MAJOR LEAF SPOT DISEASES OF BARLEY AND THEIR CONTROL USING FUNGICIDES.

BY

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A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN BOTANY AT THE UNIVERSITY OF NAIROBI.

DECLARATION

I MARY WANJIRU WANYOIKE DECLARE THAT THIS THESIS IS MY ORIGINAL WORK AND HAS NOT BEEN PRESENTED FOR A DEGREE AT ANY OTHER UNIVERSITY.

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TABLE OF CONTENTS.

LIST OF FIGURES.

LIST OF PLATES

LIST OF TABLES

DEDICATION

This thesis is dedicated to my mother Veronicah Wanjiku and my father Michael Wanyoike who are celebrating their fiftieth birthday and also to my son IAN Ndungu and my husband James Njuguna for their patience, love and understanding.

ABSTRACT

TITLE: "STUDIES ON LEAF-SPOT DISEASES OF BARLEY & THEIR CONTROL USING FUNGICIDES"

Barley leaves exhibiting disease symptoms were collected from Kenya Breweries experimental plots in Mau Narok and Naivasha. Two barley foliar fungal pathogens, *Rhynchosporium secalis* Oud and *Bipolaris sorokinianum* Sorok were isolated and characterised. Comparisons of conidia obtained from pathogenecity tests showed that the morphology of the conidia produced on seedling leaves were similar to those used in the inoculum.

The sensitivity of *R. secalis* and *B. sorokinianum* to five fungicides namely Carbendazim, Propiconazole, Frutriafol, Triadimenal and Prochloraz were tested on fungicide amended media and slide germination tests. ED_{50} values (dose for 50 percent inhibition) were calculated from the dose response curves. *R. secalis* showed no growth on agar amended with Prochloraz at all the concentrations used, while Carbendazim and Propiconazole showed superior efficacy over Frutriafol and Triadimenal. ED_{50} for the four fungicides was as follows after a three week period: Carbendazim, 3.8 ppm; Propiconazole, 14.2 ppm; Frutriafol, 239.8 ppm and Triadimenal, 271.7 ppm.

With *B. sorokinianum* all the five fungicides

used were active in inhibiting mycelial growth. Their ED_{50} were as follows after ten days of incubation; less than 50 ppm for Carbendazim and Propiconazole; Frutriafol, 50 ppm; Triadimenal, 125 ppm and Prochloraz 3.125 ppm.

Of all the fungicides tested, none gave complete inhibition of conidial germination of *B. scrokinianum* although however they were active with their ED_{50} as follows: 103 ppm Carbendazim; 80.8 ppm Propiconazole; 125 ppm Frutriafol; 117.1 ppm Triadimenal and 62.4 Prochloraz. The ED 50 for germtube inhibition were; Carbendazim, Propiconazole and Prochloraz lower than 50 ppm; Frutriafol 109.23 ppm and Triadimenal 95.6 ppm. The fungicides inhibited germtube elongation much more than conidial germination.

1.0 CHAPTER ONE

INTRODUCTION

Barley is an important cereal crop in Kenya grown mainly for brewing beer and feeding livestock. It is grown mainly in the Rift valley region and the slopes of Mount Kenya.

The most common diseases affecting barley in Kenya include the leaf scald caused by *Rhynchosporium secalis*; net blotch caused by *Pyrenophora teres*; spot blotch by *Bipolaris sorokinianum*; leaf rust by *Puccinia hordei* and Barley yellow dwarf virus. The other minor diseases include yellow rust, smuts and ear die back (*Pyricularia grisea*) (Kenya Breweries Ltd., 1990)

The control of these diseases is achieved by use of resistant cultivars and chemicals. The use of resistant cultivars is limited because there are only four cultivars available in Kenya Ahadi, Tumaini, Proctor, and Bima. Tumaini and Proctor are susceptible to all of the above diseases. Ahadi is morderately resistant to leaf rust and scald. Bima is resistant to leaf rust but very susceptible to all other diseases. As such, the use of chemicals has been the only effective method available for the control of these diseases and has been employed for many years. Fungicides

like Cercobin (Thiophanate methyl) and Bayleton (Triadimenal) have been in use in Kenya for over ten years (Kenya Breweries ltd., 1980).

It is suspected that the causal organisms are becoming tolerant to these fungicides. The suspicion has arisen as a result of complaints from farmers to Kenya Breweries Limited that the fungus concerned seems to survive even after applying manufacturers' recommended dosage at the appropriate times.

Scald and spot blotch occur most frequently together and scald is by far the most economically important barley disease in Kenya (Kenya Breweries annual report, 1986). The economic importance of barley diseases notably scald and spot blotch may be appreciated from the fact that Kenya Breweries LTD. spent 7.24 million shillings in 1986/7 in fungicides alone to control barley diseases as compared to 3.4 million in 1985/6 (Kenya Breweries annual report, 1987).

2.0 CHAPTER TWO

LITERATURE REVIEW

2.1 *Rhynchosporium secalis* Oud.

2.1.1 Taxonomy And Nomenclature

The genus *Rhynchosporium* Heinsen belongs to the Form class Deuteromycetes order Moniliales and family Moniliaceae (Barnett and Hunter, 1872). The oldest preserved material of *Rhynchosporium secalis* (Oud) Davis was apparently collected in Romendal (Norway) on barley in 1880 by Hirsch (cited in Caldwell, 1937). Oudemans (1897) first described the scald organism in June 1897, having found it on rye (*Secale cereale*) in the Netherlands. He named it *Karsonia secalis*. Davis (1919) in the United States proposed the new combination *Rhynchosporium secalis* (Oud) Davis to overcome certain nomenclatural difficulties.

The fungus has repent sterile hyphae, erect fertile hyphae with curved branches, forked and bearing spores on denticles (Mathre, 1982; Caldwell, 1937). The fungus has determinate growth and in culture, diameter of the colony hardly exceeds 40mm (Caldwell, 1937; Schien, 1960). Conidia (12-20 x 2-4 µm) Mathre, 1982; (12.6-21.6 x 2.5-4 µm) (Caldwell, 1937); and (13.6-27.2 x 3-4.5 µm) (Kajiwara, 1968) borne on cells of fertile stroma

are short, cylindric to ovate, one septate, curved in profile, often beaked at the apex and terminated by a rather obtuse mucro at the lower end (Barnett and Hunter, 1972, Mathre, 1982 and Caldwell, 1937). The lower cell is usually narrower than the upper cell (Barnett and Hunter 1972; Caldwell, 1937).

2.1.2 Symptoms And Development Of Scald

Typically, single lesions measuring 1 x 0.5cm are formed but coalesce to form larger blotches in severe infections (Davis and Fitt, 1990). In the early stages of development, lesions are of dark-bluish gray coloration with a water soaked appearance (Fowler and Owen, 1971; Caldwell, 1937 and Shipton *et al*, 1974). Such areas are often 1-20mm in length before evident collapse of the tissue occurs (Caldwell, 1937). This collapse takes place rapidly and in this stage, large numbers of lesions coalesce and the impressions of rapid scalding is created. Lesions developing separately tend to assume a lenticular shape. The scalded area soon dries and the centre assumes a light grey colour (Shipton *et al*, 1974; Caldwell, 1937).

2.1.3 Source Of Inoculum And Spread.

Primary inoculum of *Rhynchosporium secalis* may come from infected barley stable or debris. The importance of infected barley stable or debris as a

source of primary inoculum is generally recognised (Polley, 1971; Evans, 1969; Skoropod, 1959 and 1966; Caldwell, 1937 and Ayesu-offei and Carter, 1971). Primary inoculum may also be seedborne (Kay and Owen, 1973; Habgood, 1971; Skoropod, 1959). Jackson and Webster (1976) found seedborne incidence of up to 36.5 percent and demonstrated a transmission rate from seed to seedling of 26.5 percent with seedlots from previous California barley fields. Volunteer barley can also be a significant source of primary inoculum (Jenkins and Jemmet, 1967).

Soil surface is not a major source of primary inoculum because the fungus has limited survival ability in soil due to its weak competitive saprophytic ability (Shipton *et al*, 1974).

Secondary inoculum come from primary infections. The disease is spread from leaf to leaf by rain splash (Fitt *et al*, 1986; Skoropod, 1960 and Stedman, 1980). Wilkins, (1973) indicated that grasses may be important sources of primary inoculum as volunteer barley.

2.1.4 Control Of Scald.

The status of scald has changed, prompting the relatively recent interest in the disease (Shipton

et al, 1974). The change has been attributed to changes in agricultural practices such as intensification of cropping, increased acreages sown, early sowing of crops, use of combined harvesters, shallow cultivation practices and other practices which allow debris to accumulate in the soil surface (Skoropod, 1959 and 1966; Jenkins and Jemmet, 1967). Control measures therefore involve sanitation and provision of barriers to colonisation such as generalised and specific resistance and application of protectant fungicides (Shipton *et al*, 1974).

In Kenya, scald is controlled by use of fungicides such as Bayleton (Triadimefon), Cercobin, Propiconazole (Tilt), Carbendazim (Bravocarb), Triadimenal (Bayfidan), Frutriafol (Impact) and Prochloraz (Sportak) (Kenya Breweries Ltd., 1990).

Control using resistant cultivars is limited. This is because only one cultivar Ahadi is reported to be moderately resistant to scald. But it was found to be very susceptible to net blotch displaying the characteristic leaf firing symptoms and it is not recommended below 2400m above sea level (Kenya Breweries ltd., 1990).

Varietal resistance offers an effective method of controlling leaf blotch of barley but may need to be augmented by application of effective

fungicides. In Britain, Captafol, Chlorothanil, Prochloraz, Propico-nazole Tridemefon 4 Benzimidazoles (alone or in combination with other materials) all give effective control of this disease (Atwood, 1985).

2.2.1 *Bipolaris sorokinianum* Sorok.

Sorokin (1890) published the first description of *Helminthosporium sorokinianum*. Drechsler (1823) linked *Helminthosporium sorokinianum* as a probable synonym of *Helminthosporium sativum*. *H. Sativum* has subsequently been used almost to the exclusion of the prior name *H. sorokinianum* (Lutrell, 1955). Shoemaker (1959) reported that *Helminthosporium* is restricted to the generic type which form conidia both laterally and apically on the conidiophores. Graminicolous species with cylindric conidia that germinate from all cells are included in *Drechslera* Ito while those with bipolar germination are included in *Bipolaris*.

Conidiophores simple, cylindrical, darkbrown, 72-308 x 5-7 µm with basal cells 12-14µm in diameter and 3-13 Septate (Lutrell, 1955 and 1963; Mathre, 1982). Conidia (15-28 x 40-120 µm) and 3-10 septate (Mathre, 1982); (19-25 x 43-109 µm) and 6-10 septate (Lutrell, 1963), (15-20 x 60-120 µm) and 3-10 septate (Drechsler, 1923); olive brown; ovate to

oblong with rounded end and a prominent basal scar with some slightly curved (Drechsler, 1923; Lutrell, 1963 and Mathre, 1982). Cell walls smooth and noticeably thickened at the septa (Drechsler, 1923). Conidia germinate by single germtubes extending parallel with the longitudinal axis of the conidium from each of the terminal cells (Lutrell, 1963). In culture the mycelium is of dark coloration (Cook, 1962; Moore, 1972 and Drechsler, 1923).

2.2.2 Symptoms and Development Of Spot Blotch.

Lesions vary from minute to large 1-5 x 2-30mm (Mathre, 1982). The spots uniformly brown, often yellow, older lesions olive coloured due to sporulation (Drechsler, 1923; Lutrell, 1955). Spots develop on the leaves, leafsheaths at all stages of plant development. Small uniformly dark brown spots from secondary infection may continue to enlarge and coalesce longitudinally forming irregular brown stripes especially on the leaf blades as the tissues reach maturity (Cook, 1962).

2.2.3 Source Of Inoculum and Spread Of *B. sorokinianum*.

Primary inoculum may arise from mycelium or conidia on plant residues (Briggs, 1978; Cook,

1962). Inoculum may also be seedborne as indicated by the head blight phase of the disease which ultimately produces blighted seeds (Cook, 1962). *C. sativum* conidia may survive in the soil for a long period and may also serve as primary source of inoculum (Whittle, 1977). Australian experience is that it can survive on stubble in the soil for at least two years at room temperature (Buttler, 1959). Wild grasses may also serve as the initial inoculum source because nearly all are hosts (Atwood, 1985; Cook, 1962 and Lutrell, 1955).

Secondary inoculum or infection usually arise from primary lesions. Stakman *et al* (1923) found *Helminthosporium* spores at 10,500 feet over Nebraska and some at lower levels. They were as numerous as rust spores in July.

2.2.4. Control Of Spot Blotch

The organism causing spot blotch has been reported throughout the world as a highly variable pathogen causing seedling blight, footrot, spotblotch and seed infection of a number of cereals and grasses (Banttari, 1975 and Briggs, 1978). Therefore the use of pathogen free or fungicide treated seeds is suggested. In addition, rotation with non susceptible crops (ie non grass species) aids in destruction of infested residue which

reduces the level of primary inoculum. However, soil borne inoculum may negate the value of residue destruction.

In Kenya, the disease is controlled by use of fungicides. A number of them have been recommended. They include Bravocarb (recommended in 1984); Bayfidan (in 1987); Impact, (in 1989); Sportak, (in 1989); and Tilt, (in 1982) (Kenya Breweries ltd., 1990).

2.3.1 FUNGICIDES USED AND THEIR MODE OF ACTION

Five fungicides have been proven to be effective against leaf spot diseases of barley (Kenya Breweries ltd., 1990; Bateman *et al*, 1990). Carbendazim, the active ingredient of Bravocarb falls under the group of fungicides known as Benzimidazoles. It is a site specific fungicide, penetrative and it is broadspectrum (Nene and Thapliyal, 1979). In the United Kingdom, it is used to control *R. secalis* in cereals (Atwood, 1985). In Kenya it was recommended in 1984 and it controls leaf scald and spot blotch (Kenya Breweries Ltd., 1990).

Propiconazole which is the active ingredient of Tilt, Frutriafol (Impact), and Triadimenol (Bayfidan) falls under Triazoles. Prochloraz (Sportak) is a member of the imidazole group

(Waterfield and Sisler, 1988; Schwinn, 1983). Triazoles and Imidazoles interfere with ergosterol biosynthesis (Siegel, 1981). The chemicals that interfere with ergosterol biosynthesis are nitrogen containing heterocyclic compounds of varied structure. They are classified according to their ring structure with imidazole and triazole derivatives falling under heteroaromatic ring systems (Schwinn, 1983). They specifically inhibit the oxidative removal of sterol C-14 methyl groups by the cytochrome P-450 enzyme (Sherald *et al*, 1973 and Dekker, 1985). This leads to accumulation of several ergosterol intermediates (De waard and Nistelrooy, 1988). Ergosterol has been shown to be the principal sterol of lower and higher fungi and of lichens, hence the selectivity (Markly, 1960).

2.3.2. FUNGICIDE RESISTANCE.

About two decades ago, plant pathologists were not concerned with fungicide resistance. This is because in 1967, Geogopoulos and Zaracovitis reported cases of tolerance to agricultural fungicides to be very few and that the knowledge accumulated hardly justified a review. Dekker (1982) reckoned that after the introduction of systemic fungicides into practical agriculture, a

little more than a decade ago, notorious cases of resistance have occurred with Benzimidazole and thiophanate fungicides in most pathogenic fungi that were originally sensitive. Hardly two years after the above report by Geogopoulos and Zaracovitis (1967), resistance problems were reported on powdery mildew diseases of curcubits to Benomyl (Shroeder and Provridenti, 1969). Several other authors later reported resistance to Benomyl (Javed, 1980; Karumoto, 1976; Whan, 1976; Wuest *et al*, 1974).

Resistance problems with Bravocarb (Carbendazim) have been encountered with the fungus *Venturia inaequalis* in apple growing area of West Germany (Dekker and Geogopoulos, 1982). Strains of *Colletotricum coffeanum* were also found to be resistant to Bravocarb in Kenya (Okioga, 1976). Existence of naturally occurring tolerance to Bravocarb in wild isolates of the fungus *Ceratocystis ulmi* have been reported (Schreiber and Townsend, 1976 and Richmond and Pring, 1980). Bravocarb has been controlling leaf-spot diseases of Barley since it was recommended in 1984 (Kenya Breweries Limited handbook, 1990).

In view of the site specific action of ergosterol biosynthesis inhibitors, development of fungicide resistance is a serious threat. Indeed, laboratory mutants with resistance to Demethylation

inhibitors can readily be obtained (De waard and Fuchs, 1982; Kalamarakis *et al*, 1988). Nevertheless Resistance problems are rare in practice but have been reported in control of cereal and cucumber powdery mildews (De waard and Fuchs, 1982).

Atwood (1985) reckoned that decreased sensitivity have been recorded with triazoles but not clearly associated with poor control. Isolates of barley leaf blotch pathogen *Rhynchosporium secalis* from commercial crops in United Kingdom during 1986 differed significantly from those made prior to 1982; showing a 5-10 fold decline in sensitivity to triadimenol (Hollomon *et al*, 1987). Triadimenal tolerant strains of the pathogen *Pyrenophora teres* were widespread in U.K. (Sheridan and Nendrick, 1987).

Atwood (1985) stated that the risk of developing resistance is greater with fungicides which act at single site, or a limited number of sites in the fungal cell. And that fungi, resistant to one fungicide are resistant to related fungicides in the same group with the same mode of action. This has also been observed by Dekker (1985).

A number of cross-resistance cases among the ergosterol biosynthesis inhibitors have been established (Fuchs *et al*, 1977; Dekker, 1982 and De waard and Fuchs, 1982).

In Kenya some work has been done on fungicide sensitivity tests and fungicide resistance problems on coffee but none on barley or cereal crops in general. The fungal pathogens causing the major leaf spot diseases in barley have not been isolated and properly characterised in Kenya. In the field, diseases have been identified by their symptoms. Although the importance of identification using the symptoms may not be overlooked, sometimes it may be misleading in instances when asymptoms exist as has been experienced in scald (Davis and Fitt, 1990). Also sometimes different organisms may have similar symptoms (Shipton *et al*, 1973). This study was carried out in view of the above reasons.

2.4. The main objectives of this study were;

i) To isolate and characterise some of the fungal pathogens causing major leaf spot diseases of barley in kenya.

ii) To study the effect of some fungicides on *Bipolaris sorokinianum* mycelial growth *in vitro*.

iii) To assay the effect of some fungicides on germination and germtube length of *Bipolaris sorokinianum*.

iv) To assay the concentration at which maximum inhibition of colony development of *Rhynchosporium secalis* is achieved.

3.0 CHAPTER THREE

MATERIALS AND METHODS

3.1.1 Source of Fungal Pathogens

Barley leaves exhibiting scald symptoms caused by the fungus *Rhynchosporium secalis* were obtained from Kenya Breweries Limited research station, Mau Narok. The Tumaini variety which is very susceptible to leaf scald disease with distinct symptoms was used. *Bipolaris sorokinianum* was obtained from infected leaves of the Bima variety collected from the Kenya Breweries experimental plots at Naivasha and at Mau Narok. Secured diseased leaves of the Bima and Tumaini varieties were dried at room temperature and pressed between papers for future use.

3.1.2 ISOLATION OF PATHOGENS.

(a) *Rhynchosporium secalis* Oud.

Bactolima bean agar (Difco) medium was used (appendix 1). Diseased barley leaves of the variety Tumaini were surface sterilized using 70 percent alcohol which was then washed away with distilled water and incubated in sterile glass petridishes for 24hr. to initiate conidial formation. Conidia were then picked using a sterile scapel from a scald lesion and put in a sterile drop of water on a

slide. The drop was observed under a compound microscope to confirm the presence of conidia. Conidia were picked with a sterile loop and streaked on plates in a W-pattern. After five days, pink colonies characteristic of *R. secalis* were observed. Pure cultures of the fungus were obtained and maintained on lima bean agar.

(b) ***Biporalis sorokinianum*** **Sorok.**

Potato carrot agar medium was used for the isolation and culturing of the fungus (appendix 2). Preserved leaves of Bima variety were cut into small pieces after surface sterilisation and placed in a moist chamber (a petridish with a wet filter paper spread on bottom) and left for three days to allow sporulation. Under aseptic conditions, a few conidia were picked and centrally placed on the plates. About 10 plates were inoculated and kept on a bench for subsequent use.

3.1.3 Storage of Isolates

Identified fungi were preserved under sterile autoclaved water and mineral oil on agar slants. Those used in routine work were left in petri dishes and placed on benches.

3.1.4 CHARACTERIZATION AND IDENTIFICATION

Pressed diseased barley leaves were cut into

small pieces (2cm) and incubated in a moist chamber at 22(±4)°C for two days inorder to induce sporulation. The conidia and conidiophores were mounted on microscope slides and their dimensions taken using an eye piece micrometer in a calibrated microscope. For each species 100 representative conidia and conidiophores were measured from 10 collections. The length, diameter, number of septations, colour, and other major characters were recorded for each fungus.

Conidia from pure cultures were used to infect barley seedlings grown in pots. Spore suspensions were sprayed over seedlings. The inoculated plants were covered with polythene bags and kept at 100% relative humidity for 48 hours in the greenhouse. After the plants developed disease symptoms, the leaves containing spots were cut into small pieces (1cm x 1cm) surface sterilised and incubated to allow sporulation. Conidia and conidiophores were measured and major characters noted for comparison with those used as inoculum.

3.2. FUNGICIDE SENSITIVITY TESTS.

Stock solution (2000 ppm) was prepared by dissolving 0.2ml of the fungicide in 100ml of sterile distilled water under aseptic conditions. Other dilutions were done to attain a dose ratio of

two, hence, obtaining 1000, 500, 250 and 125 parts per million (ppm). This was done by drawing 10ml of stock solution and putting it in 1000 ppm marked flask containing 10ml of sterile distilled water and was then swirled to ensure complete mixing. The same was applied for the rest of the dilutions but each time starting with the highest concentration. To make 50 ppm, 8mls of 125 ppm dilution was drawn and put in a flask marked 50 ppm containing 10mls of distilled water. It was swirled and kept for later use. Similar treatments were done for all the chemicals under study.

3.2.1 Sensitivity tests with *Rhynchosporium secalis*

Lima bean agar was amended with various chemicals at concentrations of 1000, 500, 250, 125 and 50 ppm prior to pouring into petri plates. High sensitivity of this fungi to some chemicals necessitated the use of lower concentrations 1ppm, 3.125 ppm, 6.25 ppm and 25 ppm. Conidia from ten day old cultures were suspended in sterile distilled water and filtered through double layer cheese cloth. The conidial concentration was adjusted to approximately 1000 conidia per ml. One ml conidial suspension was spread on surface of solidified lima bean agar containing various concentrations of the chemical. Each treatment consisted of 12 replicates at each fungicide concentration. These were placed

on the bench at room temperature (22 ±4 °C). The number of colonies in four replicates were counted after every seven days for 21 days. Each time counting the four representative plates that had not been counted previously. After 21 days all the plates were counted and compared with the last undisturbed four replicate plates to ensure that colonies counted were not from dispersed conidia. The experiment was repeated twice.

3.3 Sensitivity tests with *Bipolaris sorokinianum*.

After autoclaving the medium (PCA), it was set to cool in a waterbath to 45 °C and 80mls of the medium was mixed with 8ml of the diluted fungicide. This was swirled slowly to ensure complete mixing and avoid air bubbles. The resultant medium was poured in 4 petri dishes.

After solidifying, the plates were centrally seeded with 8mm diameter discs cut from the periphery of ten day old PCA cultures of *Bipolaris sorokinianum*. They were sealed with parafilm to avoid contaminants and placed on a laboratory bench at 22 (±4) °C.

Diametral measurements were taken after every 24hr. for 10 days. Above was repeated for every dilution of all the fungicides tested. The experiment was repeated three times.

3.4 SLIDE GERMINATION TEST.

3.4.1. Preparation of Stock solution and moist chamber:

The chemicals were diluted at twice the desired concentration (2,000ppm). They were marked 'stock solutions'. This was because they were to be diluted later to the actual concentration after the addition of spore suspension of the test fungus.

Dessicators were used to act as moist chambers. Water was placed on the bottom and an improvised slide rack holding twelve glass slides was put inside. The water level was maintained below the slide rack. The lid and mouth glued to slide with petroleum jelly.

3.4.2 Preparation of Dilution Series

Dilution of the chemical was done at twice the desired concentration. The resultant dilutions were 2,000, 1,000, 500, 250 and 100ppm. This was done for all the test chemicals. Under aseptic conditions 1ml of each dilution was pippetted into correspondingly marked test tube. The tubes were placed in a rack together with an extra tube containing 1ml of water to act as a control.

The spore suspension was prepared not more than half an hour before setting up the experiment. Ten day old cultures were used throughout these

experiments.

Sterile distilled water was used to make the spore suspensions which were adjusted to approximately 4,000 conidia per ml. It was labelled the stock solution and kept in a shaker.

One millilitre of the spore suspension was dispensed into the test tubes containing the chemical dilution. They were shaken well to ensure complete mixing, then placed in a shaker. A 2.0 cc pipette was used to draw 2.0 cc of chemical-spore suspension and quickly placed on the slides in the moist chamber. A 2cc spore suspension was drawn and put on slides in the moist chamber to act as control. Each dilution was replicated six times. Germination count and measurement of germtube lengths was done after 72 hours. Exactly 50 spores were counted for each drop.

3.5 Analysis Of Data and Presentation of Results.

Analysis of variance was performed on the data obtained from the sensitivity and the slide germination tests. Tukey test was then used to exemplify the multiple comparison test.

The degree of inhibition of mycelial growth was calculated from the mean differences between the control and treated plates and expressed as a percentage of the former. Dosage response curves were obtained by plotting percentage inhibition

against fungicide concentration. Concentration of the fungicide required to reduce fungal growth by 50% and 90% for all the in vitro tests were calculated from the dose response curves. These curves were transformed into straight lines using the probit table (Bliss 1935). They were called ED 50 (or ED 90) in case of conidial germination and colony formation and IG 50 (inhibition of growth by 50%) for germtube inhibition and hyphal extension (Mcquiken *et al*, 1988).

4.0 CHAPTER FOUR

RESULTS

4.1.1 (a) *Rhynchosporium secalis* Oud

R. secalis was isolated from leaves of barley (*Hordeum vulgare*) in which it causes leaf blotch characterised by lesions that appear single, ovate and zonate blotches or long blotches especially near the base of the leaf blade. The lesions were of a dark-bluish-gray colour with a water soaked appearance or light gray and about 0.5-2 cm in length with the margins light gray in colour (plate 1).

Conidia hyaline, cylindric to ovate, mostly with a short apical beak, curved in profile; the lower cell narrower than the upper cell, (plate 2). Conidia from host measured 10.0-20.2 µm (15.4 µm) by 2.1-3.8 µm (3.2 µm) and one septate; those from pure cultures were 10-31 µm (17.3µm) by 2.0-3.8 µm (3.1 µm) and one septate.

It was observed that on average, the conidial length was higher in culture (mean 17.3 µm.) than on host (mean 15.4 µm.). Width however did not differ significantly.

Plate 1.

Barley leaves from the field (Mau Narok) showing symptoms caused by *Rhynchosporium secalis.*

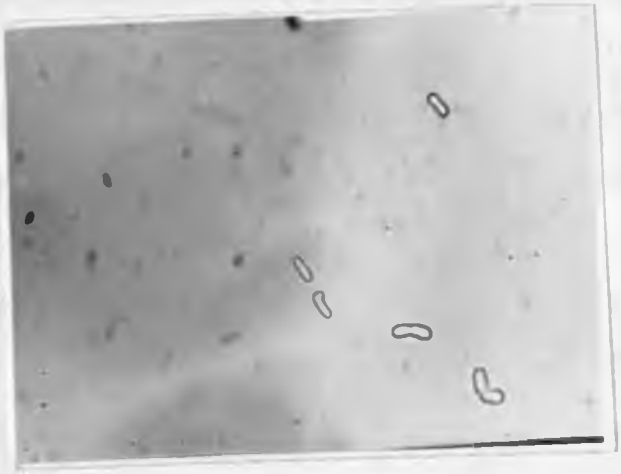


Plate 2.

Conidia of *Rhynchosporium secalis* from culture.

The number of septations remained constant for both conidia from host and in artificial medium. The diameter of culture colonies measured 15-35 millimetres (average 25 mm) in lima bean agar medium after three weeks of incubation. The fungus formed repent sterile hyphae, erect fertile hyphae bearing conidia characteristic of *R. secalis* (plate 2).

The scald symptoms were distinct after 21 days of inoculation. Conidia from pathogenecity tests measured 12-20 µm and were comparable to those from the host plant, thus, confirming the identity of the fungus as *R. secalis*.

4.12 *Bipolaris sorokinianum* Sorok

B. sorokinianum was isolated from barley leaves where it caused common seedling blight and spot blotch. The lesions varied from minute, uniformly dark brown spots to large irregular brown stripes running longitudinally on the leaf blades and leaf sheath (plate 3).

Conidiophores (plate 4) from host simple, macronematous, straight or flexuous, often geniculate, cylindrical, occasionally veruculose, darkbrown;

Plate 3.

Barley leaves from the field showing symptoms of spot blotch caused by *Bipolaris sorokinianum*.

Plate 4.

Bipolaris sorokinianum conidiophores and part of conidium on the extreme right.

74-210 µm (185 µm) by 5-8 µm (6.48 µm), 3-11 septate those from pure cultures measured 74-210 µm (180 µm) by 6-8 µm (6.84 µm) 3-11 septate.

Conidia (plate 5) acropleurogenous, simple, curved, fusiform, cylindrical, rounded at the apex, ellipsoidal, obclavate, olivaceous brown, mostly smooth, pseudoseptate; 40-120 µm (85.2 µm) by 14.3-25.1 µm (20.48 µm) 4 to 10 septations from host; those from pure cultures 53-87.7 µm (73.4 µm) by 13.8-26.6 µm (21.2 µm), 3 to 9 septate. Conidia from host longer (mean 85.2 µm) than those from artificial medium (mean 73.4 µm). Conidia from artificial medium and from host showed no remarkable differences in their widths. The average number of septations was more in conidia from host (mean 7) than conidia from cultures (mean 6). The same was true for conidiophores. The spot blotch symptoms were distinct after 3 days of inoculation. Comparisons of conidia from pathogenecity tests showed that the morphology of the conidia produced on seedling leaves were similar to those used in the inoculum (plates 6, 7 and 8).

Plate 5.

Conidium of *Bipolaris sorokinianum* from barley leaves collected from the field.

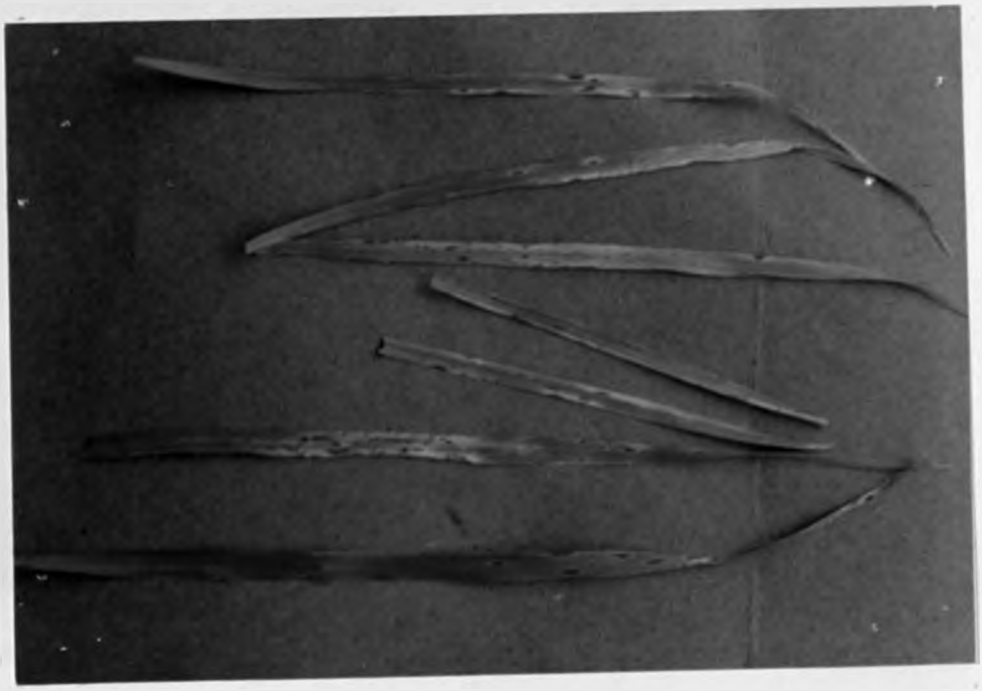


Plate 6.

Leaves of barley seedlings artificially infected with *Bipolaris sorokinianum*.

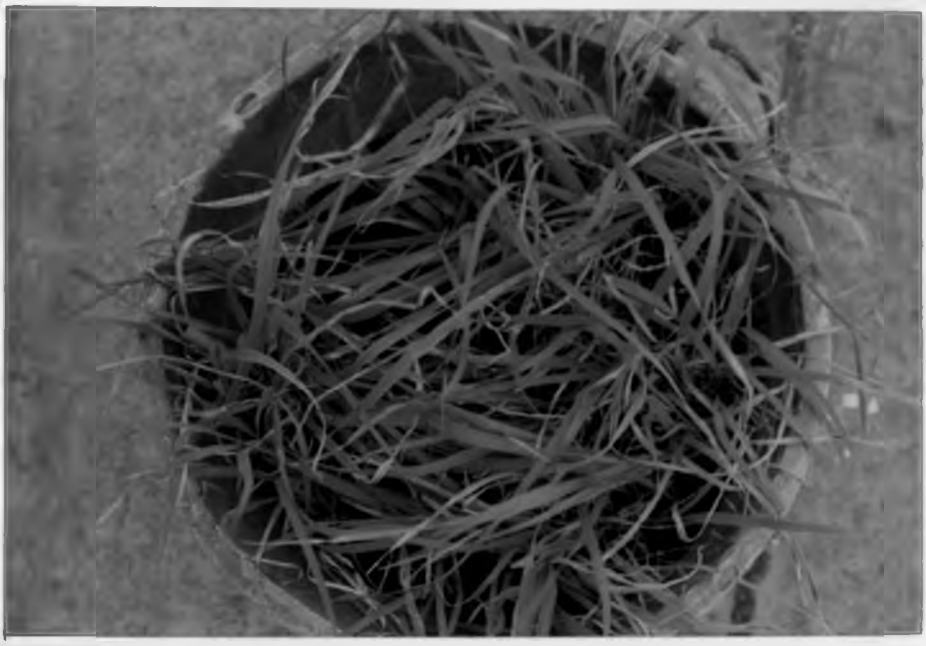


Plate 7.

Barley seedlings grown in a pot showing symptoms caused by *Bipolaris sorokinianum* after artificial inoculation.

Plate 8.

Barley seedlings which acted as control.

4.2 Fungicide sensitivity tests with *R. secalis* Oud.

Prochloraz (Sportak 45ec) totally inhibited colony formation at all the concentrations tested (plate 9). Carbendazim and Propiconazole showed superior efficacy over Frutriafol and Triadimenal (figure 1-3). The high sensitivity of this fungus to Carbendazim and Propiconazole necessitated further dilutions of these chemicals to 1ppm. There were notable differences among the fungicides (Figure 1-3).

Overall effect of the fungicide dilutions was significantly different (P 230.7, $p < .05$). Different dilutions of each individual fungicide varied significantly in their action against the test fungi. Amount of fungicide required to reduce colony formation by 50 percent (ED 50) varied with each fungicide. Carbendazim and Propiconazole were the most effective fungicides attaining an ED 50 at 1.16 ppm and 7.45 ppm respectively after one week. Higher concentrations of Frutriafol and Triadimenal were required to achieve the same effects (Table 1). Effect of time was significant in controlling colony formation of *Rhynchosporium secalis*.

Table 1. Amount of fungicide required to reduce colony formation of *Rhynchosporium secalis* by 50 and 90 percent.

Fungicide	No of incubation days	50%(ED 50) ppm	90%(ED 90) ppm
Carbendazim	after 7 days	1.16	3.60
	after 14 days	3.60	5.10
	after 21 days	3.90	5.10
Propiconazole	after 7 days	7.45	13.60
	after 14 days	14.10	26.20
	after 21 days	14.20	26.20
Flutriafol	after 7 days	51.00	80.40
	after 14 days	107.00	188.00
	after 21 days	238.80	367.00
Triadimenal	after 7 days	106.00	213.00
	after 14 days	138.00	385.00
	after 21 days	271.70	562.00

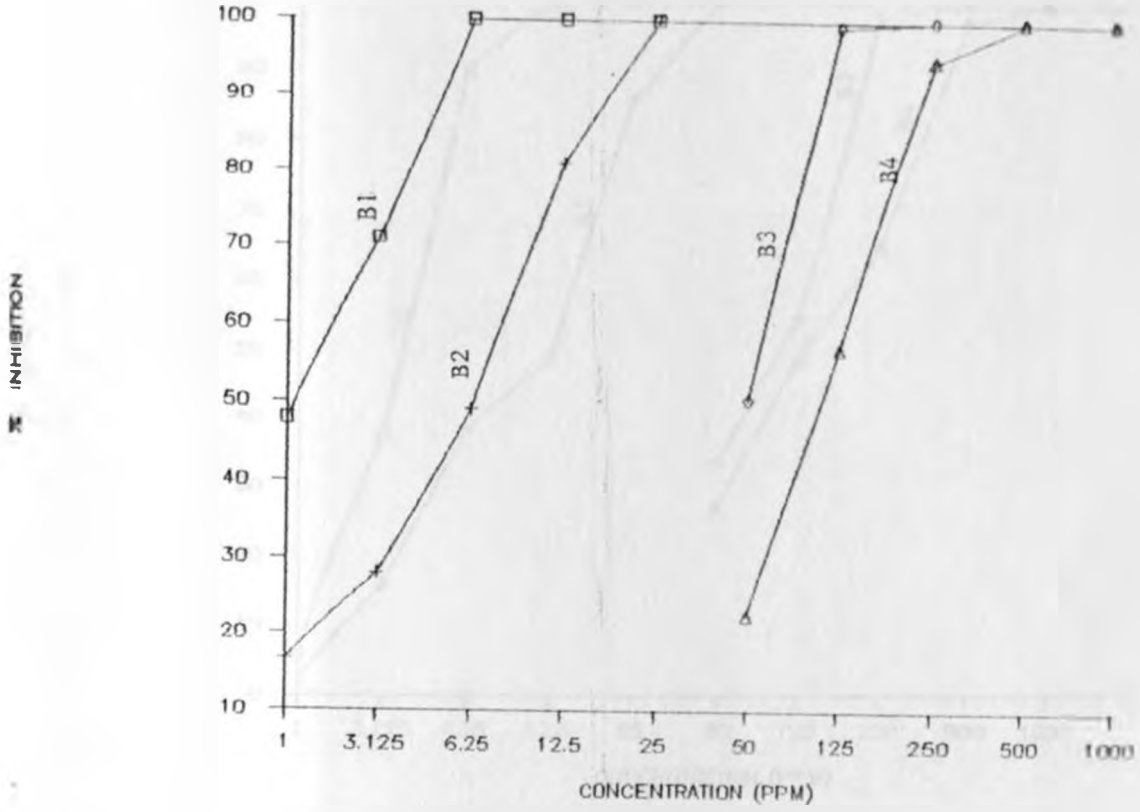


Figure 1.

Effect of fungicides on colony formation of *Rhynchosporium secalis* after incubation for one week. B1, Carbendazim; B2, Propiconazole; B3, Frutriafol; B4, Triadimenal.

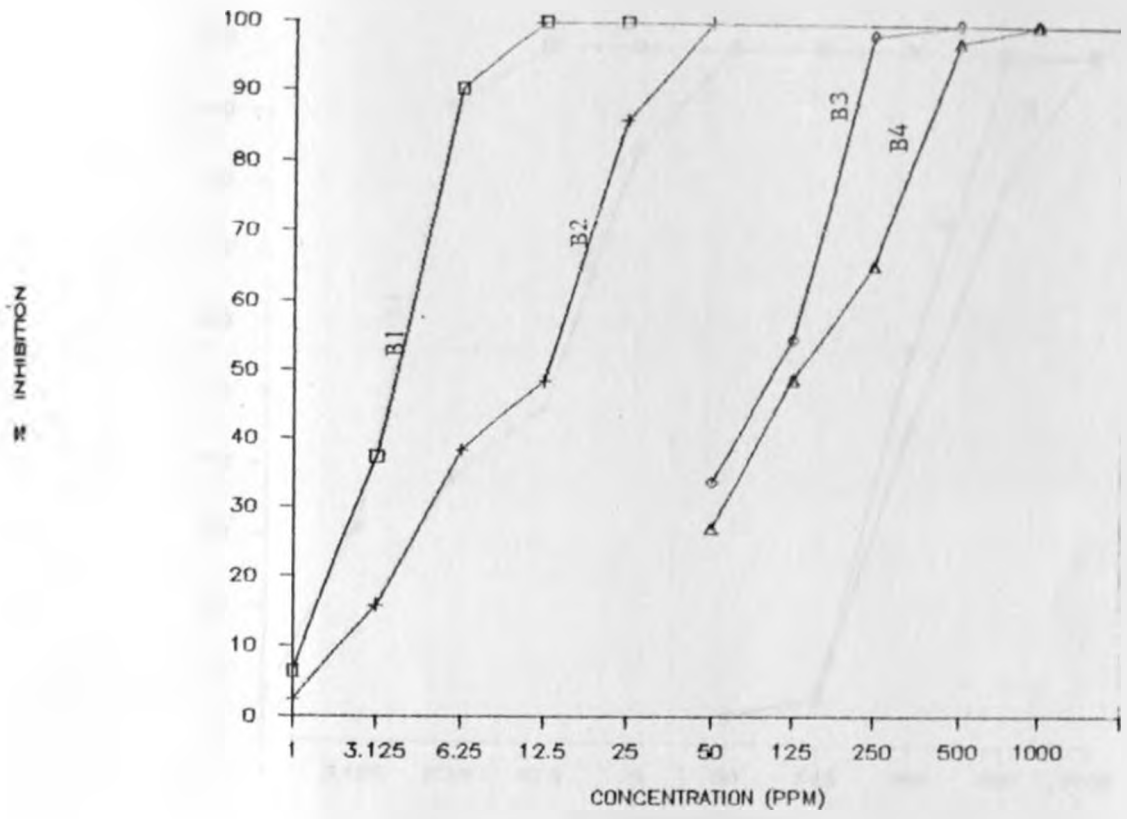


Figure 2.

Effect of fungicides on colony formation of *Rhynchosporium secalis* after incubation for two weeks. B1, Carbendazim; B2, Propiconazole; B3, Frutriafol; B4, Triadimenal.

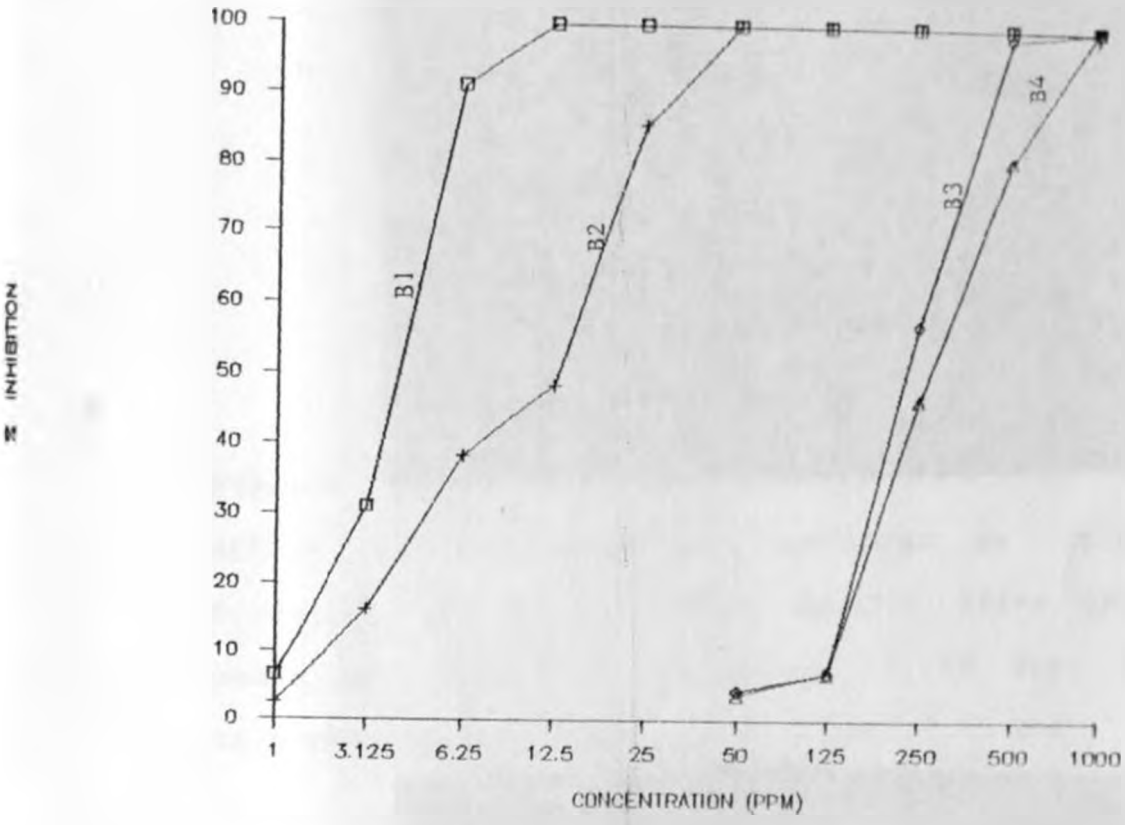


Figure 3.

Effect of fungicides on colony formation of *Rhynchosporium secalis* after incubation for three weeks. B1, Carbendazim; B2, Propiconazole; B3, Frutriafol; B4, Triadimenal.

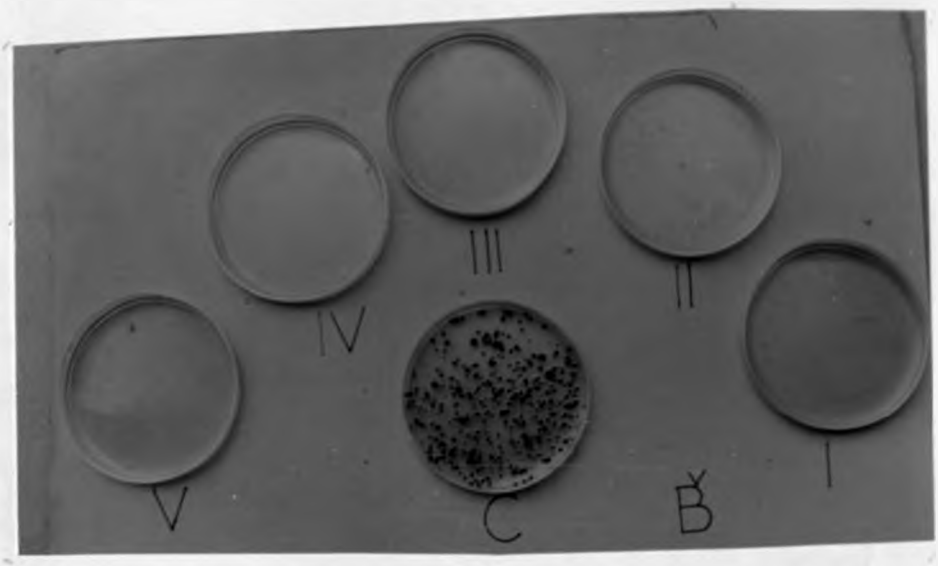


Plate 9. Effect of the fungicide Prochloraz on colony formation of *Rhynchosporium secalis* after three weeks of incubation. C, Control; I, 25 ppm; II, 12.5 ppm; III, 6.25 ppm; IV 3.13 ppm; V, 1 ppm.

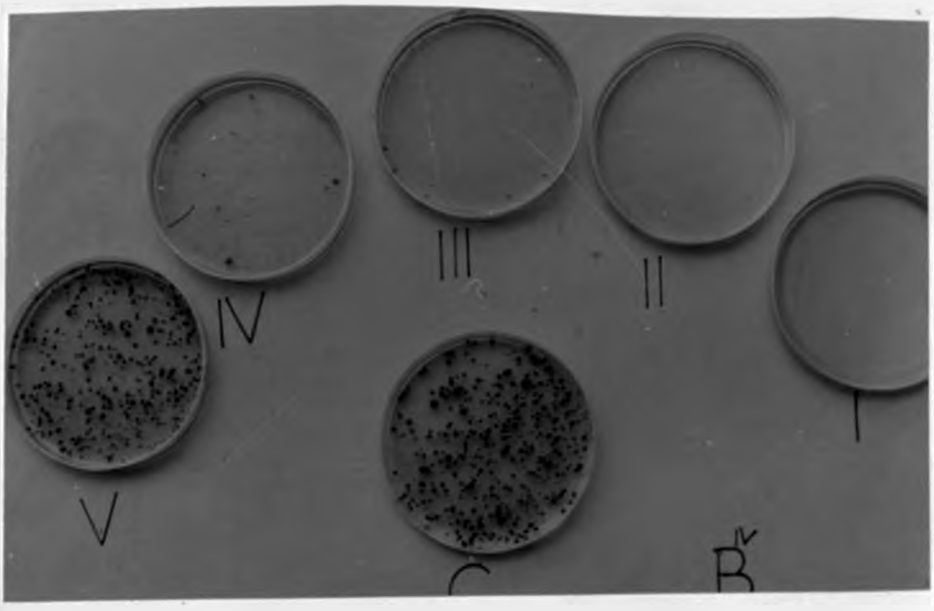


Plate 10. Effect of the fungicide Triadimenal on colony formation of *Rhynchosporium secalis* after one week of incubation. C, Control; I, 1000 ppm; II, 500 ppm; III, 250 ppm; IV 125 ppm; V, 50 ppm.

Colony formation in Propiconazole (Tilt) was progressively inhibited for the first seven days for all the dilutions except for 1ppm (Figure 4). After which the rate of inhibition of colony formation decreased up to day 14 when it became constant. But this effect of time on colony formation was not found to be significant over the three weeks (F 1.7, $p > .05$).

Triadimenol, reduced colony formation significantly in the first seven days (plate 10). For 1000 and 500ppm, inhibition was 100% by day seven and was unchanged for 1000ppm even after incubation for three weeks. However after the 7th day, the percentage inhibition decreased for 500 ppm up to day 21 where further decrease was not observed (figure 5). Effect of time was found to be significantly different over three weeks (F 30, $p < .05$).

Carbendazim (Bravocarb), (Figure 6) had 100 percent inhibition of colony formation at dilution ranges of 1000-12.5ppm after the 7 days of incubation. For 6.25ppm percent inhibition of colony formation increased up to day 14 where further delay had no effect on colony formation. On the other hand, 3.125 and 1 ppm increased up at the

same rate up to day 7 when rate of colony formation decreased differently with 1 ppm decreasing at a higher rate than 3.125 ppm (Figure 6). Incubation had a significant effect on colony formation (F 4.61, $p < .05$) and this was evident after three weeks of incubation.

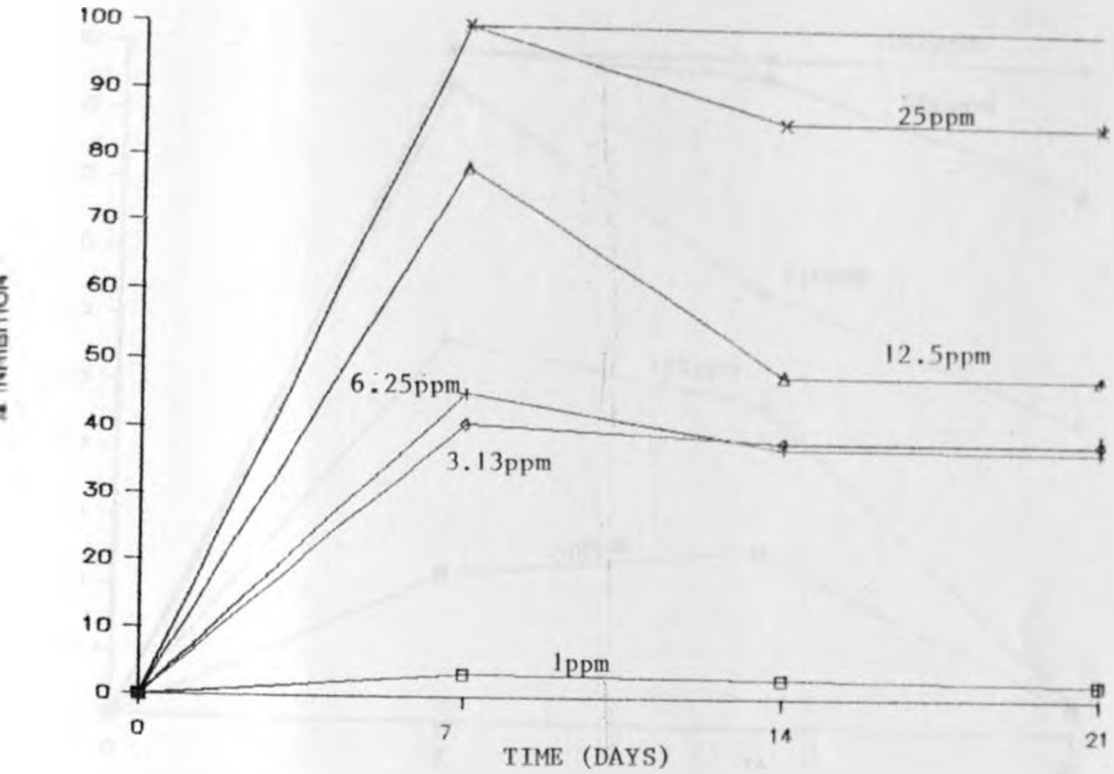


Figure 4.

Effect of various dilutions of the fungicide propiconazole with time on *Rhynchosporium secalis*.

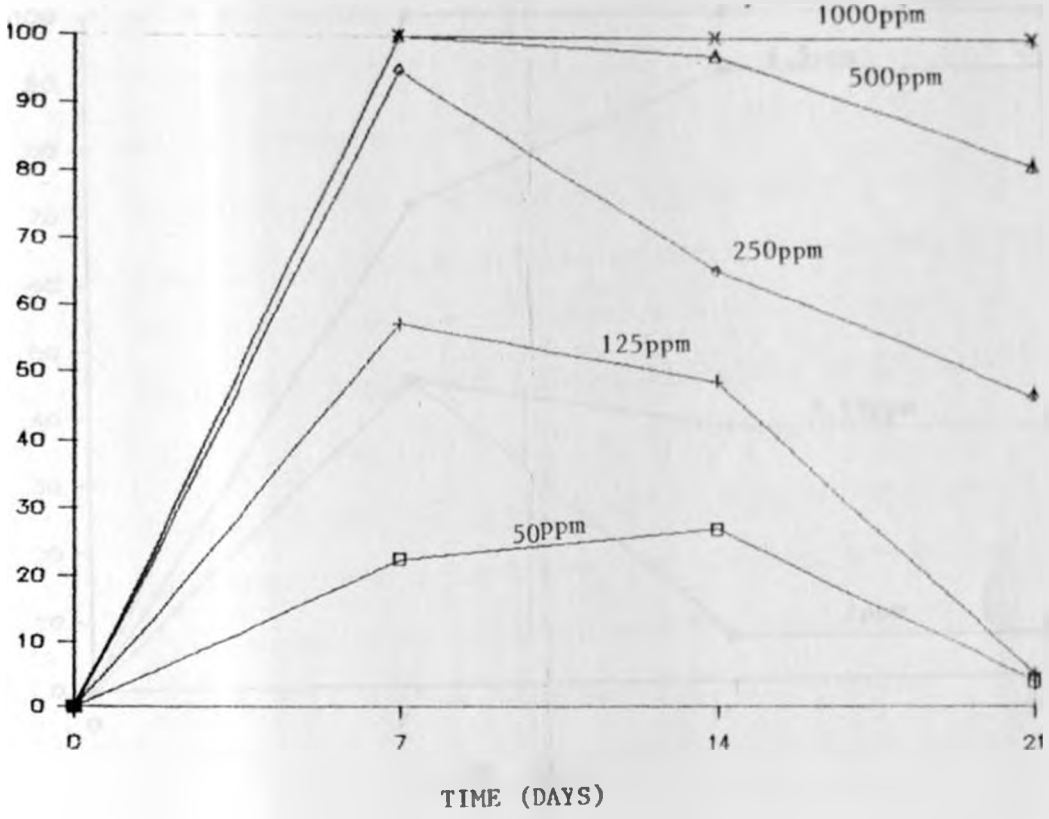


Figure 5.

Effect of various dilutions of the fungicide Triadimenal with time on *Rhynchosporium secalis*.

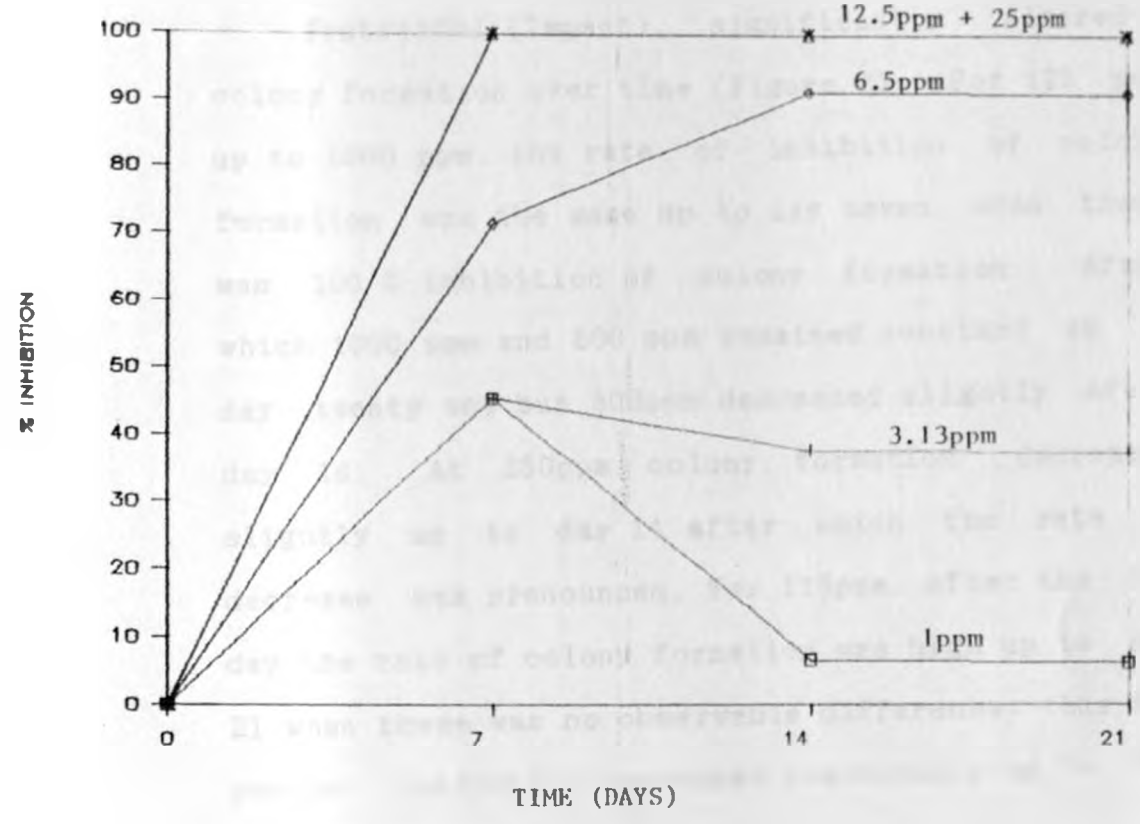


Figure 6.

Effect of various dilutions of the fungicide Carbendazim with time on *Rhynchosporium secalis*.

Frutriafol (Impact), significantly altered colony formation over time (Figure 7). For 125 ppm up to 1000 ppm, the rate of inhibition of colony formation was the same up to day seven when there was 100 % inhibition of colony formation. After which 1000 ppm and 500 ppm remained constant up to day twenty one but 500ppm decreased slightly after day 14. At 250ppm colony formation decreased slightly up to day 14 after which the rate of decrease was pronounced. For 125ppm, after the 7th day the rate of colony formation was high up to day 21 when there was no observable difference: thus the percent inhibition decreased drastically up to 7.0 percent (Figure 7).

For 50 ppm the percentage inhibition increased up to 50.2% in day seven after which it decreased drastically to 4.2%. There was significant difference between day 21 and day 7 (figure 7).

Using the fungicide calculation method by Baarscherset *et al* (1980) all the fungicides tested cause complete inhibition of colony formation (i=100%) figure 1 to 3. According to Baarscherset *et al* (1980) the dose causing maximum inhibition is referred to as Dmax and the actual or higher dose causing such total inhibition as i (table 2). Carbendazim had its Dmax at 12.5 ppm with no change

even after three weeks of incubation.

For others time doubled the Dmax after the first week of incubation. Propiconazole Dmax was 25 ppm after seven days, Frutriafol 250 ppm and Triadimenal 500 ppm (table 2).

Table 2. Fungitoxity of various fungicides on *Rhynchosporium secalis*.

	Dmax : i_b		
After:	7 days	14 days	21 days
Carbendazim	12.5:100	12.5:100	12.5:100
Propiconazole	25:100	50:100	50:100
Frutriafol	250:100	500:100	500:100
Triadimenal	500:100	1000:100	1000:100

(b: Dmax is the dose causing maximum inhibition)

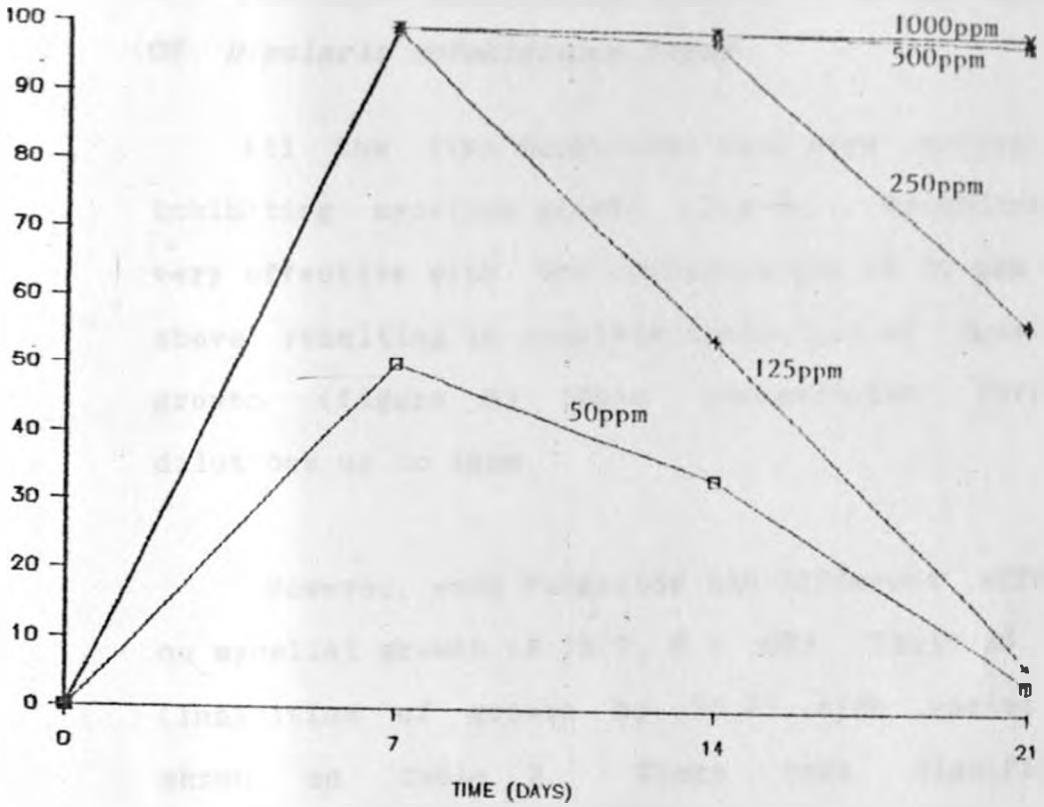


Figure 7.

Effect of various dilutions of the fungicide Frutriafol with time on *Rhynchosporium secalis*.

4.3 FUNGICIDE SENSITIVITY TESTS ON MYCELIAL GROWTH OF *Bipolaris sorokinianum* Sorok.

All the five fungicides used were active in inhibiting mycelium growth (Fig 8). Prochloraz was very effective with the concentration of 50 ppm and above resulting in complete inhibition of mycelial growth (figure 8). This necessitated further dilutions up to 1ppm.

However, each fungicide had different effects on mycelial growth (F 75.7, $P < .05$). Their IG 50 (Inhibition of growth by 50 %) also varied as shown on table 3. There were significant differences between Prochloraz, Carbendazim, Propiconazole and Triadimenol.

Table 3. Amount of fungicide required to reduce mycelial growth of *Bipolaris sorokinianum* by 50 and 90 % (ppm).

Fungicide	50%(IG 50)	90%(IG 90)
Carbendazim	<50.00	<1000.00
Propiconazole	<50.00	133.70
Frutriafol	50.00	205.40
Triadimenal	125.00	241.90
Prochloraz	3.13	20.00

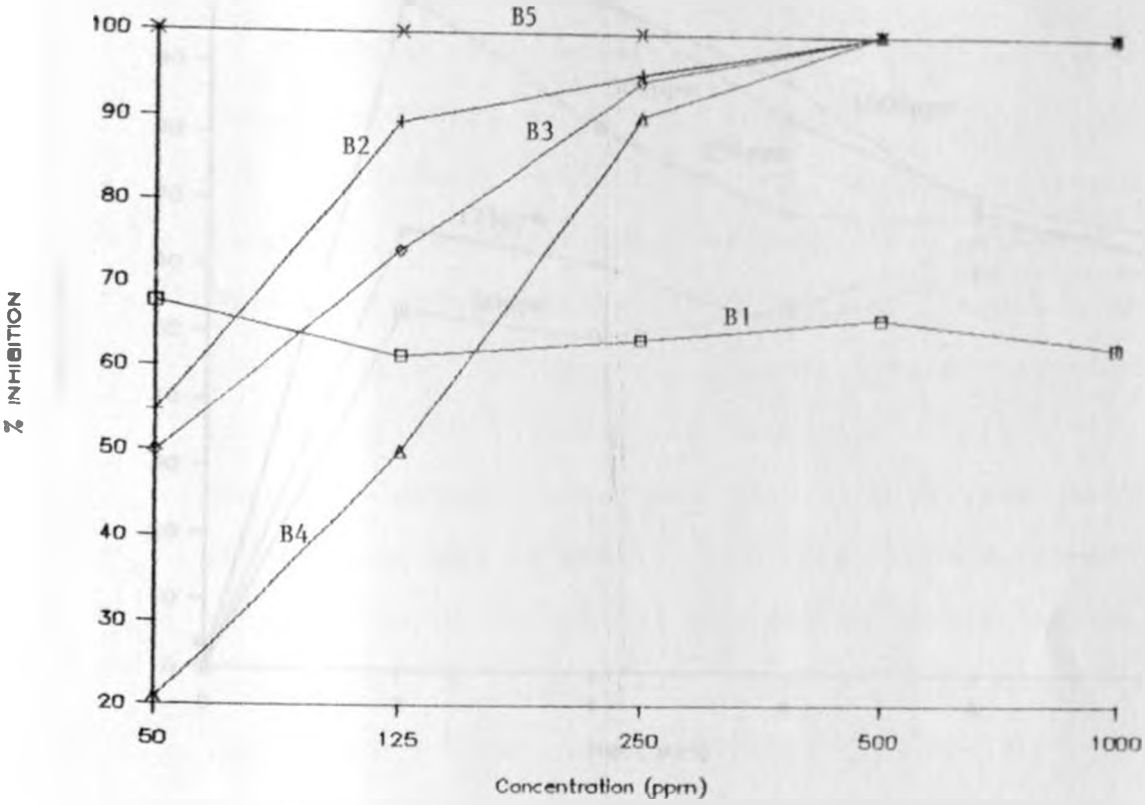


Figure 8.

Dose response curves of *Bipolaris sorokinianum* after ten days of incubation. B1, Carbendazim; B2, Propiconazole; B3, Frutriafol; B4, Triadimenal; B5, Prochloraz.

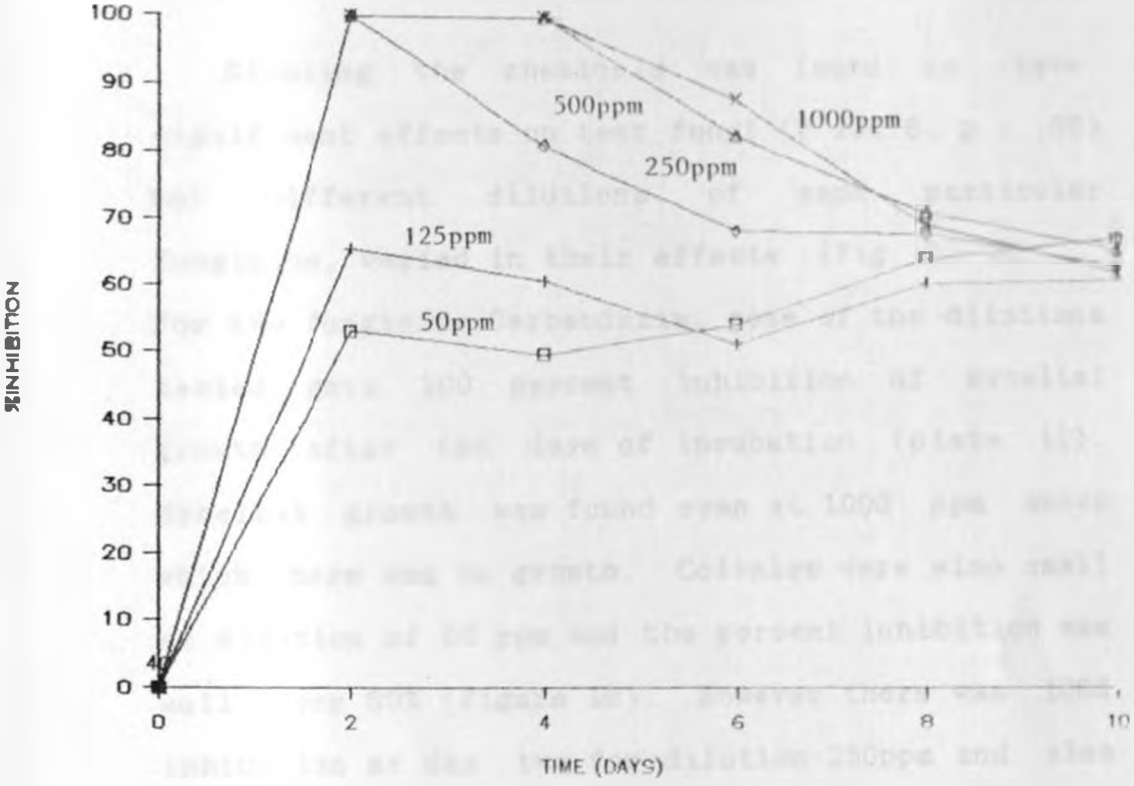


Figure 9.

Effect of various dilutions of Carbendazim on mycelial growth of *Bipolaris sorokiniana* with time.

Diluting the chemicals was found to have significant effects on test fungi (F 294.6, $p < .05$) but different dilutions of each particular fungicide, varied in their effects (Fig 10-14). For the fungicide Carbendazim, none of the dilutions tested gave 100 percent inhibition of mycelial growth after ten days of incubation (plate 11). Mycelial growth was found even at 1000 ppm above which there was no growth. Colonies were also small at dilution of 50 ppm and the percent inhibition was well over 60% (figure 10). However there was 100% inhibition at day two for dilution 250ppm and also at day four for dilution 1000 and 500ppm.

Propiconazole, Frutriafol and Triadimenol, at dilutions 1000ppm and 500ppm completely inhibited growth, (Figures 11-13). The other three dilutions significantly differed (F 11.8, $p < .05$) for Frutriafol, (F 211.43, $p < .05$) for Propiconazole and (F 384.9. $p < .05$) for Triadimenol. The critical value (q value) obtained from tukey test was significant for all dilutions tested apart for 250ppm and 125ppm in Propiconazole.

For Prochloraz, the dilutions 12.5, 6.25, 3.125 and 1ppm differed quite significantly (F 976, $p < .05$) with the critical values (q values) significant

except for dilution 12.5 and 6.25ppm (Figure 14).

Effect of time had a significant effect on mycelial growth but the response varied on individual fungicides (F 27.7, $p < .05$). Effect of time was not significant for the fungicide Prochloraz (F 0.368 $p > .05$); but was significant for the other fungicides tested. It can be observed from the response curves (Figure 10-14) that the higher the dilution the longer the lag phase.

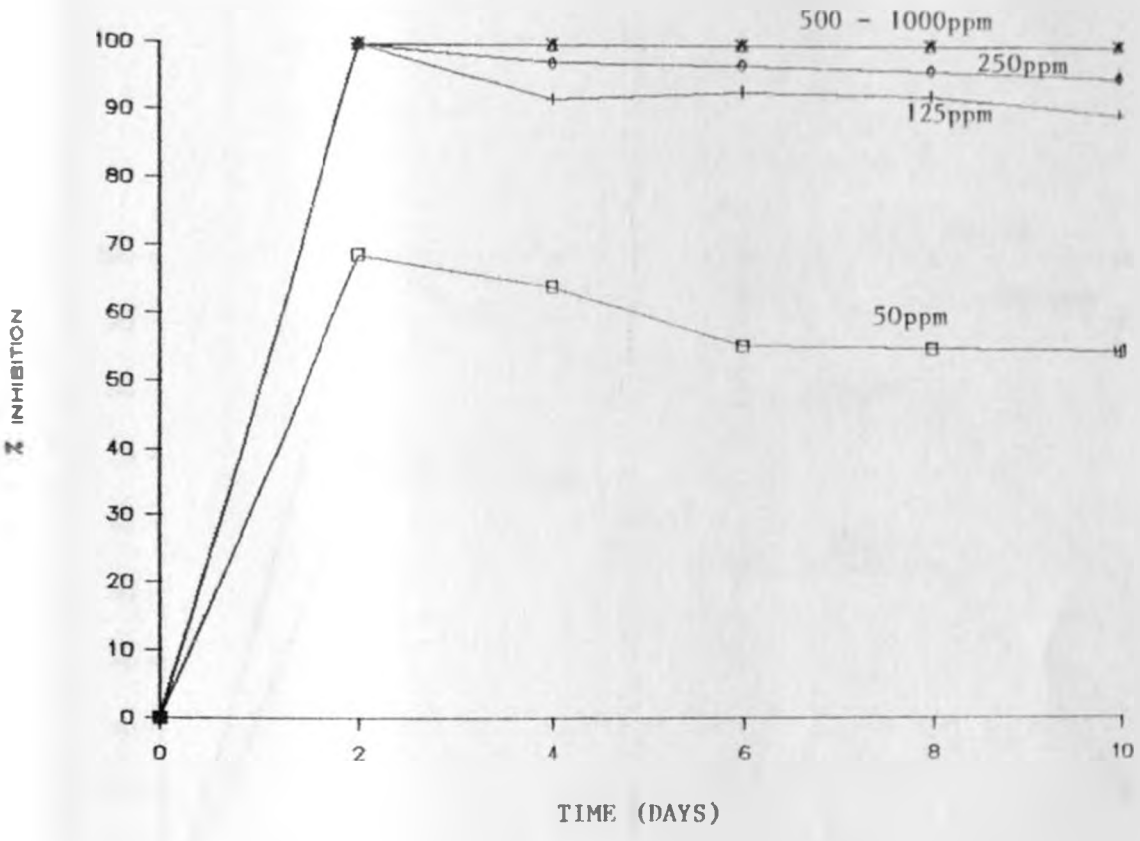


Figure 10.

Effect of various dilutions of the fungicide Propiconazole on *Bipolaris sorokinianum* with time.

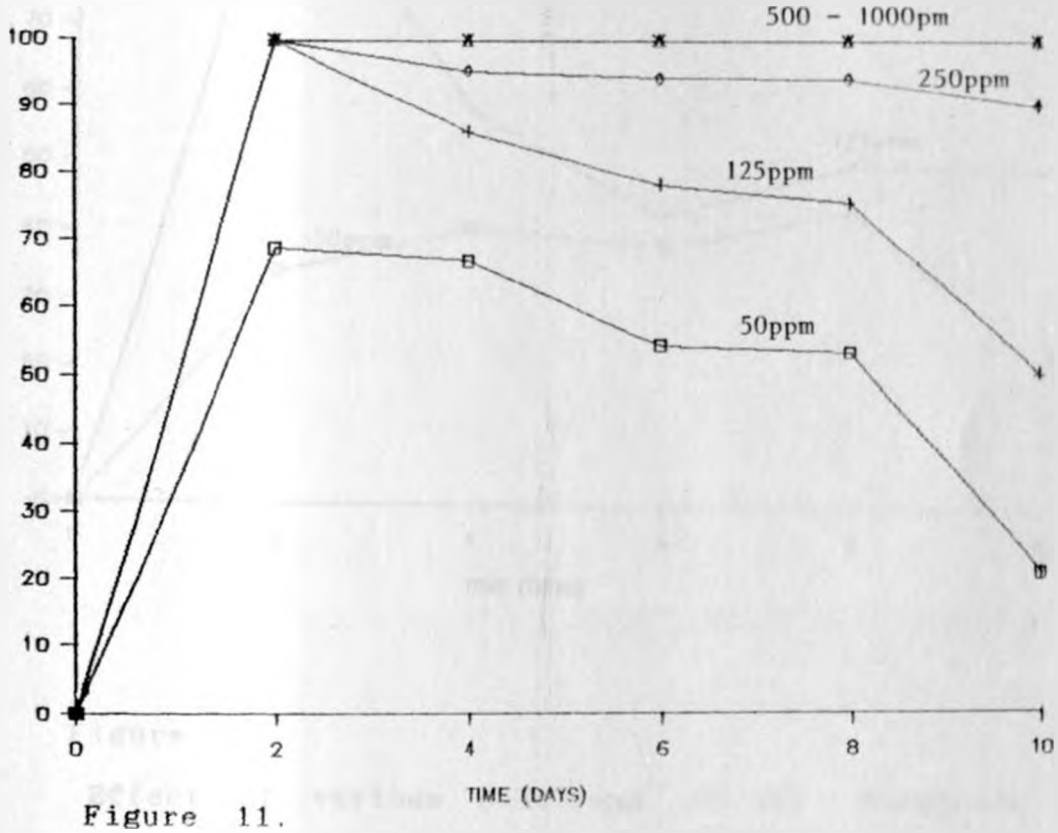


Figure 11.

Effect of various dilutions of the fungicide Frutriafol on *Bipolaris sorokinianum* mycelial growth with time.

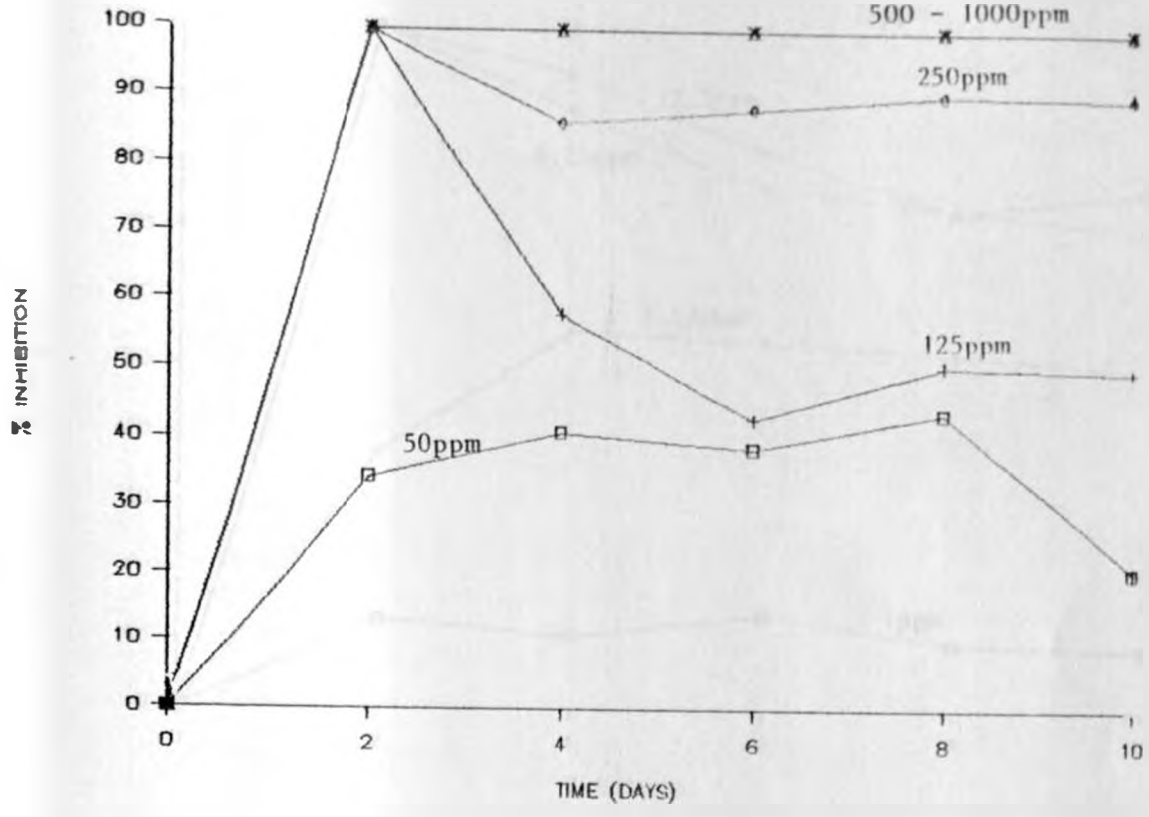


Figure 12.

Effect of various dilutions of the fungicide Triadimenal on *Bipolaris sorokinianum* mycelial growth with time.

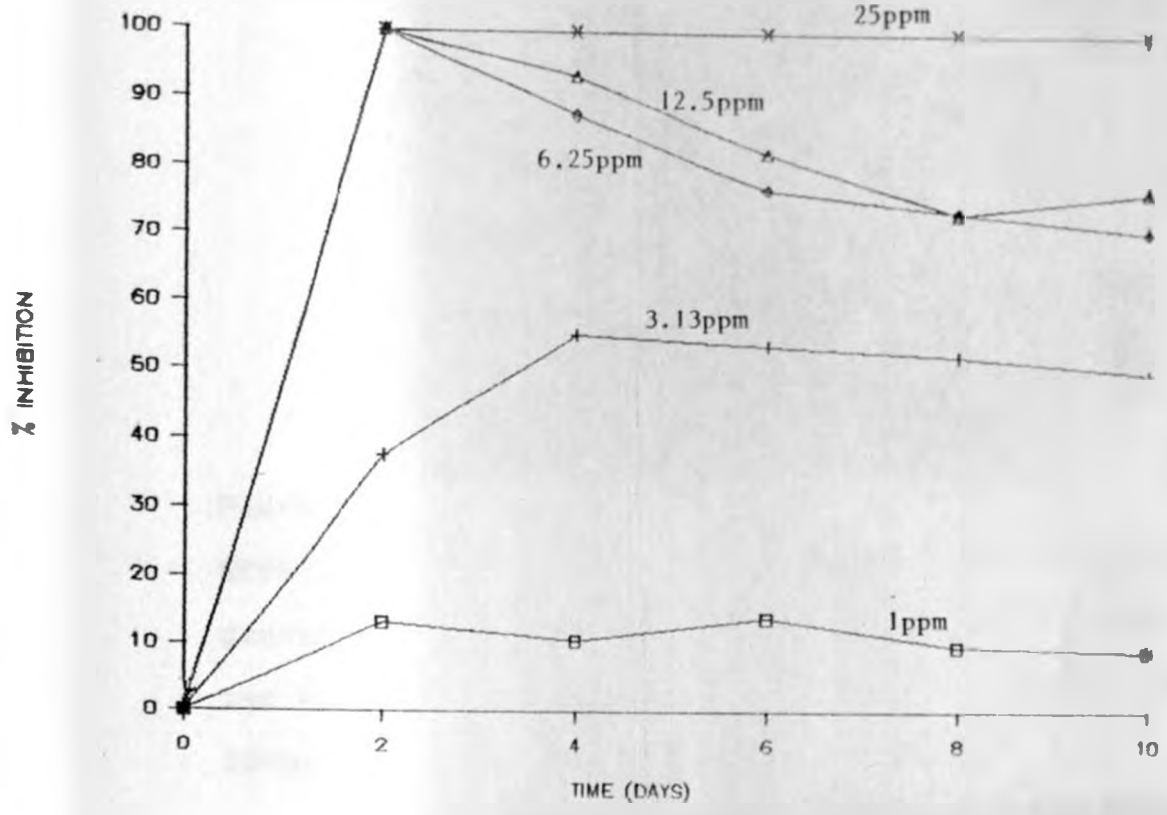


Figure 13.

Effect of various dilutions of the fungicide Prochloraz on *Bipolaris sorokinianum* mycelial growth with time.

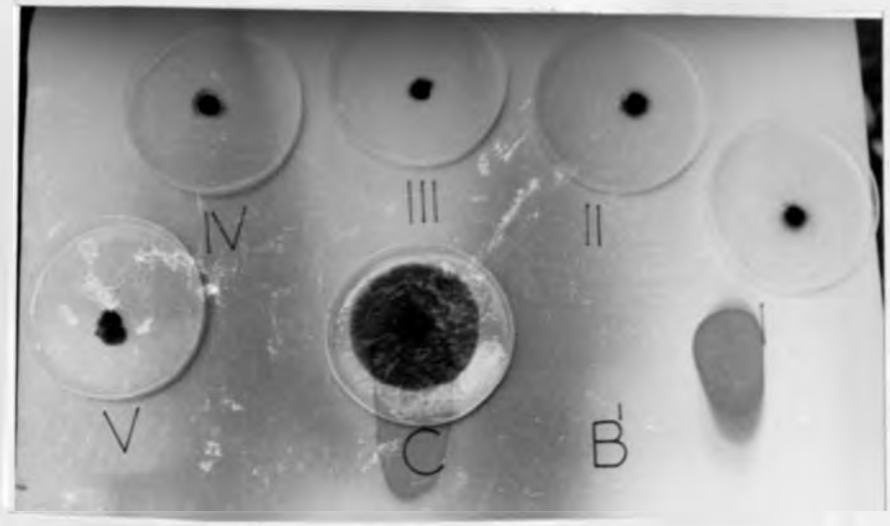


Plate 11.

Effect of the fungicide Carbendazim on mycelial growth of *Bipolaris sorokinianum* (strain I) after ten days of incubation. I, 1000ppm; II, 500ppm; III, 250ppm; IV, 125ppm; V, 50ppm and C, control.

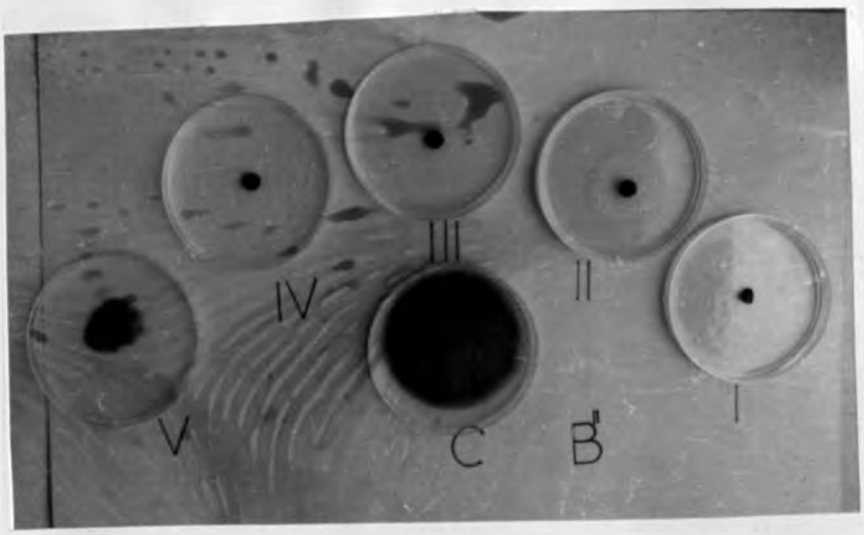


Plate 12.

Effect of the fungicide Propiconazole on mycelial growth of *Bipolaris sorokinianum* (strain I) after ten days of incubation. I, 1000ppm; II, 500ppm; III, 250ppm; IV, 125ppm V, 50ppm; C, control.

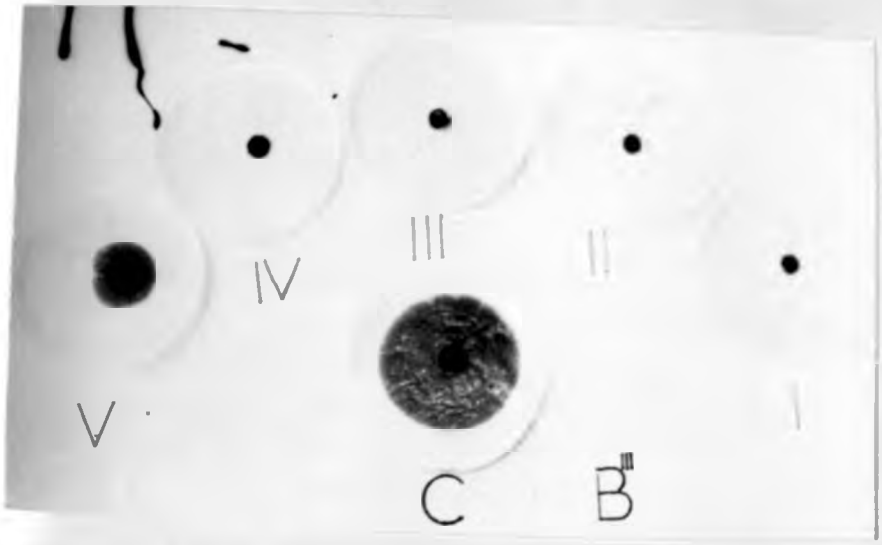


Plate 13.

Effect of the fungicide Frutriafol on mycelial growth of *Bipolaris sorokinianum* (strain I) after ten days of incubation. I, 1000ppm; II, 500ppm; III, 250ppm; IV, 125ppm V, 50ppm, C, control.

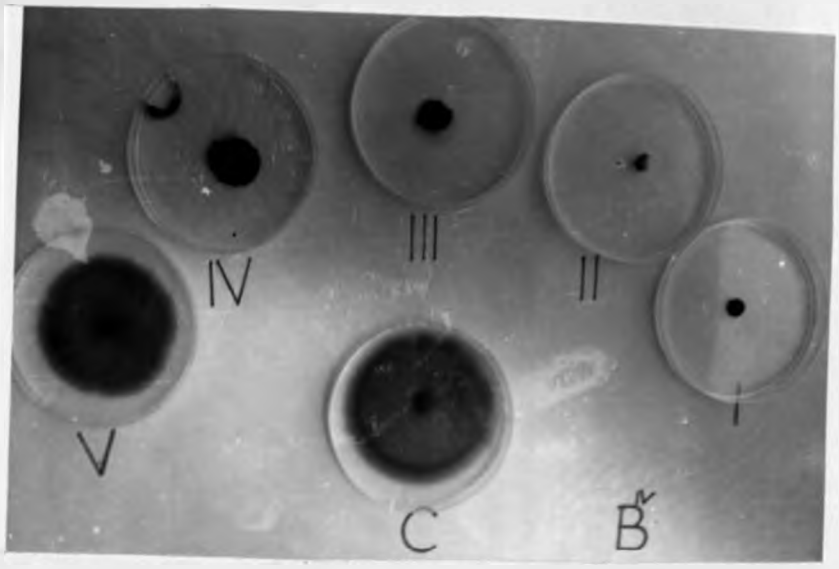


Plate 14.

Effect of the fungicide Triadimenal on mycelial growth of *Bipolaris sorokinianum* (strain I) after ten days of incubation. I, 1000ppm; II, 500ppm; III, 250ppm; IV, 125ppm V, 50ppm; C, control.

Two strains of *B. sorokinianum* were isolated from the field. The first isolate was dark greenish and grew fast in artificial media (PCA) filling the plate in approximately ten days. The other isolate however was lighter in colour and very slow in growth filling half the plate in 10 days.

The sensitivity tests also differed. The first isolate is the one that has been discussed and it is sensitive to these fungicides (plates 11-14).

However for the second isolate the sensitivity was low. Growth occurred in all the dilutions of Carbendazim (viz, 1000, 500, 250, 125 and 50 ppm). Sporulation was very heavy in the dilutions 1000, 500, and 250 ppm, making the colonies look black (plate 15a, B1).

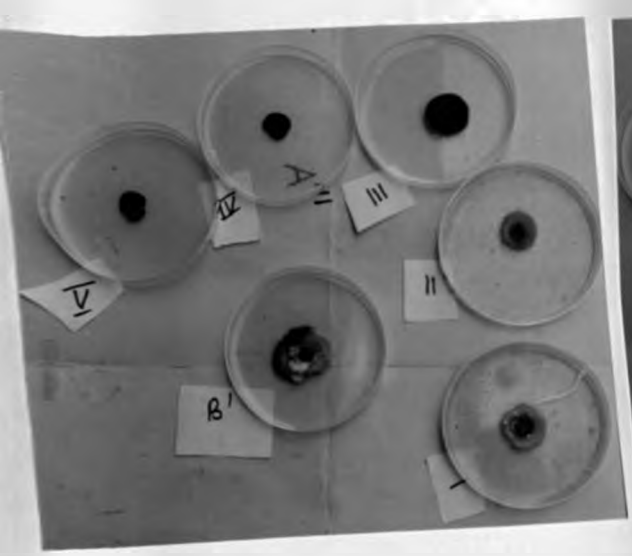
Mycelial growth occurred in all the dilutions of the fungicide Propiconazole except for 1000 ppm. However the dilutions 500, 250 and 125 ppm had very small growth with heavy sporulation making the colonies dark (Plate 15a B2).

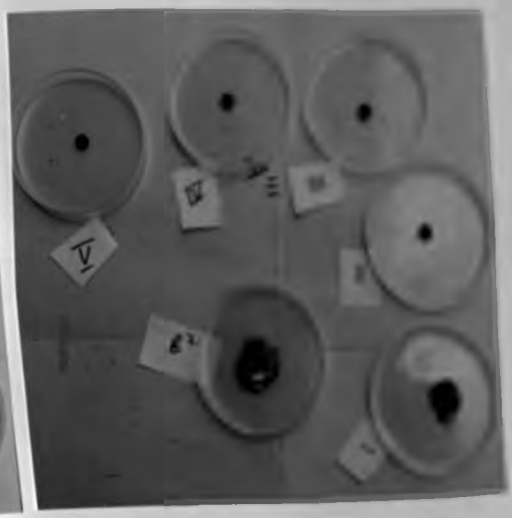
Frutriafol and Triadimenal there was growth in all the dilutions but none at 1000 ppm (Plate 15b, B3 and 15b, B4 respectively).

Prochloraz there was growth in all the dilutions used. The colonies were of the same diameter but the concentrations 125 to 1000 ppm allowed heavy sporulation (Plate 15c, B5).

Plate 15.

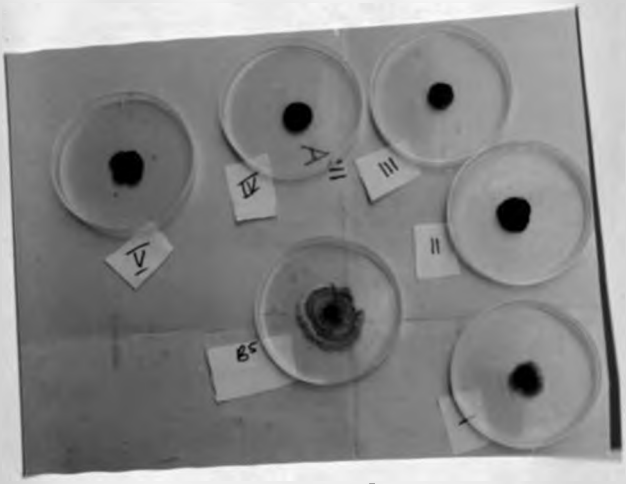
Effect of various fungicides on mycelial growth of *Bipolaris sorokinianum* (strain II) after ten days of incubation. I, 50ppm; II, 125ppm; III, 250ppm; IV, 500ppm V, 1000ppm B^x, control (where x is either 1, 2, 3, 4, or 5).





15a) Left (B1) Carbendazim. Right (B2) Propiconazole

15b) Left (B3) Frutriafol, Right (B4) Triadimenal



15c) (B5) Prochloraz.

4.4 SLIDE GERMINATION TESTS.

4.4.1 Inhibition of spore germination of *Bipolaris sorokinianum* Sorok

All the five fungicides used were active in inhibiting conidial germination of *B. sorokinianum* although none gave 100 percent inhibition (Figure 14). They also differed significantly in their action (F 6.916, $p < .05$). Of the five fungicides, Prochloraz was the most effective. This was apparent from their EC 50 (table 4).

Table 4.
Effect of fungicides on spore germination of *Bipolaris sorokinianum* Sorok.

Amount of fungicide required to reduce spore germination by 50% and 90% (ppm):		
Fungicide	50 %	90 %
Carbendazim	103.0	>1000.0
Propiconazole	80.8	571.4
Frutriafol	125.0	>1000.0
Triadimenal	117.1	540.0
Prochloraz	62.5	215.0

The different dilutions used also differed significantly with one another (F 401.2 p < .05) with all the dilutions having varied responses.

When dilutions in an individual fungicide were compared, they differed quite significantly (F2370 p < .05) with the one having the highest concentration inhibiting most. The q value from tukey test was also significant for all particular dilutions in an individual fungicide.

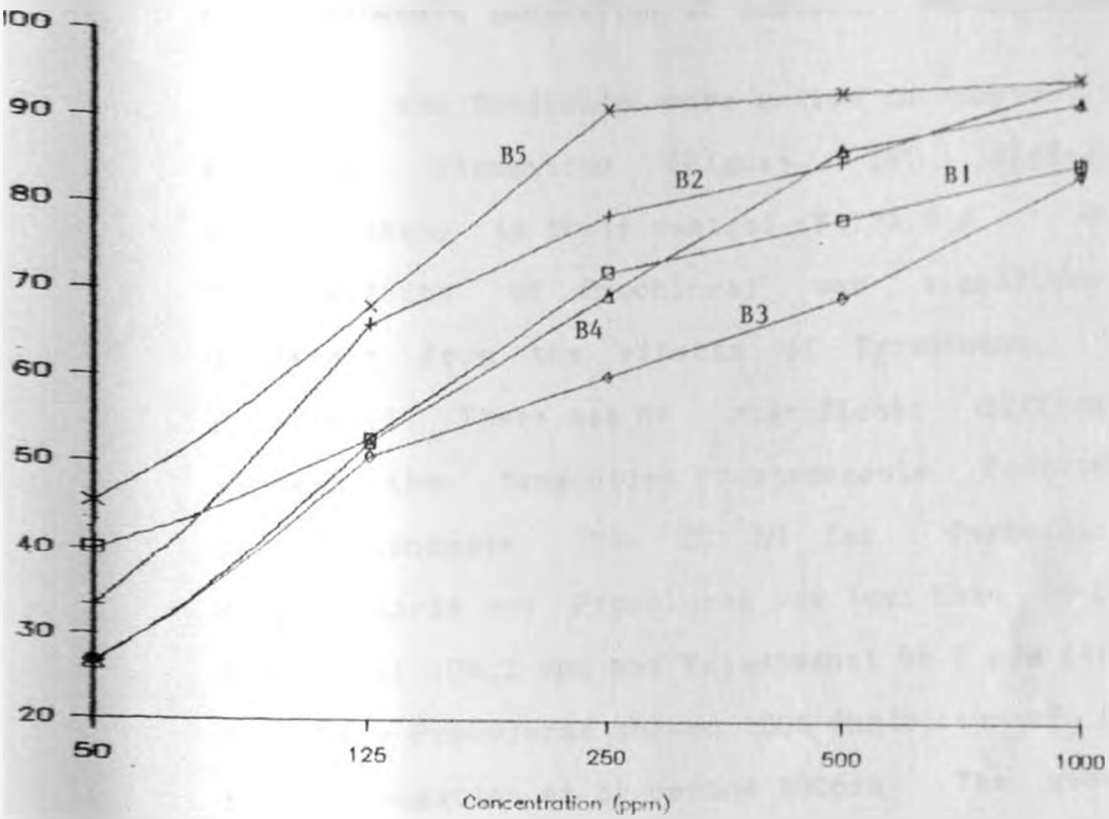


Figure 14.

Effect of fungicides on conidial germination of *Bipolaris sorokinianum*. B1, Carbendazim; B2, Propiconazole; B3, Frutriafol; B4, Triadimenal; B5, Prochloraz.

4.4.2 Germtube inhibition of *Biporalis sorokinianum*

All the fungicides were active in controlling germtube elongation (Figure 16) differing significantly in their control (F 172.3 p < .05). The effects of Prochloraz was significantly different from the effects of Triadimenal and Frutriafol. There was no significant difference between the fungicides Propiconazole, Frutriafol and Carbendazim. The EC 50 for Carbendazim, Propiconazole and Prochloraz was less than 50 ppm; Frutriafol 109.2 ppm and Triadimenol 95.6 ppm (table 5). Only Prochloraz showed 100% inhibition of germ tube elongation at or beyond 500ppm. The general effect of dilutions (50, 125, 250, 500 and 1000 ppm) for all the fungicides in inhibiting germtube elongation was also very significant (F 303.5 p < .05). Germtube inhibition was known to increase with higher concentrations of the test fungicides. Different dilutions of each test fungicide, had different effects on germtube elongation. Effect of different dilutions was significant for all the fungicides tested. Only 1000ppm and 500ppm were found to share significantly similar effects for all the chemicals tested.

Table 5. Effect of fungicides on germtube elongation of *Bipolaris sorokinianum*.

Amount of fungicide required to reduce germtube inhibition by 50 and 80 percent:		
Fungicide	50 %	80 %
Carbendazim	<50.00	194.70
Propiconazole	<50.00	461.80
Frutriafol	109.23	873.80
Triadimenal	95.60	881.50
Prochloraz	<50.00	171.60

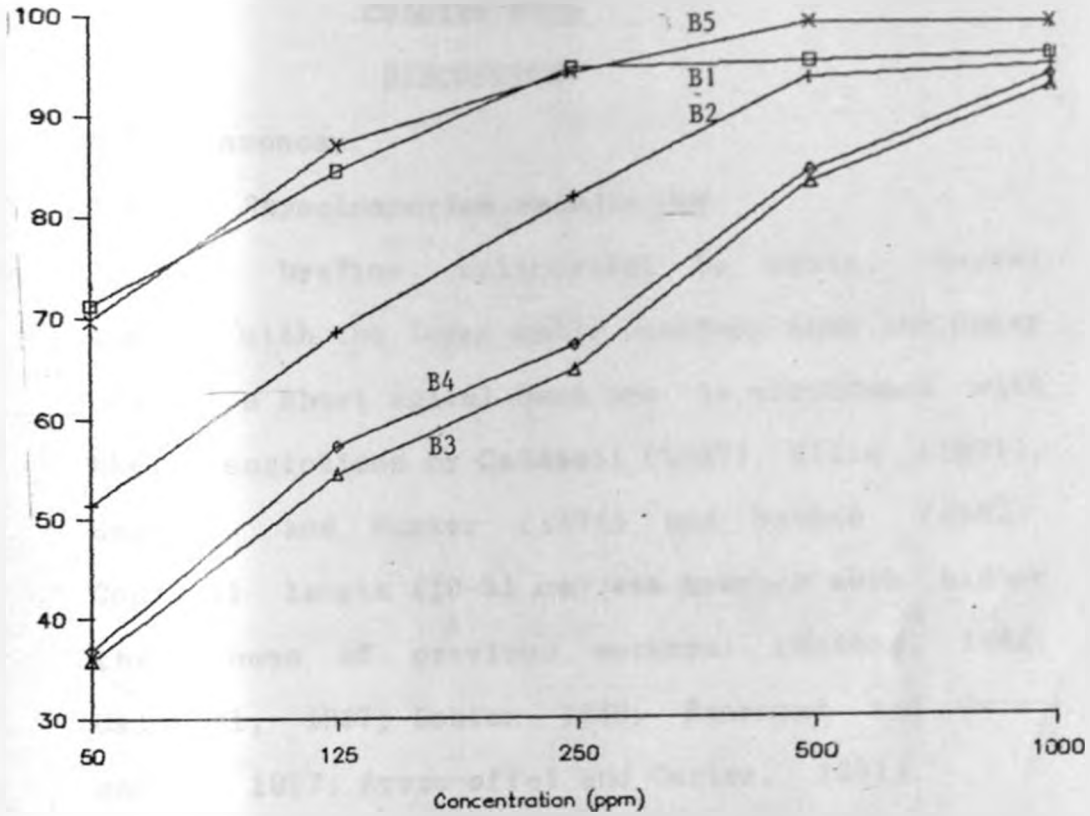


Figure 15.

Effect of various fungicides on germtube inhibition of *Bipolaris sorokinianum*. B1, Carbendazim; B2, Propiconazole; B3, Frutriafol; B4, Triadimenal; B5, Prochloraz.

5.0 CHAPTER FIVE

DISCUSSION

5.1 Taxonomy.

5.1.1 *Rhynchosporium secalis* Oud.

The hyaline, cylindrical to ovate, curved conidia with the lower cell narrower than the upper one and a short apical beak are in accordance with the descriptions by Caldwell (1937), Ellis (1971), Barnett and Hunter (1971) and Mathre (1982). Conidial length (10-31 µm) was however much higher than those of previous workers: (Mathre, 1982; Caldwell, 1937; Schien, 1960; Skoropod and Grinchenko, 1957; Ayesu-offei and Carter, 1971).

Conidia from the host plants were shorter and thicker than those from culture medium. Similar differences between isolates from the culture media and host plants have been recorded by other workers as well (Caldwell 1937, Owen 1958). This may be due to the rich Lima bean agar medium used. The colony diameter in lima bean agar averaged 25 mm much similar to those findings of Schien (1960).

R. secalis was isolated from the distinct scald lesions and therefore these lesions can be used in identifying the disease in the field. However, the characteristic scald symptoms take a long time to become distinct (21 days) and hence the need to rely on the isolation of the pathogen in order to

identify the disease early enough.

5.1.2. *Bipolaris sorokinianum* Sorok.

In accordance with Mathre (1982), Lutrell (1963), Drechsler (1923), Barnett and Hunter (1972) and Ellis (1971), conidiophores were simple, macronematous, straight or flexuous, often geniculate, cylindrical, occasionally veruculose, olivacious brown and 74-210 µm.

The average conidiophore lengths were slightly higher from host lesions than from culture media. This was also observed by Drechsler (1923). The number of septations are within the range of 3-13 given by Lutrell (1963), but Mathre (1982) recorded 6-10 septa. The conidiophore widths were comparable with those of Drechsler (1923), Mathre (1982) and Lutrell (1963).

The acropleurogenous, simple, curved, fusiform, obclavate, olivacious brown, mostly smooth, 53-120 x 13.8-26.6 µm and 3-10 septate, conidia of *B. sorokinianum* were in accordance with the descriptions by Ellis (1971), Burnett and Hunter (1972), Drechsler (1923), Lutrell (1963) and Mathre (1982).

Conidial dimensions from the artificial medium were differing with those from the host. This is

because *Bipolaris sorokinianum* is said to sporulate on artificial media and spores instead of being slender and allantoid, become shorter, thicker and nearly straight (Lutrell, 1955 and Drechsler, 1923).

Conidial germination was found to be polar. Drechsler (1923) reckoned that mature *B. sorokinianum* conidia and closely related species have two perceptibly thin places at the periphery wall, these being located at the tip and at the base of the spore, in the later case occupying a narrow zone adjacent to and surrounding the helium. Here, normal germination takes place by the production of one germtube from the thin region at the tip, or that near the helium, or more often from both. This characteristic distinguishes the pathogen from the other barley pathogens like *Helminthosporium teres* and *H. gramineanum*. This is a good characteristic because it takes about 72 hours to get the conidia to germinate and it can be exploited in distinguishing the disease in instances of doubt.

Conidia of *B. sorokinianum* was isolated from the spot blotch lesions. This, coupled with the fact that the lesions are distinct after the third day of inoculation makes the identification of the disease using the symptoms easy and reliable.

5.2. Sensitivity tests with *Rhynchosporium secalis* Oud

R. secalis was known to be inhibited at all the concentrations of Prochloraz (Sportak) tested. Gallimore *et al*, (1987) also demonstrated a high level of sensitivity of *Pseudocercosporella herpotrichroides* isolates to Prochloraz. These *in vitro* bioassays give a baseline study and provide useful basis for latter comparative studies. Should disease control failure be reported, prior information on the sensitivity of the pathogen population is needed to determine whether it is a result of the emergence of fungicide resistance.

The results in table 1 indicate that Triadimenal and Frutriafol have the highest ED 50 while Carbendazim and Propiconazole have the lowest. Hollomon *et al*, (1987) had found Propiconazole to be a more active fungicide *in Vitro* as compared to Triadimenal. He also found Carbendazim to be very effective in inhibiting isolates of *Rhynchosporium secalis in Vitro*. Frohberger (1978) found Triadimenal (Baytan) to have relatively little effect at concentrations of less than 100ppm on mycelial growth and spore germination of most economically important pathogenic fungi *in vitro* but have exceptionally good effect *in Vivo*. Therefore, *in vitro* tests cannot be exclusively used to delimit

the effect of these fungicides against the plant pathogens. _ Davidse (1982) reported that Carbendazim interferes with mitosis in fungi and they specifically inhibit microtubule assembly Leading to interferences with a great number of processes where they are involved in nuclear formation, cellular migration and organelle movement. This may explain why Carbendazim had the lowest ED 50 because colony formation is a consequence of these mitotic processes.

Triadimenal and Frutriafol (triazoles) specifically interfere with ergosterol biosynthesis and under practical conditions the developmental stage mostly inhibited is the formation and proliferation of subcuticular stroma (Sherald, 1973; Schwinn, 1983). They therefore do not have high inhibitory effects on colony formation and hence the observed high ED 50.

From their ED 50 (table 1) values, it can be seen that these fungicides are very effective in controlling *R. secalis*. The question is why they do not seem to be controlling *R. secalis* in the field. However in the field these systemic fungicides have to be transported to the site of action and they have to accumulate sufficient concentration of the active substance in order to effect the inhibition effects.

The effect of time was not significant for Propiconazole, and therefore, the fungicide was effective for a long time (figure 4). Figures 5, 6 and 7, show that the fungus was able to overcome the fungistatic effect of Carbendazim, Frutriafol and Triadimenal and therefore the number of colonies increased with time. This is especially true for the dilutions of 250 ppm and below. The explanation is that either the fungus is able to metabolise these fungicides or it is able to tolerate them with time. This may be the reason why the control of *R. secalis* in the field seems to be declining especially when there is the effect of rain, dilution of the fungicide in the host plants and the partitioning effects once inside the plant.

Table 2 shows that the fungicide causing maximum inhibition (Dmax) doubled for all the fungicides (Propiconazole, Frutriafol and Triadimenal) except for Carbendazim after the first week of incubation. This is a very encouraging result for Carbendazim whose Dmax was 100 % at 12.5 ppm which is much lower than the recommended dosage of about 1000 ppm. The low dose can provide a plant with about three weeks of protection against infection. Although there is considerable dilution of the active ingredient of systemic fungicides in the growing plants, this is very low concentration and hence can compensate for the dilution effect

(Flohberger, 1978).

Rhynchosporium secalis grows both horizontally and vertically on agar medium. On fungicide amended agar medium, the hyphae tend to make minimal contact with the medium surface. The fungicide dose reaching the aerial hyphae is therefore reduced and hence explaining why there were many poorly developed colonies. The above phenomenon has also been observed by Waterfield and Sisler (1989) while working with the fungus *Sclerotium rolfsii*. These in *Vitro* results can not therefore be exclusively used in drawing conclusions over the effects of these fungicides against *R. secalis in Vivo*.

5.3. Sensitivity tests with *Bipolaris sorokinianum* Sorok mycelial growth.

Table 3 indicates that the most active fungicide was Prochloraz followed by Carbendazim, Propiconazole, Frutriafol and finally Triadimenol. Other researchers working with either two or more of the above fungicides found the same trend (Leroux *et al*, 1988; Köller and Wuben, 1989 and Bateman *et al*, 1990). Based on these *in vitro* tests, one may be tempted to conclude that Prochloraz and Carbendazim are more effective than Frutriafol and Triadimenol. However, this might be misleading because

Triadimenal has been shown to be less effective *in vitro* than *in vivo* (Frohberger, 1978).

The results in figure 9 show that there was a lag period (2 days for dilutions below 250 ppm and 4 days for 500 and 1000 ppm) when probably the fungus was adjusting to the new environment and with time, the percentage inhibition progressively decreased. This may mean that this fungicide has fungistatic effect on *B. sorokinianum* which are overcome with time. Mercer (1971) showed that the systemic movement of thiophanate methyl fungicides (Carbendazim included) is restricted to the transpiration stream and hence the compounds are unlikely to move from a sprayed leaf into the new growth. This, coupled with the fact that the fungus is able to overcome the fungistatic effects of Carbendazim means that a latter attack of the disease will probably require a latter spray application to check its spread. Hence the importance of correct timing of the fungicide application and the monitoring of the disease in the field.

It was observed that the higher the fungicide concentration, the longer the lag phase. This is because fungistatic effect is increased with high concentration of the fungicide. Therefore the

fungus is temporarily inhibited or is adjusting to the new adverse environment and hence taking a longer time to grow.

Sheridan and Grbavac (1985) found Triadimenal to be cultivar specific in New Zealand. None of the new barley cultivars now in use in New Zealand respond to Triadimenal for net blotch control and this fungicide should never be used. In Kenya Triadimenal has been used indiscriminately against scald and Spot blotch. Little, if any, is known on its effectiveness on various barley Cultivars. A field study is therefore required to simulate the effect of this fungicide on the various cultivars being grown.

B. sorokinianum was found to have two isolates which responded differently to these fungicides. The first isolate was more sensitive and its growth was checked by these fungicides. The second isolate seemed to be insensitive to these fungicides. This may explain why the control appear to be failing in the field, especially when the insensitive isolate is the dominant one at a particular time or season. However it is still too early to conclude that there is some sort of resistance. A study is inevitable so as to ascertain whether there is an emergence of resistant sub-population . It would also be of importance to know whether the resistant sub-

population would survive and become economically significant under field conditions.

The fungicides used in this study have the same mode of action (except Carbendazim). It would mean that repeated use of these fungicides may increase selection pressure and enhance the resistant sub-population. I would, therefore, recommend to the Kenya Breweries Ltd. to advice farmers to use fungicides with different modes of action alternatively, or use recommended fungicide mixtures in controlling *B. sorokinianum*. This will reduce the selection pressure of the resistant sub-population.

5.4. Sensitivity tests with *B. sorokinianum* conidia.

The results in Table 4 indicate that none of the tested fungicides gave 100 % inhibition of conidial germination. Ishii *et al* (1990) noted that ergosterol biosynthesis inhibitors did not inhibit germination completely. Other authors have also confirmed this result (Pontezen and Scheinflug, 1989; Papas and Fisher, 1979 and Siegel, 1981). This could possibly be due to their mode of action. It is not the inhibition of the sterol C-14 demethylase itself which causes the inhibition of spore germination, mycelial growth or germtube growth, but the extent of sterol precursor

accumulation in proportion to C-14 desmethyl sterols present (Nes, 1973 and Siegel, 1981). This indicate that there is always a time lag between the onset of enzyme inhibition and the beginning of the inhibition of the germtube elongation depending on the rate of sterol biosynthesis, rate of sterol degradation and the sterol reserves of the spores. Spore germination therefore is not inhibited by triazole fungicides. This may imply that there is lack of control of *B. sorokinianum* in the field. The importance of this implication may be appreciated when the two types of strain isolated from the field are considered. For the first isolate, although control may fail at the conidial stage, the mycelial stage is appreciably inhibited. But the second isolate (which was less sensitive at mycelial stage) may be difficult to control.

Comparisons of table 4 and 5 show that spore germination required higher concentration than germtube inhibition. This has also been reported by other authors (Waterfield and Sisler, 1989; Sherald *et al*, 1974; Brandes and Paul, 1981 and Richmond and Pring, 1984). Mcquiken *et al*, (1988) reckoned that sterol biosynthesis fungicides inhibit sterol biosynthesis and cell wall formation and hence reduce cell length rather than cell number or clamp formation. Sterol synthesis is

required for cell extension growth only. So, nuclear division and clamp formation are unlikely to be affected by ergosterol biosynthesis inhibitors. This may explain why inhibition of spore germination requires high concentration of fungicides as compared to germtube inhibition. This implies that even if the fungus is not checked at conidial stage, its germtube is inhibited and hence effecting disease control in the field.

Some fungal spores have been shown to have reserves of ergosterol making the fungal spore tolerant to the fungicide but the germtube emerging is appreciably more sensitive (Waterfield and Sisler, 1989).

Measurement of germtube lengths therefore can give a more reliable data on effects of demethylation inhibiting fungicides than spore germination. This has also been observed by Ishii *et al* (1990).

CONCLUSION

From the results, it has been established that in the laboratory studies the five fungicides are effective against both *R. secalis* and *B. sorokinianum* but the reason for their ineffectiveness in the field is yet to be determined. These experiments have been done with the fungi in direct contact with the fungicides. However this is not the case in the field where the fungus is inside the host thus bringing in the issue of the dilution effects, redistribution of these systematic fungicides, and the time of the fungicide application.

B. sorokinianum had two isolates from the field differing in their sensitivity towards these fungicides. The less sensitive isolate may be the dominant one in the field at a particular season thus spraying becomes ineffective.

The leaf spot diseases caused by *R. secalis* and *B. sorokinianum* are attributed to primary infection of the seedlings, resulting from the infected seeds and to secondary infection of the growing plant. Two lines of procedure in attempting their control are indicated. To prevent primary seedling infection, the use of pathogen free seeds by all growers is recommended. Seed dressings with fungicides serve in controlling the primary seedling infection. Foliar

sprays control secondary infection. But the foliar sprays have to be timed in that they are done before symptom development. Early spraying risk being washed away by the rains or even being metabolised once inside the plant. This calls for a study which would be able to single out the disease cycle and the right time when a fungicide application will be economically viable.

All in all, the fungicides were found to have the same trend in their effect against these two pathogens. Prochloraz was the most active. However for carbendazim and propiconazole the IG 50 % (inhibition of growth by 50 %) for *Rhynchosporium secalis* were much lower as compared to those of *Bipolaris sorokinianum*.

Mycelial growth and colony formation were more sensitive than spore germination.

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APPENDIX 1

Lima Bean Agar.

Lima bean agar (Difco)	23g
Distilled water	1L

Heat to boiling to dissolve the medium. Autoclave for twenty minutes at 121°C and 15 lb pressure. Set the medium to cool in a water bath (50°C) and pour into sterile petri plates.

APPENDIX 2

Potato Carrot Agar

potato	20g
Carrot	2g
Agar	20g
Distilled water	1L

Boil the grated vegetables in distilled water for one hour. Then drain through a fine sieve. Add agar and adjust to one litre with more distilled water. Sterilise in an autoclave for twenty minutes at 121°C and 15 lb pressure. Set to cool in a water bath (50°C) and pour into sterile petri dishes.