# IDENTIFICATION, PATHOGENICITY AND MANAGEMENT OF FUNGI ISOLATED FROM CASTOR (*Ricinus communis* L.) IN SAMARU, NIGERIA

**By**

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## DECLARATION

I declare that the work in the thesis entitled 'Identification, pathogenicity and management of fungi isolated from castor **(**R*icinus communis* L.) in Samaru, Nigeria' has been performed by me in the Department of Crop Protection under the supervision of Dr (Mrs) A.B. Zarafi and Prof (Mrs) O. Alabi.

The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at any university.

| | | |
|---|---|---|
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## CERTIFICATION

This thesis entitled "IDENTIFICATION, PATHOGENICITY AND MANAGEMENT OF FUNGI ISOLATED FROM CASTOR (*RICINUS COMMUNIS* L.) IN SAMARU, NIGERIA" By Mamza, Wadiam Sebastin meets the regulations governing the award of degree of Master of Science (Crop Protection) of Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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## DEDICATION

This work is dedicated to my son Jantiku Sebastin Mamza for his patience and understanding throughout my period of study.

**ACKNOWLEDGEMENT**

I am most grateful to the Almighty God for His protection and guidance throughout this period of study. I wish to express my profound gratitude to Dr (Mrs) A.B. Zarafi, my major supervisor for her deligent and untiring supervision of this thesis. The same sincere appreciation is extended to Prof (Mrs) O. Alabi for her valuable contributions during the course of this research work. I am indebted to Dr A.A. Ochigbo, Executive Director NCRI, Badeggi and the management of NCRI for giving me the opportunity to go on study leave. I am grateful to the International Mycological Institute (IMI), Egham London for identification of the pathogen to specie level. I am grateful to Mr Auta Audu for identifying the pathogen to genus level. I am deeply indepted to Dr S.M. Misari for his encouragement and support throughout my period of study. Special thanks to Drs B.D Kashina, L.J Bamaiyi, Messrs S.E.L Alao, Abdulrahaman Ahmed, Abdullahi Maji, Usman Tanimu, James Negedu, Mrs Maria Akpa, Mrs Aishatu Hamman, Mrs J. Uwakwe and other members of Crop Protection Department whose names I am not able to list individually but contributed to the success of this work in one way or the other. My profound gratitude and thanks also goes to my husband who was so patient to take care of the family during the period I was away and for his encouragement and moral support. To my parents, Mr and Mrs A. Waba for their love and to see it fit that I am educated. My sisters and brothers who gave me the encouragement. Special appreciation to Mrs Martha Misari for her constant support and care during the period of study. Sincere gratitude to my classmate, Saluadeen Mohammed and all my friends who encouraged me during this period. Sincere appreciation to Mr Mathias Kasa for the excellent job in typesetting this thesis.

## ABSTRACT

The fungi isolated from infected castor leaves were *Fusarium* sp, *Helminthosporium* sp and *Curvularia* sp. Pathogenicity tests was conducted on the fungi and *Fusarium* sp was pathogenic on castor and its pathogenicity was confirmed using Koch's postulate. The *Fusarium* sp was later identified to be *Fusarium pallidoroseum* by International Mycological Institute (IMI) Egham, London. Plants at different ages (11, 18 and 25 days old) were inoculated using three methods (spray, smear and soil inoculation). Twenty one day old castor plants were inoculated with inoculum at different densities ($10^3$, $10^5$, $10^7$, $10^{10}$ conidia / ml and water) and assessed in order to determine at what density the pathogen can induce infection. Six fungicides were evaluated *in vitro* at three concentrations (one and half the recommended, recommended and half recommended rates) for their effect on growth and regrowth of mycelia, sporulation and conidia size of *Fusarium pallidoroseum*. Five of the fungicides tested in the laboratory were further evaluated at two concentrations (recommended and half recommended rates) for their efficacy in the management of leaf blight induced by *Fusarium pallidoroseum in vivo.* Effect of three sowing dates on disease incidence and severity was assessed on two castor types during the 2006 wet season. During the pathogenicity tests it was observed that, younger plants were more susceptible to infection than older plants. For the *in vitro* evaluation of fungicides, it was observed that all the fungicides (benomyl, benomyl + thiram, mancozeb, metalaxyl-M + thiomethoxam + difenoconazole, tricyclazole and carbendazim + mancozeb) at all the concentrations tested inhibited mycelial growth, sporulation, and reduced conidia size and mycelial regrowth of the fungus. Benomyl, benomyl + thiram and tricyclazole completely inhibited mycelial growth and sporulation

of the fungus at the three tested rates. Metalaxyl-M + thiomethoxam + difenoconazole, carbendazim + mancozeb inhibited mycelial growth and sporulation only at one and half rate but not at recommended and half recommended rates. Inhibitory effect of all the fungicides on mycelial growth and sporulation were greatest at one and half recommended rate. Benomyl, benomyl + thiram, mancozeb, carbendazim + mancozeb and tricyclazole reduced the rate of disease development on 21 - day- old inoculated castor plants in the screenhouse at recommended than at half the recommended rate. Large seeded castor sown on July 27 had less disease severity and higher yield compared with plants sown in August 3 and August 11. For the small seeded type, August 3 sown plants had high disease severity and more yield than those sown on other dates. *F. pallidoroseum* is the cause of leaf blight, seedling rot and wilt of castor in Samaru. The younger plants (11 days old) were more susceptible to infection. The evaluated fungicides can be used to control *F. pallidoroseum* on castor. Sowing late, resulted in poor seed germination thereby affecting bean yield negatively.

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# CHAPTER ONE

## 1. INTRODUCTION

Castor, *Ricinus communis* L., a monotypic genus belongs to the family Euphorbiaceae. The plant varies greatly in growth habit, colour of foliage, stem, seed size and colour, oil content and many other characteristics. It is widely cultivated in the tropics, sub tropics and warm regions for its seed from which castor oil is extracted (Purseglove, 1968; Weiss, 1971). Castor can thrive well in dry arid climate as well as areas of heavy rains and floods (Weiss, 1971). It requires low temperature (15 - 18 $^{o}$C) for emergence. A moderate temperature of 20 - 26 $^{o}$C is favourable for flowering. In its natural growing area, the plant is perennial forming a tree stature but commercially treated as annual or biennial and exhibits a bushy appearance (Atsmon, 1989; Moshkin, 1986; Weiss, 1971). Seed colours ranges from light to dark brown, with various mottling patterns. High rainfall and humidity favours leaf diseases (Hiruy and Alemaw, 1989). The important part of the crop is the seed which contains 50-55 % oil (Duke, 1983; Adefris and Nigussie, 1993; Gobin *et al.,* 2001).

Traditionally, castor oil is used in lamps lightening and in medicine. The oil is used in industries as cosmetic base, pharmaceuticals, high grade lubricant and protecting coatings in paints (Purseglove, 1968; Gobin *et al*., 2001; Duke, 1983).

Castor is attacked by many pests including insects, nematodes, fungi, bacteria and viruses (Duke, 1983). The major insect pests are the semi lopper *(Achoea janata*), capsule borer *(Dichrocrocis punctiferalis*) (Patel *et al.*, 1991; Nanda and Prasad, 1974). The most important bacterial disease is leaf spot caused by *Xanthomonas ricini* (Anon. 1971).

Fungal diseases include wilt caused by *Fusarium oxysporum* f. sp *ricini*, *r*oot rot *(Macrophomina phaseolina*), gray rot (*Botrytis ricini*) which caused yield loss of 80 - 100 % in India (Anjani *et al.*, 2004), seedling blight caused by *Phytophthora palmivora* in Hawaii (Uchida and Aragaki; 1988), leaf spot induced by *Cercospora ricinella* and Alternaria leaf spot caused by *Alternaria ricini* (Duke, 1983; Anon. 1971).

## 1.1 Justification of Study

Castor oil which is very important in industries as cosmetic base, high grade lubricant and as water resistant and protective coating in paints have suffered some set backs due to diseases. In India, yield loss of 80 - 100 % has been attributed to fungal diseases (Anjani *et al.*, 2004) and these have caused economic loss to farmers in terms of foreign exchange. In Nigeria, with the recent demand for castor oil in industries and the Federal Government in its effort to diversify the economy is encouraging its production for local industries and as a source of foreign exchange.

In view of the emerging importance of the crop and with little or no information on the various diseases affecting its growth and reproduction in Nigeria there is need to identify fungi associated with castor and the management of diseases induced by them.

## 1.2 Objectives of Study

The objectives of this study are to:

Isolate and identify fungi associated with castor and ascertain their pathogenicity.

Determine minimum infectious inoculum density of fungi on castor.

Evaluate *in vitro* and *in vivo* effects of some fungicides on the pathogen.

Determine the effect of sowing dates on disease incidence and severity.

# CHAPTER TWO

# 2.0 LITERATURE REVIEW

## 2.1 Origin and Distribution of Castor

*Ricinus communis* originated from Ethiopia and grows wild in East and North Africa (Adefris and Nigussie, 1993). It was first cultivated in ancient Egypt as far back as 4000 BC (Purseglove, 1968; Gobin *et al*., 2001). In Nigeria, castor is an indigenous source of vegetable oil used in industries due to its high oil content. However, its potentials under Nigerian conditions are yet to be fully exploited (Yayock, 1985).

## 2.2 Ecology of Castor

Castor requires warm climate and at least 140 – 180 days growing period and can be grown over a wide altitude range in the tropics and sub tropics with both low and medium rainfall (Purseglove, 1968; Weiss, 1971; Gobin *et al.*, 2001). It grows best in deep well drained soils which encourages deep and drought resistant root system (Duke, 1983; Gobin *et al*., 2001). Castor is grown on sandy and clayey soils, and thrives well on poor soils (Kulkarni, 1959; Duke, 1983).

The castor plant which takes 5 – 8 months to mature is usually sown around June – July or September – October under rainfed and irrigation respectively in India (Duke, 1983; Gobin *et al.,* 2001). The seeds are sown in rows 1 – 2 meters apart for tall varieties, and 40 - 50 cm intra row spacing for the dwarf types (Kulkarni, 1959). Soaking the seed for about 12 hours prior to sowing has been found to improve germination (Yegna, 1965).

## 2.3 Botany of Castor

The wild cultivars are short-lived perennials growing up to 9 to 12 meters tall, with well developed tap and lateral roots. The cultivated varieties are annuals with height of between 1 and 7 meters. The leaves are large laminate carried on leaf stalks coming off the main stem and branches. Castor is monoecious and is mostly wind pollinated although cross pollination is common due to activities of bees and the flower nectar is used by bees to produce excellent honey (Purseglove, 1968; Gobin *et al.*, 2001). The fruit is green when unripe, brown when matured and each capsule contains 3 seeds, which are 0.5 – 1.5 cm long (Purseglove, 1968; Duke, 1983). Each seed has a shape of a well-fed tick with colour varying from pale grey, brown or almost black with darker mottlings (Purseglove, 1968). Castor seeds contain between 50 - 55 % oil and a toxin ricin, which is poisonous, (causes blood coagulation) (C.S.I.R., 1948-1976; Duke, 1983). Upon extraction of oil, the toxin is eliminated (Duke and Wain, 1981).

## 2.4 Production and Yield of Castor

The demand for castor seeds and oil in world market has increased greatly over the years (Adefris and Nigussie, 1993). In 1960s, World production had reached over 500,000 tons with India and Brazil producing very large quantities (C.S.I.R 1948-1979). The average seed yields in pure castor stands vary from 500 kg/ha in India to 1000 kg/ha in Thailand (Gobin *et al.,* 2001). Under improved cultural management with irrigation, yields of 2,500 – 3000 kg/ha were achieved in USA (Duke, 1983; Gobin *et al.,* 2001). World production is about 25296 MT per annum with Africa producing 1863MT (FAO, 2003). The three largest importers of castor seeds and oil are the United States, France and

United Kingdom with India (1087 kg/ha), China (993 kg/ha) and Brazil (823 kg/ha) being the largest growers and exporters (FAO, 2005).

## 2.5 Economic Importance of Castor

Traditionally, castor oil is used for medicine and lightening of lamps in Ethiopia, ancient Egypt and the Mediterranean (Eshete, 1987). It is used in the industries as cosmetic base, pharmaceuticals, high grade lubricant and water resistant coating in paints (Purseglove, 1968; Duke, 1983; Gobin *et al.*, 2001). The pomace or cake residue is used as organic fertilizer (Duke, 1983).

Once toxin is removed from the seed by detoxification, the seed cake can be fed to livestocks (C.S.I.R. 1948 - 1976; Devendra and Raghavan, 1978). In Nigeria, shelled castor seeds when well processed through fermentation are used as soup condiment (Onyeike and Onwuka, 1999). The stems can be made into wall and paper board (Purseglove, 1968; Duke, 1983; Gobin *et al.*, 2001).

## 2.6 Insect Pests and Diseases of Castor

Reports from African Centre of Diversity showed that castor is tolerant to high soil pH, heat, drought, bacteria, fungi, insects, viruses among others (Duke, 1978). However, castor plant is reported to be affected by insect pests (Patel *et al.*, 1991; Nanda and Prasad, 1974; Gobin *et al.*, 2001). The insect pests are *Achoea janata* causing leaf defoliation, *Dichrocrocis punctiferalis* which attacks seed capsules, green stinkbugs, leaf-hoppers, leaf-miners and grasshoppers which feed on leaves (Duke, 1983). There was also a report on the incidence of leaf defoliator (*Achoea janata)*, capsule borer (*Dichrocrocis punctiferalis)* in Samaru, Nigeria (Anon., 1994).

The fungal diseases are Cercospora leaf spot (*Cercospora ricinella*), attacked leaves are covered with a large number of roundish or irregular spots which wither and dry up fast. Seedling blight (*Phytophthora colocasiae*) attacks leaves of seedlings and older plants, it can also infect other crops like potato, tomato, pepper, sesame which serve as collateral or alternative hosts plants (Duke, 1983; Gobin *et al.,* 2001). Anjani *et al*. (2004) reported that the major diseases of castor in India are wilt (*Fusarium oxysporum* f.sp *ricini*), root rot (*Macrophomina phaseolina*) and gray rot (*Botrytris ricini*) and they can cause 80 – 100 % crop damage. *Fusarium orthoceras* and *F. semitectum* has been isolated from the blackened and decayed roots of castor beans (*Ricinus communis*) suffering from wilt at Ashdot Ya'aqov in the Jordan Valley, where the plant is used as a windbreak in banana and orange plantations (Reichert,1940).

The most destructive diseases in the United States of America are seedling blight and leaf spots caused by *Xanthomonas campestris* pv *ricinicola* and *Alternaria ricini* respectively (Duke, 1983). Others are soil borne diseases such as root rot and charcoal rot caused by *Phymatotrichum omivorum* and *Macrophomina phaseolina* respectively (Duke, 1983). *Macrophomina phaseolina* has also been found to be the causal organism of wilt in castor in South Africa, Costa Rica and Brazil (Grezes- Besset *et al.,* 1996). In Nigeria the greatest pest threat is the capsule borer (*Dichrocrocis punctiferalis*), and the diseases are *Alternaria* leaf spot (*Alternaria* sp) and bacterial leaf spot (*Xanthomonas* sp) (Anon., 1971).

Some notable diseases caused by *Fusarium* species are crown and root rot of tomato caused by *Fusarium oxysporum* f.sp *radicis lycopersici* (Booth, 1971). Other diseases include, the Panama disease or vascular wilt of Banana or Moko disease caused by *F.*

*oxysporum* f.sp *cubense*, the vascular wilt of cotton caused by *Fusarium oxysporum* f.sp *vasinfectum* which has also been recorded on *Cajanus* spp, *Coffea* spp, *Hevea* spp, *Hibiscus* spp, *Ricinus communis*, *Solanum* spp and *Vigna* spp (Synder and Hansen, 1940). *Fusarium pallidoroseum* formally called *F. semitectum* has been reported to be pathogenic on castor causing seedling blight and leaf rot especially during adverse environmental conditions. Although the pathogen has been reported to be a weak parasite (Booth, 1971), it has caused stalk rot of maize in Australia (Francis and Burgess, 1975), pod rot and collar rot of soyabean in India (Saharan and Gupta, 1972), damping-off and root-rot of beans in United Arab Region (El-Helaly *et al.*, 1971), stump disease of coffee in Kenya (Mulinge, 1971). *F. pallidoroseum* has been isolated from sorghum showing stalk rot symptom (Hindeker and Anahosur, 1994). Also *F. semitectum* var. *majus* was identified as soil-borne pathogen of *Striga hermonthica* in the Sudan. Complete control of Striga and enhanced host growth were achieved when 10 g of inoculum per kilogram of soil was incorporated before planting (Abbasher *et al.*,1996) The pathogen is soil borne but can also be transmitted by water and seed (Booth, 1971).

## 2.7 Taxonomy and Characteristics of *Fusarium* sp

### 2.7.1 Nomenclature and taxonomy of *Fusarium* sp.

*Fusarium* sp. belongs to the order Hypocreales of the family Hypocraceae of the genus *Fusarium* with over twenty species (de Hoog *et al*., 2000). The most common species are *Fusarium solani, F. oxysporum, F. chlamydosporum, F. moniliforme, F. vasinfectum, F. pallidoroseum* and others (Nelson *et al.*, 1981).

### 2.7.2 Morphological and cultural characteristics of *Fusarium* sp.

The mycelia of *Fusarium* sp are septate or non septate and consist of condiophores which are variable. The conidiophores could be slender, short and simple or short, branched irregularly or bearing a whorl of phialides, single, or grouped into sporodochia. Phialides are cylindrical, with a small collarette, solitary or produced as a component of branching systems (Booth, 1971). Conidia are phialospores hyaline variable principally of two kinds that is macro and micro. The macro conidia are several celled slightly curved or bent at the pointed ends typically canoe shaped and has a width and length of 2.5 - 4 and 11 - 70 μm respectively (C.M.I. description, 1978; Barnnet and Barry, 1998; Sutton *et al.,* 1998). The microconidia 2-4 x 4 – 8 μm are formed on long or short simple chain, one celled, ovoid or oblong and hyaline (Barnnet and Barry, 1998; Sutton *et al.,* 1998). Chlamydospores when present are sparse in chains, thick walled, hyaline, intercalary or terminal (Sutton *et al.,* 1998; Booth, 1971). When grown on potato dextrose agar (PDA), colonies of *Fusarium* sp are usually fast growing, covering 9 cm Petri-dish within five days at 25 – 28 $^{o}$C and producing pale or brightly coloured cottony aerial mycelium (Booth, 1977). The thallus varies from whitish to yellow, brownish, pink, reddish or lilac shades (Booth, 1977; Burgess and Liddell, 1983). When grown on sabouraud dextrose agar at 25 $^{o}$C, it produces wooly to cottony, flat, spreading colonies (Sutton *et al.,* 1998).

## 2.8 Disease Inducement as Influenced by Inoculum Concentration.

Disease inducement varies with inoculum concentration as well as the pathogen involved. For instance work by Aleck and Harrisson (1978), Bain *et al.* (1990) on blackleg of potato induced by *Erwinia carotovora* development on the field, was due to

high inoculum density on the seed tubers and environmental conditions such as high soil moisture and low temperature during the growing season. In a similar work, Bdliya and Langerfeld (2005), in their study reported that blackleg of potato development was high at the higher inoculum density of $2.3x10^9$ cells/ml, though it did not differ significantly from that of the lower bacterial concentration of $2.3x10^5$ cells/ml. At 3 days after planting (DAP) incidence of tuber seed piece rot significantly increased with increase in inoculum density. There was rise in the incidence of seed piece rot at 6 days after planting (DAP) for both inoculum densities. However at 6 days after planting (DAP) there were no significant differences between the two inoculum densities. At 12 days after planting (DAP), incidence of seed piece rot reached 100 % regardless of inoculum densities.

### 2.9 Chemical Control of Some Diseases Induced by Fungi

Fungicides have been evaluated for the control of different foliar diseases of plants, both *in vitro* and *in vivo*. Benomyl (Benlate 50 WP Dupont) has been used to control sugarbeet leaf-spot caused by *Cercospora beticola* (Karaoglanidis *et al.,* 2003). The use of benomyl, carbendazim and mancozeb at three rates (10,000, 25,000 and 50,000 ppm) significantly inhibited *Physoderma maydis* (maize brown spot) (Osunlaja and Alamuta, 1999). Complete inhibition of sporangia germination was at 10,000 ppm a.i. However, carbendazim at 50,000 ppm a.i was effective in the control of the disease on field which resulted in highest grain yield.

Foliar sprays of groundnut for the control of *Cercospora* leaf spot with benomyl (Aulakh and Sunaur, 1973) and carbendazim or benomyl (Vaishnau *et al*., 1982) has been found

to reduce disease incidence and gave significant increase in number and weight of pods per plant. Similarly benomyl was found to inhibit the growth of *Fusarium moniliforme* and *Fusarium roseum in vitro* (Khanna and Chandra, 1976). It was used to control banana fruit rots induced by both pathogens. Benomyl was also effective in the control of *Colletotrichum graminicola* on sorghum (Misra and Siradhana, 1978). *In vitro* use of benomyl was found to completely inhibit the growth of *Stenocarpella maydis* at 5 g, 10 g and 20 g a. i. /ha (Marley and Gbenga, 2004). Similarly, benomyl was inhibitory on the mycelial growth of *Colletotrichum capsici* (Joshi and Wangikar, 1979; Alafiatayo, 1982). Also Adeoti (1991) reported that carbendazim and benomyl completely inhibited the mycelial growth of *Coniella musaiaensis* on PDA even at concentration as low as 12.5% the manufacturers recommended dose. Meanwhile, calixin M and mancozeb were less effective during the trial. Delp (1980) also reported that carbendazim plus maneb has been effective in controlling *Cercospora* sp on sugar beets in Greece. However, Yar'adua (1986) evaluated efficacy of some fungicides to control *Alternaria porri* on onions *in vitro* and he observed that benomyl did not completely inhibit the mycelial growth of the pathogen at half the recommended rate (500 ppm).

Mancozeb has been used to control *Alternaria* and *Septoria* leaf spots on tomato and resulted in yield increase (Rajagopal and Vidhyasekaran, 1982). Tologbonshe (1983), in his studies on *Cercospora* leaf spot of egg plant (*Solanum* spp) reported that mancozeb and benomyl completely inhibited the growth of the fungus at the three concentrations tested. Similarly, *Alternaria* leaf spot of safflower caused by *Alternaria carthani* was reported to be effectively controlled by mancozeb 0.25 % (Dithane M-45) and Bavistin 0.1 % and with resultant increased yield of 30.3 and 29.2 % respectively (Patil and

Jadhav, 1985). Emechebe (1981) reported that brown blotch *Colletotrichum capsici* of cowpea was effectively controlled at a 14-day spraying interval with benomyl, carbendazim + maneb and mancozeb. In a similar fungicide trial, Ndedu (1986) reported that high rates such as 3.36 kg/ha wettable powder (WP) of mancozeb (Dithane M45) gave better control of groundnut leafspot and rust.

Similarly, Obagwu (1997), in his findings reported that mycelial growth and sporulation of *Colletotrichum capsici* on bambaranut were completely inhibited *in vitro* even at half the recommended rates of benomyl (Benlate), mancozeb ( Dithane M-45), carbendazim + maneb (Delsene M) and copper oxychloride (Cupravit). Ramachandra, *et al.* (1989), reported the effectiveness of metalaxyl formulations (Ridomil 5 granules and Apron 35 WS) used as seed treatment in the control of rhizome rot of ginger (*Pythium aphanidermatum*). Kochman and Langdon (1986), also reported the efficacy of benomyl, benomyl + lindane, thiobendazole, iprodione on *Sclerotinia* and *Sclerotionum* pathogens of sunflower seed and showed that benomyl, thiobendazole, iprodione, were effective at all the rates used.

# CHAPTER THREE

# 3.0 MATERIALS AND METHODS

## 3.1 Isolation and Identification of Pathogen

Infected castor leaves were collected from the Institute for Agricultural Research (IAR) research fields and brought to the laboratory. The infected leaves were washed, cut into pieces, sterilized for five minutes using 0.5 % sodium hypochloride and rinsed thrice with sterile distilled water (SDW). The pieces were placed in petri dishes containing Potato Dextrose Agar with Streptomycin (PDAs) and labelled. The plates were incubated at 28 $^{o}$C. Fungal mycelia of the isolated organisms were sub-cultured on fresh PDAs to get pure culture. Pure cultures of the organisms were examined using a compound microscope and the fungi identified. Pure cultures obtained were placed in slant bottles and kept for future use. Specimen of the isolated pathogen was sent to International Mycological Institute (IMI), Egham London, for identification.

### 3.1.1 Pathogenicity tests in the screenhouse

#### 3.1.1.1 *Raising of seedlings*

Castor Seeds obtained from infected fields were sown in plastic pots (12 cm diameter) containing sterile soil at three sowing dates of seven days interval in order to have plants of varying ages,11, 18 and 25 days at inoculation. Three inoculation methods, smearing, spraying and soil inoculation were used. One stand per pot was planted and three pots were used for each inoculation method for the different plant age.

### 3.1.1.2 *Inoculum preparation and inoculation of plants*

The isolates obtained in 3.1 were grown on PDAs in the laboratory for 14 days for the organism to sporulate. Ten millilitres of sterile distilled water was added to each Petri dish and mycelial mat from the culture was harvested using a sterile scapel. The mycelia were blended in an electric blender for five minutes, 200 ml of sterile distilled water (SDW) was added in 500 ml conical flask and filtered using a double layer muslin cloth.

To avoid contamination, for each method, plants were inoculated outside the glasshouse. The seedlings of 11, 18 and 25 days old were inoculated by spraying using a hand atomizer. The next sets of plants were smeared by direct application of harvested mycelial mat on the leaves using fingertips fitted with gloves. The soil inoculation was done by pouring 20 ml of inoculum suspension at the base of the stem of each plant. The seedlings for control were treated with sterile distilled water only. The inoculated and control seedlings were covered with polythene bags to increase humidity around the plants and the pots were labelled and arranged in completely randomized design. After 24 hours, the polythene bags were removed for 20 minutes to aerate and water the plants in the mornings and evenings. Two days after inoculation the polythene bags were finally removed and symptom appearance was observed daily. At seven days after inoculation, leaves of plants showing typical symptoms of wilting, leaf spot and stem rot were collected and taken to laboratory for re-isolation of the organism and compared with the original culture to confirm Koch's postulate.

Disease incidence was taken at 7 days after inoculation (DAI) and severity was assessed at 7, 14 and 21 DAI. Disease incidence was determined by counting diseased leaves and

expressing it as a percentage of the total leaves per plant. Disease severity scores were obtained by using a 1-5 scale as described by Tarr, 1981; where:

1 = No symptoms on leaves.

2 = 1- 25 % total leaf number with symptoms.

3 = 26- 50 % total leaf number with syptoms.

4 = 51- 75% total leaf number with symptoms

5 = 76 % or more leaf number with symptoms.

### 3.2 Determination of Inoculum Density Capable of Disease Inducement.

The experiment was conducted in a screenhouse to determine the infectious inoculum density. Seeds of castor obtained from diseased fields were sown in plastic pots (12 cm diameter) containing sterile soil with each pot having two stands. Inoculum was prepared from 14 - day - old culture grown on PDAs using a sterile scapel to harvest the mycelia, blended using an electric blender and sieved with a double layer cheese cloth into a 500-ml conical flask. The spores were counted with the aid of haemocytometer and $3.6 \times 10^{10}$ spores / ml was taken as the highest density ($10^{10}$ ) and serial dilutions of the suspension were made to the desired inoculum densities of $10^3$, $10^5$, $10^7$. At 21 days, the plants were inoculated with the suspension of the inoculum at different density and those sprayed with sterile water served as control. The experiment had three replicates with one pot sown with two plants representing a replicate. Prior to inoculation, plants were sprayed with water and covered with polythene bags for 24 hours to create high humidity and inoculation was done in the evening by spraying 20 ml of the inoculum per plant using a hand atomizer. The inoculation was done outside the screen house for each of the

treatment to avoid cross inoculation. Control plants were sprayed with sterile distilled water only. The pots were labelled and arranged in a Complete Randomized Design. The inoculated and control plants were covered with polythene bags for 24 hours to maintain high humidity. After 24 hours, plants were sprayed with water and aerated for 20 minutes and by 48 hours polythene bags were finally removed. Plants were observed daily for symptoms and at seven days after inoculation disease incidence and severity were assessed.

## 3.3 Evaluation of Fungicides for the Control of Pathogen

### 3.3.1 *In vitro* evaluation of fungicides

Six fungicides were evaluated at three rates, half recommended rate, recommended rate and one and half recommended rate. The fungicides used and their recommended rates were benomyl 0.15g a.i /litre (Benlate 50 WP Dupont), benomyl + thiram 0.8g a.i / litre (Bentex T 40 WP Dupont), carbendazim + mancozeb 1.12g a.i / litre (Team 84 WP African Agro), tricyclazole 0.4 g a.i / litre (Profit 85 WP African Agro), metalaxyl-M + thiomethoxam + difenoconazole 0.04 g a.i / litre (Apron star 42 WS Sygenta) and mancozeb 0.62 g a.i/ litre (Dithane M45 80 WP Dupont). The required quantity of each fungicide for each concentration was weighed and dissolved in five millilitres of ethanol in separate beakers. When it has dissolved completely, each fungicide was made up to 100 ml with freshly prepared and autoclaved Potato Dextrose Agar (PDA) after allowing to cool to a pouring temperature of 40-45 $^{o}$C before streptomycin was added. Using sterilized McCartney bottle for each fungicide concentration, 20 ml of PDAs amended with different fungicides was measured into 9 cm diameter Petri-dishes. Each plate

including the control (without fungicide) on solidification was inoculated in the middle with 14-day-old culture of *Fusarium pallidoroseum* using 0.5 cm cork borer. The labelled Petri dishes were placed in an incubator at 28 $^{o}$C and observed daily for mycelial growth. The Petri-dishes were arranged in completely randomized design with each fungicide as treatment and their rates replicated five times. A Petri dish represented a replicate.

Radial mycelial growth was taken at seven and fourteen days after inoculation, by measuring the diameter along two perpendicular lines, from the underside of the Petri dishes. Cultural growth behaviour of the fungus in different treatment was observed. Sporulation was determined at 14 days after inoculation. For each fungicide and its various rates, conidia suspension was prepared from five Petri dishes. To obtain 10 % spore concentration, 10 ml of the initial inoculum was added to 90 ml of sterile distilled water and the spores counted under light microscope with the aid of a haemocytometer. Means of four readings were taken for each fungicide rate, and the equation; $\frac{n}{256} x4x10^{6}$ was used to calculate the total number of conidia per milliliter (Booth, 1971). Where: n = number of conidia counted in the chambers, 256 = constant volume obtained from 16x16 and $4x10^{6}$ conidia per mls = constant.

Spore size was determined by measuring the length and width of 50 conidia under a microscope with micrometer. The mycelia from each treatment were cultured on freshly prepared PDAs to evaluate the effect of the fungicides on mycelia. This was achieved by using a 0.5 cm cork borer to cut from actively growing portion of mycelia from 14- day-old culture of fungicides treated Petri dishes to the new plates. Five plates were

inoculated making five replicates for each of the treatments. At 14 days after inoculation, mycelial regrowth on the clean plates was observed and their radial growth measured.

### 3.3.2 Evaluation of fungicides *in vivo*

Five fungicides that were evaluated *in vitro* were further screened as foliar spray in the screenhouse at half the recommended and recommended rates. Castor seeds were sown in 12 cm size plastic pots filled with heat sterilised soil and watered as required until plants were 21 days old. A total of 42 pots were used for the experiment and each treatment had three pots sown with two plants with each pot representing a replicate. The pots were arranged in a Complete Randomize Design and each treatment was replicated thrice. Inoculum was prepared from 14 - day- old culture and diluted to a density of $3.6 \times 10^5$ conidia per milliliter. The seedlings were then inoculated except for control plants which were sprayed with sterile water. Seven days after inoculation disease incidence was determined before randomly grouping the pots and fungicides were applied. Fungicide applications were done separately outside the glass house, to avoid drift. Disease severity was assessed at seven days interval for four weeks starting from seven days after fungicide application (DAF). There were check plants which were inoculated with the pathogen but no fungicide was applied and the control plants sprayed with only sterile water and neither inoculation of pathogen nor fungicide applied.

## 3.4 Leaf blight Incidence and Severity on Castor Sown at Different Dates

The experiment was conducted at the Institute for Agricultural Research (IAR) farm, Samaru, Zaria ($11^o11'$N, $7^o38'$E and 686 m above sea level) in the Northern Guinea savanna zone of Nigeria. The effect of sowing dates on disease incidence and severity

was examined using two different castor types sourced locally (large seeded and small seeded). The field was ploughed, harrowed and ridged at 75 cm. The seeds were sown at three different dates of seven days interval (27$^{th}$ July, 3$^{rd}$ August and 11$^{th}$ August) during 2006 cropping season. Each seed type was sown two seeds per stand at 50 cm intra row spacing on 5 m ridges with 5 ridges making a plot. Plots were separated with unsown 1 m alley, making a total of 18 plots in a Randomized Complete Block Design and replicated three times. Three weeks after germination, the recommended rate of fertilizer, 40 kg N, 50 kg $P_2O_5$ and 40 kg $K_2O$ per hectare (Brigham, 1993) was spot applied using 15:15:15 compound fertilizer mixed with Single Super Phosphate (SSP). Hoe weeding was done at 3, 6, 9 and 12 weeks after sowing (WAS).

Germination count was done when plants were 42 days after sowing by counting the total number of germinated plants and expressing it as a percentage of the total plants in each plot. The field was observed weekly for symptom expression. Disease incidence and severity were assessed at weekly interval starting at 57 days after sowing (DAS). Disease incidence was determined by counting diseased plants and expressing it as a percentage of the total expected plants in each plot. Disease severity was assessed by scoring ten randomly selected plants in the 3 middle rows using a 1-5 scale as in 3.1.1.2. After harvest and threshing, bean yield in each plot was weighed and expressed in kilograms per hectare.

### 3.5 Statistical Analysis

All data collected were subjected to statistical analysis (SAS 2002). Where the numbers of treatments were more than six, means were separated using Student Newman Keuls (SNK) and where the treatments were less than six, Least Significant Difference (LSD) was used to compare the means.

# CHAPTER FOUR

## 4.0 RESULTS

### 4.1. Isolation, Identification and Symptoms of the Pathogen

The fungi isolated from the infected leaves were *Fusarium* sp, *Helminthosporium* sp and *Curvularia* sp. The pathogenicity tests showed that the *Fusarium* sp was pathogenic on castor. The *Fusarium* sp was later identified at International Mycological Institute (IMI) Egham, London as *Fusarium pallidoroseum* (Appendix A).

#### 4.1.1 Symptoms

The symptoms on naturally infected leaves were large yellowish brown irregular lesions extending towards the leaf margins and the leaf mid – ribs (Plate I). While on inoculated plants, the symptoms were initially light or pale green irregular lesions of less than 3 mm in diameter around the leaf margin. Later these lesions enlarged advancing towards the leaf mid rib. After 6 – 7 days the leaves became yellowish brown, wilted and finally fell off while the stem showed rot and eventual collapse (Plates II and III).

#### 4.1.2. Morphological and cultural characteristics of the *F. pallidoroseum* on PDA

When the pathogen isolated from naturally infected leaf was grown on Potato Dextrose Agar amended with streptomycin (PDAs) and kept in an incubator at 28 $^{o}$C, the mycelial growth was cottony white. The fungus was fast growing covering a 9 cm diameter petri dish in seven days. Microscopic examination of 14 - day- old culture showed a mass of hyphae which were septate, hyaline and well branched to form sporodochia. Condiophores, phialides, macroconidia and microconidia were also observed (Plate IV). The macroconidia were two to four celled, thick walled, smooth and sickle or canoe

shaped. Microconidia were one celled, smooth, hyaline, ovoid or cylindrical. Chlamydospores were present in sparse or chains and thick walled, hyaline, intercalary or terminal.



Plate I: Naturally infected castor leaf

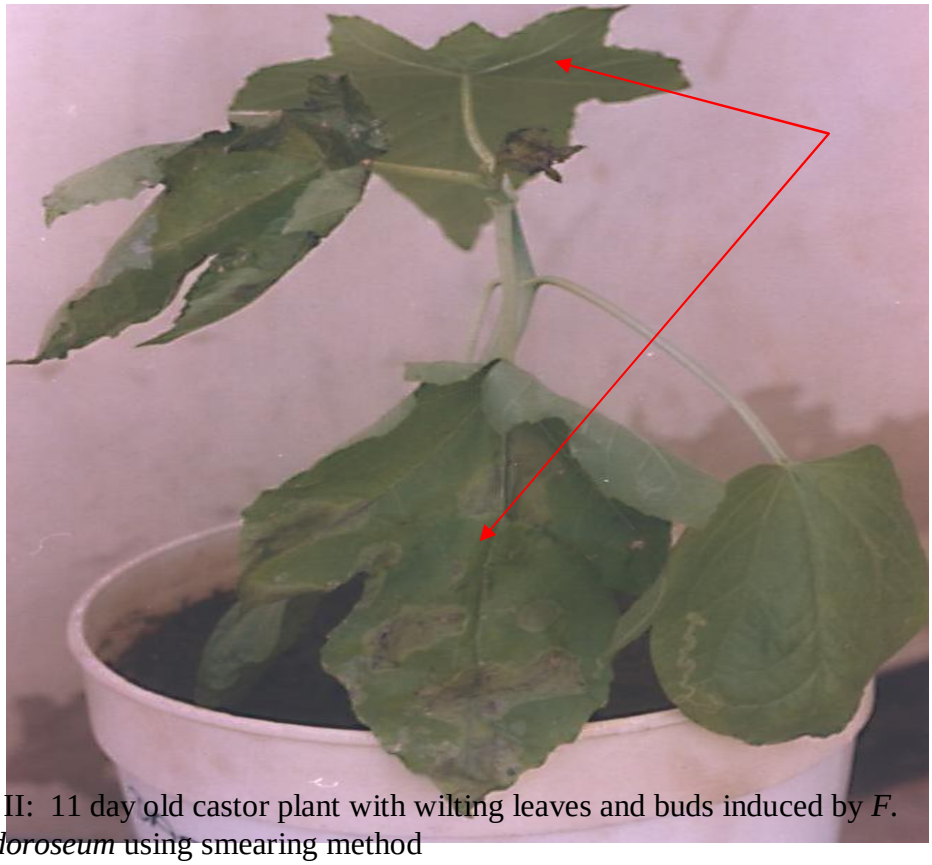


Plate II: 11 day old castor plant with wilting leaves and buds induced by *F. pallidoroseum* using smearing method



Plate III: 11 day old castor plant with stem rot induced by *F. pallidoroseum* using soil inoculation method.

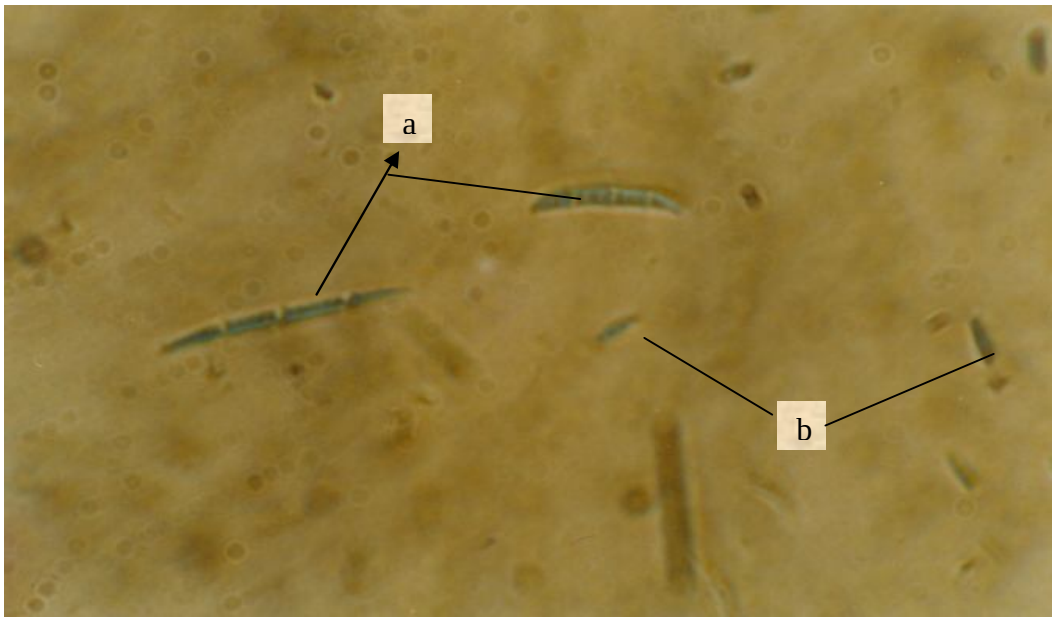



Plate IV: Macro (a) and Micro (b) conidia showing septations of *F. pallidoroseum* in culture

### 4. 1. 3 Incidence and severity of leaf blight induced by *Fusarium pallidoroseum* on castor of different ages inoculated using different methods

The results of the pathogenicity test using three methods of inoculation (spraying, smearing and soil) on castor at 11, 18 and 25 days old are shown in Tables 1 and 2 and Plates V and VI. The plants at the three ages inoculated with the three different methods were infected. For spraying method, there were significant ($P \leq 0.05$) differences in disease incidence in plants inoculated at different ages, though 11 and 18 days old which recorded the highest disease incidence were not statistically different from each other (Table 1). Control plants had significantly lower disease incidence than all the other treatments. For smearing method, there were significant ($P \leq 0.05$) differences in disease incidence between the plants inoculated at 11, 18 and 25 days old. However, plants inoculated at 25 days old were not statistically different from the control plants which had no disease. For soil inoculation, plants inoculated at 11 days old had significantly ($P \leq 0.05$) higher disease incidence than those inoculated at 18 and 25 days old. The disease incidence in control plants was not statistically different from plants inoculated at 25 days old.

Table 1: Leaf blight incidence on castor of different ages inoculated with *F. pallidoroseum* using different methods.

| Age of plant (days) | Incidence ( %) using**: | | |
|---|---|---|---|
| | Spraying | Smearing | Soil inoculation |
| 11 | 94.4 | 100.0 | 100.0 |
| 18 | 94.4 | 77.7 | 88.9 |
| 25 | 22.2 | 11.7 | 1.0 |
| Control | 1.0 | 1.0 | 1.0 |
| LSD | 72.69 * | 19.86 * | 9.1 * |

DAI= Days after inoculation

*= Significantly different ($P \leq 0.05$)

**= data was transformed using arcsin

The disease severity taken at various days after inoculation (7, 14 and 21 DAI) for the different ages of castor using different inoculation methods are presented in Table 2.

There were significant ($P \leq 0.05$) differences between the different ages of plants in the various inoculation methods. At 7 DAI there were significant differences in disease severity among the different ages of castor used. For both spraying and smearing at 7 DAI, there were no significant ($P \leq 0.05$) differences in disease severity between 11 and 18 days old plants and also between 25 days old inoculated plants and the control. Plants inoculated at 11 and 18 days old had higher disease severity when compared with 25 days old and the control plants. For soil inoculation, there were significant ($P \leq 0.05$) differences between the plants inoculated at different ages. The plants inoculated at 11 days old recorded the highest disease severity, followed by those inoculated at 18 and 25 days old respectively. The disease severity taken at 14 DAI were not statistically ($P \leq$

0 .05) different among 11 and 18 days old inoculated plants for both spraying and soil inoculation methods. On the other hand for smearing method, 25 day old plants inoculated and control plants were not statistically ($P \leq 0.05$) different, while plants inoculated at 11 and 18 days old were also not statistically different in their disease severity. At 21 DAI, the trend was similar for all the inoculation methods with 11 and 18 days old plants recording statistically higher severity when compared with 25 day old and control plants.

Table 2: Leaf blight severity on castor of different ages inoculated with *F.pallidoroseum* using different methods.

| Age of Plant (days) | Severity ( %) at**: | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | 7 DAI | | | 14 DAI | | | 21 DAI | | |
| | SP | SM | SI | SP | SM | SI | SP | SM | SI |
| 11 | 4.7 | 4.0 | 6.0 | 6.7 | 7.3 | 7.3 | 9.3 | 10.0 | 10.0 |
| 18 | 3.3 | 3.3 | 4.0 | 5.3 | 6.0 | 8.0 | 10.0 | 10.0 | 10.0 |
| 25 | 1.3 | 1.3 | 1.0 | 1.0 | 2.7 | 1.0 | 2.0 | 1.0 | 1.0 |
| Control | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| LSD | 1.6* | 1.2* | 1.9* | 1.5* | 1.5* | 2.2* | 1.96* | 0·0* | 0.0* |

*= Significantly different ($P \leq 0.05$).

**= data was transformed using arcsin

DAI = Days after inoculation.

SP – spray, SM – smear, SI – Soil inoculation

At seven days after inoculation (7 DAI), castor plants inoculated at 11 days old using smearing and soil inoculation had wilted leaves and collapsed stems while plants inoculated with spraying had only wilted leaves (plate V). Plants inoculated with smearing method at 18 days old had some unwilted leaves. For spraying and soil inoculation, the leaves were wilted and stem collapsed (Plate VI).



Plate V: Reaction of castor plants inoculated with *Fusarium pallidoroseum* after 11 days using different methods.



Plate VI: Reaction of castor plants inoculated with *Fusarium pallidoroseum* after 18 days using different methods.

### 4.2 Effect of Inoculum Density on Disease Incidence and Severity.

The effect of four inoculum densities on incidence and severity of leaf blight on castor is presented in Table 3. For disease incidence, there were no significant ($P \leq 0.05$) differences between plants inoculated with inoculum densities of $10^3$, $10^5$, $10^7$ and $10^{10}$, while the control plants sprayed with water had no disease incidence. The control plants were significantly different from those inoculated with $10^3$, $10^5$ and $10^7$ inoculum densities (Table 3).

At 7 days after inoculation (DAI) there were no significant ($P \leq 0.05$) differences in disease severity between the inoculated plants and the control although, plants inoculated with $10^{5,}$ $10^7$ and $10^{10}$ density had higher severity. Similarly at 14 and 21 DAI, there were no significant ($P \leq 0.05$) differences between plants inoculated with $10^3$, $10^5$ and $10^7$ inoculum strength with $10^3$ inducing the lowest disease severity. The control plants had no disease and were not statistically different from those inoculated with inoculum strength at $10^5$ and $10^3$ (Table 3). Plants inoculated with $10^{10}$ inoculum strength had the higest disease severity and were significantly ($P \leq 0.05$) different from those inoculated with $10^3$, $10^5$ and $10^7$ inoculum density.

Table 3: Effect of inoculum density on diseae incidence and severity.

| Inoculum density spores / ml | Incidence (%) at**: | Severity ( %) at**: | | |
|---|---|---|---|---|
| | 7 DAI | 7 DAI | 14 DAI | 21 DAI |
| $10^3$ | 22.90 | 1.33 | 1.33 | 2.0 |
| $10^5$ | 33.30 | 2.67 | 2.00 | 3.33 |
| $10^7$ | 66.70 | 2.67 | 3.33 | 3.33 |
| $10^{10}$ | 55.53 | 2.67 | 4.00 | 5.33 |
| water | 1.00 | 1.00 | 1.00 | 1.00 |
| LSD | 43.98 * | 1.7 NS | 2.5* | 2.7* |
| SE± | 13.9 | 0.54 | 0.80 | 0.81 |

*= Significantly different ($P \leq 0.05$)

**= data was transformed using arcsin

NS=Not significant ($P \leq 0.05$)

DAI = days after inoculation

### 4.3 Evaluation of Fungicides

#### 4.3.1 Effect of fungicides on the mycelial growth, sporulation, spore size and mycelial regrowth of *Fusarium pallidoroseum in vitro.*

There were highly significant ($P \leq 0.01$) differences among the fungicides on the radial mycelial growth of the fungus at 7 and 14 days after inoculation (DAI), sporulation, spore width and mycelial regrowth at 14 DAI (Table 4). Benomyl, benomyl + thiram and tricyclazole completely inhibited mycelial growth of *Fusarium pallidoroseum* at 7 and 14 DAI. Due to complete inhibition of mycelial growth by these fungicides there was no sporulation; hence data on conidia count, spore size and mycelial regrowth could not be obtained. These were followed by carbendazim + mancozeb which inhibited mycelial growth significantly more than metalaxyl-M + thiomethoxam + difenoconazole and mancozeb. Mancozeb gave the least inhibition among the evaluated fungicides but significantly inhibited mycelial growth than the control. At 14 DAI, among the fungicides that permitted some mycelial growth (mancozeb, metalaxyl-M + thiomethoxam + difenoconazole and carbendazim + mancozeb), there were significant ($P \leq 0.05$) differences in their mycelial regrowth. Mancozeb supported the highest mycelial regrowth followed by metalaxyl-M + thiomethoxam + difenoconazole. Conidia count from carbendazim + mancozeb incorporated plates was least, followed by mancozeb and metalaxyl-M + thiomethoxam + difenoconazole respectively. For conidia length, there were no significant ($P \leq 0.05$) differences in Petri dishes treated with mancozeb, metalaxyl-M + thiomethoxam + difenoconazole, carbendazim + Mancozeb and control, although longer conidia were recorded from culture grown on mancozeb treated PDAs followed by those treated with metalaxyl-M + thiomethoxam + difenoconazole, control and carbendazim + mancozeb in that order. For conidia width, there were no significant

($P \leq 0.05$) differences between mancozeb, metalaxyl-M + thiomethoxam + difenoconazole, carbendazim + mancozeb and control plates even though carbendazim + mancozeb gave wider conidia followed by the control with metalaxyl-M + thiomethoxam + difenoconazole having the least conidia width. Meanwhile, conidia from control and mancozeb treated plates were at par ($P \leq 0.05$).

The interaction between the fungicides and varying concentrations for mycelial growth at 7 and 14 DAI, sporulation, conidia width and mycelial regrowth 14 DAI were highly significant ($P \leq 0.01$) and the conidia length was significant ($P \leq 0.05$).

Table 4: Effect of fungicides on mycelial growth, sporulation, conidia size and regrowth of *Fusarium pallidoroseum in vitro*

| Fungicide | Mycelial growth (cm) at: | | Mycelial regrowth (cm) 14 DAI | Conidia count (4x10/ml) 14 DAI | Conidia length (µm) (14 DAI | Conidia width(µm) 14 DAI |
|---|---|---|---|---|---|---|
| | 7DAI | 14DAI | | | | |
| Benomyl | 0.50e | 0.50e | 0.00e | 0.0d | 0.0b | 0.0c |
| Mancozeb | 5.77b | 6.18b | 7.70b | 41.2c | 11.7a | 3.6ab |
| Benomyl+thiram | 0.50e | 0.50e | 0.00e | 0.0d | 0.0b | 0.0c |
| Metalaxyl-M + thio + difen | 2.82c | 3.01c | 5.12d | 65.2b | 10.4a | 2.8b |
| Tricyclazole | 0.50e | 0.50e | 0.00e | 0.0d | 0.0b | 0.0c |
| Carbendazim + mancozeb | 1.39d | 1.78d | 5.32c | 40.6c | 8.0a | 4.5a |
| Control | 9.00a | 9.00a | 9.00a | 168.0a | 9.1a | 3.8ab |
| Interactions(F X C ) | ** | ** | ** | ** | * | ** |
| SE | 0.092 | 0.064 | 0.064 | 8.94 | 27.88 | 5.78 |
| CV | 12.05 | 8.13 | 6.38 | 6.62 | 128.5 | 70.9 |

Values in each column with the same alphabet(s) are not significantly different from each other using Students Newman Keuls (SNK) test.

** Highly significant ($P \leq 0.01$).

*Significant ($P \leq 0.05$).

DAI= Days after inoculation.

F= Fungicide

C= Concentration

thio= thiomethoxam

difen= difenoconazole

The effect of the fungicides at different rates on mycelial growth at 7 and 14 DAI are presented in Table 5 and Plates VII, VIII and IX. There were significant ($P \leq 0.05$) differences among mycelial growth of the fungus cultured on fungicides treated plates and their different rates at 7 and 14 DAI. All the six fungicides evaluated, except mancozeb completely inhibited mycelial growth of the fungus at 7 and 14 DAI (Table 5, Plates VII, VIII and IX). Benomyl, benomyl + thiram and tricyclazole at the three rates tested completely inhibited fungal mycelial growth both at 7 and 14 DAI. Carbendazim + mancozeb and metalaxyl-M + thiomethoxam + difenoconazole at recommended and half the recommended rates reduced the growth of mycelia at 7 and 14 DAI, when compared with the control. However, at one and half the rate there was complete inhibition of mycelial growth. Mancozeb at all the rates tested partially inhibited mycelial growth at 7 and 14 DAI, though the growth were lower than those on the control plates (Table 5).

The fungicides also affected mycelial colouration on PDAs. The culture of fungus grown on fungicide-free PDAs (control plates) was cotton white and fluffy. The culture of fungus grown on metalaxyl-M + thiomethoxam + difenoconazole and carbendazim + mancozeb incorporated plates were similar to the control (Plates VII and VIII), while the growth was flat spreading, creamy white and not fluffy in PDAs mixed with mancozeb (Plate IX). For the remaining fungicides treated PDAs, there was no growth.

The effect of varying concentrations of the different fungicides on mycelial growth of *Fusarium pallidoroseum in vitro* is presented in Table 5 and Plates X, XI and XII.

For each fungicide, there were significant (($P \leq 0.05$) differences in mycelial growth between the different rates tested at 7 and 14 DAI, except for benomyl, benomyl + thiram

and tricyclazole which completely inhibited mycelial growth at all rates. The inhibitory effect of these fungicides at one and half recommended rate, recommended and half recommended rates did not differ statistically ($P \leq 0.05$). Metalaxyl-M + thiomethoxam + difenoconazole and carbendazim + mancozeb at one and half recommended rate completely inhibited mycelial growth, while at recommended and half recommended rates mycelial growth were partially inhibited. Mancozeb gave the least inhibitory effect on mycelial growth and there were no significant ($P \leq 0.05$) differences between recommended and half recommended rates, in respect to mycelial growth at 7 and 14 DAI.

Table 5: Effect of fungicides at different concentrations on mycelial growth of *Fusarium pallidoroseum in vitro* at 7 and 14 days after inoculation.

| Fungicide | Mycelial growth (cm) at: | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 7 DAI | | | | | 14 DAI | | | | |
| | One and half recommended rate | Recommended rate | Half recommended rate | Control | LSD[a] | One and half recommended rate | Recommended rate | Half recommended rate | Control | LSD[b] |
| Benomyl | 0.5c | 0.5d | 0.5e | 9.0 | 0.0* | 0.5c | 0.5d | 0.5e | 9.0 | 0.0* |
| Mancozeb | 3.8b | 6.5b | 7.0b | 9.0 | 0.8* | 4.3b | 7.1b | 7.2b | 9.0 | 0.6* |
| Benomyl + thiram | 0.5c | 0.5d | 0.5e | 9.0 | 0.0* | 0.5c | 0.5d | 0.5e | 9.0 | 0.0* |
| Metalaxyl-M + thio + difen | 0.5c | 3.3c | 4.0c | 9.0 | 0.4* | 0.5c | 3.7c | 4.7c | 9.0 | 0.3* |
| Tricyclazole | 0.5c | 0.5d | 0.5e | 9.0 | 0.0* | 0.5c | 0.5d | 0.5e | 9.0 | 0.0* |
| Carbendazim + mancozeb | 0.5c | 0.68d | 3.0d | 9.0 | 0.4* | 0.5c | 0.9d | 3.9d | 9.0 | 0.2* |
| Control | 9.0a | 9.0a | 9.0a | | | 9.0a | 9.0a | 9.0a | | |
| | * | * | * | | | * | * | * | | |
| SE± | 0.17 | 0.12 | 0.16 | | | 0.10 | 0.06 | 0.13 | | |

Values in each column with the same alphabet(s) are not significantly different from each other using Students Newman Keuls (SNK) test.

*= Significantly different ($P \leq 0.05$)

[a] and [b] = LSD used to compare values in each row at ($P \leq 0.05$)

thio= thiomethoxam

difen= difenoconazole

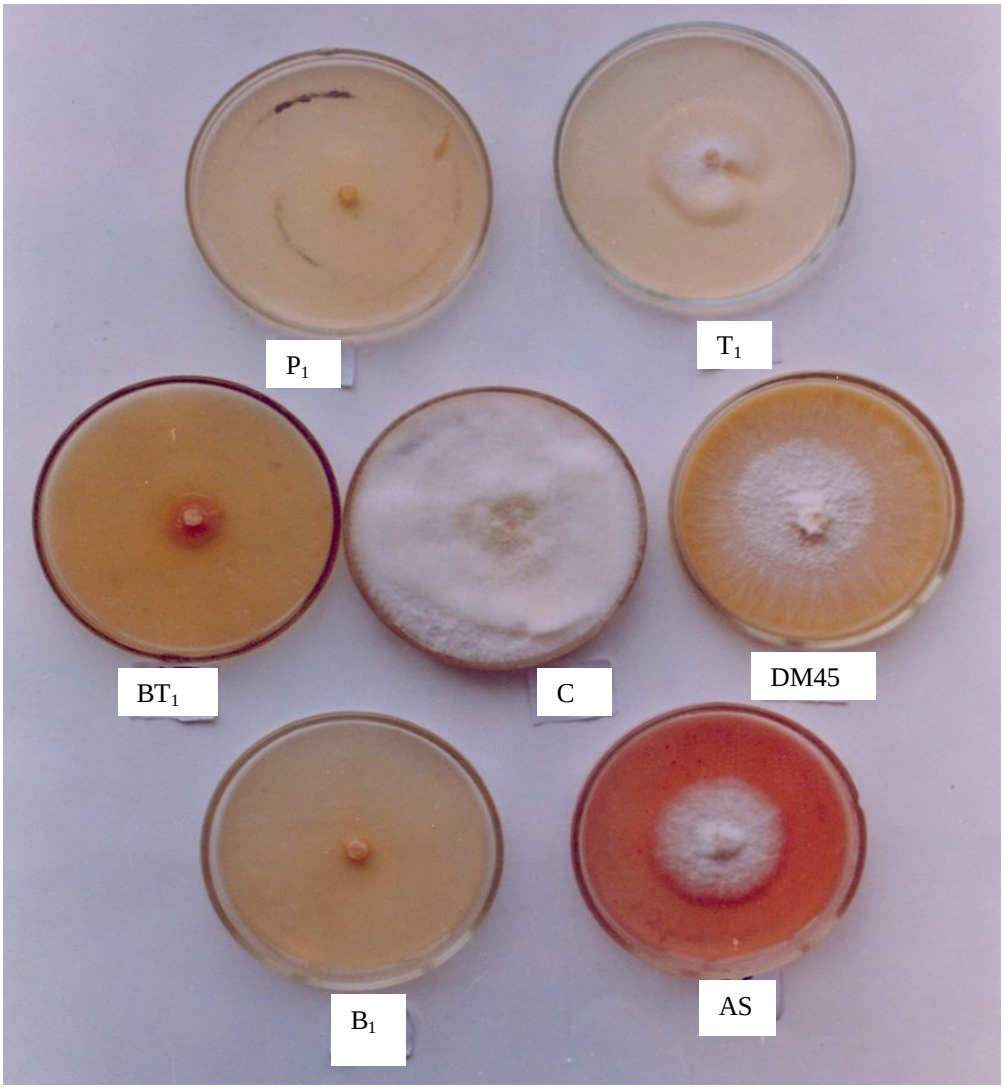



Plate VII: Effect of fungicides at half recommended rate on the mycelial growth of *Fusarium pallidoroseum* at 7 DAI.

**Key**

P = Tricyclazole
T = Carbendazim + mancozeb
DM45 = Mancozeb
B = Benomyl
BT = Benomyl + thiram
AS =Metalaxyl –M + thiomethoxam+ difenoconazole
C = control

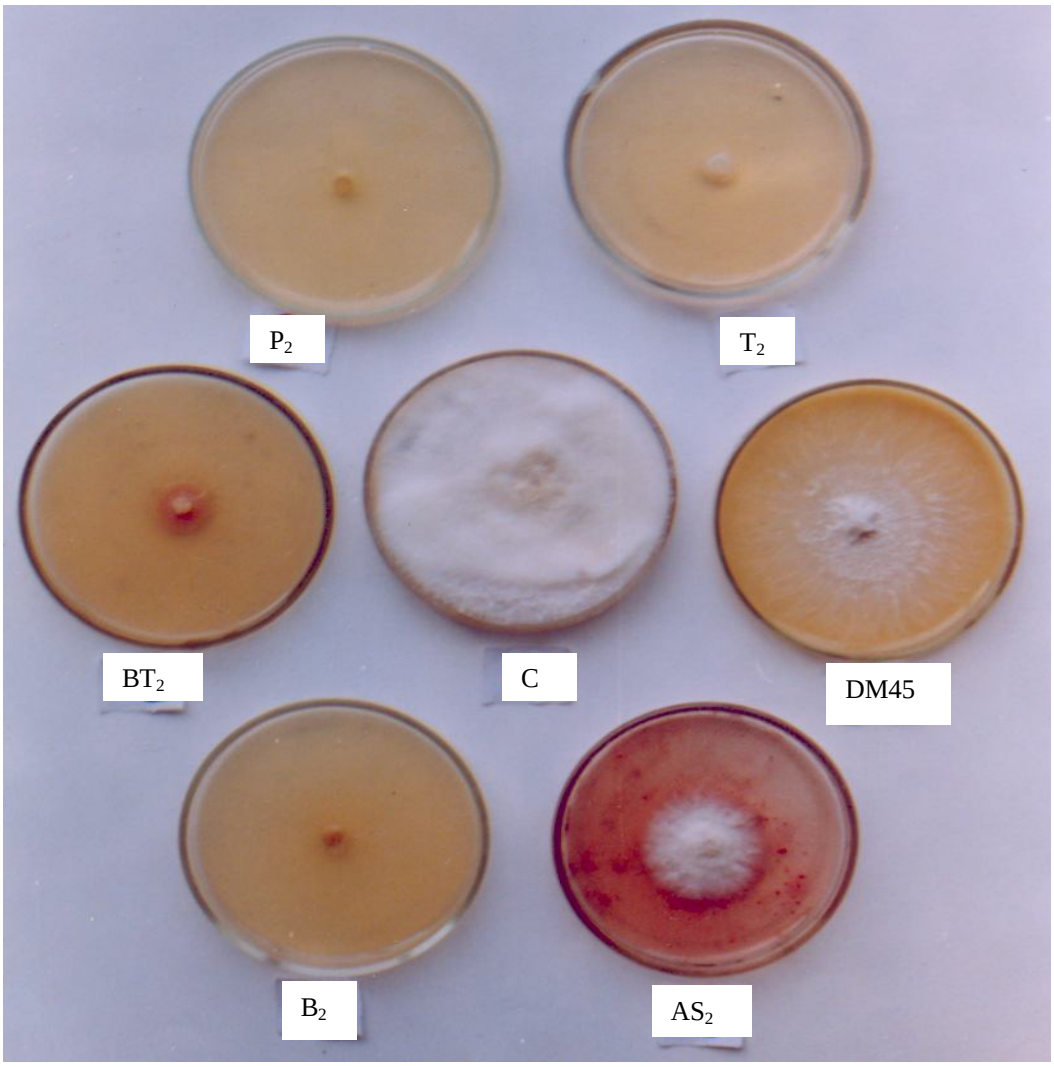



Plate VIII: Effect of fungicides at recommended rate on the mycelial growth of *Fusarium pallidoroseum* at 7 DAI.

**Key**

P = Tricyclazole
T = Carbendazim + mancozeb
DM45 = Mancozeb
B = Benomyl
BT = Benomyl + thiram
AS = Metalaxyl-M + thiomethoxam + difenoconazole
C = control

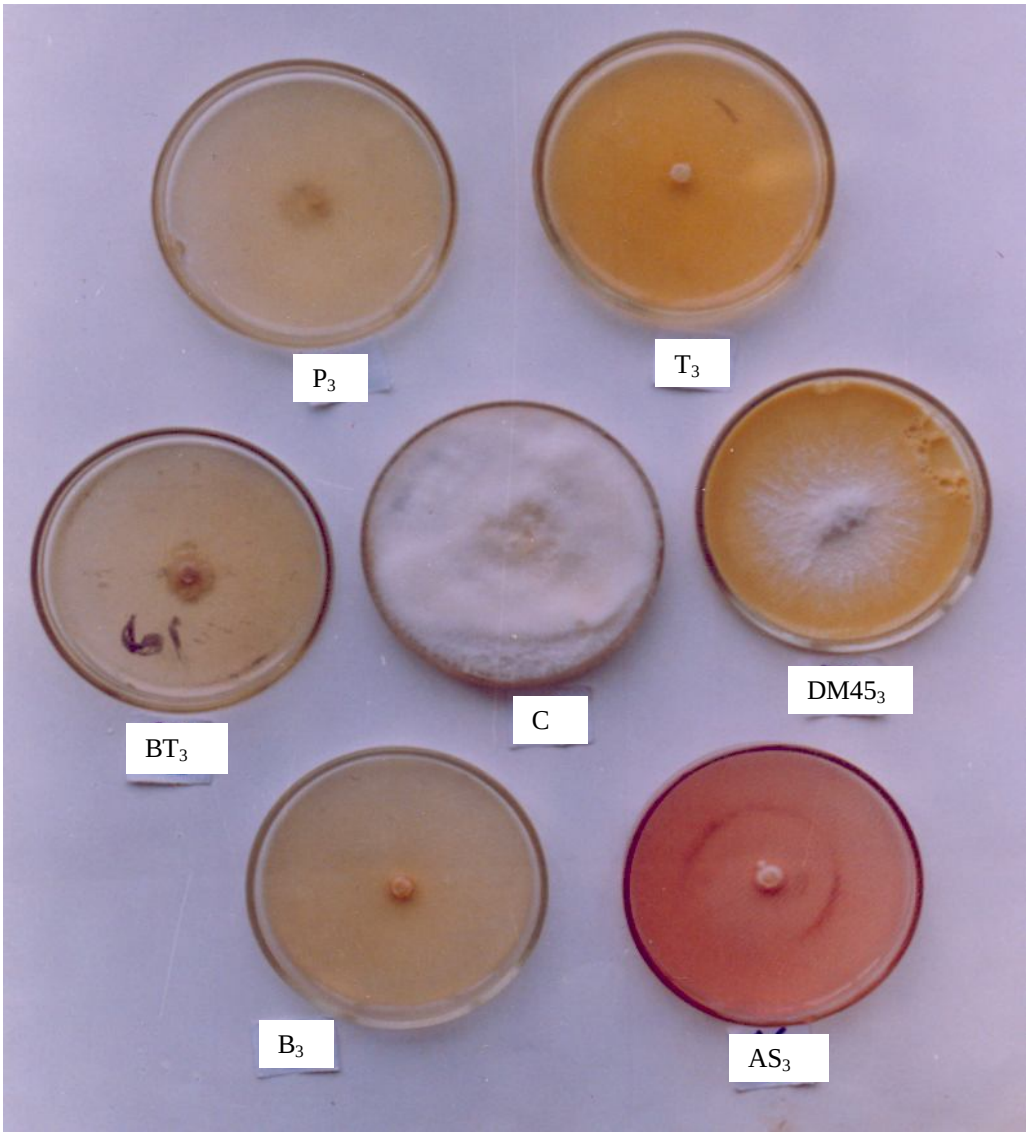



Plate IX: Effect of fungicides at one and half recommended rate on the mycelial growth of *Fusarium pallidoroseum* at 7 DAI.

**Key**

P = Tricyclazole
T = Carbendazim + mancozeb
DM45 = Mancozeb
B = Benomyl
BT = Benomyl + thiram
AS = Metalaxyl-M+ thiomethoxam + difenoconazole
C = control

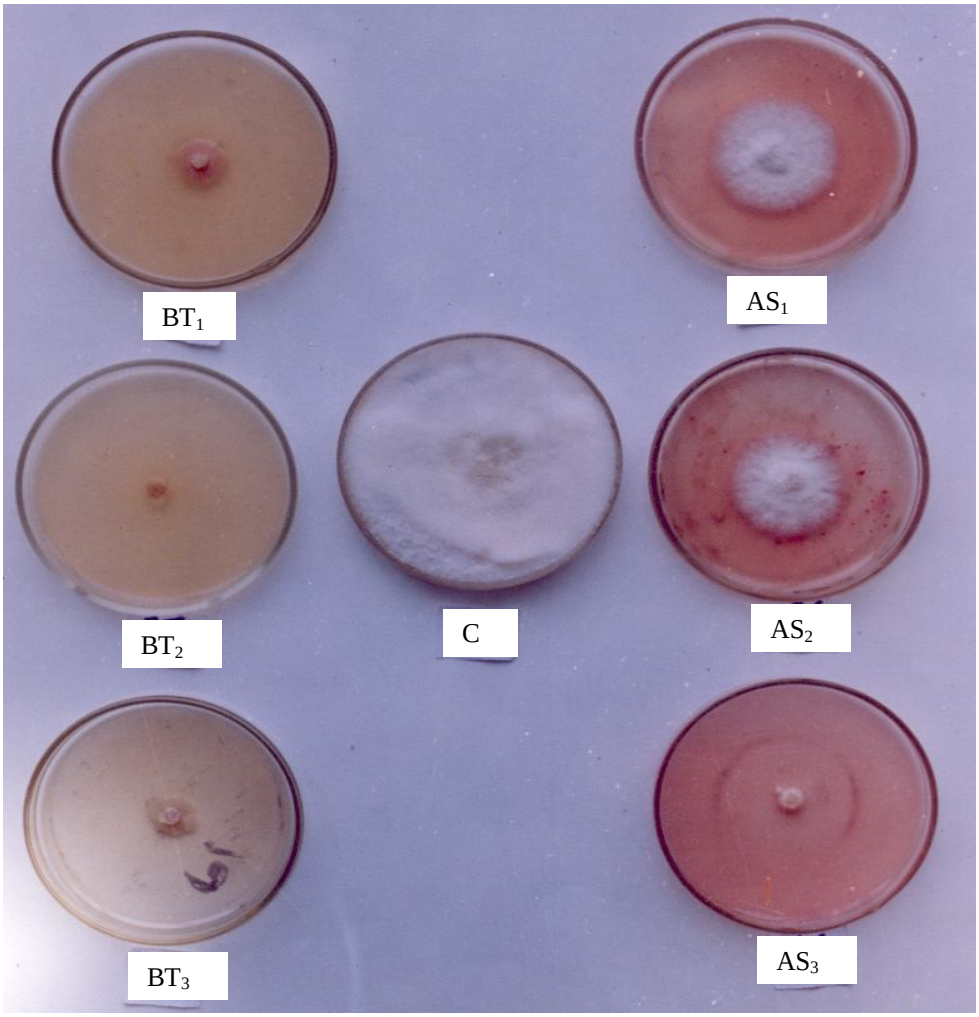



Plate X: Effect of benomyl + thiram (BT) and metalaxyl-M + thiomethoxam + difenoconazole (AS) at varying concentrations on mycelial growth of *Fusarium pallidoroseum*, 7 DAI.

**Key**
C = control
$BT_1$ = half recommended rate
$BT_2$ = recommended rate
$BT_3$ = one and half recommended rate
$AS_1$= half recommended rate
$AS_2$= recommended rate
$AS_3$=one and half recommended rate

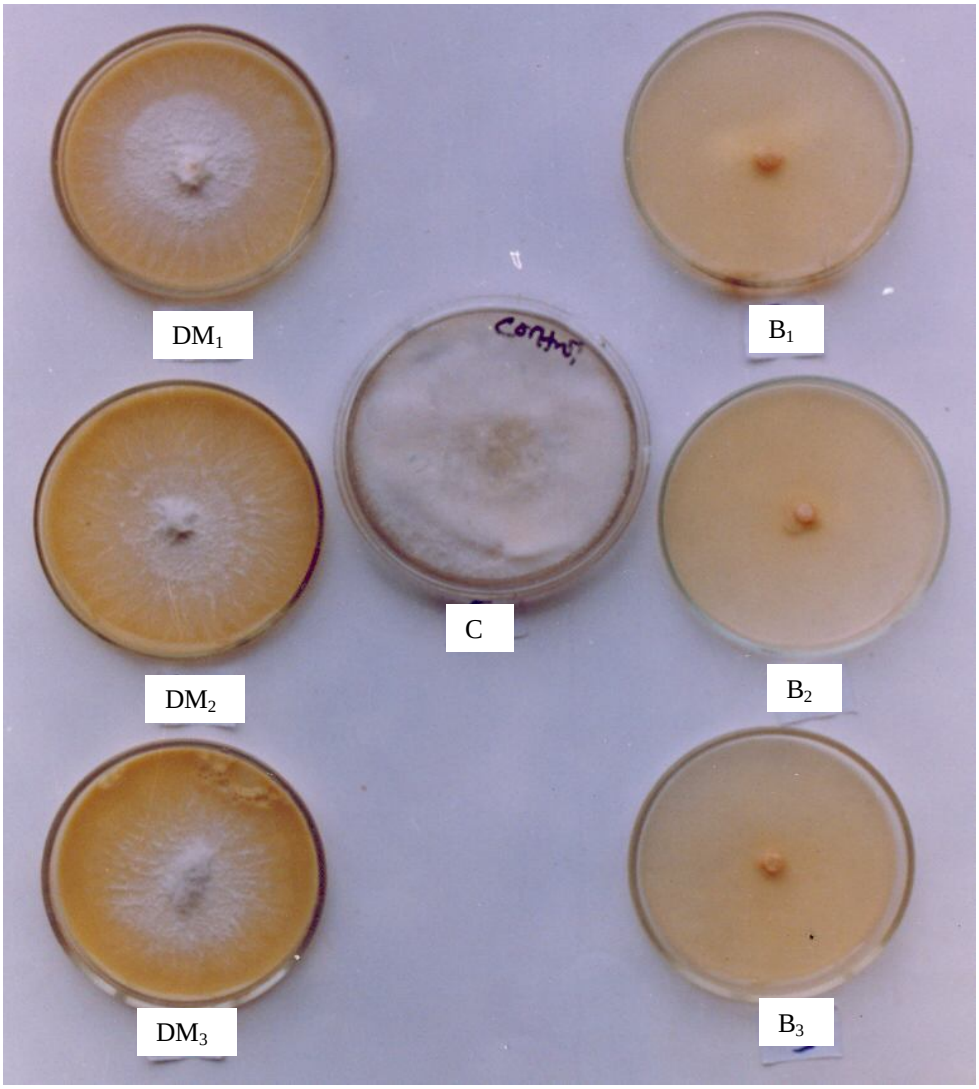



Plate XI: Effect of mancozeb (DM) and benomyl (B) at varying concentrations on the mycelial growth of *Fusarium pallidoroseum*, 7 DAI.

**Key**
C = control
$DM_1$ = half recommended rate
$DM_2$ = recommended rate
$DM_3$ = one and half recommended rate
$B_1$= half recommended rate
$B_2$= recommended rate
$B_3$=one and half recommended rate

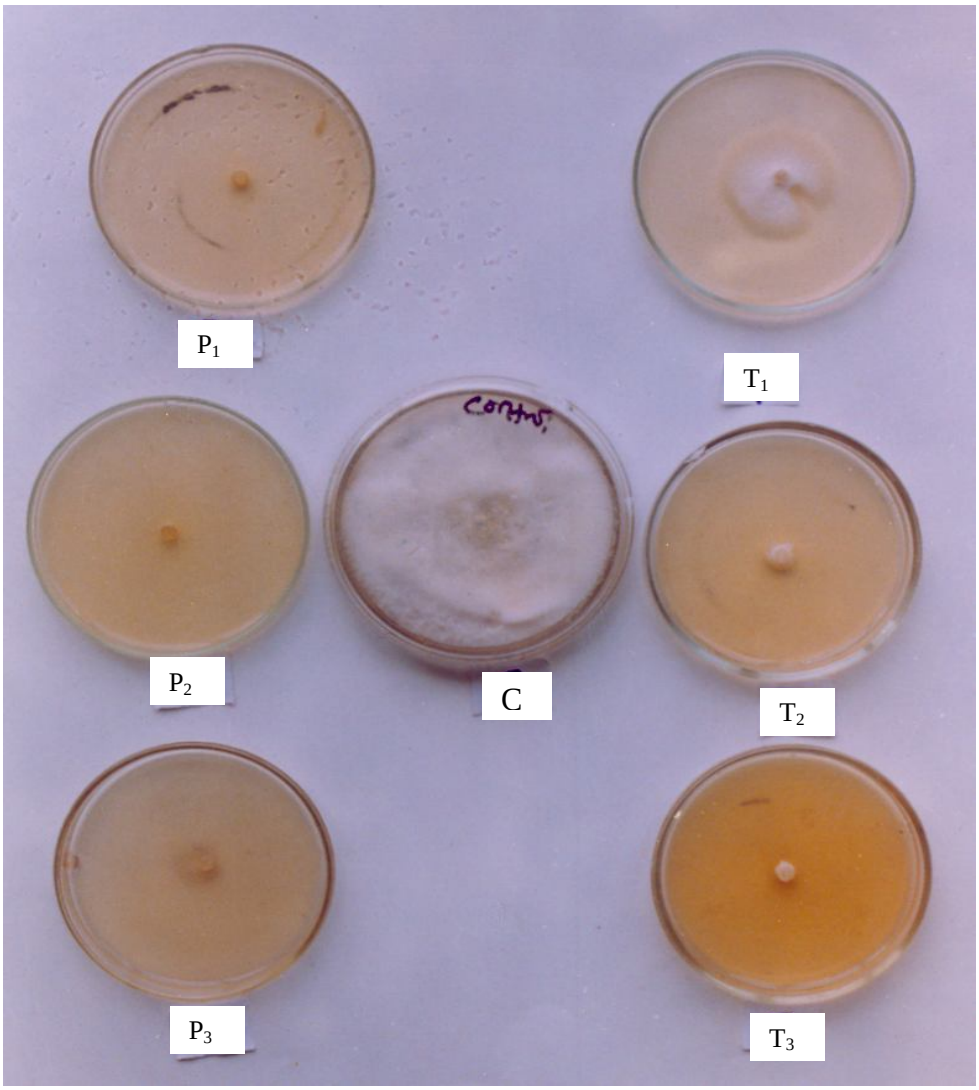



Plate XII: Effect of tricyclazole (P) carbendazim + mancozeb (T) at varying concentrations on mycelial growth of *Fusarium pallidoroseum*, 7 DAI.

**Key**
C = control
$P_1$ = half recommended rate
$P_2$ = recommended rate
$P_3$ = one and half recommended rate
$T_1$= half recommended rate
$T_2$= recommended rate
$T_3$=one and half recommended rate

The effects of fungicides at varying concentrations on sporulation of *Fusarium pallidoroseum in vitro* are summarized in Table 6. There were significant ($P \leq 0.05$) differences among the different rates in relation to sporulation. All the rates of the different fungicides evaluated significantly inhibited sporulation of the fungus when compared with the mycelia on the control plates. Benomyl, benomyl + thiram and tricyclazole completely inhibited sporulation at all the rates (one and half recommended rate, recommended and half recommended rates). The inhibitory effect of these fungicides did not differ statistically ($P \leq 0.05$). On the other hand mancozeb and carbendazim + mancozeb could not stop sporulation completely at the three rates used even though sporulation decreased as the rate increased. Metalaxyl-M + thiomethoxam + difenoconazole completely inhibited sporulation at one and half recommended rate, while at recommended and half recommended rates the sporulation was low.

Table 6: Effect of varying concentrations of fungicides on sporulation of *Fusarium pallidoroseum in vitro* at 14 days after inoculation

| Fungicides | Conidia /ml ($4x10^6$) | | | | |
|---|---|---|---|---|---|
| | One and half recommended rate | Recommended rate | Half recommended rate | Control | LSD[a] |
| Benomyl | 0.0c | 0.0d | 0.0e | 168.0 | 0.0* |
| Mancozeb | 31.7b | 38.5c | 53.5d | 168.0 | 8.1** |
| Benomyl + thiram | 0.0c | 0.0d | 0.0e | 168.0 | 0.0* |
| Metalaxyl-M + thio +difen | 0.0c | 92.6b | 103.1b | 168.0 | 5.3** |
| Tricyclazole | 0.0c | 0.0d | 0.0e | 168.0 | 0.0* |
| Carbendazim + mancozeb | 0.0c | 36.6c | 57.0c | 168.0 | 9.5** |
| Control | 168.0a | 168.0a | 168.0a | | |
| SNK | ** | ** | ** | | |
| SE± | 5.93 | 6.15 | 2.96 | | |

Values in each column with the same alphabet(s) are not significantly different from each other using Students Newman Keuls (SNK) test ($P \leq 0.05$).

[a] = Values in each row was compared using LSD at ($P \leq 0.05$)

thio =thiomethoxam

difen = difenoconazole

The effect of fungicides at varying concentrations on conidia dimensions is presented in Table 7. The differences among the fungicides and the different concentrations in relation to conidia lenght were statistically significant ($P \leq 0.05$). At one and half recommended rate most of the fungicides (benomyl, benomyl + thiram, metalaxyl-M + thiomethoxam + difenoconazole, tricyclazole and carbendazim + mancozeb), prevented mycelial growth and spore production, hence spore size could not be obtained. Mancozeb at one and half recommended rate produced the longest conidia although it was not significantly ($P \leq 0.05$) different from those conidia produced in plates with mancozeb at recommended and half recommended rates. Metalaxyl-M + thiomethoxam + difenoconazole at recommended rate produced longer conidia when compared with those on half recommended rate and the control plates, but they were not statistically ($P \leq 0.05$) different from each other. Carbendazim+mancozeb at recommended and half recommended rates produced conidia which did not significantly differ from each other and the control in length. Generally, the conidia produced in fungicides treated PDAs, were longer than those produced in the control plates.

The fungicides at varying concentrations reduced the conidia width (Table 7). Benomyl, benomyl + thiram, tricyclazole at one and half recommended rate, recommended and half recommended rates completely inhibited conidia production. Mancozeb at one and half recommended rate, recommended and half recommended rates produced conidia which were not statistically ($P \leq 0.05$) different from each other and the control in width. Though at one and half recommended rate the conidia were wider which was followed by the control, half recommended and recommended rates having the least width. Metalaxyl-M + thiomethoxam + difenoconazole produced no conidia at one and half recommended

rate while at recommended and half recommended rate conidia width was not statistically ($P \leq 0.05$) different with the conidia produced in the control. Similarly, carbendazim + mancozeb at one and half recommended rate inhibited conidia production, while at recommended and half recommended rates conidia width reduced and were not statistically diferent from each other as well as the control. At recommended rate conidia width was wider followed by half recommended rate having the least. For each fungicide tested there was significant differences between the concentrations.

Table 7: Effect of fungicides and concentrations on conidia length and conidia width of *Fusarium pallidoroseum in vitro* at 14 DAI.

| Fungicides | Conidia length ( μm) | | | | | Conidia width (μm) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | One and half recommended rate | Recommended rate | Half recommended rate | Control | LSD[a] | One and half recommended rate | Recommended rate | Half recommended rate | Control | LSD[b] |
| Benomyl | 0.0b | 0.0b | 0.0a | 10.4 | 4.2* | 0.0b | 0.0a | 0.0a | 3.8 | 1.23* |
| Mancozeb | 18.1a | 14.3b | 12.4a | 10.4 | 12.6NS | 3.9a | 3.3a | 3.5a | 3.8 | 2.69NS |
| Benomyl + thiram | 0.0b | 0.0b | 0.0a | 10.4 | 4.2* | 0.0b | 0.0b | 0.0b | 3.8 | 1.23* |
| Metalaxyl-M + thio + difen | 0.0b | 20.5a | 10.8a | 10.4 | 12.6* | 0.0b | 4.2a | 4.3a | 3.8 | 2.83* |
| Tricyclazole | 0.0b | 0.0b | 0.0a | 10.4 | 4.2* | 0.0b | 0.0b | 0.0b | 3.8 | 1.23* |
| Carbendazim + mancozeb | 0.0b | 11.3ab | 12.9a | 10.4 | I4.6NS | 0.0b | 5.6a | 3.7a | 3.8 | 2.47* |
| Control | 10.4a | 10.4ab | 10.4a | | | 3.8a | 3.8a | 3.8a | | |
| SNK | * | * | NS | | | * | NS | * | | |
| SE± | 12.20 | 18.65 | 17.54 | | | 2.93 | 3.73 | 3.02 | | |

Values in each column with the same alphabet(s) are not significantly different from each other using Students Newman Keuls (SNK) test ($P \leq 0.05$).
*Significantly different ($P \leq 0.05$)
[a] = values in each row are compared using Least Significant Difference (LSD) ($P \leq 0.05$)
[b] = values in each row are compared using Least Significant Difference (LSD) ($P \leq 0.05$).
DAI = days after inoculation
thio = thiomethoxam
difen = difenoconazole

There were significant differences among the fungicides at different concentrations in relation to mycelial regrowth at 14 days (Table 8). Among the six fungicides tested, benomyl, benomyl + thiram and tricyclazole completely inhibited mycelial regrowth at the three rates tested. Metalaxyl-M + thiomethoxam + difenoconazole and carbendazim + mancozeb completely inhibited mycelial regrowth only at one and half recommended rate. At recommended and half recommended rates, metalaxyl-M + thiomethoxam + difenoconazole and carbendazim+mancozeb supported mycelial regrowth and did not differ statistically ($P \leq 0.05$) from each other. Mancozeb at all the three rates tested did not completely inhibit mycelial regrowth and was statistically ($P \leq 0.05$) different from the control.

The effect of varying concentrations of fungicides on mycelial regrowth is presented (Table 8). Benomyl, benomyl + thiram and tricyclazole completely inhibited the mycelial regrowth at the three concentrations tested. The inhibitory effect of these fungicides were not significantly ($P \leq 0.05$) different at all the concentrations tested. Metalaxyl-M + thiomethoxam + difenoconazole and carbendazim + mancozeb at one and half recommended rate significantly ($P \leq 0.05$) inhibited mycelial regrowth each compared to other concentrations. Mancozeb gave the least inhibitory effect on mycelial regrowth at all the three rates although they were statistically ($P \leq 0.05$) different with the control. For all the fungicides, the inhibitory effect decreased with increase in concentrations. The control was significantly different from even the lowest rate in all the fungicides tested, except for carbendazim + mancozeb.

Table 8: Effect of fungicides at varying concentrations on mycelial regrowth of *Fusarium pallidoroseum in vitro* at 14 DAI.

| Fungicide | Mycelial regrowth (cm) | | | | |
|---|---|---|---|---|---|
| | One and half recommended rate | Recommended rate | Half recommended rate | Control | LSD[a] |
| Benomyl | 0.0c | 0.0c | 0.0c | 9.0 | 0.0* |
| Mancozeb | 7.0b | 8.1b | 8.2b | 9.0 | 0.3* |
| Benomyl + thiram | 0.0c | 0.0c | 0.0c | 9.0 | 0.0* |
| Metalaxyl-M + thio + difen | 0.0c | 8.0a | 8.2b | 9.0 | 0.6* |
| Tricyclazole | 0.0c | 0.0c | 0.0c | 9.0 | 0.0* |
| Carbendazim + mancozeb | 0.0c | 7.4b | 8.6a | 9.0 | 0.4* |
| Control | 9.0a | 9.0a | 9.0a | | |
| SNK | * | * | * | | |
| SE± | 0.01 | 0.07 | 0.09 | | |

Values in each column with the same alphabet(s) are not significantly different from each other using Students Newman Keuls (SNK) test ($P \leq 0.05$).

[a] = values in each row are compared using Least Significant Difference (LSD) ($P \leq 0.05$)

DAI = days after inoculation

thio = thiomethoxam

difen = difenoconazole

### 4.3.2. Efficacy of varying fungicide concentrations on *Fusarium pallidoroseum in vivo*

When 21-day-old castor plants were inoculated with the pathogen, the disease incidence at 7 DAI was 3 %. Disease severity recorded at seven days interval after fungicides applications are presented in Figures 1 and 2.

Disease severity for fungicides at recommended rate significantly ($P \leq 0.05$) varied with fungicides (Figure 1). Check plants (inoculated but not treated with fungicide) had the highest disease severity followed by plants sprayed with carbendazim + mancozeb. The disease rapidly increased with time and by 21 days after fungicide application (DAF) the disease got to its peak in the check plants where no fungicide spray was done. All the fungicides tested reduced disease development on plants, with all recording lower disease severity than check plants. Among the plants sprayed with fungicides, those sprayed with carbendazim + mancozeb had the highest severity, followed by those sprayed with tricyclazole. Plants sprayed with mancozeb had the same disease severity with the control plants at 7 and 14 DAF, after which its efficacy decreased as expressed by increase in disease severity recorded at 21 and 28 DAF. The next most effective fungicides were benomyl, benomyl + thiram and mancozeb. Plants sprayed with these fungicides recorded lower disease severity at all scoring periods (7, 14, 21 and 28 DAF) although plants sprayed with benomyl recorded lower disease severity than those sprayed with benomyl + thiram and mancozeb at 14, and 21 DAF. Both benomyl and benomyl + thiram were at par at 7, 21 and 28 DAF and the plants recorded the lowest disease severity than all plants with other fungicides.

At half recommended rate, there were significant ($P \leq 0.05$) differences among the tested fungicides (Figure 2). At this rate, all the fungicides sprayed on the plants were not as effective as the recommended rate in reducing disease severity at all scoring periods with the exception of mancozeb. Among these fungicides tested, mancozeb controlled the disease better at all the scoring dates although, the disease severity increased with time. Disease severity of check plants were lower than those sprayed with carbendazim + mancozeb. Benomyl + thiram and tricyclazole at 7 DAF were at par but at 14, 21 and 28 DAF, tricyclazole reduced disease severity more than benomyl + thiram. Meanwhile, benomyl could not reduce disease spread on plants throughout the scoring periods (7, 14, 21 and 28 DAF) as there was increase in disease severity and it was a similar trend with carbendazim + mancozeb at 14, 21 and 28 DAF.

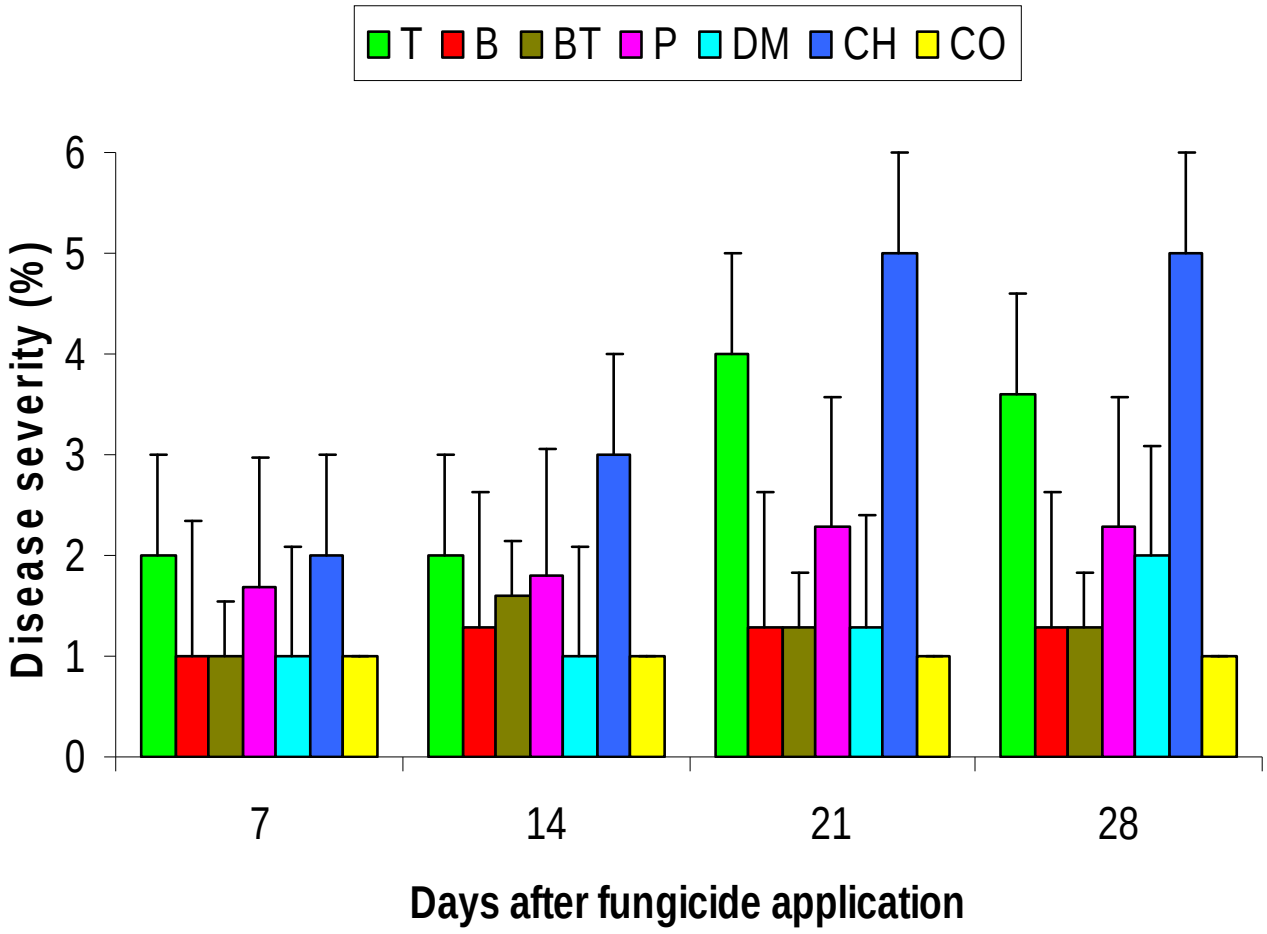



Figure 1.Effect of fungicides at recommended rate on *Fusarium pallidoroseum in vivo.*

**KEY**
Bar = $LSD_{0.05}$
T = Carbendazim+mancozeb (Team)
B = Benomyl (Benlate)
BT = Benomyl+thiram (Bentex T)
P = Tricyclazole (Profit)
DM = Mancozeb (Dithane M45)
CH = Inoculated but no fungicide
CO = No inoculation nor fungicide

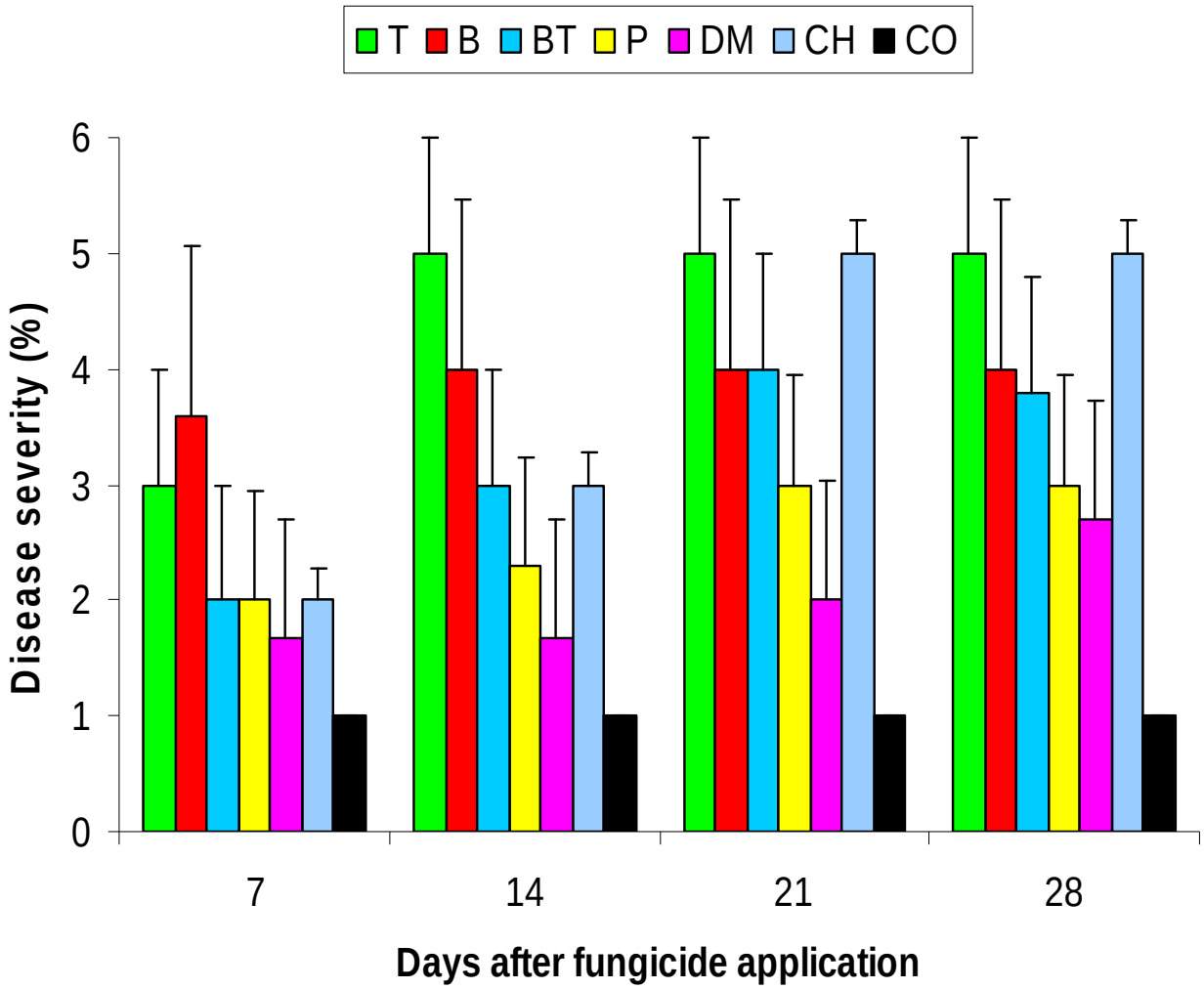



Figure 2. Effect of fungicides at half recommended rate on *Fusarium pallidoroseum in vivo.*

**KEY**
Bar = LSD $_{0.05}$
T = Carbendazim+mancozeb (Team)
B = Benomyl (Benlate)
BT = Benomyl+thiram (Bentex T)
P = Tricyclazole (Profi)
DM = Mancozeb (Dithane M45)
CH = Inoculated but no fungicide
CO = No inoculation nor fungicide

### 4.4 Effect of Sowing Dates on Incidence and Severity of Leaf blight.

For each of the two types of seeds used, there were no significant ($P \leq 0.05$) differences in disease incidence and severity between the sowing dates (Table 9). For large seeded castor, plants sown in August 11 (late) had high leaf blight incidence when compared with those sown in July 27 and August 3, with July 27 sown plants having the least incidence. However, small seeded castor sown in August 3 had higher disease incidence followed by plants sown in August 11 and July 27, with July 27 sown plants having low incidence at all scoring periods. Large seeded castor type had higher disease incidence than small seeded castor throughout the season.

For leaf blight severity, there were no significant ($P \leq 0.05$) differences between plants sown at different dates (Table10). The disease severity was consistently high in large seeded castor than in small seeded castor. For plants sown in July 27, disease severity in small seeded castor was lower than in large seeded castor except at 85 DAS. However, for plants sown in August 3, small seeded castor had higher disease severity than large seeded except at 71, 100 and 107 DAS. For the August 11 sown plants, the two seed types had the same disease severity score at 57, 85, 93, 100 and 107 DAS. Disease severity was highest on August 3 sown plants then followed by August 11 and July 27 sown plants respectively, although the differences were not significant ($P \leq 0.05$).

Table 9: Effect of sowing dates on incidence of leaf blight in two castor types.

| Sowing dates | Disease incidence (%) at: | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 57 DAS | 64 DAS | 71 DAS | 78 DAS | 85 DAS | 93 DAS | 100 DAS | 107 DAS |
| | Large seeded castor | | | | | | | |
| July 27 | 70.8 | 70.8 | 65.5 | 69.5 | 60.9 | 64.2 | 58.1 | 55.4 |
| August 3 | 74.1 | 75.9 | 83.1 | 81.9 | 81.9 | 85.2 | 75.5 | 73.0 |
| August11 | 81.5 | 78.1 | 91.5 | 83.7 | 82.2 | 82.2 | 61.9 | 61.8 |
| LSD | 64.5 | 56.4 | 29.6 | 41.8 | 31.3 | 24.6 | 32.9 | 28.6 |
| | NS | NS | NS | NS | NS | NS | NS | NS |
| | Small seeded castor | | | | | | | |
| July 27 | 34.0 | 34.0 | 34.0 | 34.0 | 34.0 | 34.0 | 34.0 | 34.0 |
| August 3 | 63.3 | 62.8 | 62.8 | 67.0 | 31.0 | 67.0 | 64.8 | 59.5 |
| August11 | 43.8 | 61.8 | 60.5 | 79.1 | 82.7 | 82.7 | 13.4 | 69.0 |
| LSD | 64.3 | 96.5 | 97.9 | 80.4 | 91.1 | 98.5 | 92.6 | 87.0 |
| | NS | NS | NS | NS | NS | NS | NS | NS |

NS= Not significant ($P \leq 0.05$)

DAS = Days after sowing.

Table 10: Effect of sowing dates on severity of leaf blight in two castor types.

| Sowing dates | Disease severity (%) at: | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 57 DAS | 64 DAS | 71 DAS | 78 DAS | 85 DAS | 93 DAS | 100 DAS | 107 DAS |
| | Large seeded castor | | | | | | | |
| July 27 | 2.0 | 2.0 | 3.3 | 3.3 | 2.0 | 2.0 | 2.0 | 2.0 |
| August 3 | 2.0 | 2.0 | 2.7 | 2.7 | 2.0 | 2.0 | 2.0 | 2.0 |
| Aug. 11 | 2.0 | 2.7 | 2.7 | 2.0 | 2.0 | 2.0 | 2.0 | 2.0 |
| LSD | 0.0 | 1.5 | 1.8 | 1.5 | 1.5 | 0.0 | 0.0 | 0.0 |
| | NS | NS | NS | NS | NS | NS | NS | NS |
| | Small seeded castor | | | | | | | |
| July 27 | 1.3 | 1.3 | 2.7 | 3.3 | 2.0 | 1.3 | 1.3 | 1.3 |
| Aug. 3 | 2.3 | 2.3 | 2.3 | 3.0 | 2.3 | 2.3 | 1.7 | 1.7 |
| Aug. 11 | 2.0 | 2.0 | 2.0 | 2.7 | 2.0 | 2.0 | 2.0 | 2.0 |
| LSD | 2.2 | 2.2 | 3.3 | 5.2 | 3.1 | 2.2 | 0.9 | 0.9 |
| | NS | NS | NS | NS | NS | NS | NS | NS |

NS= Not significant ($P \leq 0.05$).

DAS = Days after sowing.

### 4.4.1 Effect of sowing dates and disease severity on yield of two castor types in 2006.

The results (Table 11) show germination percentage, disease severity and bean yield of castor at different sowing dates. There were no significant ($P \leq 0.05$) differences between the germination percentages at all sowing dates and between the two castor types. July sown large seeded castor had higher germination percentage than the two August sowing dates. August 11 sown plants had the least germination percentage. On the other hand, for small seeded castor, August 3 sown plants had higher germination percentage than July 27 and August 11 sowing dates. August 11 sowing had low germination although there were no significant ($P \leq 0.05$) differences among the sowing dates used. Disease severity recorded for the large seeded castor sown in the different dates through the season was not significant ($P \leq 0.05$), although August sown crop had the least disease severity. For the large seeded castor, the yields from all the three sowing dates were not statistically ($P \leq 0.05$) different, July 27 sown plants had high bean yield while August 11 sown plants had low yield. However, the yields of plants sown in August 3 were higher than those sown in August 11 although there were no statistical ($P \leq 0.05$) differences between them. Similarly, in small seeded castor, there were no statistical ($P \leq 0.05$) differences between the yields of the plants sown in the three dates though the yield of August 3 sown castor were higher when compared with July 27 and August 11 sowing dates. For the small seeded castor however, the July 27 sown crop had the lowest disease severity. The bean yields were lower in plants with low germination.

Table 11: Effect of sowing dates and disease severity on bean yield in two castor types.

| Sowing date | Germination (%) | Severity (%) | Yield (kg/ha) |
|---|---|---|---|
| | | Large seeded | |
| July 27 | 57.33 | 2.3 | 1556.4 |
| August 3 | 52.67 | 2.3 | 888.5 |
| August 11 | 50.67 | 2.2 | 464.5 |
| LSD | **32.6NS** | **0.38 NS** | **1167.8 NS** |
| | | Small seeded | |
| July 27 | 38.67 | 1.86 | 625.3 |
| August 3 | 42.00 | 2.2 | 1024.4 |
| August 11 | 34.00 | 2.2 | 545.0 |
| LSD | **19.9NS** | **0.68 NS** | **2145.6 NS** |

NS = not significant at ($P \leq 0$ .05).

# CHAPTER FIVE

# DISCUSSION

This study has shown that, *Fusarium pallidoroseum* isolated from infected leaves is the causal organism of leaf blight and wilt of castor seedlings in Samaru. This is in line with the reports of Anjani *et al.* (2004), Booth (1971), Nelson *et al.* (1981) and Anon. (2006), who independently reported, that wilting in castor is induced by *Fusarium pallidoroseum* formally *semitectum*. Even though the pathogen has been confirmed by International Mycological Institute (IMI), Egham London, the cultural and morphological characteristics observed in 14 - day-old culture on PDAs at 28 $^{o}$C was similar to those described by Booth (1971) and Ellis (1971). The differences observed in conidia sizes; for the isolated 10 - 20 x 3 - 4.2 µm was obtained while in the literature 17 - 35 x 3.5 - 4 µm was reported could be as a result of media used which were different from what the previous workers used. Booth (1971) used Potato Sucrose Agar (PSA) at 25 $^{o}$C and recorded the conidia size of *F. pallidoroseum* to be 17 - 35 x 3.5 - 4 µm. In this study the size of conidia were 10 - 20 x 3 - 4.2 µm at 28 $^{o}$C on Potato Dextrose Agar (PDA). The observed variation in size could be attributed to varying culture media and temperature.

In the pathogenecity study, it was observed that the younger plants at 11 and 18 days old were more susceptible to infection for the three inoculation methods than 25 day - old plants. This agrees with Agrios (2005), who reported that plant age is important in disease infection and young plants are more susceptible. He also reported that plants in their reaction (susceptibility or resistance) to disease depend largely on age. For instance in *Pythium* related diseases such as damping off and root rots, downy mildews, bacterial blights and viral infections, the host plants are susceptible only during the growing period

and become resistant at maturity. Also depending on the particular plant - pathogen combination, the age of the host plant at the time of arrival of the pathogen may affect considerably the development of infection and of an epidemic. The greater the number of pathogen propagules within or near fields of host plants, the higher the infection rate. In a similar situation Shukla and Chand (1975) reported that younger leaves of sesame were more susceptible to bacterial leaf blight infection and attributed this to more stomata, higher nitrogen and moisture content than in older leaves.

At high inoculum density of $10^{10}$spore / ml, the severity was higher than when the dilution was increased from $10^{10}$ to $10^{7}$ $10^{5}$ and $10^{3}$ spores / ml. This implies that severity increased with increase in inoculum concentrations. This is in agreement with the work of Bdliya and Langerfeld (2005), who found that the development of potato blackleg disease induced by *Erwinia carotovora*, was higher at higher inoculum density of $2.3x10^{9}$ cells /ml, though it did not differ significantly from that of the lower bacterial concentration of $2.3\ x10^{5}$ cells /ml.

In fungicide evaluation, the effects of benomy, benomyl + thiram, mancozeb, metalaxyl – M + thiomethoxam + difenoconazole, tricyclazole and carbendzim + mancozeb on radial mycelial growth, sporulation, spore size and mycelial regrowth of *Fusarium pallidoroseum* isolated from castor are being reported for the first time. This study has shown that the above fungicides have significant and varying inhibitory effect on radial mycelial growth, sporulation, spore size and mycelial regrowth of the pathogen. Benomyl, benomyl + thiram and tricyclazole had the greatest inhibitory effect on mycelial growth and sporulation. Inhibitory effect of benomyl against many plant pathogenic fungi has

been reported; Khanna and Chandra (1976) reported the efficacy of benomyl in controlling the growth of *Fusarium moniliforme* and *Fursarium roseum in vitro*. In their research, 100 ppm of benomyl was used to control banana fruit rots induced by both pathogens. Marley and Gbenga (2004) demonstrated the efficacy of benomyl to control *Stenocarpella maydis* on maize *in vitro*. Alafiatayo (1982) also reported the efficacy of benomyl for the control of *Colletotrichum capsici in vitro*. *In vitro* evaluation of benomyl by other researchers showed that the fungicide inhibited mycelial growth and sporulation of *Verticillium* sp (Sivaprakasan and Rajagopalan, 1974), *Colletotrichum gloeosporiodes* and *Sclerotium rolfsii* (Shimfe, 1984) and *Curvularia eragrostidis* (Bawa, 1992). On the contrary, Yar'adua (1986) in his *in vitro* study of fungicides found that 500 ppm concentration of benomyl did not inhibit the sporulation of *Alternaria porri* completely. In a similar study, benomyl at low concentrations (0.5 and 1.0 ppm) stimulated mycelial growth of *Helminthosporium* spp, the causal organism of leaf spot of kentuckay blue grass (Jackson, 1970). Mancozeb at all the three rates tested did not completely inhibit mycelial growth and sporulation of *Fusarium pallidoroseum in vitro*. Although report showed that it has been effective on other fungal pathogens. Obagwu (1997) reported that at half the recommended rate mancozeb, benomyl, carbendazim+ maneb and thiram completely inhibited the growth and sporulation of *Colletotrichum capsici* causal organism of brown blotch of bambaranut *in vitro*. Singh *et al.* (1974) reported that mancozeb was effective against *Curvularia lunata.* However, mancozeb has been reported to enhance sporulation of *F. oxysporum* (Agrawal *et al.*, 1974). Similarly, in this study, mycelial growth on mancozeb treated PDAs had longer and wider (larger) spores than the control plates. Kumar and Sharma (1996) reported that carbendazim + maneb,

benomyl and thiram were able to completely inhibit mycelial growth of *C. capsici in vitro* even at lower doses than recommended rates. Thiram gave the best seed control of seed borne *C .capsici.* Also Adeoti (1991) reported that carbendazim and benomyl completely inhibited the mycelial growth of *Coniella musaiaensis* on PDA even at concentration as low as 12.5 % the manufacturers recommended dose. Meanwhile, calixin M and mancozeb were less effective on the fungus.

In this study, benomyl, benomyl+thiram, tricyclazole, metalaxyl–M + thiomethoxam + difenoconazole had high inhibitory effect on mycelial growth and sporulation of *Fusarium pallidoroseum* rather than spore size which is in agreement with the results of previous researchers working independently on different plant pathogenic fungi. Singh (1983) reported that application of metalaxyl on Pearl millet plant infected with *Sclerospora graminicola* was effective in controlling the disease. Bawa (1992) reported the efficacy of metalaxyl + carboxin + furathiocarb on mycelial growth and sporulation of *Curvularia eragrostidis in vitro* which increased with increase in concentration. In this study, depending on the concentration benomyl, benomyl + thiram, tricyclazole, metalaxyl–M + thiomethoxam + difenoconazole tricyclazole and carbendazim + mancozeb had complete and partial inhibitory effect on mycelial growth, sporulation and mycelial regrowth of *Fusarium pallidoroseum in vitro*. Carbendazim + mancozeb effectively reduced mycelial growth at recommended and half the recommended rates, while tricyclazole completely inhibit mycelial growth and sporulation at all the concentrations.

In the *in vivo* study, the evaluated fungicides benomyl, benomyl +thiram, carbendazim + mancozeb, tricyclazole and mancozeb at recommended rate were able to reduce disease severity than at half of the recommended rate. The effectiveness of mancozeb and benomyl at the recommended rate in this study agrees with the reports of Tologbonshe (1983). In his study, mancozeb and benomyl completely inhibited the growth of *Cercospora* leaf spot on egg plant at three different concentrations used. Similarly, Karaoglanidis *et al*. (2003) reported the effectiveness of benomyl in controlling leaf-spot caused by *Cercospora beticola* on sugar beet. Emechebe (1981) reported the effective control of cowpea brown blotch disease using benomyl, carbendazim + maneb and mixture of benomyl and mancozeb in the field. In this study, benomyl at the recommended rate was able to reduce leaf blight severity on castor. Similarly, Osunlaja and Alamuta (1999), also reported the use of benomyl, carbendazim and mancozeb at three rates (10,000, 25,000 and 50,000 ppm) which significantly inhibited *Physoderma maydis* (maize brown spot) on maize plant. Delp (1980) also reported that use of carbendazim plus maneb has been effective in controlling *Cercospora* on sugar beets in Greece.

Mancozeb had the lowest inhibitory effect on *F. pallidoroseum in vitro,* but it was the most effective in reducing the severity of leaf blight on castor *in vivo*. In contrast, all the other fungicides tested had inhibitory effect on this pathogen *in vitro,* than *in vivo*.

Adeoti (1991) obtained a similar result and reported that the effectiveness of the fungicides during the *in vitro* trial than in the *in vivo* may be attributed to the direct close contact between the fungus and the fungicides in agar plate. However, the ineffectiveness of a fungicide in inhibiting mycelial growth *in vitro* may not necessarily be the same on

the field. For instance, it has been demonstrated in an experiment by Davis and Dimond (1952) that some systemics which are fungicidally inactive *in vitro* could initiate antifungal effect *in vivo*.

In the present study, the effect of sowing dates on incidence and severity of leaf blight did not show a definite trend. The differences observed in disease parameters may be due to changes in weather which affects disease development. At 71 and 78 DAS, disease incidence increased from 78 % to 91 % and from 61 % to79 % for large and small seeded castor respectively at August 3 sowing date. This was due to fluctuations in temperature and relative humidity in the 2006 raining season (Appendix C). Large seeded castor sown in August 11 and small seeded sown in August 3 had high disease incidence when compared with July 27 sown plants. It could likely be the low disease incidence in early sown crop was due to low inoculum level and the drier weather early in the season. On the contrary, Jidda *et al.* (2005) reported the lowest incidence and severity of smut on Pearl millet when sowing was delayed. At high temperatures of 26-27.5 $^{o}$C and daily rainfall recorded from mid - August to end of September, the leaf blight incidence was high (Appendix C). In a related work, Wang *et al.* (1999) reported the contrary, that the development of symptoms of *Fusarium* wilt in Australian cotton was enhanced at a moderate temperature range of 18 – 23 $^{o}$C, but suppressed at a higher temperature range of 28 – 33 $^{o}$C. Also high relative humidity has been reported to increase disease incidence and severity of angular leaf spot and anthracnose on beans (Koech and Whitebread, 2000). In this study, there were no significant differences in leaf blight severity for all the sowing dates although severity at 71 and 78 DAS for both large and small seeded castor types sown in July 27 was higher. High leaf blight severity of 3.3 % and 2.7 % for large

and small seeded castor respectively, observed in July 27 and August 3 sown plants could be attributed to higher germination percentage recorded on the plots sown in these particular dates which created a humid microenvironment. This type of environment favours disease development (Agrios 2005). Although report showed that late planting of castor in India recorded reduced incidences and severities of diseases and pests (Kulkarni, 1959), which is contrary to the findings of this study. Favourable weather conditions, particularly persistent wet weather, wind driven rain splash in August and September encouraged rapid disease spread and development. In this study there were no significant differences between sowing dates and bean yields obtained from castor sown at different dates. The higher bean yield of July 27 sown large seeded plants when compared with plants sown at August 3 and 11 could be due to early sowing and good germination percentage. In a similar situation, Odo and Bibinu (1998) reported that, there was significant increase in grain yield of early sown Pearl millet / legume mixtures. Poor seedling germination observed in castor might have affected bean yield to some extent. Similarly, this agrees with the works of Jones and Wells (1998), who reported that poor seed germination and early seedling damage reduced plant populations in north-eastern cotton producing region of USA. On the other hand, small seeded plants sown at August 3 had higher yield when compared with July 27 and August 11 sowing. The reduction in yield observed in both varieties during the late planting of August, could be due to reduced germination percentage. In a similar work, Okoro (2005) reported that planting of soybean around August 4 suffered reduced germination percentage and yield compared to early sowing of July. An early planted crop escapes severe infection resulting in increased yield. Similarly, works of Adeoti and Popoola (2004) and Shafshak *et al.* (1987), reported

that early and mid July planting dates of cotton were more favourable for better seedling emergence, less incidence of *Alternaria* leaf spot and better seed cotton yield than planting in late July and early August. Bange *et al.* (1997) attributed the higher yield of cotton in some months to more favourable environmental conditions such as high radiation, high light interception and high radiation use efficiency. Even though there was variation in seed yield for both seed types, other factors could play a role such as seeds genetic make up, climatic conditions, time of planting and soil nutrient. It is recognized, however that yield is a complex parameter and other factors, besides disease may be involved.

# CHAPTER SIX

## SUMMARY AND CONCLUSION

The fungus inducing leaf blight and wilt on castor leaf was isolated, identified and its pathogenicity confirmed in the glasshouse. The fungus was identified at Crop Protection Department IAR, ABU Zaria as *Fusarium* sp which was confirmed at the International Mycological Institute (IMI), Egham London, as *Fusarium pallidoroseum*. The cultural and morphological characteristics were same as those described by CMI. During the pathogenicity tests 11 and 18 days old castor seedlings inoculated with the three methods of inoculation (spray, smear and soil) were most susceptible. High disease severity was observed in castor seedlings inoculated with high inoculum density.

The effect of varying concentrations of fungicides namely benomyl, benomyl + thiram, metalaxyl-M + thiomethoxam + difenoconazole, tricyclazole and carbendazim + mancozeb on mycelial growth, sporulation, spore sizes and mycelial regrowth of *Fusarium pallidoroseum* was evaluated *in vitro* and with the exception of metalaxyl-M + thiomethoxam + difenoconazole in the *in vivo*. All the concentrations of the fungicides tested significantly inhibited mycelial growth and sporulation. Benomyl, benomyl + thiram, tricyclazole had the greatest inhibitory effect on mycelial growth, sporulation and mycelial regrowth of the fungus. Mancozeb gave the least inhibitory effect on *Fusarium pallidoroseum in vitro*, while carbendazim + mancozeb and metalaxyl-M + thiomethoxam + difenoconazole gave partial inhibition. Mancozeb controlled disease better than all the other fungicides during *in vivo* evaluation.

In the field study conducted during the 2006 wet season at Samaru, the effect of three sowing dates on incidence and severity of leaf blight caused by *Fusarium pallidoroseum*

on castor was low with no significant differences between the sowing dates.

From the study, the following conclusions can be made:-

(1) Leaf blight and wilt of castor is induced by *Fusarium pallidoroseum.*

(2) The younger plants (11 days old) were more susceptible to infection.

(3) At high inoculum density of $3.6 \times 10^{10}$ the disease severity was higher at 7 DAI.

(4) The six chemicals evaluated *in vitro* had inhibitory effect on mycelial growth, sporulation, spore size and mycelial regrowth of *F. pallidoroseum* and their effect increased with concentration. For the *in vivo* trial all the fungicides at recommended rate gave better results by reducing leaf blight severity on castor plants than at half the recommended rate.

(5) Sowing late, August 11 resulted in poor seed germination thereby affecting bean yield.

From the above conclusions, *Fusarium pallidoroseum* on castor in Samaru can be controlled using fungicides. Other types of disease management like cultural control practices involving sowing dates and spacing, breeding for resistant varieties can also be evaluated so as to develop an effective control package for the disease.

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## Appendix A

IMI Identification for *F. pallidoroseum*

| | |
|---|---|
| ***Global Plant Clinic***<br>**www.globalplantclinic.org**<br>***email*: plantclinic@cabi.org** | **CABI Bioscience UK Centre,** Bakeham Lane, Surrey<br>*tel*: (+44) 1491 829080 *fax*: (+44) 1491 829100<br>**Tropical Virus Unit** ,Rothamsted Research, Harpenden<br>*tel.* (+44) 1582 763133 *fax.* (+44) 1582 760981<br>**Central Science Laboratory Sand** Hutton, York<br>*tel* (+44) 1904 46 20 00 *fax:*(+44) 1904 462111 |

**Final Report**
**Enquiry Number 074/07**

| | | | |
|---|---|---|---|
| **SENDERS DETAILS:** | | **ENQUIRY DETAILS:** | |
| **Name and Address** | Mrs Alabi<br>Department of Crop Protection,<br>Faculty of Agriculture<br>Ahmadu Bello University,<br>Zaria, Nigeria | **Date of Report:** | August 07 |
| | | **Enquiry Type:** | |
| **Country** | Nigeria | | |
| **Samples** | **Castor** | | |

| GPC ref | Ref | Host | Part | Site | Identifications |
|---|---|---|---|---|---|
| W10338 | A | Castor | Culture | Samaru Zaria | *Fusarium pallidoroseum*<br>*Fusarium* sp (IMI 395559) This culture is contaminated with bacteria and problematic to identify |
| W10339 | B | Castor | Culture | Samaru Zaria | *Fusarium pallidoroseum* |
| W10340 | C | Castor | Culture | Samaru Zaria | *Fusarium pallidoroseum*<br>*Fusarium* sp (IMI 395558) The culture is contaminated with bacteria which makes identification very difficult, and no conidiogenous cells were seen which are critical for diagnosis. The conidia bear some resemblance to those of *Fusarium lateritium*, but this identification is very tentative |
| W10341 | D | Castor | Culture | Samaru Zaria | *Fusarium pallidoroseum* |

**Appendix B**

List of Fungicides, Manufacturers Rates and Trade names used in the Laboratory and Screenhouse for their Efficacy on *F. pallidoroseum*

| Fungicide | Manufacturers Rate | Trade name |
|---|---|---|
| Benomyl | 0.15 g a.i /litre | Benlate 50 WP Dupont |
| Benomyl + thiram | 0.8 g a.i / litre | Bentex T 40 WP Dupont |
| Carbendazim + mancozeb | 1.12 g a.i / litre | Team 84 WP African Agro |
| Tricyclazole | 0.4 g a.i/ litre | Profit 85 WP African Agro |
| Metalaxyl-M + thiomethoxam + difenoconazole | 0.04 g a.i/ litre | Apron star 42 WP Sygenta |
| Mancozeb | 0.62 g a.i / litre | Dithane M45 80 WP Dupont |

## Appendix C

## DAILY WEATHER DATA FOR 2006 RAINY SEASON

| | July | | | August | | | September | | | October | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S/N | Rainfall (mm) | Temperature (⁰C) | Relative Humidity | Rainfall (mm) | Temperature (⁰C) | Relative Humidity | Rainfall (mm) | Temperature (⁰C) | Relative Humidity | Rainfall (mm) | Temperature (⁰C) | Relative Humidity |
| 1 | 3.6 | 24 | 80.0 | - | 27.0 | 61.5 | - | 26.0 | 77.5 | - | 25.0 | 94.5 |
| 2 | - | 25.5 | 68.0 | 18.4 | 25.0 | 72.0 | 5.4 | 27.0 | 74.0 | 1.1 | 25.5 | 69.0 |
| 3 | - | 27.5 | 71.5 | - | 23.5 | 84.0 | 10.7 | 24.5 | 68.0 | 10.8 | 27.0 | 66.5 |
| 4 | - | 27.5 | 71.0 | - | 24.0 | 78.0 | 29.8 | 24.5 | 82.5 | - | 24.0 | 74.0 |
| 5 | - | 27.0 | 67.0 | - | 25.0 | 74.0 | - | 24.0 | 79.0 | 5.5 | 25.5 | 79.5 |
| 6 | - | 27.0 | 69.0 | 30.0 | 26.5 | 73.0 | - | 26.0 | 76.0 | - | 26.5 | 80.0 |
| 7 | - | 25.0 | 76.5 | - | 25.0 | 76.0 | 20.1 | 25.5 | 71.5 | 3.5 | 27.0 | 63.0 |
| 8 | - | 26.5 | 72.5 | - | 24.0 | 84.0 | - | 23.5 | 90.0 | - | 28.0 | 63.0 |
| 9 | 8.0 | 26.0 | 69.5 | - | 25.5 | 71.5 | 4.8 | 23.5 | 83.0 | 0.9 | 27.0 | 70.5 |
| 10 | 6.2 | 25.0 | 78.5 | 26.4 | 26.0 | 79.5 | - | 25.0 | 81.5 | - | 27.5 | 62.5 |
| 11 | - | 25.0 | 59.5 | - | 23.5 | 82.0 | 20.1 | 26.5 | 73.5 | - | 28.0 | 69.0 |
| 12 | 43.8 | 27.0 | 68.5 | - | 25.0 | 75.0 | 15.0 | 25.5 | 78.0 | - | 26.5 | 74.5 |
| 13 | - | 25.5 | 68.5 | 6.9 | 26.5 | 71.0 | 24.9 | 23.5 | 88.0 | - | 27.5 | 53.5 |
| 14 | 21.6 | 26.0 | 74.5 | 1.0 | 25.0 | 66.5 | 30.5 | 24.0 | 79.5 | - | 26.0 | 58.0 |
| 15 | - | 24.5 | 73.5 | - | 24.0 | 74.0 | - | 24.5 | 75.0 | - | 25.5 | 60.5 |
| 16 | 8.7 | 27.0 | 70.5 | - | 26.0 | 70.5 | - | 26.0 | 68.0 | 6.7 | 28.5 | 65.5 |
| 17 | 40.0 | 25.5 | 69.0 | 31.5 | 26.5 | 72.0 | - | 27.5 | 71.0 | - | 29.0 | 64.5 |
| 18 | 50.3 | 24.5 | 83.5 | - | 26.0 | 71.0 | 0.6 | 27.0 | 63.0 | - | 28.0 | 58.5 |
| 19 | 7.4 | 25.5 | 76.5 | - | 26.0 | 70.0 | 13.9 | 26.5 | 75.5 | - | 27.0 | 64.0 |
| 20 | - | 24.0 | 79.5 | 4.1 | 26.5 | 65.5 | 21.1 | 26.0 | 87.0 | - | 28.5 | 68.0 |
| 21 | 163 | 25.5 | 74.5 | 33.5 | 24.0 | 83.0 | - | 25.0 | 78.5 | - | 27.0 | 62.0 |
| 22 | 8.8 | 25.0 | 72.5 | 2.0 | 24.0 | 95.0 | 1.3 | 27.0 | 70.5 | - | 27.0 | 54.0 |
| 23 | 9.4 | 25.5 | 77.0 | 1.2 | 24.0 | 95.0 | 18.4 | 26.0 | 77.5 | - | 28.5 | 50.0 |
| 24 | 4.7 | 24.0 | 74.5 | - | 25.0 | 70.5 | 0.7 | 24.5 | 74.5 | - | 28.0 | 50.5 |
| 25 | 3.4 | 25.5 | 85.0 | - | 24.5 | 77.0 | - | 27.0 | 70.5 | - | 28.5 | 49.0 |
| 26 | - | 25.5 | 76.0 | 10.9 | 25.5 | 67.5 | - | 26.5 | 65.0 | - | 27.0 | 47.5 |
| 27 | - | 27.0 | 70.0 | 203 | 24.5 | 77.0 | - | 26.0 | 67.5 | - | 23.0 | 49.0 |
| 28 | - | 27.0 | 69.0 | - | 25.0 | 84.0 | - | 25.5 | 69.5 | - | 23.5 | 46.0 |
| 29 | - | 26.0 | 71.0 | 20.5 | 25.0 | 77.0 | 57.7 | 27.0 | 67.5 | - | 23.0 | 33.0 |
| 30 | - | 26.5 | 64.0 | 8.8 | 24.5 | 73.0 | - | 24.5 | 71.0 | - | 23.5 | 44.0 |
| 31 | - | 25.5 | 63.5 | 6.5 | 25.5 | 75.5 | - | - | - | | 21.5 | 29.0 |
| **Total** | **232.2** | **798.5** | **2244.5** | **222.0** | **782** | **2344.5** | **275.0** | **742.0** | **2253.5** | **28.5** | **818.5** | **1872.5** |
| **Mean** | | **25.8** | **72.45** | | **25.0** | **75.5** | | **24.73** | **75.1** | | **26.4** | **60.4** |

**Appendix D**

## Anova Tables

Anova Table for Pathogenecity

| Source | Df | Incidence for Spraying method | | | |
|---|---|---|---|---|---|
| | | Sum of squares | Mean square | F-value | Pr>F |
| Treatment | 3 | 25276.40 | 8425.47 | 1.00 | 0.440 |
| Error | 8 | 67219.55 | 8402.44 | | |
| Total | 11 | 92495.95 | | | |

Incidence for Smearing

| Source | Df | Sum of squares | Mean square | F-value | Pr>F |
|---|---|---|---|---|---|
| Treatment | 3 | 21333.77 | 7111.26 | 64.51 | 0.0001 |
| Error | 8 | 881.86 | 110.23 | | |
| Total | 11 | 22215.63 | | | |

Incidence for Soil Inoculation

| Source | Df | Sum of squares | Mean square | F-value | Pr>F |
|---|---|---|---|---|---|
| Treatment | 3 | 26380.23 | 8793.41 | 379.72 | 0.0001 |
| Error | 8 | 185.26 | 23.16 | | |
| Total | 11 | 26565.49 | | | |

Anova for severity using Spraying Method at 7 DAI

| Source | Df | Sum of squares | Mean square | F-value | Pr>F |
|---|---|---|---|---|---|
| Treatment | 3 | 26.92 | 8.97 | 11.96 | 0.0025 |
| Error | 8 | 6.00 | 0.75 | | |
| Total | 11 | 32.92 | | | |

Anova for severity using smearing method at 7 DAI

| Source | Df | Sum of squares | Mean square | F-value | Pr>F |
|---|---|---|---|---|---|
| Treatment | 3 | 19.58 | 6.53 | 15.67 | 0.0010 |
| Error | 8 | 3.33 | 0.42 | | |
| Total | 11 | 21.92 | | | |

Anova for severity using soil Inoculation method at 7 DAI

| Source | Df | Sum of squares | Mean square | F-value | Pr>F |
|---|---|---|---|---|---|
| Treatment | 3 | 54.00 | 18.00 | 18.00 | 0.0006 |
| Error | 8 | 8.0 | 1.00 | | |
| Total | 11 | 62.00 | | | |

Anova for severity using spraying method at 14 DAI

| Source | Df | Sum of squares | Mean square | F-value | Pr>F |
|---|---|---|---|---|---|
| Treatment | 3 | 77.67 | 25.89 | 38.83 | 0.0001 |
| Error | 8 | 5.33 | 0.67 | | |
| Total | 11 | 83.00 | | | |

Anova for severity using smearing method of 14 DAI

| Source | Df | Sum of squares | Mean square | F-value | Pr>F |
|---|---|---|---|---|---|
| Treatment | 3 | 65.85 | 21.86 | 32.79 | 0.0001 |
| Error | 8 | 5.53 | 0.67 | | |
| Total | 11 | 70.91 | | | |

Anova for severity using soil Inoculation at 14 DAI

| Source | Df | Sum of squares | Mean square | F-value | Pr>F |
|---|---|---|---|---|---|
| Treatment | 3 | 134.00 | 44.67 | 33.50 | 0.0001 |
| Error | 8 | 10.66 | 1.33 | | |
| Total | 11 | 144.66 | | | |

Anova for severity using Spraying Method at 21 DAI

| Source | Df | Sum of squares | Mean square | F-value | Pr>F |
|---|---|---|---|---|---|
| Treatment | 3 | 202.25 | 67.42 | 62.23 | 0.0001 |
| Error | 8 | 8.66 | 1.08 | | |
| Total | 11 | 210.91 | | | |

Anova for severity using smearing method at 21 DAI

| Source | Df | Sum of squares | Mean square | F-value | Pr>F |
|---|---|---|---|---|---|
| Treatment | 3 | 243.00 | 81.00 | 99999.99 | 0.0 |
| Error | 8 | 0.00 | 0.00 | | |
| Total | 11 | 243.00 | | | |

Anova for severity using soil Inoculation method at 21 DAI

| Source | Df | Sum of squares | Mean square | F-value | Pr>F |
|---|---|---|---|---|---|
| Treatment | 3 | 243.00 | 81.00 | 99999.99 | 0.0 |
| Error | 8 | 0.00 | 0.00 | | |
| Total | 11 | 243.00 | | | |

Anova for disease incidence using Inoculum density

| Source | Df | Sum of squares | Mean square | F-value | Pr>F |
|---|---|---|---|---|---|
| Treatment | 4 | 8183.63 | 2045.91 | 3.50 | 0.0491 |
| Error | 10 | 5843.58 | 584.36 | | |
| Total | 14 | 14027.21 | | | |

Anova for disease severity at 7 DAI

| Source | Df | Sum of squares | Mean square | F-value | Pr>F |
|---|---|---|---|---|---|
| Treatment | 4 | 8.27 | 2.06 | 2.38 | 0.1208 |
| Error | 10 | 8.66 | 0.86 | | |
| Total | 14 | 16.93 | | | |

Anova for disease severity at 7 DAI

| Source | Df | Sum of squares | Mean square | F-value | Pr>F |
|---|---|---|---|---|---|
| Treatment | 4 | 20.00 | 5.00 | 2.59 | 0.1016 |
| Error | 10 | 19.33 | 1.93 | | |
| Total | 14 | 39.33 | | | |

Anova for disease severity at 21 DAI

| Source | Df | Sum of squares | Mean square | F-value | Pr>F |
|---|---|---|---|---|---|
| Treatment | 4 | 32.00 | 8.00 | 3.68 | 0.0445 |
| Error | 10 | 22.00 | 2.20 | | |
| Total | 14 | 54.00 | | | |

Anova for mycelial growth at 7 DAI showing interaction of fungicides and concentrations

| Source | DF | Sum of Squares | Mean Square | F- value | Pr>F |
|---|---|---|---|---|---|
| Treatmant | 6 | 975.113 | 162.518 | 1282.08 | 0.0001 |
| Concentration | 2 | 35.360 | 17.680 | 139.47 | 0.0001 |
| Trt x Conc | 12 | 62.339 | 5.194 | 40.98 | 0.0001 |
| Error | 84 | 10.648 | 0.126762 | | |
| Total | 104 | 1083.461 | | | |

Anova for mycelial growth at 14 DAI showing interaction of fungicides and concentrations.

| Source | DF | Sum of Squares | Mean Square | F- value | Pr>F |
|---|---|---|---|---|---|
| Treatmant | 6 | 995.284 | 165.880 | 2708.78 | 0.0001 |
| Concentration | 2 | 39.225 | 19.612 | 320.27 | 0.0001 |
| Trt x Conc | 12 | 68.101 | 5.675 | 92.67 | 0.0001 |
| Error | 84 | 5.144 | 0.061238 | | |
| Total | 104 | 1107.755 | | | |

Anova for conidia count at 14 DAI showing interaction of fungides and concentrations

| Source | DF | Sum of Squares | Mean Square | F- value | Pr>F |
|---|---|---|---|---|---|
| Treatmant | 6 | 194813.04 | 32468.84 | 3651.96 | 0.0001 |
| Concentration | 2 | 5340.98 | 2670.49 | 300.37 | 0.0001 |
| Trt x Conc | 12 | 16031.69 | 1335.9743 | 150.26 | 0.0001 |
| Error | 40 | 355.63 | 0.0612 | | |
| Total | 62 | 216561.24 | | | |

Anova for conidia lengh at 14 DAI showing interaction of fungides and concentrations

| Source | DF | Sum of Squares | Mean Square | F- value | Pr>F |
|---|---|---|---|---|---|
| Treatmant | 6 | 2574.90 | 429.15 | 8.28 | 0.0001 |
| Concentration | 2 | 225.68 | 122.84 | 2.18 | 0.1201 |
| Trt x Conc | 12 | 1846.97 | 153.91 | 2.97 | 0.0018 |
| Error | 84 | 4146.93 | 51.83 | | |
| Total | 104 | 1766.299 | | | |

Anova for conidia width at 14 DAI showing interaction of fungides and concentrations

| Source | DF | Sum of Squares | Mean Square | F- value | Pr>F |
|---|---|---|---|---|---|
| Treatmant | 6 | 368.38 | 61.39 | 27.57 | 0.0001 |
| Concentration | 2 | 9.25 | 4.64 | 2.08 | 0.1321 |
| Trt x Conc | 12 | 61.78 | 5.14 | 2.31 | 0.0137 |
| Error | 84 | 178.17 | 2.22 | | |
| Total | 104 | 628.90 | | | |

Anova for mycelial regrowth at 14 DAI showing interaction of fungides and concentrations

| Source | DF | Sum of Squares | Mean Square | F- value | Pr>F |
|---|---|---|---|---|---|
| Treatmant | 6 | 1344.006 | 224.001 | 3657.87 | 0.0001 |
| Concentration | 2 | 109.537 | 54.768 | 894.36 | 0.0001 |
| Trt x Conc | 12 | 307.612 | 25.634 | 418.60 | 0.0001 |
| Error | 84 | 5.144 | 0.0612 | | |
| Total | 104 | 1766.299 | | | |

Anova for carbendazim+ mancozeb at half recommended rate during *in vivo* experiment

| Source | Df | Sum of squares | Mean square | F-value | Pr>F |
|---|---|---|---|---|---|
| Treatment | 3 | 36.00 | 12.00 | 12.00 | 0.0025 |
| Error | 8 | 8.00 | 1.00 | | |
| Total | 11 | 44.00 | | | |

Anova for Benomyl at half recommended rate during *in vivo* experiment

| Source | Df | Sum of squares | Mean square | F-value | Pr>F |
|---|---|---|---|---|---|
| Treatment | 3 | 9.00 | 3.00 | 1.00 | 0.4411 |
| Error | 8 | 24.00 | 3.00 | | |
| Total | 11 | 33.00 | | | |

Anova for benomyl+thiram at half recommended rate during *in vivo* experiment

| Source | Df | Sum of squares | Mean square | F-value | Pr>F |
|---|---|---|---|---|---|
| Treatment | 3 | 44.00 | 14.66 | 6.29 | 0.0169 |
| Error | 8 | 18.66 | 2.33 | | |
| Total | 11 | 62.66 | | | |

Anova for mancozeb at half recommended rate during *in vivo* experiment

| Source | Df | Sum of squares | Mean square | F-value | Pr>F |
|---|---|---|---|---|---|
| Treatment | 3 | 13.33 | 4.44 | 6.67 | 0.0144 |
| Error | 8 | 5.33 | 0.66 | | |
| Total | 11 | 18.66 | | | |

Anova for tricyclazole at half recommended rate during *in vivo* experiment

| Source | Df | Sum of squares | Mean square | F-value | Pr>F |
|---|---|---|---|---|---|
| Treatment | 3 | 4.91 | 1.63 | 2.19 | 0.1678 |
| Error | 8 | 6.00 | 0.75 | | |
| Total | 11 | 10.91 | | | |

Anova for check during *in vivo* experiment

| Source | Df | Sum of squares | Mean square | F-value | Pr>F |
|---|---|---|---|---|---|
| Treatment | 3 | 81.00 | 27.00 | 27.00 | 0.0002 |
| Error | 8 | 8.00 | 1.00 | | |
| Total | 11 | 89.00 | | | |

Anova for control during *in vivo* experiment

| Source | Df | Sum of squares | Mean square | F-value | Pr>F |
|---|---|---|---|---|---|
| Treatment | 3 | 0 | 0 | 99999.99 | 0.0 |
| Error | 8 | 0 | 0 | | |
| Total | 11 | 0 | | | |

Anova for carbendazim + mancozeb at recommended rate during *in vivo* experiment

| Source | Df | Sum of squares | Mean square | F-value | Pr>F |
|---|---|---|---|---|---|
| Treatment | 3 | 86.33 | 28.77 | 10.79 | 0.0035 |
| Error | 8 | 21.33 | 2.66 | | |
| Total | 11 | 107.66 | | | |

Anova for benomyl at recommended rate during *in vivo* experiment

| Source | Df | Sum of squares | Mean square | F-value | Pr>F |
|---|---|---|---|---|---|
| Treatment | 3 | 2.25 | 0.75 | 0.47 | 0.7091 |
| Error | 8 | 12.66 | 1.58 | | |
| Total | 11 | 14.91 | | | |

Anova for benomyl + thiram at recommended rate during *in vivo* experiment

| Source | Df | Sum of squares | Mean square | F-value | Pr>F |
|---|---|---|---|---|---|
| Treatment | 3 | 9.66 | 3.22 | 1.07 | 0.4131 |
| Error | 8 | 24.00 | 3.00 | | |
| Total | 11 | 33.66 | | | |

Anova for mancozeb at recommended rate during *in vivo* experiment

| Source | Df | Sum of squares | Mean square | F-value | Pr>F |
|---|---|---|---|---|---|
| Treatment | 3 | 11.00 | 3.66 | 11.00 | 0.0033 |
| Error | 8 | 2.66 | 0.33 | | |
| Total | 11 | 13.66 | | | |