	17	0(0)
15	14	0(0)
20	16	9(56)
21-24* *	16	14(93)
25	16	15(94)
26	15	14(93)
30	16	0(0)

* each figure is an average of five .
* * room temperature

Appendix VIb

pH	Total spore count*	No. germinated*
2	15	5(33)**
3	17	8(47)
4	14	13(93)
5	13	10(78)
5.6	14	10(71)
6	16	11(69)
7	15	10(67)
8	13	8(62)
Distilled (5.35) water	14	12(86)

* each figure is an average of five.

** Numbers in brackets are percentages of germinated spores.

Appendix VII: Effect of media growth and sporulation of *P. angolensis* at room temperature (21-24°C).

Dry mycelial weight (mg)

Reps	Orange leaf extract		Potato dextrose		Corn meal		Potato carrot	
	Solid	Liquid	Solid	Liquid	Solid	Liquid	Solid	Liquid
1	580	771	501	721	492	622	471	441
2	562	756	544	734	491	641	472	412
3	591	761	532	736	488	652	450	444
4	593	791	501	691	476	593	471	423
5	582	734	510	702	491	616	474	424
Mean	582±11	763±19	518±17	717±18	488±6	624±21	468±9	429±13

Incubation period for solid media = 3 weeks

Incubation period for liquid media = 2 weeks

pH change of liquid media:

	Initial	Filtrate
Orange leaf extract	5.4	6.4
Potato dextrose	5.9	6.2
Potato carrot	6.1	6.15
Cornmeal	6.1	6.2

Appendix VIII: Effect of different light conditions on growth and sporulation of *P. angolensis* after 14 days incubation at room temperature (21-24°C).

Dry mycelial weight (mg).

Reps	24 hours white light	24 hours darkness	24 hours red light	24 hours blue light	12 hours light/ 12 hours darkness
1	612	541	599	561	582
2	645	552	576	550	578
3	606	534	584	562	584
4	599	571	563	550	583
5	634	553	568	581	560
Mean	619±17	550±13	578±13	561±11	577±9
Sporulation	Nil	Nil	Nil	Nil	Nil

Appendix (X) Character Score of some fungi

Character	Fungi				
	A	B	C	D	E
I COLONY					
Effuse	+	o	+	*	*
Velvety	+	+	+	*	*
Immersed	+	+	+	*	*
Olivaceous brown	+	+	+	*	*
II Stroma					
Erumpent	*	+	+	+	*
Substomatal	*	+	o	+	+
III Setae and hyphopodia					
Present	o	o	o	*	*
Absent	+	+	+	*	*
IV Conidiophores					
Macronematous	+	+	+	+	+
Mononematous	+	+	+	+	+
Caespitose	+	+	+	+	+
Unbranched	+	+	+	+	+
Loosely branched	+	o	+	o	*
Straight	+	+	+	+	+
Flexuous	+	+	+	+	+
Smooth	+	+	+	+	+
Olivaceous brown	+	+	+	+	+

		A	B	C	D	E
V	Conidiogeneous cells					
	Polyblastic	+	+	+	+	+
	Intergrated	+	+	+	+	+
	Terminal becoming intercalary	+	+	+	+	+
	Sympodial	+	+	+	+	+
	Cylindrical	+	+	+	+	+
	Cicatrized	+	+	+	+	+
VI	Ramoconidia					
	Present	+	o	*	*	*
	Absent	o	+	*	*	*
VII	Conidia					
	Dry	+	+	+	+	+
	Single	+	+	+	+	+
	Catenulate	+	+	o	+	+
	Acropleurogenous	+	+	+	+	+
	Hyaline	+	+	+	+	+
	Smooth	+	+	+	+	+
	Ellipsoidal	+	+	+	+	+
	Broadly fusiform	+	+	+	+	+
	Rounded ends	+	+	+	+	+
	Truncated ends	+	+	+	+	+
	Pigmented	+	+	+	+	+
	Septations	+	+	+	+	+

Key

+ = Present

o = Absent

* = Description not given in literature

A = *Phaeoramularia muntanola*

B = Kenyan isolate

C = *Phaeoisariopsis griseola*

D = Nigerian isolate

E = CMI descriptions of *P. angolensis*

Appendix IX log Table

ppm	log.
0.01	-2.0
0.025	-1.6 ($\bar{2}$.4) = LD_{50} Dithane M45
0.05	-1.3 ($\bar{2}$.7)
0.1	-1.0
0.2	-0.7 ($\bar{1}$.3)
0.25	-0.6 ($\bar{1}$.4) = LD_{50} Antracol
0.5	-0.3 ($\bar{1}$.7)
1	0
1.5	0.18
2	0.3
10	1.0
100	2.0
1000	3.0
2000	3.3
3000	3.48
3162	3.5 = LD_{50} Kocide 101
3548	3.55 = LD_{50} Copper Oxychloride
4000	3.60
5000	3.70