EFFICACY OF SELECTED BOTANICALS AND MICROBIAL AGENTS AS ECO-FRIENDLY PROTECTANTS AGAINST *Fusarium verticillioides* (Sacc.) NIRENBERG EAR-ROT DISEASE OF MAIZE, *Zea mays L.*

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ABSTRACT

Fusarium verticillioides is a major fungal pathogen of maize causing rots in seed, root, stalk and ear; seedling blight and fumonisin mycotoxin contamination of grains. Use of chemicals is being discouraged due to its human and environmental health hazards. Information on eco-friendly control method against the pathogen is scanty. Therefore, the efficacies of selected botanicals and microbial agents as eco-friendly control against *F. verticillioides* were determined.

Six Quality Protein Maize (QPM) varieties (ART/98/SW5, ART/98/SW4, ART/98/SW6, OBATANPA, ILE-1-OB, TZPB) and ACR/99/TZL (local check) were evaluated in the field to identify susceptible varieties used as seed treatments in two planting seasons in Randomised Complete Block Design (RCBD) with three replicates. Seedling Blight (SB), Lodging after Tasselling (LT) and percentage ear-rot at maturity were assessed using standard procedures. Aqueous extracts of *Tithonia diversifolia*, *Mirabilis jalapa*, *Senna occidentalis* and *Physalis angulata* were evaluated in the laboratory in Completely Randomised Design (CRD) and on field in RCBD for fungicidal activities against *F. verticillioides* at 30.0%, 25.0%, 20.0%, 15.0%, 10.0% and 5.0% (w/v). Four microbial agents: *Trichoderma pseudokoningii*, *T. harzianum*, *Pseudomonas fluorescens* and *Bacillus subtillis* were separately bioassayed at four levels of concentration, alongside carbamate in the laboratory in CRD and the effective levels of concentration were used on the field in RCBD as protectants against *F.verticillioides*. ILE-1-OB, ART/98/SW5 and ACR/99/TZL seeds were treated with *S.occidentalis* at 10.0% and *T. pseudokoningii* at 7.1 x 10^8 spores/mL using three coating methods [Cooked-cassava Starch Slurry (CSS), Water Suspension (WS) and Powder Treatment (PT)] were used alongside carbamate (0.5 g/L). Data were taken on mycelial growth inhibition (%), germination (%) and yield (kg), and subjected to descriptive statistics and ANOVA at p=0.05.

Variety ART/98/SW5 with 15.0% ear-rot; 13.0% SB and 18.0% LT was rated highly resistant. Other five varieties had >30.0% LT; >20.0% ear-rot and SB, rated moderately susceptible. Aqueous *S. occidentalis* at 10.0% caused most significant mycelial growth inhibition (20.1%) followed by *M. jalapa* (16.7%) at 25% concentration. Inhibitory property of *S. occidentalis* produced 3.1% yield increase compared with control. Conversely, *T. diversifolia* stimulated rather than inhibit mycelial growth of *F.verticillioides* by 1.9% at 25.0% concentration resulting in yield reduction by 1.4%. Similarly, *P. fluorescens* at 1.0x10^9 cfu/mL significantly inhibited mycelial growth by 51.8% followed by *T. pseudokoningii* at

7.1×10^8 spores/mL by 44.7%; which indicates high inhibitory potential against *F.verticillioides* in the field. Carbamate inhibited mycelia by 34.6% and 10.0% yield increase. Most effective coating method was CSS with significantly lowest incidence of ear-rot (15.0%) after carbamate (3.2%) followed by WS (20%) and PT (30%). *Trichoderma pseudokoningii* as microbial agent had highest effect (18.0%) on maize germination followed by (15.3%) *P. fluorescens*, (13.3%) *T. harzianum* and (1.6%) *B. subtilis*. *Senna occidentalis* and *M. jalapa* had significant effect of 11.1% and 8.3% respectively on maize germination compared with control, while *T. diversifolia* 3.0% and *P. angulata* 0.9%.

Use of resistant variety-ART/98/SW5, aqueous extract of *Senna occidentalis* at 10.0% concentration and *Trichoderma pseudokoningii* at 7.1×10^8 spores/mL provided best treatment of *Fusarium verticillioides* of ear-rot disease of quality protein maize. These treatments could be incorporated into integrated management of the pathogen.

CERTIFICATION

I certify that this work was carried out by Mrs. Oluwafolake Adenike AKINBODE at the Department of Crop Protection and Environmental Biology, University of Ibadan, Ibadan and the Institute of Agricultural Research and Training (I. A. R & T), Ibadan, Nigeria.

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CHAPTER ONE

INTRODUCTION

Maize (*Zea mays* L) is the third most important cereal crop in the tropics (CIMMYT, 2004). It is an important grain crop in the world due to its ability to produce economic yields under the marginal production conditions of low soil fertility and management (FAO, 2005). Total world production of maize in 2011 was estimated at 817,110,509 MT. Nigeria's share of the production is 9, 180, 270 MT (FAOSTAT, 2011). It is a food crop of economic significance and nutritional importance in the human diet; as a major staple food of the people, animal feed, fodder crop and raw materials for industries. Demands for maize have always exceeded its supply in Nigeria, indicating the need for higher productivity in terms of quantity and quality (Smale *et al*., 2011).

Conventional maize is a poor-quality staple food; unless consumed as part of a varied diet – which is beyond the means of most people in the developing world. But, Quality protein maize (QPM), new release of maize variety(ies), produces 70 to 100% ore of lysine and tryptophan and yields 10% more grain than most varieties of tropical maize (Vasal, 2000). These two amino acids allow the body to manufacture complete proteins, thereby eliminating wet-malnutrition (kwashiorkor). In addition tryptophan can be converted in the body to niacin, which theoretically reduces the incidence of pellagra. QPM offers 90% of the nutritional value of skim milk (typical Composition for Skim Milk Powder Lactose 49.5 – 52.0%, Protein 34.0 – 37.0%, Ash 8.2 – 8.6%, Moisture 3.0 – 4.0%, fat 0.6 – 1.25% and essential Amino acids (g/100g protein): Isoleucine 2.19, Leucine 3.54, Lysine 2.87, Methionine 0.91 and Phenylalanine 1.75), the standard for adequate nutritional value (Hugo, 2000; Olakojo *et al*., 2007; Upadhyay *et al*., 2009). These qualities make QPM to be more preferred and highly recommended for consumption.

Maize is highly susceptible to both pests and parasites that cause pre- and post harvest losses which leads to great economic losses in Nigeria (Samuel *et al*., 2011). *Fusarium* ear rot of maize is among the destructive diseases in many areas of the country and most maize growing areas in the world. Anon (1985) reported a range of 5 – 40% infected fields in the tropics. Ear rot reduces crop yield directly by destroying

the grains, and the mycotoxin produced due to this disease causes food contamination which is poisonous to human and livestock.

Maize is most commonly colonized crop by *Fusarium verticillioides* (Leslie and Summerell, 2005).

This fungus is common in African countries and Nigeria is not an exception (Leslie and Summerell, 2005). The pathogen causes ear and stalk rot of maize. Stalk rot causes stalk breakage and lodging thereby making harvesting difficult and consequently reducing grain yield, while ear rot reduces plant yield directly by destroying the grain quality. Leslie *et al.* (1990) reported that *F. verticillioides* colonizing maize tissues are resident in roots, stalks, ear and most other plant parts. Symptomless (latent) infection can exist throughout the plant, and seed-transmitted strains of the fungus can develop systemically to infect the kernels (Munkvold and Desjardins, 1997). Diseases of maize associated with *F. verticillioides* include seed rot, root rot, stalk rot, kernel or ear rot, and seedling blight (Bacon and Nelson, 1994; Bacon and Hinton, 1996). It is an important contributor to maize seedling disease, and for the induction of leaf lesions indicative of foliar maize diseases (USDA, 2007 Annual Report). Fumonisin is a mycotoxin produced by this pathogen in the grains.

The fungus can exist as a saprobe or an endophyte. The grains from the symptomless infected plants enter the food chain of animal and man (Bacon and Hinton, 1996) with the potential to produce a mycotoxin that adversely affects the consumers (Macdonald and Chapman, 1997; Leslie and Summerell, 2005). The mycotoxin occurs anywhere this fungus proliferates, causing chemical pollution of biological origin and setting (Bacon *et al.*, 1992; Merrill *et al.*, 1996a, b). Cooney *et al.* (2001) reported that *Fusarium* infection is generally a problem in grain crops. Drepper and Renfro (1990) reported that the high incidence of *Fusarium* species can be attributed to favourable weather conditions (during the rainy season when relative humidity is high), for the fungus to develop and this occurs during grain filling, at harvest, and not in the store. In Nigeria, the public is generally unaware of the hazard posed by this fungus on any infected grains either symptomatic or asymptomatic (Bacon *et al.*, 1992). There is little information at farmers' level on how to reduce or eradicate its presence in maize either at pre- and /or post-harvest.

The necessity of quality maize for cultivation and storage for later use, has led to various efforts aimed at controlling this disease of maize, which will subsequently reduce the incidence of mycotoxin accumulation in maize seeds thereby acting as a

way to minimize the hazard due to mycotoxin contamination. The approaches to the control of pathogens of maize have taken various forms such as the use of chemicals, cultural control strategies and integrated methods (MacDonald and Chapman, 1997). These control measures sometimes have various setbacks even at the farmer's level of operation in Nigeria.

The use of chemicals has long-term effects on the health of the populace, the natural environment, sometimes; pathogens build up of resistance to it. The World Health Organization linked several deaths of people and those that suffer acute health conditions to pesticide poisoning (GOAN, 1999; Coulibaly and Lowenberg-DeBoer, 2002).

No effective control strategy has been reported as efficient till date. This has raised the interest in developing alternative methods of control from nature. Nature has a lot of plants and microorganisms for use in natural crop protection for a cleaner and safer environment. This is the use of botanicals and biological control methods. This requires little skill; it is a cheap and readily available control measure. Little effort has been made to investigate the method, time of application of the botanicals and bioagents on maize stalk, as well as the toxin inactivation in maize using this control strategy especially in Nigeria (Sobowale *et al*., 2007). The development of environmentally friendly strategies for disease management requires an understanding of suitable, available, environmentally friendly antagonistic micro-organisms and botanicals, whose efficient application and assessment methods could inactivate the pathogen on maize. A good grasp of these aspects would contribute to better management of the disease, reduce contamination thereby producing cleaner, safer maize seeds, enhance higher yields and invariably increase the farmers' income.

The plants tested in this study are *Tithonia diversifolia*; *Physalis angulata, Senna occidentalis,* and *Mirabilis jalapa* which are said to have fungicidal properties. They are known for their risk-free effect on beneficial organisms and have been used to treat human infections but, not much documented on plant diseases. Also, beneficial antagonistic organisms isolated from the soil (*Trichoderma harzianum, T. pseudokoningii, Pseudomonas fluorescens and Bacillus subtilis*) were used in this study.

Currently, there is the need to obtain information that would contribute to the protection of maize for better management against *F. verticillioides*. Various formulations have been used to control pathogens through manipulation of the

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environment, host, or antagonists, or by mass introduction of one or more antagonists (Adekunle *et al.,* 2001). These methods include the use of fungal spores (Harman *et al*., 1980), suspensions of bacteria (Paulitz, 1992), powdery preparations of fungal mycelium (Latunde-Dada, 1993), and the use of botanicals (Bilgrami *et al*., 1980; Chauhan and Joshi, 1990; Peluola, 2005). There is also the need to try new formulations for effective application of the botanicals and bioagents, for enhanced maize production at affordable cost, which will bring better earning to the resource-poor African farmers. The risks associated with the use of synthetic fungicides have stimulated interest in the development of natural sources of fungicides. Therefore, the objectives of this study were to:

1. evaluate the antagonistic ability of four (4) microbial agents and the antifungal potentials of four (4) botanicals on *Fusarium* ear rot pathogen of maize,

2. assess application methods of the bioagents and botanicals with a view to developing seed treatments for the use of the maize farmers,

3. identify and devise appropriate formulations of these bioagents and botanicals as seed treatments *in vitro* and *in vivo,* and

4. compare the effects of the botanicals and the microbial agents, with a standard synthetic fungicide on ear rot pathogen of maize to assess the suitability of both methods for the use of the farmers.

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CHAPTER TWO

2.0   LITERATURE REVIEW

2.1   Origin, taxonomy and distribution of maize

Maize (*Zea mays*) is the most commonly cultivated cereal crop in Nigeria and worldwide. It belongs to the family Poaceae (IITA, 1982). Maize is a versatile crop grown over a range of agro climatic zones. It is grown from 58^0N to 40^0S, from below sea level to altitudes higher than 3000 m, and in areas with 250 mm to more than 5000 mm of rainfall per year (Shaw, 1988; Dowswell *et al*., 1996) and with a growing cycle ranging from 3 to 13 months (CIMMYT, 2000). It is an annual crop with long leaves and tassel. The shoot carries the cob at maturity on only the short branch. Maize has high variation of cob forms, colours and sizes. It is grown for its seed, as a staple crop and as an industrial material in agro-based industries. The taxonomy of maize given as;

Kingdom: Plantae

Division: Magnoliophyta

Class: Lilliopsida

Tribe: Maydeae

Order: Poales

Family: Poaceae

Genus: *Zea*

Species: *Zea mays* L.

2.2   Agronomy and production of maize

Maize exhibits hypogeal type of germination at a rate of usually 90%, provided the conditions for germination are satisfied. Seed germination is easy when the conditions for germination are favourable. Chemical changes activate growth in the embryo axis (Manyong *et al*., 2000). The radicle elongates and emerges from the seed coat within 2 or 3 days. Shortly after, the plumule also begins to elongate and additional leaves begin to form inside the developing seedling (called coleoptiles after it breaks out of the seed). The time taken between planting and emergence depends mainly on the temperature conditions which may vary from the normal (4 -5 days to 15days) under cool conditions (IITA, 1982). After a vegetative stage, ending at the 20

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to 23rd foliage leaf, tassel initiation starts between 25 and 35 days from planting when the plants are about knee-high and the eighth leaf is fully emerged (IITA, 1982). Soon after tassel initiation, a period of rapid elongation starts and the plants accumulate dry matter and plant nutrients very rapidly, placing heavy demands on the root system to supply water and nutrients (Belfield and Brown, 2008). The roots also grow rapidly and the entire surface soil is filled with the root system. Ear development begins within a week of tassel formation. The number of rows that would develop on the ear is reduced if nitrogen becomes limiting at this stage of development. The potential number of ovules on the ear is also determined by six weeks after emergence. Pollen shedding takes place five to six days after tassel initiation (Eckebil, 1994). .

The tassel produces a large number of pollen grains which are shed over a period of five to eight days. The pollen shed begins from the middle of the central spike and spreads over the entire tassel. After pollination and ovule fertilization, the silks turn brown and within the next two weeks the kernels grow very rapidly. The embryo starts developing and the leaf initials are laid down (IITA, 1982). Seminal roots are also initiated while the embryo matures and dry matter accumulation ceases. The moisture content of the grain at this stage is usually about 35%. When the cob reaches dry state, called physiological maturity stage, the plants could be harvested at this stage without any loss in the grain yield. If drying facilities are not available, harvesting is delayed until the moisture content of the grain is reduced to 15 percent. The rate of drying of the plant depends on the weather conditions (IITA, 1982). In Nigeria, maize is usually intercropped with legumes such as cowpea, groundnut and tuber crops like yam, sweet potato (IAR&T, 2005).

The crop tolerates a wide range of climates from warm temperate to tropical. Young plants grow better with 50-60% of tropical solar radiation and under full daylight and may not grow well on altitude of 1,500metres above sea level. However, in hot dry areas, maize do well if irrigated. *Zea mays* are much suited to a humid tropical climate and often grow in semi-cultivated, i.e. having been introduced into a climatic situation, maintain themselves fairly successfully by their normal means of reproduction (Belfield and Brown, 2008).

In Nigeria, the crop is principally cultivated in the wet season and at times during dry season under irrigation between latitude 10°N and 12° 30'N of Northern-Guinea and the Sudan Savanna ecological zones. The mean temperature range for

night and day in these areas is above 21.1°C and 32.2°C respectively. Rainfall in the areas varies from 600mm to 1300mm, with one peak in July/August (Eckebil, 1994).

Most countries in the world grow maize. Use of maize is increasing globally. Maize production in Nigeria has been on the increase and with increased area under cultivation. Still, there is a wide gap between the potential and the actual yield due to constraints caused by pests and diseases.

2.3   Maize utilization in Nigeria

Maize is used for three main purposes: as a staple human food, as feed for livestock and as raw material for many agro-industries. It is a staple food of the people in Nigeria. Maize is used in beverage and ethanol production. It serves as a source of income for the resource – poor farmers particularly women (IAR&T, 2005). Maize is a crop par excellence for food, feed and industrial utilization. The composition of edible portion of maize include the water free portion of the kernel contains about 77 percent starch hence a good material for starch and glue. Its main nutritional content is carbohydrate, 2% sugar, 9% protein, 5 % fat, 5 % pentosan, and 2 % ash (IITA, 1982, AICRP on Maize, 2007). There is great variation among different strains of maize in the content of protein and fat. The proportion of crude protein may be as high as 15% and as low as 6%. The high carbohydrate content and some other essential nutrients make it suitable for the poor people in the developing countries (IITA, 1982). However, it is deficit in essential amino acid, lysine and d tryptophan. To overcome this deficiency, quality protein maize (QPM) with sufficiently higher quantity of lysine and tryptophan have been developed. The crop can be eaten cooked or roasted, processed as 'ogi' and feed for livestock. The crop is sometimes used as stakes for legumes and yam when intercropped. Maize is used in cowpea rotation for the control of *Striga hermonthica*, a parasitic plant that is devastating cereals production (Quin, 1997; Singh *et al*., 1997).

2.4   Constraints to maize production

Despite the importance of this crop, there are many constraints to its production. Constraints such as the climatic change (Olaoye, 1999; Adejuwon, 2004), edaphic factors (Henao and Baanante, 2006), pests and diseases (Ismaila *et al*., 2010) poor varieties (Idem and Showemimo, 2004), weed (Ciotola *et al*., 1995, IAPPS,

2007). Diseases and pests are primary constraints to maize production, which cause reduction in yield and quality of seed. Not all the diseases and pests occur in the same region or at the same time. Every region has specific diseases and pests, which are of major importance in reducing maize yields (IAR&T, 2005).

Maize is attacked by a large number of diseases, which cause severe losses of the crop in the field. Holliday (1990) defined disease as "a condition where the normal functions are disturbed and harmed". Disease by pathologists is a continuing relationship between a pathogen and its host plant in which the pathogen obtains nutrition while the host is damaged i.e., its growth, fertility, survival and yield are affected.

Several maize diseases have been reported by many researchers, while some are important in certain regions and seasons. Among the major groupings of maize diseases are the physiological disorders, viral, fungal, bacterial and to a little extent nematode diseases (Prescott *et al*., 2000). Fungal diseases have common names such as stalk rot, foliar blight, and leaf spot. But, the most useful criterion for describing a disease is by the organism causing it (Bosland and Votava, 2000). Maize is susceptible to a number of fungi. The important and prominent of all the fungal diseases of maize in terms of its economic importance worldwide is ear rot caused by *Fusarium verticillioides* (Leslie and Summerell, 2005; Afolabi, 2007).

2.5 Common maize diseases

The diseases of maize are caused by fungi, bacteria and viruses. Some of the common bacterial diseases of maize include bacterial leaf blight and stalk rot caused by *Pseudomonas avenae* subsp. *avenae* Manns; bacterial leaf spot caused by *Xanthomonas campestris* pv. *holcicola* (Elliott); Bacterial stalk and top rot caused by *Erwinia carotovora* subsp. *carotovora;* bacterial stripe caused by *Pseudomonas andropogonis* (Smith) Stapp; Goss's bacterial wilt and blight (leaf freckles and wilt) caused by *Clavibacter michiganensis* subsp. *nebraskensis* (Vidaver & Mandel). There is the Stewart's disease (bacterial wilt) caused by *Erwinia stewartii* (Smith) Dye (McGee, 1994).

The fungal diseases are anthracnose leaf blight and anthracnose stalk rot, caused by *Colletotrichum graminicola*; Aspergillus ear and kernel rot caused by *Aspergillus parasiticus*; Banded leaf and sheath spot caused by *Rhizoctonia solani*

Kühn; Black kernel rot caused by *Botryodiplodia theobromae* Pat; Brown spot caused by, *Physoderma maydis*; Curvularia leaf spot caused by *Curvularia lunata*. Diplodia ear rot, stalk rot, seed rot and seedling blight are caused by *Diplodia maydis* (Berk.) Sacc. Downy mildew caused by *Peronosclerospora maydis* (Racib.) C.G. Shaw; Fusarium ear and stalk rot induced by *F. moniliforme* J. Sheld. var. *subglutinans* now known as *F. verticillioides* (Wilke *et al*., 2007).

Others include *Pythium* stalk rot caused by *Pythium aphanidermatum* (Edson) Fitzp., *Rhizoctonia* ear, root and stalk rot caused by *Rhizoctonia solani* Kühn; Rust caused by *Puccinia sorghi*; Southern leaf blight caused by *Dreschlera maydis*. Storage rots are caused by *Aspergillus* spp., *Penicillium* spp. and other fungi. Parasitic higher plants that compete with maize in the field are the S*triga asiatica* (L.) Kutze commonly called *S. lutea* Lour. Also *S. hermonthica* (Del.) Benth is a major constraint to maize production (Karaya *et al*., 2012).

The virus and virus-like diseases are Corn lethal necrosis, Virus complex (Maize chlorotic mottle virus [MCMV] and Maize dwarf mosaic virus [MDMV] A, B, D and E, Maize chlorotic dwarf, Maize chlorotic dwarf virus (MCDV), Maize chlorotic mottle, and Maize chlorotic mottle virus (MCMV), maize leaf fleck (MLFV), cucumber mosaic, Johnsongrass mosaic, maize bushy stunt, maize pellucid ringspot, fine striping disease. Other maize viral diseases are ring mottle, rough dwarf, sterile stunt and maize streak (List of Maize diseases, the free encyclopedia http://en.wikipedia.org/wiki/List_of_maize_diseases, page was last modified on 17 October 2009 at 08:47).

2.6 Ear rot of maize caused by *Fusarium verticillioides*

Maize production is greatly affected by the incidence of diseases, resulting in huge economic losses. The *Fusarium* ear and stalk rot is caused by F*usarium verticillioides* (Reid *et al*., 2002). *Fusarium* attacks on maize are among the destructive diseases of maize in many areas of Nigeria. Anon (1985) reported a range of $5 - 40\%$ losses infected in maize fields especially in the tropics. Ear rot reduces plant yield directly by destroying the grains, while stalk rot causes stalk breakage and lodging; making harvesting difficult, leading to yield losses and contamination which is dangerous to human health. About 123 deaths were reported in Eastern part of Kenya as a result of this (Leslie and Summerell, 2005).

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Maize is most commonly colonized by *Fusarium verticillioides* which was formerly known as *Fusarium moniliforme*. This fungus is common in Africa with up to 95% of *Fusarium* recovered in a field (Leslie and Summerell, 2005). Leslie *et al.* (1990) reported that maize tissues are usually colonized by *F. verticillioides* resident in roots, stalks, and most other parts of the plant.

2.7   Spread and Distribution of ear rot disease

Fungi in the genus *Fusarium* are the most common causes of ear and kernel rot diseases of maize. The importance of these diseases has been recognized for many decades, but they remain difficult problems because high levels of genetic resistance have not been discovered and incorporated into high-yielding, agronomically desirable hybrids. The complexity of developing resistant maize hybrids is related to the multi-faceted epidemiology of *Fusarium* diseases. Ear rot is the common name for the disease caused by several species of the fungus *Fusarium*. The major incitant is *F. verticillioides* syn. *F. moniliforme* (White, 2004). *F. verticillioides* is rated as one of the most destructive pathogens of the crop (King, 1981; White, 2004). *Fusarium verticillioides* (Sacc.) Nirenberg is a common pathogen of maize causing root, stalk, and ear rots worldwide (Munkvold and Desjardins, 1997) and is more widespread in tropical and subtropical regions. The disease is often more severe particularly on monocropped maize, and spreads rapidly during cool and wet weather (Ochor *et al.*,1987; Vigier *et al.*,1997) where it causes considerable damage to maize plant parts. Ear rot is a major economic concern to maize producers and the processing industry due to the losses in grain yield and quality.

Losses due to stalk and ear rot in maize of 50-100% had been reported (McGee, 1988; Chungu *et al.*, 1997; Pascale *et al.*, 2002).The pathogen responsible is seed-borne, seed transmitted and in about 40% of the seeds of maize and persists in crop debris (McGee, 1988).

Plate. 2.1: Intact and split kernels covered with white or pinkish-white mold are typical symptoms of ear rot infection by *F. verticillioides*. Source: Munkvold *et al*. (1999).

2.8   Mode of Infection by causal organism

Fusarium verticillioides (synonym, *Fusarium moniliforme* Sheldon; teleomorph, *Gibberella moniliformis* [synonym, *Gibberella fujikuroi* mating population A]) is the most commonly reported fungal species infecting maize (Koehler, 1960; Miller *et al*., 1983; Munkvold and Carlton, 1997). *F. verticillioides* can be found in plant residues in almost every maize field at harvest, yet the disease symptoms vary widely and range from asymptomatic infection (latent infection) to severe rotting of all plant parts. In many cases diseased and asymptomatic plants occur in the same field planted with a genetically uniform host. Environmental conditions, water availability (Christensen and Wilcoxson, 1966; Lawrence *et al*., 1981; Desjardins *et al*., 1996a, b; Merrill *et al*., 1996a, b; Kulisek and Hazebroek, 2000), and the genetic background of the plant and the pathogen (Koehler, 1959; Salazar and Vargas, 1977; Kellerman *et al*., 1990a, b) may all be important factors in disease development.

Infection of maize by *F. verticillioides* can occur via several routes. The most commonly reported method of kernel infection is through airborne conidia that infect the silks (Drepper and Renfro, 1990; Headrick and Pataky, 1991; Leslie *et al*., 1992; Marasas, 1996). After invasion through the silks, the fungus infects the kernels, but usually only a small percentage of the infected kernels become symptomatic (Meadows, 2000). Another proposed infection pathway is systemic through the seed (Gelderblom *et al.,* 1988). Systemic infection can start from fungal conidia or mycelia that are either carried inside the seeds or on the seed surface. The fungus develops inside the young plant, moving from the roots to the stalk and finally to the cob and kernels.

Movement of the fungus from infected seeds to the stalk and kernels has been established in a number of studies, for instance as reported by Sobowale *et al*. (2001). Kedera *et al*. (2001) used vegetative compatibility (vegetative compatibility groups) as a marker to track specific strains and obtained evidence that the fungus moves from the seeds to kernels by recovering the isolate inoculated into the seeds from approximately 10% of the kernels. Similar findings were reported by researchers, who also used vegetative compatibility groups to track fungal movement in the plant (Marasas, 1996; Munkvold and Carlton, 1997; Meadows, 2000). These researchers also showed that although infected seeds may contribute to kernel infection, local infection via silks is the main pathway of kernel infection. Systemic infection also may result from

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inoculum that survives in crop residues in the soil; however, the relative importance of soilborne and seed-borne inocula as the cause of systemic infections is not known (Koehler, 1960; Payne *et al.*, 1988).

The pathogen *F. verticillioides* enters naturally by penetrating the host through mechanical wounds or punctures of the sucking insect (vector). The infection process is briefly described as follows: under favourable conditions, a spore of *Fusarium* germinates in - hours and forms an appressorium in close contact with the host surface. From this appressorium, thin penetration tube passes through the cuticle into thick hyphae. At first, the fungus invades the parenchymatous tissue slowly, penetrating the cell walls by thin tubes, which suggests that this is done by mechanical pressure. On colonizing the cells, the surrounding hyphae are formed which penetrate cells in all directions. When resistant varieties are used, the cuticle and epidermis are invaded in a similar way. The fungus seldom advances beyond one or two cells and these die promptly (Carlile *et al*., 2004).

Between 4 and 5 days of infection, the invaded tissues collapse and die, and discoloration is soon apparent in the injured area. The disease appears on all the above ground parts of the maize plants. The fully developed lesions of the rot are dark and sunken, with numerous acervuli bearing spore masses arranged in concentric rings (Carlile *et al*., 2004).

2.9   Factors affecting disease development and mycotoxin production

One of the most important factors that determine the level of disease and mycotoxin accumulation is genetic resistance of maize hybrids. Hybrids differ significantly in this trait, for Fusarium ear rot (Clements *et al*., 2003). In addition, physical traits of hybrids, such as husk coverage (Warfield and Davis, 1996), are related to disease susceptibility. Genetic variability in the pathogen is a relatively unexplored factor that also may contribute to variability in disease and mycotoxins (Melcion *et al*., 1997; Carter *et al*., 2002). *Fusarium* ear rot is favoured by distinctly different conditions. Fusarium ear rot is more common in warmer and drier areas (Miller, 1994; Bottalico, 1998), with the optimum temperature of about 30^0C (Mar´ın *et al*., 1999; Reid *et al*., 1999) especially the grain-filling period (Marasas *et al*., 2001). Several lines of evidence indicate that drought stress is associated with elevated levels of *F. verticillioides* infection and fumonisin accumulation in kernels (Miller, 2001). These results are consistent, in that a dry period before or during grain filling

Plate 2.2: Healthy maize cob (left) and infected maize cob with multiple infection by insect attack and *Fusarium verticillioides* (right). Source: Munkvold *et al*. (1999).

favours more severe Fusarium ear rot and higher levels of fumonisins. There may be an interaction between drought stress, insect populations, and Fusarium ear rot and fumonisins.

Various efforts to synthesize the effects of environment on *Fusarium* ear diseases of maize have been limited due to series of constraints**.** Vigier *et al*. (1997) developed a regression model for ear rot incidence (caused by *F. verticillioides*) based on July rainfall and an examination of ear damage and incidence of fungal infection. This model did not have predictive capabilities, since it relied on assessment of the infected ears for physical injury and kernel infection. A mechanistic model of post-inoculation fungal growth was developed by Stewart *et al*. (2002). This model comprised differential equations relating growth rates of *F. verticillioides* to temperature, relative humidity and precipitation, and to a non-linear silk function that described changes in silk susceptibility over time (Munkvold, 2003). The key determinants of fungal growth were temperature and ear wetness, which were estimated using precipitation and relative humidity data. This model effectively predicted ear rot severity following inoculation with a coefficient of determination of 0.89. However, its predictive capability is limited by the lack of assessment of pre-infection events (Stewart *et al*., 2002). Conditions for growth of *F. verticillioides* and production of fumonisins on solid corn kernels *in vitro* have been studied extensively by Mar´ın *et al*. (1999) who developed a polynomial regression describing fumonisin production as a function of temperature and water activity. They reported optimal conditions of 30^0C and 0.97 *a*w for fumonisin B1 production by *F. verticillioides*. Other studies confirmed 30^0C as the optimal temperature for *F. verticillioides* growth and fumonisin production, (Mar´ın *et al*., 1995).

There were significant interactions between the effects of temperature and water activity. Growth and fumonisin production for fungi were greatly reduced at *a*w values around 0.92. Germination of fungi was optimal around 30^0C, but this was affected by water activity (Mar´ın *et al*., 1996); the minimum *a*w for germination was 0.88, and the species germinated rapidly at 0.94 and above. These results have relevance to predicting fumonisin development in field-drying grain, but have not been tested as such. *Fusarium* species that infect maize kernels clearly encounter other fungi, including other *Fusarium* species, and competition among these fungi can have a distinct effect on the eventual levels of infection and mycotoxin contamination. In mixed field inoculations, *F. verticillioides* interfered with the growth of *F.*

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graminearum and markedly reduced the levels of deoxynivalenol in grain compared to *F. graminearum* alone (Reid *et al*., 1999). *F. verticillioides* growth and fumonisin levels were affected inconsistently by the presence of *F. graminearum*. Competition among *Fusarium* species and with other fungi has also been studied on irradiated grain (Velluti *et al*., 2000; Mar´ın *et al*., 2001).

F. verticillioides* produces several toxins that have potential toxicity for humans and domesticated animals. The most significant of these toxins produced by *F. verticillioides* are the fumonisins (Chamberlain *et al*., 1993; Munkvold and Carlton, 1997). Since fumonisins can be detected in symptomatic and asymptomatic maize kernels, control of fumonisin contamination in maize has become a priority area in food safety research (Boling *et al.*, 1963), and some guidelines for maximum fumonisin levels in human food and animal feeds have been issued (Gulya *et al*., 1980). The presence of fumonisins in asymptomatic grain means that a better understanding of the asymptomatic endophytic portion of the life cycle of *F. verticillioides* is importantly needed (Merrill *et al*., 1996a, and b). Work with genetically tractable strains has suggested that the limiting step in the movement of the fungus from the seeds to the upper parts of the plant is the transition of the fungus from the seedling crown to the stalk (Kellerman *et al*., 1990a, b; Meadows, 2000). However, the data generated by such analyses are limited to isolation of the fungus from different tissues and do not provide a clear picture of the colonization of the plant by the fungus. Moreover, not all experiments have produced the same results (Salazar and Vargas, 1977).

Light and electron microscopy have been used to obtain more detailed information on the infection of maize by *F. verticillioides* (Salazar and Vargas, 1977; Bacon *et al*., 1992, Meadows, 2000). These methods provide information on events such as seed infection and root penetration, but the data are limited to particular time points. More information on plant fungal development may be obtained by tracing transgenic isolates that express reporter genes. Work with ß-glucuronidase-expressing isolates has provided some insight into the *F. verticillioides*-maize interaction (Boling *et al*., 1963; Scott and King, 1984); however, the tissue sectioning and other experimental manipulations required to monitor ß-glucuronidase activity limit its utility in such analyses. The green fluorescent protein (GFP) has become a commonly used tool in the analysis of fungus-plant interactions. Spores and hyphae of GFP-expressing fungal isolates can be identified by fluorescence microscopy in intact

tissues or tissue sections without extensive manipulation and provide highly informative data on processes of plant colonization (Fajemisin, 1982; Huss and Leslie, 1993; Leslie, 1996; Klittich *et al*., 1997; Dowden, 2001).

2.10   Epidemiology and dissemination of maize ear rot disease

The fungus, *F. verticillioides* is seed-borne; seed transmitted and found on soil surface or infected plant debris (McGee, 1988). It is also dispersed by rain splash, air currents and contact.   Insects that burrow into the stalk have been reported to contribute to the heavy losses of maize in the tropics by creating ingress to the fungal pathogen (McGee, 1988). It survives the dry season in infected seeds and either on the soil surface or buried (McGee, 1988).

Once established, splashing and wind-driven rains are means of further spread. These cause wounds on the host through which the air blown, spores enter to further penetrate the host tissues (McGee, 1988). There is the record of much increase in severity of the disease in rainy season (McGee, 1988).

Cool temperatures favour the infection (McGee, 1988). The infection process starts with the initial infection, death of the epidermal cell, while the growth of the pathogen continues. The secondary necrotrophic hyphae produced kills and destroy the tissues quickly. The severity of the disease is influenced by age of the host, and degree of infection. This commonly occurs on young tissues and during silking.

Fusarium verticillioides and other species causing Fusarium ear rot are dispersed primarily as microconidia, although macroconidia also act as infectious propagules. Microconidia typically are more numerous and more easily wind-dispersed than macroconidia. Propagules of *F. verticillioides*, are common in the air within fields of maize (Gillette, 1999), and also at locations distant from maize fields. Ooka and Kommedahl (1977) estimated that viable spores of *F. verticillioides* (reported as *F. moniliforme*) travelled as much as 300–400 km.

Insects play a key role in the dispersal of *F. verticillioides*. Several insects have been implicated in the dispersal of *F. verticillioides*, including European corn borers (*Ostrinia nubilalis*), sap beetles (*Carpophilus* spp. and *Glischrochilus quadrisignatus*), western flower thrips (*Frankliniella occidentalis*), and corn rootworm beetles (*Diabrotica* spp.) (Gilbertson *et al*., 1986; Dowd, 1998). Several lepidopteran and coleopteran species were also associated with increased ear rot and *F.*

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verticillioides infection in Africa (Cardwell *et al*., 2000). European corn borer larvae can acquire spores of *F. verticillioides* from leaf surfaces and transport them to kernels (Sobek and Munkvold, 1999). Adult rootworm beetles and sap beetles commonly carry spores of *F. verticillioides*. Rootworm beetles feed on maize silks, where spores of either *Fusarium* species may be deposited and cause kernel infection.

Sap beetles are attracted to maize ears that have been damaged by other insects such as the European corn borer or corn earworm. In addition, they are attracted to volatile compounds produced by *F. verticillioides* (Bartelt and Wicklow, 1999). The beetles, therefore, may be well-situated for acquiring *Fusarium* spores from infested plant material and delivering them to wounded kernels, which are very susceptible to infection. Attraction of insects to *F. verticillioides*-infected plant material may not be limited to sap beetles. Schulthess *et al*. (2002) reported that several lepidopteran and coleopteran insect species were more numerous on *F. verticillioides*-inoculated plants than on non-inoculated control plants.

2.11   Symptoms of Fusarium ear and stalk rot disease

Fusarium verticillioides causes Fusarium ear and stalk rot; it is most common in dry, warm areas. It is particularly severe if it begins just before tasseling (McGee, 1988). Wilted plants remain standing when dry, and small, dark-brown lesions develop in the lowest internodes. When infected stalks are split, the phloem appears dark brown, and there is a general conspicuous browning of tissues. In the final stages of infection, pith is shredded and surrounding tissues become discolored (Leslie, 1991; CIMMYT, 2004). During August, lesions were found at nodes higher up the stem. They are well defined as a dark-brown or black discoloration of the stalk decay generally starts at the calyx end and spread to the stalk. The decay dries, eventually affecting the whole stalk which often showed a concentric line pattern. Pink sporodochia appears on the older stem lesions and also on the affected stalks. The infected maize plants show slight yellowing of the foliage and vascular discoloration prior to the death of the plant (Leslie, 1996).

The Fusarium ear rot of maize has intact or split kernels covered (Fig. 2.1) with white or pinkish white mould are typical symptoms of ear rot infection by *F. verticillioides*. The fungus can cause disease at all developmental stages of the plant, in some cases without causing symptoms (Munkvold *et al*., 1997).
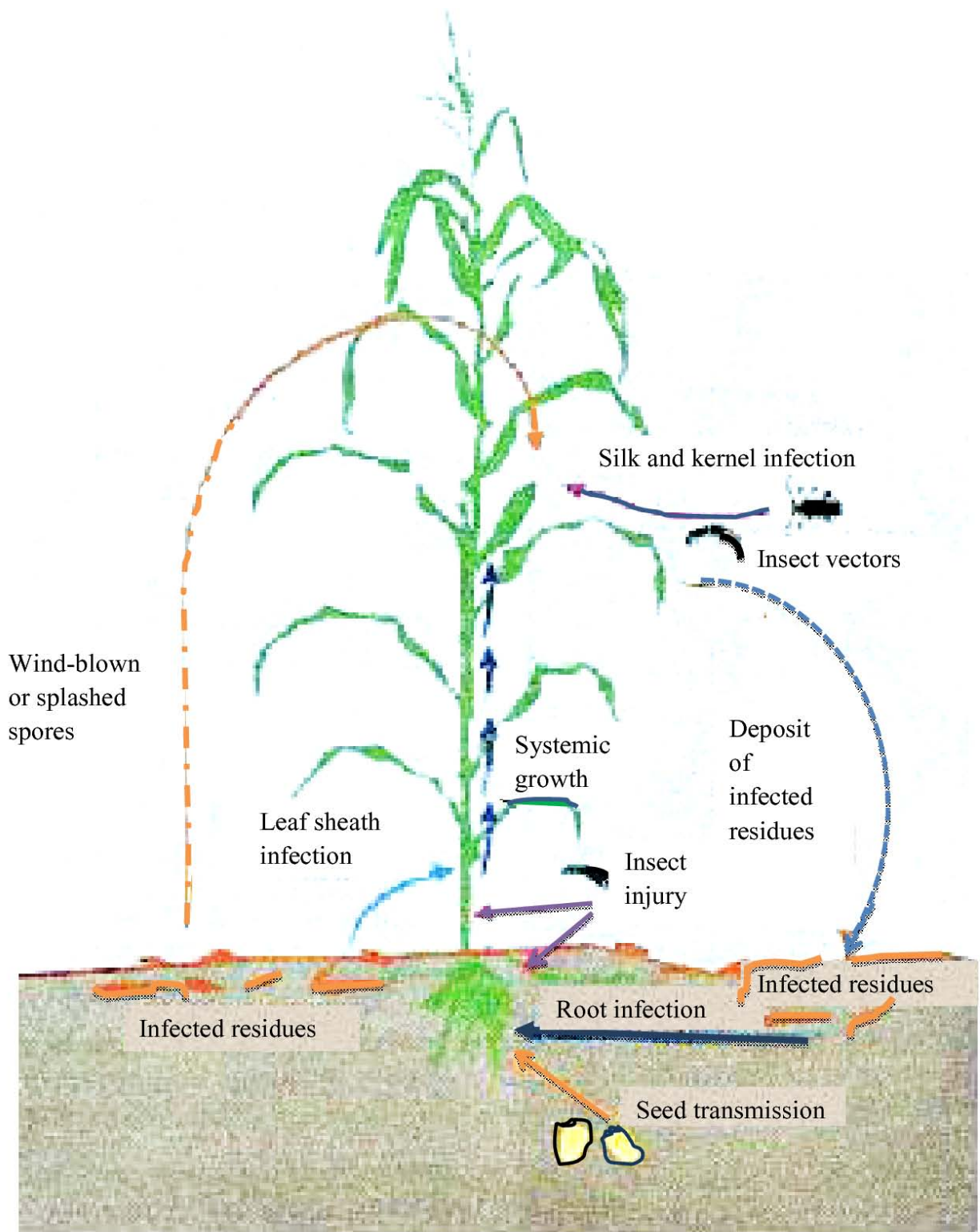
Fig. 2.1: Disease cycle of *F. verticillioides* on maize showing various infection pathways. Source: Munkvold and Desjardins (1997).

Plate 2.3: Intact and split kernels covered with white or pinkish white mould are typical symptoms of ear rot infection by *F. verticillioides.* Source: Munkvold and Hellmich (1999).

2.12   Economic damages and losses due to ear rot disease

It has been established that there is a negative correlation between the incidence and severity with yield parameters. Significant stalk rot may however lead to significant loss in yield and lower biomass (Schaafsma *et al*., 1993; Munkvold and Desjardins, 1997; Vigier *et al*., 1997). When accurate assessment and quantification of yield loss, the incidence of stalk rot in maize is made, a quick control measure is inevitable to reduce the inherent yield loss and toxin contamination by this pathogen (Merrill *et al*., 1996b).

Ear rot by *F. verticillioides* has become a serious threat to the production of maize. This pathogen caused up to 60 percent yield losses in commercial maize fields (Bankole *et al*., 2003; 2007). In addition to its effects on yield and seed quality, *F. verticillioides* can produce several toxic metabolites, of which fumonisins are the most prevalent (Leslie *et al*., 2005). This situation is further complicated by the common occurrence of fumonisins in symptomless infected kernels (Bullerman and Tsai, 1994; Munkvold *et al*., 1997). Previous studies have reported *F. verticillioides* to be the most predominant *Fusarium* species in West Africa (Bankole *et al*., 2003; Bankole and Mabekoje, 2004; Fandohan *et al*., 2005). Fumonisins are a group of structurally related mycotoxins that are common contaminants of maize-based foods and feeds worldwide, and the most abundantly found fumonisins in naturally contaminated foods and feeds are FB1, FB2, and FB3 (Rheeder *et al*., 2002). Although a cause-and-effect relationship has not been clearly demonstrated, evidence exists of potentially serious human and animal health problems associated with consumption of maize products contaminated with fumonisin. These problems include human oesophageal cancer (Chu and Li, 1994). Neural tube defects in newborns (Stack, 1998), leukoencephalomalacia in horses (Kellerman *et al*., 1990), liver cancer in rats (Gelderblom *et al*., 1994), and neurodegeneration in mice (Osuchowski *et al*., 2005).

2.13   Control of maize ear rot disease

Breeding for resistance to maize diseases appears to be the most efficient means of disease control, just as it is done for other crops (Afolabi, 2007). Screening for resistance in the field is done at various stages of crop development, on the basis of phenotypic disease symptoms expressed by plants, which are naturally infected by the disease. The economic value of resistant varieties is roughly equated with the saved cost of chemicals, time, labour and any other risks involved. The possibility of the

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pathogen overcoming the resistance already built up in plants is the main problem of using resistant varieties (Afolabi, 2007).

In attempts to make maximum use of local knowledge and scientific know-how, the experience of recent decades has made it clear that single crop protection measure has its limitation; hence crop protection should be viewed from a holistic perspective. The agro-ecological diversity, erratic rainfall pattern, soil erosion and nutrient depletion and differences in crop's ability to tolerate or resist pests exposes crop to pest attack, which calls for integrated disease management. Moreover, the farmers live in diverse socio-economic situations and may have different production goals. Thus, appropriate crop protection strategies for small scale farmers can only result from a thorough understanding of the natural, technological and socio-economic conditions with their interrelationships (McGee, 1988).

In these situations it is decisive that farmers understand the principles of crop protection and adapt, rather than adopt, promising pest management techniques to their situations. Preventive crop protection must be the first step in plant protection. Managing the farming system towards a high degree of self-regulatory mechanisms which reduce the need for curative crop protection is the most intelligent and economic way to cut down expenses (Stoll, 2000).

Preventive crop protection practices are employed to counteract the nature and behaviour of the pest, to encourage the activity of antagonistic organisms or to strengthen plants to resist or tolerate effect of the diseases. To practice preventive crop protection successfully, knowledge of biological factors such as the soil, the plants, the pest, the agro-ecosystems and the farming system are all essential in order to develop appropriate crop protection strategies (Stoll, 2000). The principles of preventive crop protection include the knowledge of agro-ecosystems (the agricultural ecosystem and the environment) in which it is imbedded are the first factors which determine the pest pressure upon crops. This entails series of questions which help the farmers and extension agents to make correct observations and decisions. Healthy plants and soils play a significant role on the host plant resistance. These correlate directly with diseases infection. A plant which is undernourished is in a state of imbalance and is more prone to infection. There is a close relationship between the physiology of a plant which is also determined by the site, the soil structure, nutrients and the type of agricultural practice (Stoll, 2000).

Plants in natural environments depend entirely on their own defences against pathogens (an indication of how effective the natural defences can be). With the exclusive orientation of past breeding programmes on high yields, many of these protective properties have been lost. In traditional cultivation systems, farmers have long been familiar with a wide choice of local plants which were appropriate to their particular needs, to particular sites and pathogen control. An advantage of resistant plants is that, pathogens are less vigorous and more easily killed by adverse environmental conditions (Manyong *et al*., 2000)

To breed high yielding varieties with multiple pest and disease resistance under low management conditions and with high nutrient content which are acceptable to consumers is costly and are usually lengthy in process (Hillocks *et al.,* 1996).

2.14 Biological control of fungal pathogens

Biological control involves the use of living organisms to control plant pathogens. It is often referred to as biocontrol. The mechanism according to Baker and Cook (1974) involves the reduction of inoculum density or disease producing activities of a pathogen or parasite in its active or dormant state. This is accomplished using one or more organisms naturally or through manipulation of the environment. The host or antagonists or by mass introduction of one or more antagonists including bacteria, fungi and actinomycetes known to produce antibiotics is referred to as biological control (Cook, 1991). *Trichoderma spp* have been used successfully to suppress damping-off fungi and *Coniothyrium miniatans*, which under field conditions attack species of the essential pathogenic genus, *Sclerotinia* (Burge, 1988). Adekunle *et al*. (2001) also reported that seed treatment with three species of *Trichoderma* successfully suppressed *M. phaseolina in vitro* and gave about 65% reduction in sclerotial population in screenhouse trials. Appropriate formulation was considered necessary for implementation of biocontrol agents (Lumsden and Lewis, 1989).

Several formulations had been evaluated such as dust formulation for and *Gliocladium* mixed with inert carrier for soil application (Lewis and Papavizas, 1991), seed treatment with conidia of *Trichoderma harmatum* (Bon.) Brainier (Harman *et al*., 1980; Chet, 1987) dust preparation of conidia from *Gliocladium virens* Miller (Gidden and Foster) as seed treatment against damping-off and Fusarium wilt. The results compared favourably well with benomyl in treated fields (Kang and Kim, 1989). Other biocontrol agents include *Penicillium spp, Gliocladium, Bacillus subtilis, B. cereus* and

Pseudomonas fluorescens. They antagonize plant pathogens on or near the surface of the hosts such as seeds or seedling (Blakeman and Fokkema, 1982). Mechanisms employed in biological control are many and complex. According to Howell (2003), not all these mechanisms are found in a single biological control agent; hence hybridization of different strains or species is required to combine these beneficial characteristics. He therefore suggested isolation of biocontrol agents from the locality where it is expected to function as disease control agent.

This ear rot disease of maize has not received much attention in terms of suppression of disease occurrence or total eradication by way of control measures (Bankole and Adebanjo, 2003). The high inoculum pressure in farmers' fields which has risen to very high incidence and severity of these diseases have given rise to the investigation of methods of disease management that will be suitable for its control (Jouany, 2007). These control measures are the use of cultural control, fungicide application, the use of resistant varieties, quarantine and sanitary measures. Adequate cultural practices can help to control the diseases (Stuckey *et al*., 1993). Crop rotation of 2-3 years and field sanitation are sufficient to eliminate the fungus on debris (FAO, 2007). Mixed cropping could afford protection from rapid epidemic development and inoculum build-up as reported by Adebitan and Ikotun (1996) for cowpea.

Since many of the first degree infections arise from infected seeds, production of disease-free seeds plays a major role in disease control. One of the most successful means of enhancing crop productivity is to ensure that quality seeds are sown. Seed quality connotes freedom from disease-causing propagules or high germinability and vigour. Due to the fact that the seeds are liable to damage by pathogens, farmers are often forced to sow poor quality seed which results in poor and reduced seedling stand/ha. Qualitatively, the seed that is infected by pathogens or contaminated by disease vectors performs poorly (Shivas and Beasley, 2005).

The level of such infection, infestation or contamination on the seed lot also does affect the germination percentage. All these are considered in seed health. To determine the suitability of the seed lot for use, the germinability of seeds, the seed health and viability are important among other factors. The treatment of seeds with antagonistic fungi has been used successfully to protect the seeds from different diseases such as damping-off disease caused by *Pythium* spp (Lutchmeah and Cooke, 1985) and *Rhizoctonia solani* (Howell, 1982). The need to adopt more of these

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antagonistic fungi becomes more important with the current shift from conventional food production to organically produced food.

Formulation procedures for biocontrol agents are dependent on adequate and effective biomass formation, which must be handled carefully in order to maintain viability at the end of processing and application. For a biological agent to be effective, it has to remain active during the period when the host plant is susceptible to the disease (Paulitz, 1992; Taylor *et al*., 2004).

Seed dressing is a technology appropriate for African farming systems, and cottage industry production levels units have been shown to be potentially economically feasible for meeting the demands of small-scale requirements. This may also provide sales revenues to producers.

Adekunle *et al*. (2001) highlighted that the feasibility of local biopesticide production with *Trichoderma* depends on several factors; the raw materials, adhesive and production substrate, need to be cheap and readily available. The *Trichoderma* isolate would have to be quite robust and grow quickly on the local substrate. The risk of inadvertently increasing potential human pathogens along with the *Trichoderma* must be very low while the dose response should not be too stringent, safeguards measure would have to be developed to avoid over-dosing or under-dosing so as not to naturally pollute or overload the ecosystem, or to allow the pathogens to develop resistance to biocontrol agents.

2.15 Biological agents of interest in this study

The biological agents that are the microorganisms of benefit to the environment used in the study have been found to have antagonistic potentials against other organisms (pathogens). The *Trichoderma* species are known to suppress infection of root by soilborne pathogens like *Macrophomina phaseolina*, *Rhizoctonia solani*, *Fusarium* species and *Pythium* species on various crops (Howell, 1982; Lutchmeah and Cooke, 1985; Ehteshamul-Haque *et al*., 1990; Adekunle *et al*., 2001; Benítez *et al*., 2004). Species of *Trichoderma* also have growth promoting capabilities that may or may not be integral to biological control (Yedidia *et al*., 1999; Benítez *et al*., 2004; Dubey *et al*., 2007). *T. harzianum* has shown effective control of root infecting fungi and root-knot nematodes (Spiegel and Chet, 1998; Sun and Liu, 2006). *T. harzianum* isolated from rhizome rot suppressive soils reduced the disease and increased plant growth and yield (Ram *et al.,* 1999). It has been reported that many *Trichoderma*

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species has an innate and/or induced resistance to many fungicides but the level of resistance varies with the fungicide (Omar, 2006).

2.16 The use of Botanicals in fungal diseases control

It is assumed that a rich soil and the various methods of cultural control are the right steps in crop protection. However, in many cases, even when farmers apply sound cultivation practices, additional curative measures are needed to protect the crop to an acceptable level. Plants with pest-controlling properties may then be an option. The use of plant extracts to control destructive disease and disease vectors is a current global trend. Rotenone (*Derris* spp.), nicotine and pyrethrins have been used considerably on subsistence as well as commercial scale. The advocacy for its use has been put into the development and promotion of plant-based products for the control of pests (Stoll, 2000). A science-based approach is one that uses formal steps to discover and determine efficacy, and attempts to either produce the botanical pesticide on a commercial scale or synthesize it for broader use in commercial agriculture. To develop the crops' biomass by using other beneficial organisms either to suppress the pathogens' growth or to boost the nutrient available for crop use.

There has been a considerable effort by action-oriented researchers and farmers to develop practical and low cost methods of botanical control. Bearing in mind the factors of importance for small farmers, plants for pest control should ideally possess characteristics such as ability to be effective at a rate of maximum 3-5% plant material based on dry weight. They should be easy to grow and require little space and time for cultivation or procurement. The material should pose no hazard to wildlife, humans or the environment. The preparation should be simple, not time-consuming nor highly technical in applications and should not be phytotoxic or decrease the quality of a crop, i.e. taste or texture (Stoll, 2000).

Natural pesticides are safer alternatives to synthetic toxic chemicals currently in use. There have been several researches for decades on the use of botanicals. Chauhan and Joshi (1990) reviewed the use of botanicals against plant pathogens. Also Dixit and Tripathi (1982) reviewed active ingredients of the antifungal components. Several plants have been evaluated for their fungitoxic properties and as possible alternative to synthetic fungicide currently in use (Awuah, 1989; Chauhan and Joshi, 1990; Enikuomehin *et al*., 1998; Ekpo, 1999). Crude steam distillate from *Ocimum*

gratissimum sprayed onto infection courts on detached cocoa pods moments after inoculation with *Phytophthora palmivora* completely inhibited the pathogen and blackpod lesion development on 75% of the infection courts (Awuah, 1994).

The use of plant extracts from tobacco (*Nicotiana tabacum*), *Derris elliptica*, (a leguminous plant), pyrethrum (*Chrysophylum cinerariefolium*) and neem *Azadiracta indica* are well known. Although, they act generally as insecticides, studies revealed their fungicidal, nematicidal, bactericidal and viricidal properties. Enikuomehin *et al*. (1998) reported the effective control of *Sclerotium rolfsii*, using ash from some tropical plants. Ash samples from *Delonix regia* stem wood; *Mangifera indica* leaf and *Vernonia amygdalina* leaf were most effective. Anthracnose disease of mango *Colletotrichum gloeosporioides* was effectively controlled with 2% eucalyptus oil and 10% castor oil in water (Pal *et al*., 1988). A significant reduction was obtained when compared with the other plant extracts tested. It has been shown that extracts from garlic bulb, mango, ginger and lantana leaves compared favorably well with the conventional fungicide, carbendazim (Ngeze, 2001).

2.17 Botanicals of interest in this study

2. 17. 1 *Mirabilis jalapa* **L.**

The common names of the plant are Clavillia, 4°clock, jalap, bonina, boa-noite, beauty of the night, morning rose, and marvel of Peru. In Yoruba language, it is called Ododo ota or Tanaposo. The taxonomist characterize *Mirabilis jalapa* to be of

> Kingdom: *Plantae*
> Division: *Magnoliophyta*
> Class: *Magnoliopsida*
> Order: *Caryophyllales*
> Family: *Nyctaginaceae,*
> Genus: *Mirabilis*
> Species: *M. jalapa* L*.*

It is a perennial plant growing to 0.6m by 0.45m in height and canopy width respectively. It is hardy. It flowers anytime from July to October, and the seeds ripen from August to October. It produces beautiful flowers that usually open around 4 o'clock in the afternoon – hence its common name*, four* o'clock*.* It is a popular ornamental plant grown worldwide for the beauty of its flowers (which can be white, red, pink, purple, or multicolored) and their sweet fragrance. The flowers are

hermaphrodite (have both male and female organs) and are pollinated by insects. The plant prefers light (sandy), medium (loamy) and heavy (clay) soils and requires well-drained soil. The plant prefers acid, neutral and basic (alkaline) soils it can grow in semi-shade (light woodland) or no shade. It requires moist soil (Yang, 2001).

The leaves are the edible part of the plant. The tender young leaves are cooked as a vegetable. The leaves are an emergency food, only eaten when all else fails. An edible crimson dye is obtained from the flowers and is used for coloring cakes and jellies. The seed is crushed and used as a pepper substitute (Duke, 1985). The parts usually used in the plant are the leaves, root and flowers.

The plant is employed medicinally apart from the fact that it is used as an ornamental plant. The indigenous Peruvian people use a root decoction as a diuretic; Indians put the flowers in baths to treat colds and flu. In Brazil, the powdery form is inhaled, dried flowers as a snuff for headaches, and use the root decoction to wash wounds and to treat such skin afflictions as leprosy. The seeds are also crushed to use as a peppery condiment on foods, and the grated tuberous root in cold water and are drunk for intestinal parasites (Kusamba *et al*., 1991). The roots of the plant are ground into a paste with black pepper and taken orally for conjunctivitis. The juice of the leaves is applied to fungal infections of the skin.

These indigenous practices impelled clavillia's presence in herbal medicine systems around the world. In Mexico, the entire plant is decocted and used for dysentery, vaginal discharge, infected wounds, and bee and scorpion stings. In Peru, the plant and/or tuber is used as diuretic, laxative, and purgative. The juice of the flower is used to clear herpes lesions and earaches. In Brazilian herbal medicine, a paste is made of the leaf and flower and applied to infections of the skin such as itchiness, eczema, herpes, skin spots and skin infections (Caceres *et al*., 1991). The juice of the root is dropped into the ear for earaches. Brazilians also use the root to combat worms, intestinal parasites, leucorrhea, hydropsy, diarrhea, dysentery, abdominal colic, syphilis, and liver infections. In the United States, the plant is used for mumps, bone fractures, and as an abortifacent to childbirth (Perry, 1980; Caceres *et al*., 1991).

Phytochemical analysis of clavillia shows that it is rich in many active compounds including triterpenes, proteins, flavonoids, alkaloids, and steroids (Yang, 2001). A group of amino acid-based proteins coined *Mirabilis jalapa* proteins (MAPs); these chemicals are produced in the seeds, roots, and young shoots, and help to protect

the plant against various plant viruses and soil-borne fungi (Grainge and Ahmed, 1988; Wong *et al*., 1992). The new phenolic compounds in clavilla found by researchers in 2001 demonstrated the *in vitro* action against the yeast *Candida albicans* (Yang, 2001). A hot water extract of the flower, leaf, and root of clavillia has shown antifungal activity in another *in vitro* study (Taylor, 2005). In early research, the root of clavillia (in water and ethanol extracts) also demonstrated mild uterine stimulant actions in rats, and antispasmodic actions in guinea pigs.

Clavillia, the lovely, sweet-smelling ornamental, has also earned its place in herbal medical practices around the world; its array of biological activities continues to support its continued use worldwide. More findings from this study regarding clavillia's power and versatility will likely explain more of its use as an antifungal for farmers' use (Rehm and Espig, 1991).

Plate 2.4: *Mirabilis jalapa* plant

2.17.2 *Senna occidentalis (L.)* **Link**

Senna occidentalis is of

> Kingdom: Plantae
>
> Division: *Magnoliophyta*
>
> Class: *Magnoliopsida*
>
> Order: *Fabales*
>
> Family: *Fabaceae*
>
> Sub-family: *Caesalpinioideae*
>
> Genus: *Senna*
>
> Species: *S. occidentalis.*

The common names Fedegoso, fedegosa, brusca, Negro coffee, coffee weed, coffee senna. In Yoruba language, it is called "rere" or "ako rere".

It is believed to have originated from Tropical America with its distribution at the coastal plain, also found weedy in disturbed areas and waste areas. It grows naturally, without being cultivated. *Senna occidentalis* is a very leafy malodorous tropical weedy shrub, whose seeds have been used as an adulterant for coffee. The plant can be better described as branched, annual herb; leaves alternate, pinnately divided, without a conspicuous gland at the base of the leaf stalk; leaflets of 4-5pairs; stipules usually not persistent and not conspicuously striated, flowers yellow, 5-parted; fruit flattened (Tona *et al*., 2004).

It is an erect tropical annual herb with leathery compounded leaves growing up to 5 − 8m high. The seed pods are dark brown and curve slightly upward; the seeds are olive-brown and flattened on both ends. Indigenous to Brazil, it is also found in warmer climates. It is sometimes called coffee senna (Tona *et al*., 2004). It is botanically classified as *Senna occidentalis* and its synonym is *Cassia occidentalis*. The seeds, which are in long pods, can be roasted and made into coffee-like beverages. The leaves and flowers can be cooked and are edible.

Fedegoso has been used as natural medicine in the rainforest. Its roots, leaves, flowers, and seeds have been employed in herbal medicine round the world. The seed is bitter and has purgative properties; it is also used as diuretic, liver detoxifier, as a hepatotonic (balances and strengthens the liver) (Elujoba *et al*., 1989). Further, it is used against whooping cough and convulsion. The seeds are brewed into coffee-like beverage for asthma and the flower infusion is used for bronchitis. The roots are considered a tonic, fever reducer and used for menstrual and uterine problems, and

constipation in babies (Elujoba *et al.*, 1989). The fresh and/or dried leaves of fedegoso are crushed or brewed into a tea and applied externally for skin disorders, wounds, skin fungus, parasitic skin diseases, abscesses, and as analgesic and anti-inflammatory natural medicine (Feng *et al*., 1962; Perry, 1980).

The leaf extracts have demonstrated an anti-inflammatory, hypotensive, smooth-muscle relaxant, antispasmodic, weak uterine stimulant, vasoconstrictor, and antioxidant activities in laboratory animals (Tona *et al*., 2004). The Senna/fedegoso plant is well known for a group of chemicals with purgative and strong laxative actions called anthraquinones.

Plate 2.5: S*enna occidentalis* plant (A) and flower and seed pod (B).

2.17.3 Mexican Sunflower – *Tithonia diversifolia* **(Hemsley). A. Gray**

Mexican sunflower is of

    Kingdom: Plantae

    Division: Magnoliophyta

    Class: Magnoliopsida

    Order: Asterales

    Family: Asteraceae

    Genus: *Tithonia*

    Species: *T. diversifolia.*

The common names are tree marigold, Mexican sunflower, Nitobe chrysanthemum. This plant is an invasive weed in Nigeria (Ogunyemi, S., personal comm.). *Tithonia diversifolia* commonly called Mexican sunflower, is a common shrub (weed) native to Central America but has become naturalized in many tropical countries, including Nigeria. A common weed found in small bushes around houses, villages, roadsides and abandoned fallow lands. It has bright yellow and conspicuous flowers. The vegetative parts of the plant produce large, blossom and attractive flowers that varies in size depending on soil fertility and rainfall.

A wide variety of natural products are used in the treatment of common infections in traditional medicine in most developing countries. *T. diversifolia* had been used in traditional medicine for the treatment of various ailments. The hot water extract of the aerial parts of the plant is used for the treatment of malaria in Guatemala, Taiwan, Mexico and Nigeria. An oral decoction of the leaves and stem is used to cure hepatitis in Taiwan and gastrointestinal disorders in Kenya and Thailand (Johns *et al*., 1995). Also, the infusion of the leaves is used for the treatment of measles in Cameroon (Kamdem *et al*., 1986), while the dried leaves are applied externally on wounds in Costa Rica (Kuo and Chen, 1997).

A decoction of the flowers is used for the treatment of skin eczema (Gurib-Fakim *et al*., 1996). Extracts of the various parts of the plant have been reported to exhibit antimalarial (Madureira *et al*., 2002), anti-inflammatory (Rungeler *et al*., 1998), anti-proliferation (Gu *et al*., 2002), insecticidal (Hongsbhanich *et al*., 1979) analgesic and anti-inflammatory and antibacterial (Bork *et al*., 1996) activities. Few reports exist on its pesticidal properties. However, nematicidal properties were reported in tea plantation when the soil was amended with wild sunflower in Sri Lanka (UNDP 2004). Extracts from sunflower also controlled root rot of phaseolus beans in

Plate.2.6: *Tithonia diversifolia* leaves and flowers

Western Kenya (www.bioline.org). Other reports are its improving effect on soil fertility in Kenya (Nziguheba *et al*., 2002), and as supplement to phosphorus in maize (Thor Smestad *et al*., 2002).

2.17.4 Mullaca - *Physalis angulata* L.

Physalis angulata is of

Kingdom: Plantae

Division: Angiosperms

Class: Eudicots

Order: Solanales

Family: Solanaceae

Genus: *Physalis*

Species: *P. angulata.*

The common names: cut leaf groundcherry, wild tomato, camapu, Cape gooseberry, and winter cherry. The common name in South America is Mullaca. In Yoruba language, it is called "Koropo".

It is an annual indigenous herb to many parts of the tropics. It can be found on most continents in the tropics, including Africa, Asia, and the Americas. It grows up to 1m high, bears small, cream-colored flowers, and produces small, light yellowish-orange, edible fruit sometimes referred to as Cape gooseberry. The fruit is about the size of a cherry tomato, and its tomatoes like, it contains many seeds the fruit contains; spontaneous clumps of plants can be found along river banks and just about anywhere the soil is disturbed and the canopy is broken (allowing enough sunlight to promote its rapid growth) (Anon,1998).

It has long held a place in natural medicine in the tropics where it grows. The plant parts mostly used are the leaves, roots and whole plant (Ayensu, 1981). Due to the narcotic properties in the fruits and leaves, they decoct into anti-inflammatory and disinfectant for skin diseases. Also, leaves and/or roots are used for earache, liver and gallbladder problems, malaria, hepatitis, diabetes and rheumatism, jaundice. The fruit promote fertility while the tea of the whole plant prevents miscarriages (Taylor, 2004).

Plate 2.7: *Physalis angulata* flowers, fruits and leaves

CHAPTER THREE

MATERIALS AND METHODS

3.0   LABORATORY EXPERIMENTS

3.1   Location and experimental sites

Studies were conducted in the Laboratories, Screenhouse and Research fields of the Institute of Agricultural Research and Training (IAR&T), Obafemi Awolowo University, Moor Plantation, Ibadan and the Pathology Laboratory of the Department of Crop Protection and Environmental Biology, University of Ibadan, Ibadan, Nigeria. In addition, the facilities and technical skills of staff of the Pathology Laboratory of the International Institute of Tropical Agriculture, IITA, Ibadan and Departments of Botany and Microbiology, University of Ibadan, Ibadan were employed in this study.

3.2   Sources of materials

Quality Protein Maize varieties used in these studies were developed and supplied by the Institute of Agricultural Research and Training (IAR&T) Ibadan, Nigeria. The plant species used as botanicals were the Mexican sunflower (*Tithonia diversifolia)*, *Physalis angulata, Senna occidentalis,* and *Mirabilis jalapa* were collected from the Practical Year Training Programme plots of the Faculty of Agriculture and Forestry, University of Ibadan, Ibadan and IAR&T, Ibadan. The antagonistic microorganisms (*Trichoderma harzianum, T. pseudokoningii, Pseudomonas fluorescens* and *Bacillus subtilis*) used in this study were isolated from soil in maize growing areas and kept in the stock culture collections of the Pathology Laboratory of the Institute of Agricultural Research and Training (IAR&T).

3.3   Sterilization of glassware and other materials

The glasswares used were washed in detergent and rinsed with tap water and left to dry. Petri dishes were placed in canisters while the pipettes were wrapped with aluminum foil. All glasswares were sterilized in an oven at $160^{o}C$ for 3 hours, while the inoculating needle was sterilized by flaming in a spirit lamp. Liquid media and the distilled water were sterilized in an autoclave at $121^{o}C$ for 15 minutes (1.05 kg/cm^2 pressure). The inoculating chamber (Laminar flow) was sterilized by swabbing with

70% alcohol. Also, an ultra violet light in the inoculating chamber was put on for 30 minutes before use. All isolations and inoculations on agar media were carried out in the sterile inoculating chamber. Corkborer of 5 mm diameter was used for fungal mycelia inoculation. Metal materials were sterilized by dipping in alcohol and heated to red hot over a spirit lamp before use. All laboratory studies were carried out under aseptic conditions.

Maize seeds were sterilized by dipping in 10% sodium hypochlorite (NaOCl) for 1 minute and rinsed in five changes of sterile distilled water. The seeds were then placed on sterile paper towel in a laminar flow cabinet for 10 -15 minutes to air dry before planting.

3.4   Preparation and sterilization of culture growth media

The selected pathogen and biocontrol agents were cultured on potato dextrose agar (PDA) as needed depending on the type of organism to grow. For the preparation of PDA, 39 g of PDA powder was suspended in 1litre of distilled water. These were prepared routinely. Similarly, 28 g Nutrient agar (NA) powder was suspended in 1litre of distilled water. The solutions were dispensed into 250 ml conical flasks. Before sterilizing, the conical flasks were capped tightly with non-absorbent cottonwool and wrapped with aluminum foil. The flasks were then sterilized for 15 minutes at 1.05 kg/cm^2 (121^{0}C) in an autoclave.

After the autoclave has cooled and pressure gauge was at zero, the lid was opened and the flasks were removed and kept in a Gallenkamp water-bath at a controlled temperature of 45^{0}C before pouring into 9-cm diameter Petri dishes. Shortly before it was poured into the Petri plates, 1.25 g of streptomycin powder was released in 1 litre of molten PDA to make an antibacterial medium for non-bacterial growth. The cooling agar was dispensed in 15 ml amounts into sterile glass Petri dishes.

3.5   Collection and isolation of the pathogen

Maize plants showing symptoms of ear rot were collected from the Institute of Agricultural Research and Training (I.A.R & T) and International Institute of Tropical Agriculture (I.I.T.A) fields. After thorough washing of the diseased parts with running tap water, small pieces (3 mm) of the infected parts were cut at the boundary of the healthy and infected tissues. These were later surface sterilized for 1 min in 10%

NaOCl solution (with 5.25% chlorine content), rinsed in 5 changes of sterilized distilled water and dried with sterile paper towel. These were then placed on solidified PDA in Petri dishes and incubated at room temperature $(28 \pm 2)^{\circ}$C for 7 days. Observation was made daily for any mycelial growth. Pure cultures were obtained by subculturing into fresh plates. Stock cultures were maintained on agar slants in McCartney bottles and stored at 4°C in the refrigerator.

Test biocontrol organisms (*Trichoderma harzianum, T. pseudokoningii, Pseudomonas fluorescens* and *Bacillus subtilis*) used in this study (i.e. the antagonists) were from soil collected in maize growing areas (rhizosphere) of the Institute Agricultural Research and Training, IAR&T and the soil samples collected were taken to Phytopathology Laboratory of the Crop Protection and Environmental Biology Department, University of Ibadan (UI) for isolation of the organisms. These were maintained on antibacterial PDA by adding 0.67 g of streptomycin powder to 500 ml of PDA medium for the fungal growth and PDA for bacterial growth.

3.6   Fungal mycelial growth

A flame-sterilized 5 mm diameter cork borer was used to cut mycelia discs randomly from 7 day old pure cultures of fungi. The mycelial discs were aseptically transferred to the centre of cooled PDA contained in Petri dishes with the aid of a flame-sterilized mounted inoculating needle, the bottom of the Petri dishes were marked with 2 perpendicular lines passing through the centre. The plates were incubated at $(28 \pm 2)^{\circ}$C for 7 days, and the radial growth was measured along the perpendicular lines and the mean calculated for each isolate.

3.7   Preparation of spore suspension of target organism

Pure cultures of the pathogen were sub-cultured from stock cultures. The pathogen was given 7 days to be fully grown. The contents of the Petri dishes of organism were then scooped out into a Warring blender, which contained a litre of sterile distilled water, and a drop of Teepol detergent (for spore dispersal) was blended for about 45 seconds. The spores were then strained off the mycelia and media fragment by using a double layer of muslin cloth placed inside a sterile funnel. The spore suspensions were poured into a garden sprayer for subsequent inoculation in the

field (Adebitan, 1991). A Haemocytometer (Fig 3.1) was used to determine the concentration of spore suspension.

Haemocytometer was used thus: spore suspension was prepared first. The surfaces of the haemocytometer and cover-slip were carefully cleaned before use. Care was taken to ensure that all surfaces were completely dried using non-linting tissue. Then the coverslip was centred at the centre on the haemocytometer. Spore suspensions of 9 microliters were pipetted into one of the two counting chambers. The suspension was thoroughly, but gently, mixed before drawing the samples. The chambers were slowly filled and steadily by avoiding injecting bubbles into the chambers and not be overfilled or underfill the chambers. The spores were then counted thus: all of the spores in each of the four 0.1 mm³ corner squares labelled A through D (Figure 1). It was noted not to count the spores touching the top or left borders and the spores touching the bottom or right borders. The spores were calculated by counting the spores in the four corner squares using the equation: spores/ml = (n) x 10^4, where: n = the average cell count per square of the four corner squares counted. For instance: If the calculated average (n) of spores in the four 1 mm corner squares of the haemocytometer is 30: = (n) x 10^4 spores /ml = 30 x 10,000 = 300,000 spores /ml. Diagrammatical representation of the haemocytometer slide used for the spore count is on the next page.
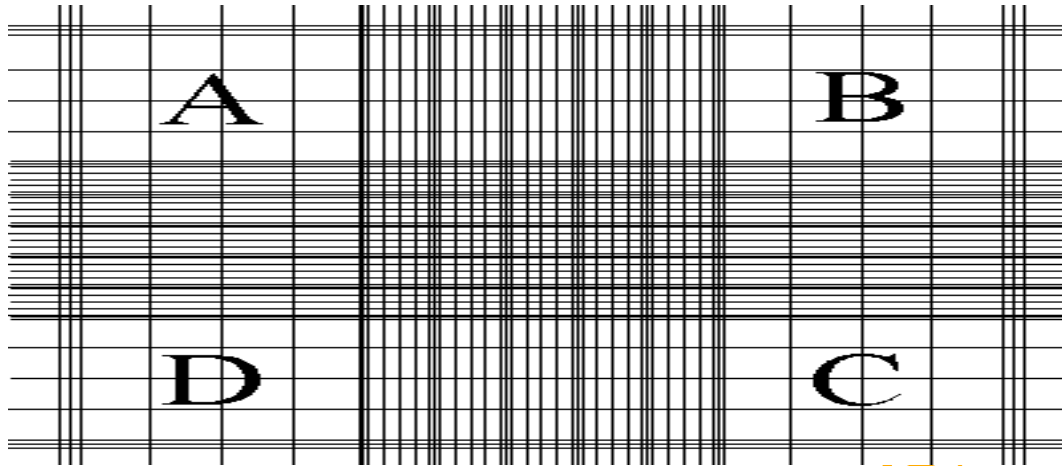
Fig. 3.1: Diagrammatical representation of the heamocytometer slide used for the spore count

3.8   Pairing of antagonists with the pathogen

The antagonists were paired with the pathogen at different locations and times on the plates with the target organism. The pathogen was placed at four peripheral positions with the antagonists individually at the centre. Also, the pathogen and antagonists were placed at two peripheral locations in opposite direction, about 1cm from the plate wall to prevent contamination and were done in this order:

- The pathogen was introduced 24 hours before the antagonist
- The pathogen was introduced simultaneously with the antagonist
- The pathogen was introduced 24 hours after the antagonist

The dixenic cultures of 3 replicates were incubated at 28-30°C for one week and observations on the growth of the organisms were taken from 48 hours of inoculation and at 24 hours interval. The interaction of antagonists and the pathogen was observed and recorded.

3.9   Extraction of plant samples

Fresh leaves and stems of the selected plant samples (*Tithonia diversifolia)*; *Physalis angulata, Senna occidentalis,* and *Mirabilis jalapa*; were washed under running tap water and spread out on the laboratory bench at room temperature of about 28 ± 2^0C for drying until they became crisp. The dried and fresh plant parts were pulverized into powder and concoction respectively by blending with Binatone blender. All samples were blended for 6-10 minutes. For each sample, 25, 50, and 75 g powder samples were extracted in 100 ml-distilled water as solvent. The suspensions were heated over water-bath at 70^0C for 1 hour, and then filtered by using Whatman No1 filter paper and the filtrate stored in the refrigerator at a temperature of 4^0C until required. The extracts were kept as stock solutions for fungitoxicity test, and according to Onalo (1999), the prepared extracts were used within 48 hours of preparation.

3.10   Test for fungitoxicity of the botanicals

3.10.1 Test of the efficacy of the botanicals.

In vitro studies were carried out on 1.25 g streptomycin incorporated into 1litre potato dextrose agar (PDA). The concentrations of the extracts were prepared by extracting the weights of plant materials in 100 ml by adding the 10 ml volume of

molten PDA and dispensed into each Petri dish that was gently swirled. About 10 ml of molten PDA at 45°C, was dispensed into each Petri dish which already had 5 ml of botanical extracts (this was done for plant sample used). These were gently swirled to the left and right directions to ensure even dispersion. Mycelial plugs were taken with sterile cork borer of 5 mm diameter from the margin of the vigorously growing target pathogen of one week-old culture and were inoculated at the center of each Petri dish. The plates were then incubated at 28-30°C for 7 days. Observation of the fungitoxic effects were made daily for 5-7 days. Photographs of salient results were taken.

The fungicide used as the check for the studies was benomyl, 50% active ingredient with trade name (Benlate R) belonging to the benzimidazole group. The Benlate solution was prepared from the stock according to the manufacturer's direction. A weight 0.5 g of Benlate powder was poured into 1litre of sterile distilled water and shaken thoroughly. It was applied at 0.5 g a.i/litre of water and sterile distilled water which served as the chemical control.

The radial growth of each fungus was measured at 24-hour interval after 48hours of incubation for seven days; the growth was measured from the perpendicular lines drawn on the reverse side of the plates. The effect of the different plant extracts on the growth of *F. verticillioides* was thus investigated, laid out in a 4 x 3 x 2 factorial experiment in a Completely Randomised Design (CRD) (4 types of plant samples: *T. diversifolia, P. angulata, M. jalapa* and *S. occidentalis*; 3 levels of the botanicals at 75, 50 & 25%; 2 nature of plant samples used: dry and wet).

According to Awuah (1989), the fungitoxicity percentage was expressed as the difference in mycelial growth in control plate (M1) in which only PDA was dispensed and mycelial growth in extract (M2), which was divided by mycelial growth in control plate multiplied by 100.

i. e. MP = $\dfrac{M1 - M2}{M1}$ X 100

Where MP = % inhibition of mycelial growth

M1 = mycelial growth in control plate

M2 = mycelial growth in extract or fungicide.

3.10.2 Minimum inhibitory concentration of the botanicals

Minimum inhibitory concentration (MIC) as described by Ejechi and Souzey (1999) is the lowest concentration of the extract that can prevent fungal growth. This helped to guide in the selection of effective concentration of botanicals and most suitable agroecology for the production of bioactive substances from the plants. Extracts were rated based on their inhibitory effects using the scale.

-0% = stimulatory (ST)

0% = no effect (NE)

1 -20% = slightly effective (SE)

21 -40% = moderately effective (ME)

41 – 60% = effective (EE)

61% and above = highly effective (HE)

3.11 Effect of interaction of both botanical and biocontrol agents on the pathogen.

In vitro studies were carried out on PDA. Minimum inhibitory concentrations of plant extract were prepared. Five millimeter aliquot of extracts of the botanicals was dispensed into 9 cm- diameter Petri dishes. Ten millilitre of molten PDA (45^0C) was dispensed into each Petri dish and gently swirled in a clockwise direction to ensure even dispersion of extracts. The target pathogen of mycelial agar plug size of 5 mm was used to inoculate the poisoned PDA at the side of the plate and the biocontrol agents were placed at the other side of the plate away from the target organism. The plates were incubated at 28 -30°C for seven days after inoculation. Observations on the effects of the combination started at 48 hours of incubation and were recorded till 7 days of incubation. Control treatment was set up singly for both bioagents and botanicals i.e poisoned agar without the biocontrol agents paired with the pathogen.

SCREENHOUSE EXPERIMENTS

3.12   Raising of maize plants for screenhouse trials

The soil used in the screenhouse was sterilized in a hot air oven at 270^0C for 72 hours and was allowed to cool for a day, packed into 20 litres plastic pots and moistened before sowing. The seeds were sown into autoclaved soil and kept in the screenhouse at 27-32°C and watered regularly on daily basis.

3.13   Pathogenicity test of the target pathogen on maize

Pathogenicity test was carried out to make sure that the isolate obtained are pathogenic and able to induce same disease symptoms on healthy maize plants. The experiment was conducted at the screenhouse of Institute Agricultural Research and Training (IAR&T), Obafemi Awolowo University, Moor Plantation, Ibadan.

3.13.1 Inoculation of the target pathogen

Two plants per pot in three replicates of each of maize varieties were inoculated with spore suspensions of 0.5 x 10^6, 1.0 x 10^6, 1.5 x 10^6, 2.0 x 10^6 spore/ml using a 500 ml hand master sprayer by spraying to runoff, this method was to check the pathogen's infective level. The inoculated plants were kept humid by covering them for 24 hours, with transparent polyethylene bags for spore germination and induction of infection. The inoculation was done at the seedling stage at 4 WAP. Symptomatic plant parts were cut, taken to the laboratory, surface sterilized in 10% sodium hypochlorite, NaOCl for 3 mins and incubated on wet blotter and PDA at a temperature of 25°C in alternating cycles of 12 hours artificial daylight and darkness. Fungal growth was examined using a compound microscope. Identification was on the basis of descriptions by Mordue (1971).

3.14. Test of effective inoculum level of the antagonists

First, the seeds were surface sterilized in 0.5% NaOCl for 1 minute and then rinsed in 5 changes of sterile distilled water. The mycelial mats of the antagonists were cultured on Potato Dextrose Agar (PDA), dried in an oven at 30°C and blended in a sterilized Gallenkamp blender set (which had been thoroughly washed and rinsed with NaOCl) for 1 min. These mycelial mat powders were stored in sterilized Petri dishes and refrigerated at 4°C before use. One gram each of the powdered mycelia of the

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Trichoderma species was mixed in 100, 200, 300 and 400mls of 5% sucrose solution in sterile distilled water containing 20 ml of 5% Tween 80 to make up the different inocula levels.

P. fluorescens were isolated from the maize rhizosphere, maintained on PDA. Cell suspension (100μl) of *P. fluorescens* was inoculated into sterile Erlenmeyer flasks containing 125 ml of PDA. The bottles were placed horizontally and incubated at 28 ± 2 ∘C under a 12: 12 h light: dark photoperiod. The culture in PDA (48 hour old) was centrifuged at 10 000 × *g* for 5 min. The pellets were resuspended in sterile distilled water, washed twice and suspended again in sterile distilled water. The density of the cells in the suspension was adjusted to 10^9 cfu/ml by using a UV-visible spectrophotometer (Hitachi, Japan).

3.15 Germination test of antagonist-treated maize seeds

Seeds treated (as explained in section 3.16, paragraph 2 and 3) with the antagonists were planted at least 2 seeds per pot in replicates of 3 pots. Two grams of ground mycelial mat of the pathogen was added (at planting) per ten (10) kilogram of sterile soil, mixed thoroughly, to induce the disease caused by this pathogen. The germination percentage of these seeds was counted on the 7th, 14, 21, 28 days after planting. Observation was carried out till harvest.

3.15.1 Effect of the inoculum treatments on maize germination

One gram of ground mycelia of each of the species of *Trichoderma, B. subtilis* and *P. fluorescens* was suspended in 100, 200, 300 and 400 ml of distilled water to determine the inoculum level in each. *T. harzianum* (C1: 1.8 x10^{11}, C2: 1.7 x10^{10}, C3: 2.3 x 10^9, C4: 7.8 x 10^8 spores/ml respectively) had a higher spore count than *T. pseudokoningii* (C1: 2.7 x10^{10}, C2: 5.3 x 10^8, C3: 7.1 x 10^7, C4: 1.5 x 10^7 spores/ml respectively). *B. subtilis* concentration in sterile distilled water were C1: 1 x 10^9, C2: 1 x 10^8, C3: 1 x 10^7, C4: 1 x 10^7 cfu/ml respectively and *P. fluorescens* (C1: 1 x 10^{10}, C2: 1 x 10^9, C3: 1 x 10^8, C4: 1 x 10^7 cfu/ml respectively). These were done for fungi and the bacteria used in this study to obtain the colony forming units for each organism and inoculum level at which seed decay, seedling blight due to *F.verticillioides* will not have effect on the seeds planted despite the fact that the seeds were planted in infested soils with the mycelia of *F. verticillioides.*

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3.15.2 Effect of botanicals on the target pathogen (*F. verticillioides*)

Extracts of leaves of fresh and dry selected botanicals for this study were incorporated into PDA plates were then inoculated with 5 mm disc of the pathogen from young culture of *F.verticillioides.* The concentration of the botanicals in the agar were calculated as follows 5, 3 and 1 ml incorporated into 15 ml PDA growth media gave 5/15 of 90 g, 75 g and 30 g; 3/15 of 100 g, 75 g and 25 g, and 1/15 of 37.5 g proportion of the botanical in the agar. The corresponding results of the calculations were the concentration of the botanicals incorporated into the agar. Therefore, the final concentrations of the selected botanicals 5 ml of 90 g is 30.0%, 5 ml of 75 g is 25%, 3 ml of 100 g is 20.0%, 3 ml of 75 g is 15.0%, 5 ml of 30 g is 10.0%, 1 ml of 25 g is 5.0% and 1 ml of 37.5 g is 2.5%, the benomyl 5/15 of 0.5 g/l is 0.00017% in the 15 ml agar growth media.

3.16 Application methods of the bioagents and botanicals as seed treatment

First, the ILE 1-OB maize variety that was found to be most susceptible to ear rot attack was used for the screenhouse trial to detect the best seed treatment method. The varieties of maize that are susceptible (ILE – 1 OB), moderately resistant (ACR 99TZL) and resistant (ART/98/SW5OB) were used on the field using the best seed treatment. The seeds were surface sterilized in 10% NaOCl for 1 min and then rinsed in five changes of sterile distilled water. The seeds were then treated with four, 4 bioagents, four, 4 botanicals and chemical (Benlate). The seeds were then soaked in the inocula of bioagents for 10 minutes and air-dried for 12 hours before planting (Adekunle *et al.,* 2001). The experiment was done in Complete Randomised Design CRD of 4 x 3 factorial laid out for the bioagents trials and 4 x 3 factorial laid out for the botanicals trials.

The production of the *Trichoderma species* on solid substrate of cassava starch was carried out thus: Cassava starch was purchased from local market. A quantity of 20 g of the starch was placed in a 250 ml beaker containing 100 ml distilled water to cook/boil the starch. Cooked cassava starch was used as an adhesive for the mycelial suspensions (Adekunle, 1998). One gram of mycelial mat of the two *Trichoderma* species were then added to it in separate flasks. These were mixed with 50 g of maize seeds, mixed, shaken, drained and left in the laminar flow for 12 hours to dry before planting.

One gram of mycelial mat of the two *Trichoderma* species were mixed in 200 and 300 ml of sterile distilled water for *T. harzianum* and *T. pseudokoningii* respectively, maize seeds of 50 g were then added separately. The spore suspensions that were tested for germination percentage were got from the 1 g of the antagonists were suspended in 100, 200, 300 and 400 mls of sterile distilled water respectively for the spores and cells count of each antagonists were done for each and tested on the maize seeds for its individual effects on germination. The maize seeds were planted in sterile soil which has been infested with 2 g of *F. verticillioides*. The seeds were soaked for 10 minutes before they were removed to a paper towel to dry in the laboratory at ambient temperature for 12 hours before planting.

One gram of the ground mycelial mat powder of the two *Trichoderma* each were mixed in 10 ml sterile distilled water , these were then added to 50 g of maize seeds, shaken and the seeds were left for 10 minutes before they were removed to a paper towel to dry in the laboratory at ambient temperature before planting. Seeds were also treated with benomyl (Benlate WP 50) a fungicide at a rate of 0.5 g a.i/ 50 g of seeds using same method.

The procedure of formulations for seed treatments for the *Trichoderma* species were as stated above are summarized as

- Cooked cassava starch method: 20 g starch + 100 ml H_20 + 1 g antagonist(s) + 50 g of seeds

- Powder coating method: 10 ml of distilled H_20+ 1 g of antagonist(s) + 50 g of seeds

- Water suspension method: 200, 300 ml of dis. H_20 + 1 g of antagonist(s) + 50 g of seeds

- Chemical control: 50 g of seeds + 0.5 g benomyl (Benlate WP 50)

- Control : no treatment

The formulation of *P. fluorescens* for seed treatment, method used by Nayaka *et al*. (2008) was adopted for this study with some modifications. Cassava starch was purchased from a local market (Oja Apata). A quantity of 20 g of the cassava substrate was placed in a 250 ml flask containing 100 ml distilled water to cook/boil. The contents were mixed thoroughly using a glass rod until all the substrate particles were

evenly moistened and no lumps were present. Flasks were then inoculated with 1 ml of *P. fluorescens* containing 10^9 cfu/ml. The contents were shaken well to disperse the inoculum evenly. The seeds were left for 10 minutes before they were removed to a paper towel to dry in the laboratory at ambient temperature before planting.

For the water suspension method, 100 ml of sterile distilled water was poured in 250 ml flask; 50 g of maize seeds were added. The contents were shaken well to disperse the inoculum evenly, and the seeds were soaked for 10 minutes before they were removed to a paper towel to dry in the laboratory at ambient temperature for 12 hours before planting.

For the powder method, cultured *P. fluorescens* on PDA was kept in the oven to dry at 30^0C for 24 hours. These were ground into powder. One 1 gram of 10^9 cfu/ml was released into 10 ml of sterile distilled water and these were added to 50 g of seeds and the whole mixture was shaken for 10 mins before they were removed and kept to dry at ambient temperature for 12 hours before planting.

The procedure of formulations for seed treatments for the *P. fluorescens* are as stated above are summarized as

- Cassava cooked starch method: 20 g starch + 100 ml H_2O + 1 ml of *P. fluorescens* containing 10^9 cfu/ml + 50 g seeds

- Powder coating method: 10 ml of distilled H_2O+ 1 mL of *P. fluorescens* containing 10^9 cfu/ml + 50 g of seeds

- Water suspension method: 200 ml of dis. H_2O + 1 ml of *P. fluorescens* containing 10^9 cfu/ml + 50 g of seeds

- Chemical control: 50 g of seeds + 0.5 g benomyl (Benlate WP 50)

- Control : no treatment

For the botanical treatments, the botanicals used were *Tithonia diversifolia, Mirabilis jalapa, P. angulata* and *Senna occidentalis*

The cooked cassava starch of 100 ml of sterile distilled water with 20 g cassava starch was used as an adhesive for the botanicals (Adekunle, 1998). A weight of 75 g of the botanicals was added to it in separate flasks. These were mixed with 50 g of maize seeds, and left in the laminar flow for 12 hours to dry before planting.

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For the water suspension for the botanicals treatments, the dry powder plant samples of 75 g were soaked in 100 ml of sterile distilled water, filtered and the substrate was used to soak 50 g of maize seeds were then added separately. The seeds were soaked for 10 minutes before they were removed to a paper towel to dry in the laboratory at ambient temperature for 12 hours before planting.

The ground dry botanicals were used here. Each sample of 75 g were weighed and poured on 50 g of maize seeds, 10 ml sterile distilled water was added for adhesion. The seeds were left for 10 minutes before they were removed to a paper towel to dry in the laboratory at ambient temperature before planting. Seeds were also treated with benomyl (Benlate WP 50) a fungicide at a rate of 0.5 g a.i/ 50 g of seeds using same method.

The procedure of formulations for seed treatments for the botanicals as stated above are summarized as

- Cooked cassava starch method: 20 g starch + 100 ml H_2O + 75 g of botanical(s) + 50 g maize seeds

- Powder coating method: 10 ml of distilled H_2O+ 75 g of botanical(s) + 50 g of seeds

- Water suspension method: 100 ml of sterile dis. H_2O + 75 g of botanical(s) + 50 g of seeds

- Chemical control: 50 g of seeds + 0.5 g benomyl (Benlate WP 50)

- Control : no treatment

3.17 FIELD STUDIES

Field studies were carried at the Southern research farm of Institute of Agricultural Research and Training, IAR&T, Ibadan, Nigeria.

3.17.1 Reaction of Quality protein maize varieties to Fusarium ear rot in the field

Five (5) QPM and one non QPM varieties were planted for two years (under natural infestation and towards the peak of the severity and incidence of the disease on the field) to check for the response of the selected varieties for their

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resistance to ear rot disease. The varieties used were ART-98-SW5-OB, ART-98-SW4-OB, ART-98-SW6-OB, ILE-1-OB, OBATAMPA, TZPB-OB and ACR 99TZL, the non QPM variety.

This experiment was carried out in 2007 and 2008 planting seasons; the experimental design was Randomised Complete Block Design with 3 replicates. All agronomic practices were carried out on the field (such as weeding which was done twice at 4 weeks and 8 weeks after planting; fertilizer application of NPK 20-10-10 and Urea; thinning was done at 2 weeks after planting) and data were taken on each maize variety. The incidence, severity of lesions and disease infection index were taken at 2,4,6,8 weeks till harvest. The reaction of the host plant to disease was classified into susceptible, moderately susceptible and resistant. The severity assessments of maize disease were based on diseased symptoms observed on inoculated plants in the greenhouse and the field trials. The results were subjected to analysis of variance (ANOVA) for statistical differences in the varietal tolerance.

3.17.1a Ear rot incidence:

The plants were observed for ear rot development and rated for disease when they showed any one of the typical ear/kernel rot symptoms such as a powdery or cottony-pink mould growth on the infected kernels. Experiment was conducted twice. The disease severity was rated on a 1 -7 scale according to Reid *et al*. (1996). Disease severity ratings were recorded on a 1–7 scale,

where 1 = < 25%

2 = 25 - 35%

3 = 36 – 45%

4 = 46 – 55%

5 = 56 – 65%

6 = 51 – 75%

and 7 = >75% of the seedling blight, kernels visibly mouldy, stalk/ear rot. Ear rot incidence was determined by counting the total number of cobs and total number of infected cobs per plot. Data on percentage incidence of ear rot was subjected to analysis of variance, and DMRT was used to evaluate the significance of the differences between the control and the treatments.

3.17.2   Field trials on the efficacy of seed treated with bioagents and botanicals on the target organism.

For the field experiment, the efficacy of seed treated with the microbial antagonistic agents and plant extracts were determined as it relates to yield. The resistant variety ART-98-SW5-OB, susceptible ILE-1 OB and a local check ACR 99TZL (variety commonly planted by farmers) were used for these trials. The seeds of maize were surface sterilized with 10% NaOCl for 1 minute, rinsed in sterile distilled water and dried on paper towel. Planting in the field was done by direct sowing of the maize varieties that have been coated using the method described in section 3.16. The plot was 6 x 4 m^2 consisted of 8 rows with 25 cm spacing within rows and 75 cm spacing between rows, the treated seeds were planted using Randomised Complete Block Design with 2 rows of 2.5 m (25 cm spacing within rows and 50 cm between rows and 1 m away from the plots) which acted as spreader rows for natural infection.

Maize varieties were replicated three times in plots. Weeding was done with the use of herbicide (Paraforce at 3.5 to 4.0 l/ha and Primextra at 3.5 to 4.0 l/ha) and manually at various growth stages. Observations on growth and development of seedling were done after planting. The experiment had an untreated control and chemical control.

3.17.2a The observations on the effect of the treatments on yield

After maturity, maize cobs were harvested, dried and threshed. The grains were weighed separately, and the grain yields in the plots with different treatments were compared with that of the control. The data were subjected to ANOVA using SAS statistical package and the test for significant difference in the treatment means of each parameter using Duncan Multiple Range Test, DMRT, and a correlation analysis was done to ascertain the relationship between the disease severity and yield parameters.

The number of plants, which were infected among the total number of plants in a plot per treatment were counted to determine the total number of disease infection on each treatment and maize genotype, and scored as stated below, which was used to calculate the percentage infection. A plant having slight or severe symptoms of disease were considered infected.

3.17.2b Observations for the Ear and stalk rot disease incidence

The incidence of the disease in each test was calculated as the percentage of infected plants in each test varieties trial in the greenhouse and field experiments. The lodged or rot was recorded periodically by considering the total number of plants lodged. This scoring was for the various varieties with the test pathogen.

A statistical analysis was done using SAS ANOVA to test for significant differences in treatment means of each of the parameters. A correlation analysis was done to ascertain the relationship between the disease severity and yield parameters; days to tasseling, cob number, seed weight, plant character and disease resistance.

CHAPTER FOUR

4.0　　　　　　RESULTS

4.1　GROWTH PATTERN OF THE TARGET ORGANISM

On the third day after inoculation, mycelial growth of *F. verticillioides* was 3.1 cm in diameter and by the 7th day the whole plate was covered (Plate 4.1). Mycelia were fluffy and pinkish-white viewed from the top of the plate whilst the underside of the plate was brown in color. A change in colour of the mycelia started from the oldest portion of the fungus from 7 days, which turned light pink in colour (Plate 4.1). Repeated sub-culturing did not affect the cultural characteristics.

4.2　GROWTH PATTERN OF THE ANTAGONISTS

The antagonists tested in this study were *Trichoderma pseudokoningii, T. harzianum, Bacillus subtilis* and *Pseudomonas fluorescens.* The mycelial growth of *T. pseudokoningii* 48 hours after inoculation was 2.1 cm in diameter and the whole plate was covered by 5 days after inoculation. The whole of the culture was powdery and dark green with scanty whitish mycelia, fully grown by 5 days after inoculation. The underside of the plate has greenish-yellow secretion into the growth medium (Plate 4.2).

By the 3rd day after inoculation, the mycelial extension of *T. harzianum* was 3.8 cm in diameter. The diameter of the mycelia extension was 9.0 cm by the 7th day. The mycelia were very fluffy white in 3 days of incubation (Plate 4.3) with observed change in the colour of the white fluffy mycelia to dark green, giving a light-green sporulating center at 7 days of incubation (Plate 4.4).

Forty-eight hours after inoculation, the colony of *P. fluorescens* was 1.4 cm in diameter. Colony diameter increased to 2.6 cm by the 7th day after inoculation (Plate.4.5). The colony was dirty brown with smooth edge and produced brownish pigmentation into the growth medium at the underside of the plate.

The *B. subtilis* grew on nutrient agar as well as on Potato Dextrose Agar (PDA) to a diameter of about 1.8 cm in 48 hours after inoculation. The colony was mucoid with rugged raised surface (Plate 4.6). It produced translucent off-white colonies with

serrated edges. There was an increase in colony size by the 7th day to 3.2 cm in diameter. In all, repeated subculturing did not affect the cultural characteristics.

4.3   PAIRING OF THE ANTAGONISTS AND TARGET ORGANISM

4.3.1 *F. verticillioides* **paired with** *Trichoderma pseudokoningii* **on the same plate**

The antagonist *T. pseudokoningii* had fast growth effect on the pathogen, *F. verticillioides*. Within 96 hours, the antagonist invaded the whole plate including the point that was inoculated with *F. verticillioides*, with the appearance of a yellowish - green diffusible substance into the growth medium (Plate 4.7).

Seven days after pairing, the growth of *F. verticillioides* was restricted probably due to colonization of its mycelia by the pathogen (Plate 4.7), two weeks later the fungus grew to 2.3 cm in diameter. The growth of *F. verticillioides* increased with increase in incubation period in order to escape the antagonist (Plate 4.7). *T. pseudokoningii* showed little inhibitory action on the pathogen.

4.3.2 *F. verticillioides* **paired with** *Trichoderma harzianum* **on the same plate**

The growth of *T. harzianum* had antagonistic effect on *F. verticillioides*. The two organisms had uninterrupted growth till after 3 days of pairing (inoculation). But by the 7th day of observation the antagonist had really inhibited the mycelial growth of the pathogen, leaving a thin zone of inhibition. The observation at 2 weeks after inhibition showed *T. harzianum* as an antagonist which had mutual interaction with the pathogen because the pathogen was able to grow again after a while (Plate 4.8).

It was observed that by the 3rd day the colour of the pathogen started to change into deep purple colour and *P. fluorescens* too had a darker, dirty brown colour (Plate. 4.9). Two weeks after inoculation, the color of *F. verticillioides* had a deeper purple and very few white mycelia. The colour of *P. fluorescens* became darker brown probably due to metabolite secreted by the pathogen in an attempt to overcome that of the antagonist. The pairing showed a clear zone of inhibition of the pathogen's growth, which was maintained throughout the period of 2 weeks of observation.
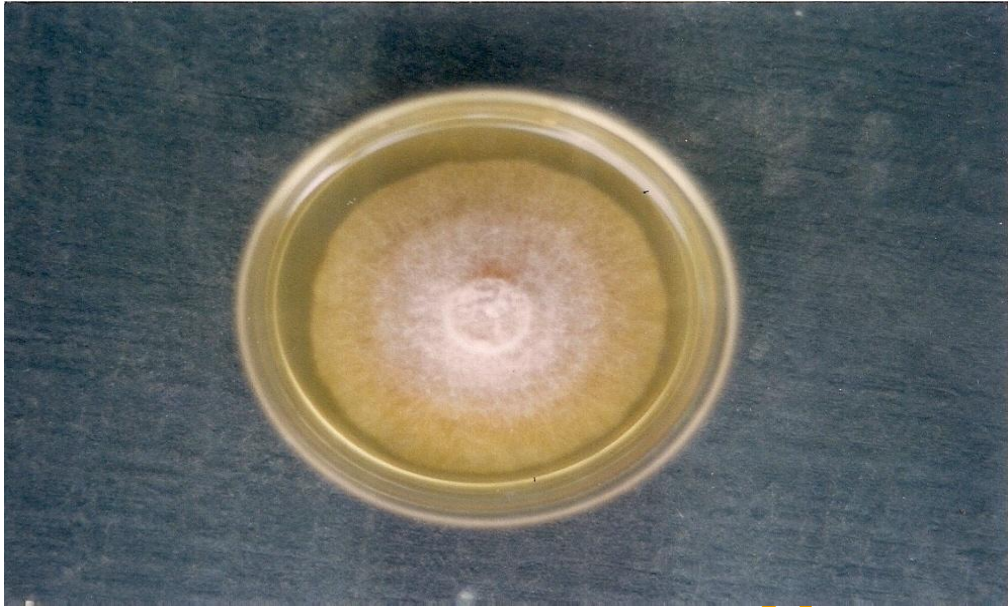
Plate. 4.1: A seven-day old culture of target organism *Fusarium verticillioides* on PDA. Diameter of growth was 80mm.
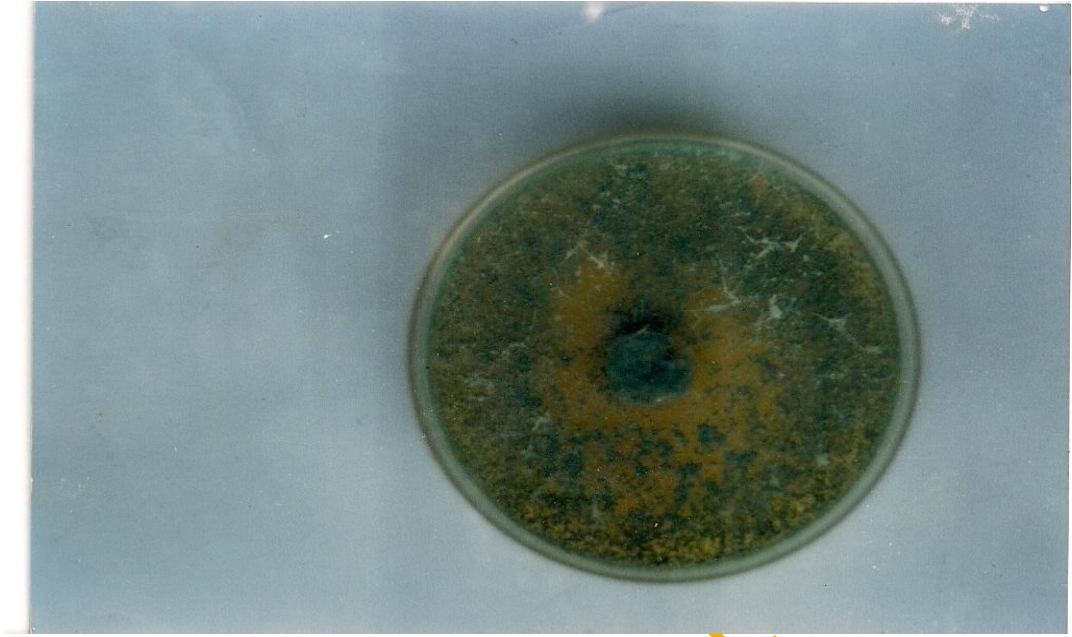
Plate 4.2: A seven-day old culture of antagonist, *Trichoderma pseudokoningii* on PDA.
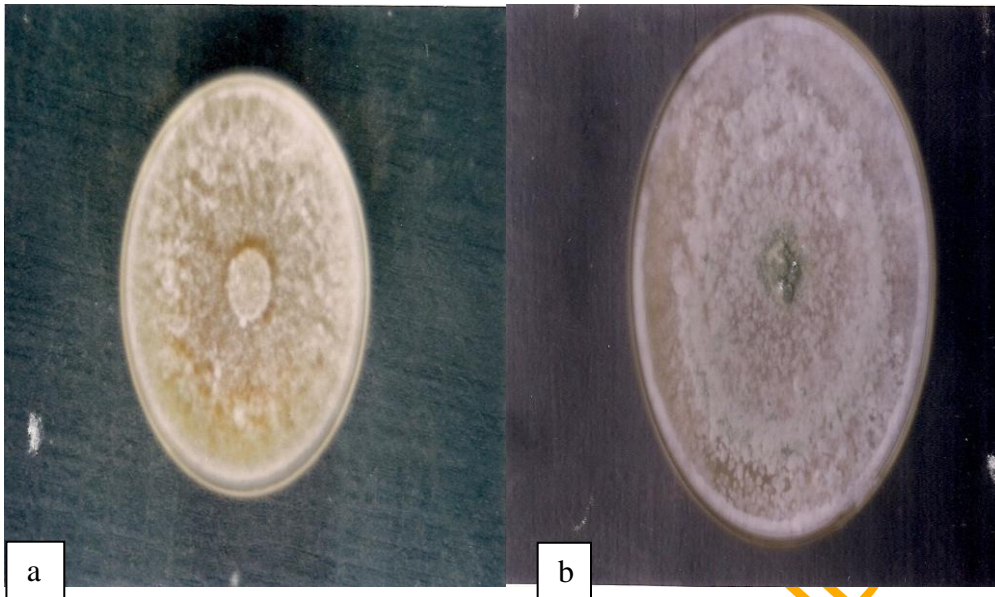
Plate 4.3a: A three-day old culture of an antagonist *Trichoderma harzianum* on PDA in a 45mm Petri dish. Plate 4.3b: A seven-day old culture of antagonist, *Trichoderma harzianum* on PDA in 90mm Petri dish, showing the fungus turning green.

Plate 4.5: (a) A seven-day old culture of *Pseudomonas fluorescens* on Nutrient agar medium and **(b)** A seven-day old culture of *Bacillus subtilis* on Nutrient agar medium.

4.3.3 *F. verticillioides* **paired with** *Pseudomonas fluorescens* **on the same plate**

The antagonist *P. fluorescens* grew to 0.5 cm in diameter by the third day after pairing with *F. verticillioides*, which stopped mycelial growth of the pathogen when the fungus had developed to a diameter of 1.4 cm. There was no extension in the mycelial growth of the pathogen at least 7 days after pairing. The antagonist maintained its 0.5 cm diameter; the sporulation and mycelial extension of the pathogen were affected.

It was observed that by the 3rd day the colour of the pathogen started to change into deep purple colour and *P. fluorescens* too had a darker, dirty brown colour (Plate. 4.9). Two weeks after inoculation, the color of *F. verticillioides* had a deeper purple and very few white mycelia. The colour of *P. fluorescens* became darker brown probably due to phytotoxic metabolite secreted by the pathogen in an attempt to overcome that of the antagonist. The pairing showed a clear zone of inhibition of the pathogen's growth, which was maintained throughout the period of 2 weeks of observation.

4.3.4   *F. verticillioides* **paired with** *Bacillus subtilis* **on the same plate**

Two days after pairing, the diameter of growth of *B. subtilis* was 0.6 cm while that of the pathogen, *F. verticillioides* averaged 0.8 cm in diameter and inhibition zone of 3.2 cm had developed. By the 7th day after pairing, the antagonist *B. subtilis* had increased in growth/size to 1.0 cm which resulted in a further sparse growth of the pathogen mycelia (Plate 4.10) leaving a thin zone of inhibition after 2 weeks of observation. Slight colour change was observed on the pathogen as incubation period increased.

4.4   **Effect of the antagonists on growth of the pathogen**

The four antagonists were separately paired with the target organism (pathogen) to determine the best antagonist and its efficacy on the pathogen. When the fungi (species of *Trichoderma*) were paired with the pathogen, *T. pseudokoningii* was found to have fast growing effect on the pathogen when paired together.

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T. harzianum parasitized the pathogen, the pathogen competed with its growth and *T. harzianum* displayed competitive action on the pathogen (Plate.4.8).The two bacteria used inhibited the growth of the pathogen. The antagonist, *P. fluorescens* had the higher inhibitory action with 51.8% while *B. subtilis* was less. The results of the statistical analysis (Table 4.1) showed that there was no significant difference in the treatment with all antagonists at early stages of incubation except with *P. fluorescens* which was significantly different from others at 95% level of significance.

Table 1 shows that *Pseudomonas fluorescens* had the highest inhibitory action of 51.75%, *Trichoderma pseudokoningii,* 44.74%, *Trichoderma harzianum* inhibitory action was 35.28% while *Bacillus subtilis* had the least inhibitory effect of 32.46%.

There was no significant effect on the pathogen due to the four treatments (antagonists) at 48 hours of incubation. There was however, significant difference due to the treatments on the pathogen at 72 hours and as the incubation period increased.

Plate 4.7: A seven-day old culture of *Trichoderma pseudokoningii* (antagonist) at the center and *F.verticillioides* (pathogen) inoculated simultaneously at the four equidistant peripheral points of the plate.
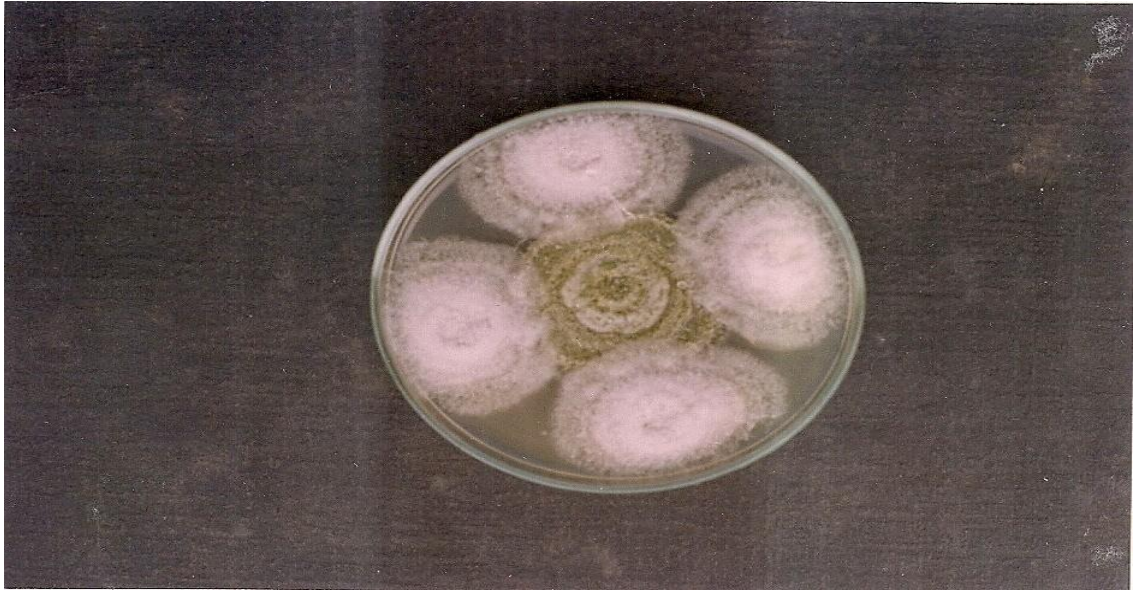
Plate 4.8: A week old (7 day) dixenic culture of *Trichoderma harzianum* (Antagonist) and *F. verticillioides* (pathogen) at four equidistant peripheral points of the plate.

Plate 4.9: A week old dixenic culture of *Pseudomonas fluorescens* (antagonist) with *F.verticillioides* at the periphery of the plate. Showing purple color from whitish pink colour of the target pathogen, *F.verticillioides* and brown color change of the antagonist, *P. fluorescens* which was slightly brown initially, acting at a distant.

Plate 4.10: A week old dixenic culture of *Bacillus subtilis* (antagonist) paired at the centre with the pathogen, *F.verticillioides* at the peripheral points of the plate. This plate shows the *B. subtilis* as it exhibited its antagonistic effect on the density of mycelia of *F.verticillioides* with a slight colour change and scanty mycelia.
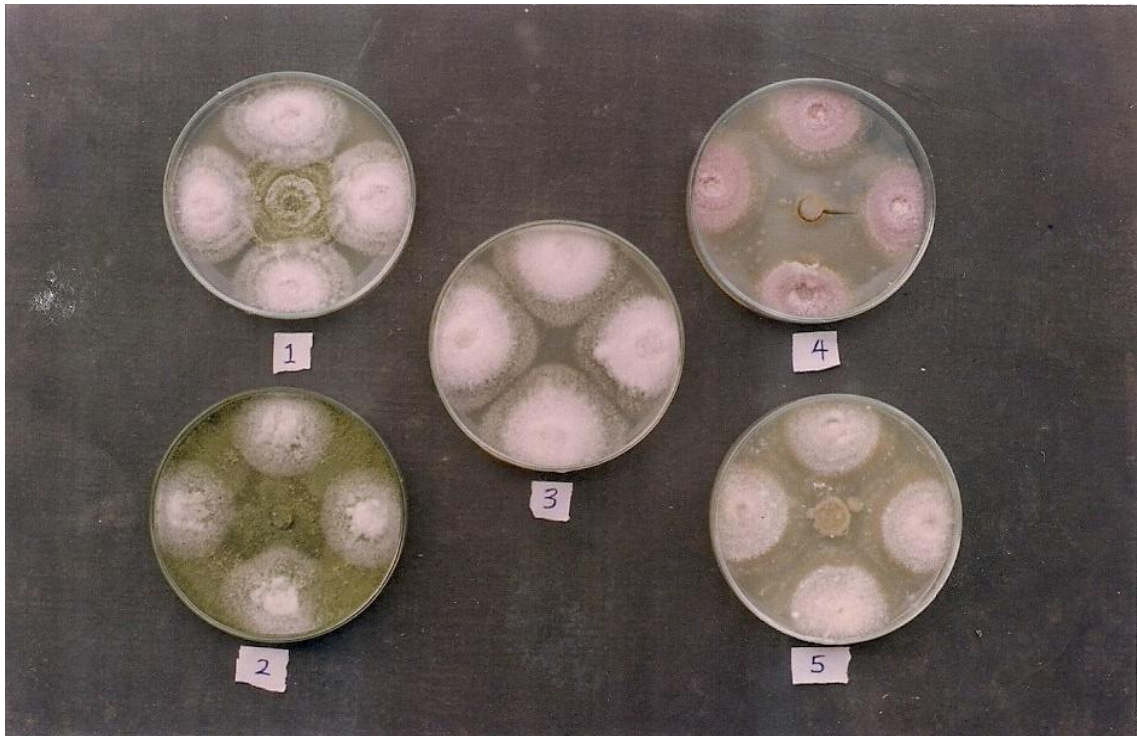
Plate 4.11: Two week-old dixenic cultures

1: Antagonism between *T.harzianum* at the centre and *F. verticillioides* at the periphery. There was no zone of inhibition of growth of the target organism. Both fungi grew at almost the same pace.

2: The interaction between *T. pseudokoningii* (centre) and *F .verticillioides* (periphery). *F. verticillioides* was invaded by the faster growing fungus *T. pseudokoningii* 3 days after inoculation but grew over the invaded area after 7 days of inoculation.

3. Control plate of *F. verticillioides* inoculated at the periphery to show the normal rate of growth of the fungus.

4: An inhibition zone of 3.20 cm was produced when *P. fluorescens* (centre) was paired with *F. verticillioides* (periphery). *F. verticillioides* probably secreted a metabolite to overcome the effect of the suspected toxic metabolite secreted by *P. fluorescens* into the medium resulting in both organisms changing colours.

5: Antagonism between *B. subtilis* (centre) and *F.verticillioides*. A thin zone of inhibition was established seven (7) days after inoculation.

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Table 4.1: Effect of antagonists on growth diameter of *F. verticillioides*

Antagonists	Incubation time (hours)							
	48		72		96		120	
	Mean growth	Inhibition (%)	Mean growth	Inhibition (%)	Mean growth	Inhibition (%)	Mean growth	Inhibition (%)
T. pseudokoningii	4.30a	18.90	5.20c	23.50	5.80cd	22.70	6.30bc	44.74
T. harzianum	5.30a	0.00	6.50ab	4.40	6.60bc	12.00	7.40a	35.10
B. subtilis	5.10a	3.80	6.30bc	7.40	7.00b	6.70	7.70a	32.46
P. fluorescens	4.80a	19.90	5.40bc	11.40	5.50cd	26.70	5.50c	5.50
Control	5.30a	3.80	6.80a	7.40	7.50a	6.70	9.00a	32.46
Mean	4.96	9.28	6.04	10.82	6.48	14.96	7.18	30.05
S.E (0.05)	0.87		0.25		0.52		0.61	

* Means of 3 replicates (cm). Means in the same column followed by the same letter are not significantly different at 0.05 level (LSD).

4.5 Effect of different pairing times of the antagonists with the pathogen

4.5.1 Pairing of the antagonists 24 hours before the pathogen

4.5.1a *Trichoderma pseudokoningii* inoculated 24 hours before *F. verticillioides*

When *T. pseudokoningii* was inoculated 24 hours earlier than *F. verticillioides,* It grew very fast covering the entire 90 mm Petri plate 5 days after pairing. The pathogen, *F. verticillioides* grew at an average of 0.7 cm (7mm) diameter at the early stage of incubation. The pathogen's mycelia were overgrown completely by the antagonist by the 7th day. The pathogen had mycelial extension of 3.00 cm at the end of the observation period (Table 4.2a & b).

4.5.1b *Trichoderma harzianum* introduced 24 hours before *F. verticillioides*

The antagonist, *T. harzianum* grew luxuriously and restricted the growth of the pathogen to its point of inoculation with mycelial growth mean of 1.3 cm. By the 5th day, the antagonist had suppressed the growth of the pathogen. The pathogen's growth on the 7th day of incubation was 2.2 cm (Table 4.2).

4.5.1c *Pseudomonas fluorescens* introduced 24 hours before *F. verticillioides*

When *P. fluorescens* was inoculated earlier than *F. verticillioides,* the bacterium grew very slowly covering 1.1 cm of the Petri plate seven days after pairing. The pathogen *F. verticillioides* grew to 2.8 cm diameter on the Petri plates. The pathogen's mycelia were pinkish to light purple color towards the antagonist by the 7th day. There were zones of inhibition by the antagonist on the pathogen. The inhibition zone was 5.2 cm (Table 4.2a & b).

4.5.1d *Bacillus subtilis* inoculated 24 hours before *F. verticillioides*

When *B. subtilis* was introduced 24 hours before the pathogen, *F. verticillioides,* the bacterium grew fast covering 1.30 cm within seven days of pairing. The pathogen *F. verticillioides* grew to 3.40 cm diameter in the Petri plates. The pathogen's mycelia were pinkish white, not as fluffy as those of the control at the 7th day (Table 4.2a & b).

Table 4.2: Pairing of each antagonist 24 hours before the pathogen

1St Trial	Time of incubation (hrs) of *F. verticillioides**					
Antagonists	48	72	96	120	144	168
T. harzianum	0.70b	0.90b	1.30c	1.60d	1.90e	2.20d
T. pseudokoningii	0.70b	1.20b	1.80b	2.60b	2.80c	3.00c
B. subtilis	0.80b	1.10b	1.90b	2.80b	3.10b	3.40b
P. fluorescens	0.70b	1.30b	1.70b	2.20c	2.50d	2.80c
Control	2.40a	4.50a	6.90a	8.86a	8.86a	9.00a
Mean	1.06	1.80	2.72	3.61	3.83	4.08
LSD	0.304	0.488	0.269	0.308	0.285	0.281
2nd Trial						
T. harzianum	0.50b	0.90b	1.10b	1.40e	1.73e	2.03c
T. pseudokoningii	0.90b	1.10b	1.86b	2.33c	2.70c	3.00c
B. subtilis	0.70b	1.00b	1.70b	3.00b	3.30b	3.60b
P. fluorescens	0.50b	1.10b	1.50b	1.86d	2.16d	2.46d
Control	2.16a	4.30a	6.36a	8.70a	8.86a	9.00a
Mean	0.95	1.68	2.50	3.45	3.74	4.01
LSD	0.448	0.853	1.259	0.420	0.257	0.225

*means of 3 replicates (cm). Means in the same column followed by the same letter are not significantly different at 0.05 level (LSD).

4.5.1e Pairing of the antagonists 24 hours before the pathogen

In the first trial (Table 4.2a) at 48 hours of incubation after the pairing of the antagonists with the pathogen, *F.verticillioides,* there was no significant difference for all the antagonists used but the control was significantly different from all, as its growth was not hindered in any ways; the mycelia grew well. The same trend of no distinct difference in the growth of the pathogen was observed at 72 hours. *T. harzianum* paired 24 hours before the pathogen at 96 hours of incubation had significant effect on the pathogen compared with others, it had the best inhibitory effect on the pathogen at that hour, the other three organisms had no significant differences among themselves, still the control had the highest mycelia growth. The same trend was observed at 120 hours, but at this hour, *P. fluorescens* restricted the growth of the pathogen significantly better from the effect of *T. pseudokoningii* and *B. subtilis* which are not significantly different from one another. The effect of the *B. subtilis* was significantly different from all antagonists being the weakest antagonist. There was significant difference in *T. pseudokoningii* and *P. fluorescens*, effect on the pathogen. *T. harzianum* maintained its inhibitory effect on the pathogen.

For the second trial (Table 4.2b), at 48 hours of incubation after the pairing of the antagonists with the pathogen, there was no significant difference in the treatments except for the control which was significantly higher from the other plates that had the pathogen and individual antagonists paired together. The same observation was made at 72 and 96 hours of incubation. At 120 and 144 hours, the antagonists reacted significantly in different ways to the pathogen. *T. pseudokoningii* was the most effective followed by *P. fluorescens* which was significantly different from all and from each other. At 168 hours there was no significant difference between *T. harzianum* and *T. pseudokoningii* but not as effective as *P. fluorescens*, whose action on the pathogen was significantly different in all.

4.5.2. Pairing of the antagonists simultaneously with the pathogen
4.5.2a *Trichoderma pseudokoningii* inoculated simultaneously with *F.verticillioides*

T. pseudokoningii grew fast, stopped the pathogen's growth at an average of 2.90 cm diameter by the third day of incubation. The antagonist sporulated on mycelia of *F. verticillioides*. The pathogen later grew to 4.16 cm at later hours of incubation (Table 4.3).

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4.5.2b *Trichoderma harzianum* introduced simultaneously with *F. verticillioides*

When both pathogen and antagonist were simultaneously introduced, *T. harzianum* grew fast, almost touching the *F. verticillioides*. Fifth day of incubation there was no clear zone of inhibition. By 7th day of pairing, *T. harzianum* had completely grown over *F. verticillioides,* and the pathogen's growth was 3.8 cm (Table 4.3).

4.5.2c *Pseudomonas fluorescens* inoculated simultaneously with *F. verticillioides*

When *F. verticillioides* was simultaneously inoculated with *Pseudomonas fluorescens,* the antagonist grew to 0.70 cm in diameter and stopped the pathogen's growth which was 2.50 cm in diameter by the third day of incubation. This was maintained throughout the period of observation. The deep purple coloration of the pathogen mycelial was observed at the termination of the experiment. There was 4.26 cm zone of inhibition (Table 4.3).

4.5.2d *Bacillus subtilis* paired simultaneously with *F. verticillioides*

During simultaneous inoculation of *F. verticillioides* with *B. subtilis, B. subtilis* grew fast and stopped the pathogen's growth at 2.03 cm diameter by the third day of incubation. The pathogen grew to 4.23 cm with a thin zone of inhibition 7 days after pairing (Table 4.3).

Table 4.3: Growth of *F. verticillioides* when paired with the antagonists simultaneously

1st trial	Time of incubation (hrs) of *F. Verticillioides (cm)*					
Antagonists						
	48	72	96	120	144	168
T. harzianum	1.10b	2.30b	2.70bc	3.10b	3.40c	3.80c
T. pseudokoningii	0.90b	1.40c	2.90b	3.46b	3.76b	4.16b
B. subtilis	1.00b	1.30c	2.03d	3.50b	3.83b	4.23b
P. fluorescens	0.90b	1.43c	2.50c	3.60b	3.90b	4.26b
Control	2.00a	3.40a	5.10a	7.03a	8.90a	9.00a
Mean	1.18	1.96	3.04	4.13	4.75	5.09
LSD	0.269	0.357	0.328	2.374	0.322	0.325
2nd Trial						
T. harzianum	1.00b	2.00b	2.60b	3.20b	3.50b	3.93b
T. pseudokoningii	0.70c	1.20c	2.70b	3.10b	3.40b	3.80b
B. subtilis	0.90bc	1.10c	1.80c	3.20b	3.50b	3.90b
P. fluorescens	1.00b	2.10b	2.70b	3.30b	3.60b	3.40b
Control	2.10a	3.10a	4.70a	7.20a	7.50a	7.86a
Mean	1.14	1.90	2.90	4.00	4.30	4.57
LSD	0.281	0.453	0.438	0.430	0.430	0.420

*Means of 3 replicates (cm).

Means in the same column followed by the same letter are not significantly different at 0.05 level (LSD).

4.5.2e Pairing of the antagonists simultaneously with the pathogen

In the first trial (Table 4.3), there was no significant difference at 48 hours of incubation for all the treatments. At 72 hours there was no significant difference for *T. pseudokoningii, B. subtilis* and *P. fluorescens*' effect against the pathogen. But, *T. harzianum* was significantly different at that time compared with the other treatments. For the first time, there was caused significant reduction by *B. subtilis* at 96 hours on the pathogen compared with the other treatments. There was no significant difference in the *T. harzianum and P. fluorescens* at 96 hours on the pathogen as the two microbial agents did not differ in their action against the pathogen. And in a way, *T. harzianum* and *T. pseudokoningii* were not significantly different, they grew in an attempt to inhibit the pathogen's growth at 120 hours of incubation despite the significant differences observed at 96 hours, there was no significant difference at this time, as the trend as observed in earlier times of incubation were followed at this hour for each pairing. *T. harzianum* distinguished itself at 144 and 168 hours of incubation, in the sense that it caused zones of inhibition at these times of 3.50 cm and 3.93 cm of the pathogen which made *T. harzianum* a promising antagonist against *F. verticillioides.*

At the second trial of the simultaneous pairing of the antagonists with the pathogen (Table 4.3), there was no significant difference in the effect of each antagonist against the pathogen. However, *P. fluorescens* still showed inhibitory potentials against the pathogen with about 40% reduction of *F. verticillioides'* growth due to secretion of metabolites in the growth media.

4.5.3. Pairing of the antagonists 24 hours after the pathogen

4.5.3a *Trichoderma pseudokoningii* inoculated 24 hours after F.*verticillioides*

With the introduction of the pathogen before the antagonist, *T. pseudokoningii* grew much faster than the pathogen in all Petri plates. By the third day after pairing, radial growth of *F. verticillioides* was 2.10 cm diameter which was overgrown by *T. pseudokoningii*. By the 7th day of pairing, *T. pseudokoningii* had filled the plate, sporulating on the pathogen's mycelia. The mycelia growth of *F. verticillioides* was 4.53 cm after a week of observation (Table 4.4).

4.5.3b *Trichoderma harzianum* **introduced 24 hours after** *F. verticillioides*

The earlier introduction of *F. verticillioides* than *T. harzianum* into the plate resulted in *T. harzianum* growing fast, making contact with the pathogen when it was at 2.43 cm. *T. harzianum* significantly inhibited the growth of *F. verticillioides* to 3.90 cm (Table 4.4).

4.5.3c *Pseudomonas fluorescens* **inoculated 24 hours after** *F. verticillioides*

With the introduction of the pathogen before the antagonist, *Pseudomonas fluorescens* grew much slower than when it was introduced before the pathogen and simultaneously with the pathogen in all Petri plates. By the third day after pairing, mycelial growth of *F. verticillioides* was 2.40 cm diameter. The pathogen's mycelia sporulated much better than when the other two methods of pairing used. There were zones of inhibition by this antagonist. The fungus growth was limited to 3.53 cm. The inhibition zone was 3.07 cm.

4.5.3d *Bacillus subtilis* **inoculated 24 hours after** *F. verticillioides*

The pathogen, *F. verticillioides* introduced 24 hours before the antagonist, *B. subtilis* grew to 1.00 cm in all Petri plates. By the third day of pairing, growth of *F. verticillioides* was 2.46 cm diameter. By the 7th day after pairing, *F. verticillioides* grew to 4.06 cm on the average in diameter.

4.5.3e Pairing of the antagonists 24 hours after the pathogen

In the first trial (Table 4.4) of the pairing with the antagonists introduction 24 hours after the pathogen, *F. verticillioides* has been in culture, at 48 hours of incubation, *P. fluorescens'* effect on the pathogen was significantly different and not significantly different from *T. pseudokoningii, B. subtilis* and *P. fluorescens* at 48 hours. There were no significant differences at 72 and 96 hours of incubation but, *P. fluorescens* at 96 hours was significantly different.

The second trial, 48 hours of incubation gave significant effect of the antagonists on the pathogen. *P. fluorescens* gave a significant inhibitory effect on the pathogen followed by *T. harzianum* and *T. pseudokoningii*. At all the times of observation, *P. fluorescens'* effect on the pathogen was distinct. Next to its effectiveness was *T. harzianum*

Table 4.4: Growth response of *F. verticillioides* when paired with the each antagonist after 24 hours of the pathogen's inoculation

1st trial	Time of incubation (hrs) of *F. verticillioides*					
Antagonists	48	72	96	120	144	168
T. harzianum	1.40b	1.80b	2.30b	3.10c	3.50c	3.90c
T. pseudokoningii	1.10c	2.03b	2.50b	3.70b	4.13b	4.53b
B. subtilis	1.20bc	1.90b	2.46b	3.23c	3.66bc	4.06bc
P. fluorescens	1.00c	1.40b	1.90c	2.40d	3.13c	3.53c
Control	2.30a	4.30a	6.30a	8.70a	8.90a	9.00a
LSD	0.269	0.674	0.393	0.357	0.539	0.533
2nd Trial						
T. harzianum	1.00c	1.60bc	2.06bc	2.80c	3.20c	3.60c
T. pseudokoningii	1.00c	1.80b	2.30b	3.60b	4.00b	4.40b
B. subtilis	1.30b	1.50bc	2.23bc	3.00c	3.40c	3.80c
P. fluorescens	0.80d	1.33c	1.76c	2.10d	2.50d	2.90d
Control	2.10a	3.90a	6.00a	8.20a	8.60a	8.8ba
LSD	0.181	0.393	0.467	0.514	0.514	0.44

Means of 3 replicates (cm). Means in the same column followed by the same letter are not significantly different at 0.05 level (LSD).

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4.6 Effect of the three pairing methods of antagonists on the pathogen

The pairing method of introducing the antagonists 24 hours before the pathogen showed no significant difference at 48, 72 and 96 hours (Fig. 4.1). At 120 hours, the treatments were significantly different from each other at p = 0.05. The effect of *T. harzianum* on the pathogen was significantly different from the other antagonists. It gave the highest inhibitory effect on the pathogen; *F. verticillioides* growth was 1.40 cm. This effectiveness as an antagonist was followed by *P. fluorescens* which limited the growth of the pathogen to 1.86 cm. There were significant differences in the growth of *F. verticillioides* due to the effect of *B. subtilis* and *T. pseudokoningii* to 3.00 cm and 2.33 cm respectively.

At 144 hours the action of *T. harzianum* was significantly different from all the antagonists tested against the pathogen's growth was 1.73 cm on the average. This action was followed by the *P. fluorescens* with *F. verticillioides*' growth as 2.16 cm. Similar trend was observed at 168 hours after pairing. *T. harzianum* was the most effective antagonist among all the antagonists tested against *F. verticillioides* when the antagonists were introduced 24 hours before the pathogen.

Simultaneous pairing of the pathogen with the antagonists showed that *T. harzianum* was still the most effective followed by *P. fluorescens* then *T. pseudokoningii* and the least effective was *B. subtilis*.

The 24 hours pairing after the pathogen has been in the growth media showed that *T. harzianum* was still the most effective at all times of incubation which make the antagonist a promising biocontrol agent against *F. verticillioides*. The fungus was most effective in the experiment against the pathogen at all the three pairing times. The introduction of the antagonist before the pathogen *in vitro* was observed to be the best followed by the simultaneous pairing and the least effective (though effective too it was better than the control) was the introduction of the antagonist 24 hours after the pathogen. It was observed that all the antagonists tested had good inhibitory potentials on the pathogen, *F. verticillioides*.
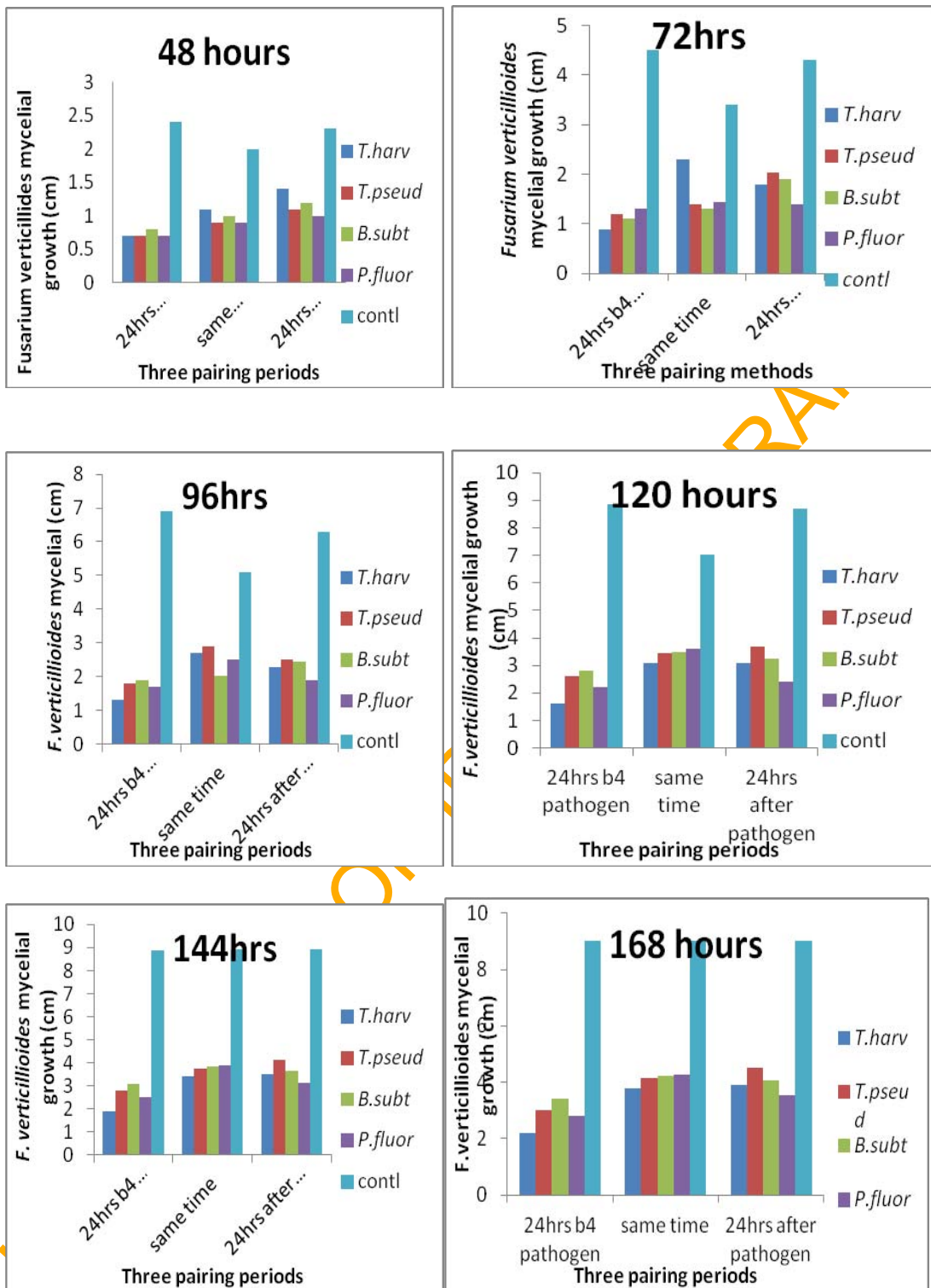
Fig. 4.1: Comparison of the effect of the antagonists on the pathogen, *F.verticillioides* at the three different pairing periods. (24hrs b4 = 24 hours before the pathogen, 24hrs after = 24 hours after the pathogen; Contl = Control; *B. subtilis = B. subtilis; T. harv = T. harzianum, P. fluor = P. fluorescens*)

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4.7   Effect of different locations of the antagonists with the pathogen

4.7.1a Simultaneously placed laterally at the two extreme opposite direction pairing of the antagonists and the pathogen

The simultaneous placed laterally at the two extreme opposite direction pairing of each of the antagonists with the pathogen showed that each of the antagonists reacted to the pathogen differently. In the first trial (Table 4.5), it was observed that at 48 hours all the organisms (each of the antagonists and the pathogen, *F. verticillioides*) had their normal growth rate. At 72 hours, *T. pseudokoningii,* due to its fast growth nature was observed to have overgrown the pathogen. The pathogen in its own case grew well enough, probably due to the distance from the antagonist; it was still overgrown by the mycelia of the antagonist. At 168 hours of incubation, the mycelia of *F. verticillioides* tried to regrow, to overgrow the antagonist mycelial on it. *T. pseudokoningii* (the antagonist) overgrew *F. verticillioides* with visible dark greenish color of the antagonist on the pathogen's mycelia. However, as the incubation time increased (as the pairing got older together on the same plate), the pathogen's mycelia were seen on that of the antagonist.

The growth of *F. verticillioides* was really scanty. *B. subtilis*, with its slow growth inhibited the growth of the pathogen with an observed slight colour change to little pinkish mycelia. It was the most effective at this hour. It reduced the growth of the pathogen of all the antagonists tested. *T. harzianum* paired with *F. verticillioides* simultaneously placed laterally at the two extreme opposite direction had their growth untampered with at both 48 and 72 hours of incubation. There was no interaction of their mycelia growing on one another, and no color change was observed. From 96 to 168 hours of incubation, *T. harzianum* had no visible effect on the *F. verticillioides*, they both cohabited very well. *T. harzianum* may not be a good antagonist against *F. verticillioides* when pairing in the opposite direction. It would be better for the T. *harzianum* to be in close contact for it to be effective as an antagonist against *F.verticillioides*. *F. verticillioides* paired in the opposite direction with *T. harzianum* showed more fluffiness of the pathogen. This was the case throughout this trial with the pairing of these two organisms (*T. harzianum* and *F. verticillioides*) paired in opposite direction.

P. fluorescens, a very slow growing organism gave the pathogen an opportunity to express itself. At 48 hours the colony expansion of *P. fluorescens* was minimal, with dark brown colour, which gave the pathogen an opportunity for wider growth as this

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was not different from the control. The effect of secretion normally attached to *P. fluorescens* was observed as from 72 hours, there was pathogen's mycelia extension but the fluffiness was not seen and the color started changing, to purplish. This was observed till after a week of incubation.

Statistically, the simultaneous opposite pairing of the antagonists with the pathogen showed that there was significant difference in the effect of *T. harzianum* compared with *T. pseudokoningii* and *B. subtilis*. *B. subtilis* allowed a higher mycelial extension of the pathogen but not significantly different from *P. fluorescens* and control at 48 hours of incubation (Table 4.5). At 72 hours, *F. verticillioides*' growth when paired with *T. harzianum* was significantly higher than the other treatments. *B. subtilis* and *T. pseudokoningii* were able to control the growth of this pathogen significantly at this hour differently; there was no significant difference in *P. fluorescens* and control. *T. harzianum, P. fluorescens* and control were not significantly different in effect on *F. verticillioides* at 96 hours of incubation, but *B. subtilis* significantly inhibited the growth of the pathogen to 5.40 cm, followed by *T. pseudokoningii*'s effect on it to 6.10 cm. At 120 hours, *B. subtilis* still maintained its potency by inhibiting the growth of the pathogen to 5.63 cm on the average. The same trend of action on the pathogen was observed at 168 hours. *B. subtilis* reduced the growth of the pathogen to 6.33 cm; there was no significant difference in the growth of the pathogen with *T. harzianum* and *P. fluorescens*.

In the second trial (Table 4.5), the two bacteria used had significant effect on the pathogen at 48 hours. Followed by *T. pseudokoningii* at 72 hours there was no significant difference in the effect of *T. pseudokoningii, B. subtilis* and *P. fluorescens* on *F. verticillioides*. *T. harzianum* promoted the growth of the pathogen. *T. pseudokoningii* and *P. fluorescens* were not significantly different in effect from each other at 96 hours of incubation different compared with the other antagonists, as it gave lower mycelia extension of the pathogen. *T. harzianum* gave higher mycelia extension than even the control. The trend of the growth of the pathogen as inhibited by the antagonists showed *B. subtilis* as a weak antagonist against the *F. verticillioides*.

The second trial gave weak result of the antagonist against the pathogen. In the first and second trials, the effect of the antagonists as in the other experiments earlier carried out were not as effective in this experiment. This is probably due to the location of the pathogen and the antagonists individually against it. They were kept distant from each other which gave each organism the opportunity to express itself and

also the secretion of metabolite into the media did not travel well enough to inhibit the pathogen. The location of the antagonist against the pathogen is the shortcoming of this method when pairing.

4.7.1b Simultaneous pairing of the antagonists at the centre and the pathogen at the four equidistant edges

The pairing with the antagonist at the centre and the pathogen at the four equidistant edges showed *T. pseudokoningii,* being a fast growing organism had a significant effect on the pathogen, unlike the other antagonists. At 48 hours of incubation, *T. pseudokoningii* white mycelia have extended towards the pathogen with the greenish colour of the older mycelia at the point of inoculation. At 72 hours of incubation, *T. pseudokoningii* showed parasitic/antagonistic effect on the pathogen which was significantly different from the other treatments. The mycelia overgrew the mycelia of the pathogen, *F.verticillioides*. As the incubation period increased, it was observed that the growth of *F.verticillioides* was stopped at 120-144 hours of incubation. But a change was observed at 168 hours of incubation. *F.verticillioides* mycelia were rejuvenated with an attempt to overcome the suppression by the antagonist. *T. pseudokoningii* gave the best inhibitory effect at that hour followed by *P. fluorescens*. Similar effect/action was noticed at 120, 144 and 168 hours with *T. pseudokoningii* exhibiting hyperparasitic effect on the pathogen all through this experiment where it was place at the centre surrounded by the pathogen at four equidistant away from it.

At 48 hours of incubation, the effect of *P. fluorescens* at this hour on the pathogen was lower than *T. pseudokoningii*'s effect. This was due to the slow growth of the bacterium. *P. fluorescens* was next in effectiveness with 6.10 cm at 96 hours. As the incubation period increased, the colour of the *P. fluorescens* started getting brown by the day, the growth of *F. verticillioides* was hindered and colour change was observed, from the light pink to dark pink /purple color at 120 hours till the experiment was terminated. *T. harzianum* and *B. subtilis* were not significantly different in action from each other at 48 and 72 hours. *T. harzianum* was observed to have mutualistic growth with the pathogen. *T. harzianum* was 6.10 cm though weak at this period in inhibiting the pathogen's growth. As the time of incubation increased, a thin line of inhibition was established which was observed as from 120 hours of incubation.

Table 4.5: Growth of *F. verticillioides* when paired laterally at the extreme ends of the plate with each antagonist in simultaneous opposite direction to the pathogen

1st trial   Radial mycelial growth of *F. verticillioides* (cm) at Time of incubation (hrs)

Antagonists	48	72	96	120	144	168*
T. harzianum	5.80a	6.80a	7.00a	7.70b	8.06b	8.46b
T. pseudokoningii	4.80b	5.50c	6.10b	6.60c	6.90d	7.26c
B. subtilis	4.90b	5.10d	5.40c	5.63d	5.93e	6.33d
P. fluorescens	5.00ab	6.00b	6.90a	7.40b	7.73c	8.13b
Control	5.10ab	6.30b	7.20aa	8.96a	9.00a	9.00a
Mean	5.12	5.94	6.52	7.25	5.59	7.83
LSD	0.841	0.363	0.438	0.300	0.315	0.338
2nd Trial						
T. harzianum	5.20a	6.00a	7.00a	7.40a	7.70a	8.06a
T. pseudokoningii	4.40c	4.90c	5.40c	6.20b	6.43b	6.90a
B. subtilis	4.00d	5.00c	6.30b	7.20a	7.50a	7.90a
P. fluorescens	4.00d	4.80c	5.30c	6.00b	6.33b	6.70b
Control	4.80b	5.30b	5.90b	6.10b	6.43b	6.76b
Mean	4.48	5.20	5.98	6.58	6.87	7.26
LSD	0.304	0.215	0.414	0.293	0.354	0.289

*Means of 3 replicates (cm). Means in the same column followed by the same letter are not significantly different at 0.05 level. * Values are means of the growth of *F.verticillioides* when paired with the respective/ corresponding antagonists.

The fluffiness of the pathogen was also reduced. *B. subtilis* and the control were not significantly different from each other till 96 hours of incubation, but there was slight color change the *F.verticillioides* when paired with the antagonist at this hour. *B. subtilis* effect on *F.verticillioides* 'growth 6.83 cm and significantly lower to the effect of *T. pseudokoningii* and *P. fluorescens* at 96 hours of incubation. In the second trial (Table 4.6), it was obvious that *T. pseudokoningii* was an efficient antagonist against *F. verticillioides* when it was placed at the centre of the plate.

4.7.1c Simultaneous pairing of the antagonists at the edge and the pathogen at the centre

The first trial of pairing antagonists individually and separately at the four equidistant edges of the plate and *F. verticillioides* at the centre showed *P. fluorescens* not significantly different at 48 hours of incubation from *B. subtilis* but distinctly significant throughout the experiment. Effect *B. subtilis* and *T. harzianum* was not significantly different from one another but different compared with the effect of *T. pseudokoningii* and *P. fluorescens* on *F. verticillioides.* The growth 9.00 cm of *F. verticillioides* in this trial was reduced to 6.10 cm by *P. fluorescens*, 6.90 cm by *T. pseudokoningii*, and no significant difference in *T. harzianum* 8.06 cm and *B. subtilis* 8.30 cm. In this trial, all the antagonists proved effective against the pathogen, but *P. fluorescens* distinguished itself with its action by inhibiting the growth of the pathogen from a distance (Table 4.7). This may be due to the metabolite probably secreted into the media. Apart from the fast growth of *T. pseudokoningii*, a greenish-yellow secretion was also observed in the media with this antagonist, this may probably be the metabolite that was also employed along with the fast colonizing effect of the antagonist on the pathogen.

For the second trial (Table 4.7), *P. fluorescens* and *T. pseudokoningii* distinguished them in that they were able to inhibit the growth of the pathogen throughout the incubation period. At the end of this trial, all the antagonists tested against *F.verticillioides*, inhibited its growth. But, *B. subtilis* was ineffective as such; it had pathogen's growth of 8.00 cm while the control plates had a mean of 8.20 cm.

Table 4.6: Radial mycelial growth of *F. verticillioides* when paired with the each antagonist simultaneously with the antagonists at the centre and the pathogen at the edge

1st Trial   Radial mycelial growth of *F. verticillioides* (cm) at time of incubation (hrs*)

	48	72	96	120	144	168*
T. harzianum	5.50a	5.70bc	6.10b	6.60bc	6.80bc	7.10bc
T.pseudokoningii	4.50b	4.70d	5.06d	5.33d	5.53d	5.80d
B. subtilis	5.40a	5.60bc	6.83a	7.00b	7.20b	7.50b
P. fluorescens	5.10a	5.30c	5.60c	6.10c	6.33c	6.63c
Control	5.50a	6.70a	7.00a	8.56a	8.76a	8.00a
Mean	5.20	5.60	6.11	6.71	6.92	7.00
LSD	0.430	0.354	0.435	0.690	0.690	0.690
2nd Trial						
T. harzianum	5.30ab	5.66b	6.40b	6.80c	7.00c	7.30c
T. pseudokoningii	4.40c	4.80c	5.10e	5.60e	5.80de	6.10e
B. subtilis	5.60a	5.90ab	6.90ab	7.40b	7.60b	7.90b
P. fluorescens	4.60c	5.00c	5.40c	6.00d	6.20d	6.50d
Control	5.00b	6.00a	7.06a	8.76a	8.90a	9.00a
Mean	4.98	5.47	6.17	6.75	7.10	7.36
LSD	0.363	0.308	0.559	0.393	0.363	0.354

Means of 3 replicates (cm), indicating the growth of *F.verticillioides* when paired with the corresponding antagonist. Means in the same column followed by the same letter are not significantly different at 0.05 level. * Values are means of the growth of *F.verticillioides* when paired with the respective/ corresponding antagonists.

4.8 Effect of the pathogen on maize plant during pathogenicity test

F. verticillioides which was isolated from maize ear was found to be pathogenic to maize plants when tested for pathogenicity. Pure culture of the organism was obtained and compared with the identified pure culture from IITA.

The localized infection produced by *F. verticillioides* on the maize plants was lodging and stem rots. The rot produced by *F. verticillioides* was white pinkish on the stalks which were not fast spreading but covered the most part of the stalk within 2 weeks of inoculation.

4.9 Effect of the inoculum treatments on maize seeds germination
4.9.1 Percentage (%) germination of maize seeds treated with *T. pseudokoningii*

At 7 days after planting, C3 (7.1 x 10^7 spores/ml) gave 77% germination which was significantly higher than the other concentrations and even the synthetic fungicide, benomyl as control. C2 (5.3 x 10^8 spores/ml) gave 64% germination which was significantly different from benomyl used as chemical control. The least germination percentage (57%) was observed on C4 (1.5 x 10^7 spores/ ml), which was the same as the control of 57% germination. At 2 weeks after planting, C2 was still the best concentration for the control of *F. verticillioides* in the soil. The effect of benomyl was higher than the other treatments used, as it had 73% germination. The vigour of maize plants in C3 was stronger than the other concentrations. It gave 80% germination.

The control plants gave weak stands and had seedling blight which is symptomatic *F. verticillioides* infection. At 28 days after planting, the seedling survival in C3 pots, with 80% germination was higher than the benomyl treatment that gave 73%. C1 and C2 were not significantly different from one another with 75% germination each. They were higher than the control and the benomyl (Table 4.8). This result showed that the effect of benomyl was not immediately felt on the plants in the pots.

In the second trial at 28 days after planting, C3 of *T. pseudokoningii* had 75% maize germination which was higher and better than the benomyl treated plants that had 73% germination (Table 4.8). The C4 of *T. pseudokoningii* had 60% germination and was weaker in germination compared with the control plants which had 72% germination of maize seeds planted in it. It was observed at 7 days after planting that

the percentage germination for the control plants were 69% and increased to 72% at the end of the experiment.

The best stand for *T. pseudokoningii* was obtained when the seeds were treated with 7.1 x 10^7 spores/ml mycelia suspension. The two trials consistently gave 76.38% on the average. Table 4.8 shows that there were significant differences due to the treatments in all plant stands.

For seeds treated with *T. pseudokoningii,* initial percentage germination for 7.1 x 10^7 and 2.7 x 10^{10} spores/ml were not significantly different from the Benomyl-treated seeds. The germination from the antagonist-treated and Benomyl-treated seeds were significantly higher than those of the control. The same went for the 2nd experiment. *T. pseudokoningii* had the best/highest germination percentage at 7.1 x 10^7 spores/ml in both experiments. In the 1st and 2nd experiments 80 and 75% were the final stands of maize in the screenhouse which were not significantly different from the stands/germination from other concentrations (Table 4.8). It can be inferred from this result that C3 (7.1 x 10^7 spores/ml) will be concentration at which *T. pseudokoningii* will be most effective in *in vivo* applications, C1 (2.7 x 10^{10} spores/ml) and C2 (5.3 x 10^8 spores/ml) are also good, effective but the concentration as low as C4 (1.5 x 10^7 spores/ml) will not be effective against the seed and soil borne pathogen, *F.verticillioides*. In the control, there was 69% initial germination and 72% final plant stand. C2 was a good control measure against *F. verticillioides* infested soil or seed borne, a good alternative to the use of fungicide. C1 too can also be due in lieu of the C2 treatment.

Benomyl (fungicide) application had 71.88% average maize stands in the 1st and 2nd trials. While the control had 61% germination in the first trial, and 40% in the second trial (Table 4.8).

Table 4.7: Simultaneous pairing of the antagonists at the edge and the pathogen at the centre

1st Trial	mycelial growth of *F. verticillioides* (cm) at time of incubation (hrs)					
Treatments	48	72	96	120	144	168*
T. harzianum	5.30a	6.50ab	6.60c	7.40b	7.70b	8.06b
T.pseudokoningii	4.30c	5.20c	5.80d	6.30c	6.60c	6.90c
B. subtilis	5.10ab	6.30b	7.00b	7.70b	8.00b	8.30b
P. fluorescens	4.80b	5.40c	5.50d	5.50d	5.80d	6.10d
Control	3.30a	6.80a	7.50a	8.96a	8.96a	9.00a
Mean	4.56	6.04	6.48	7.17	7.41	7.67
LSD	0.335	0.335	0.381	0.318	0.318	0.261
2nd Trial						
T. harzianum	5.00a	6.10a	6.80a	7.60a	6.00c	6.30c
T.pseudokoningii	4.10c	4.80d	5.20c	5.70c	6.30b	6.60c
B. subtilis	4.90a	5.60b	6.70a	7.40a	7.70a	8.00a
P. fluorescens	4.40b	5.00cd	5.20c	5.60c	5.90c	6.20c
Control	4.90a	5.20c	5.50b	6.00b	7.90a	8.20a
Mean	4.66	5.34	5.88	6.46	6.76	7.06
LSD	0.269	0.335	0.269	0.293	0.293	0.293

* Means of 3 replicates (cm), showing the growth of *F. verticillioides* when paired with the antagonists correspondingly. Means in the same column followed by the same letter are not significantly different at 0.05 level (LSD).

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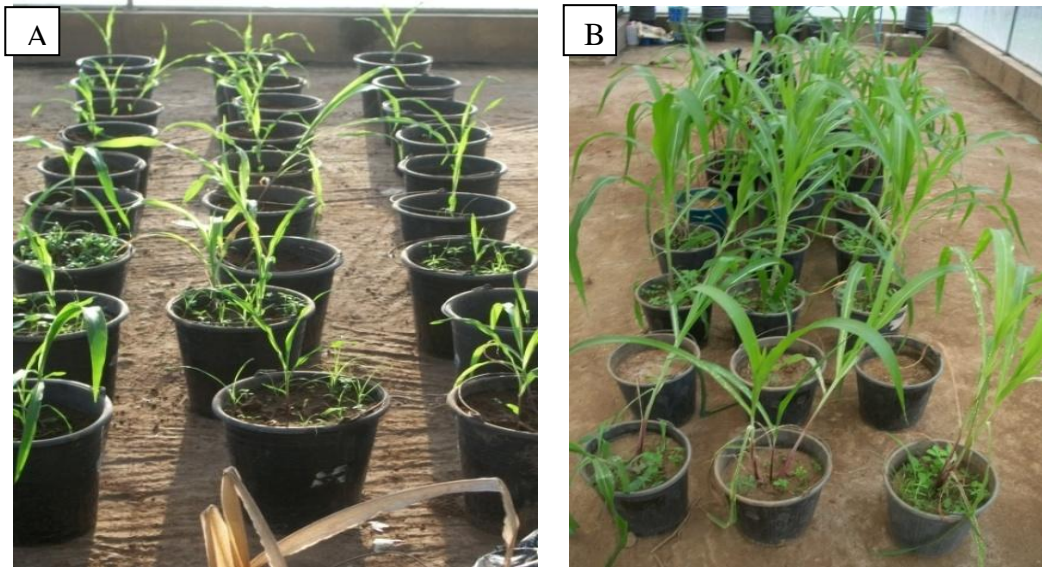
Plate 4.12: Germinated maize seeds planted in *F. verticillioides* infested soil in the screenhouse at (A) 2 weeks and (B) 5 weeks after planting.

Table 4.8: Percentage germination of maize seeds treated with *T. pseudokoningii* at different spore concentrations

1st trial	Days after planting			
T. pseudokoningii	Germination 7	percentage 14	(%) 21	28
C1	72.00b	75.00b	75.00b	75.00b
C2	64.00c	71.66c	75.00b	75.00b
C3	77.00a	80.00a	80.00a	80.00a
C4	57.00d	60.00d	60.00e	60.00e
Benomyl	70.00b	73.00bc	73.00c	73.00c
Control	57.00d	72.00c	72.00d	72.00d
Mean	66.16	71.94	72.50	72.50
S.E (0.05)	3.432	2.218	0.726	0.726
2nd Trial				
C1	70.00a	71.66a	74.00ab	74.00ab
C2	55.00b	64.00b	70.00d	70.00d
C3	72.00a	72.00a	75.00a	75.00a
C4	49.00c	60.00c	60.00e	60.00e
Benomyl	70.00a	73.00a	73.00bc	73.00bc
Control	69.00a	72.00a	72.00c	72.00c
Mean	64.16	68.77	70.66	70.66
S.E (0.05)	3.081	1.827	1.452	1.452

C1 = 2.7 x 10^{10} spores/ml; C2 = 5.3 x 10^{8} spores/ml;
C3 = 7.1 x 10^{7} spores/ml; C4 = 1.5 x 10^{7} spores/ml
Means of 3 replicates (cm). Means in the same column followed by the same letter are not significantly different at 0.05 level.

4.9.2 Percentage germination of maize seeds treated with *T. harzianum*

At 7 days after planting (DAP), benomyl treated seeds gave the highest germination percentage of 70% which was not significantly different from C2 (1.7 x 10^8 spores/ml) treated seeds with 64% germination. This C2 with 64% germination was not significantly different from C3 (2.3 x 10^7 spores/ml) treated seeds of 54% germination. At 14 days after planting, benomyl treated seeds' germination percentage increased to 76.66% which was still not significantly different from C2 that also increased to 72% seed germinated. C2 was not significantly different at 14 DAP from C1 (1.8 x 10^{10} spores/ml) with 60.66%, and C4 (7.8 x 10^7 spores/ml) with 58%. C4 was 64% and C3 was 69% germination which was not significantly different from each other but were significantly lower when compared with the other concentrations at 21 days after planting. The effect of benomyl and C4 were not significantly different from one another but were significantly higher than the other treatments considered at 28 days after planting (Table 4.9).

The second trial of effect of the different concentrations of *T. harzianum* on *F. verticillioides* infested soil showed C2 with 63% germination not different (at P $\leq$ 0.05) from benomyl treatment of 60% (Table 4.9b), both were significantly different from the others; C1 55% and C3 53%, the least effective was C4 with 45% at 7 days after planting. Throughout this trial, C4 was the least effective though better than the control. C1 63.66%, C2 65% and benomyl 64% were significantly the same and gave the best germination percentage at 28 days after planting.

C2 of 1.7 x 10^8 spores/ml of *T. harzianum* gave germination percentage of maize seeds of 74.50 % on the average in the first trial. The second trial followed a similar trend (Table 4.9b). C2 gave an average initial maize stands (at 7 days) of 64% and 84% in the final stand (at 28 days) in the first experiment. The C3, 2.3 x 10^7 spores/ml had average stand of maize of 60.87% in the two experiments. For the 7.8 x 10^7 spores/ml, C4, initial maize stands at 7 days was 50% germination and the final stand at 28 days was 64% germination in the first trial, while 45% and 48% were respectively recorded in the second experiment.

Treatment of maize seeds with *T. harzianum* mycelial mat of 1.7 x 10^{10} spores/ml and T. *pseudokoningii* mycelial mat of 7.1 x 10^{10} spores/ml in both experiments were not significantly different from each other. The germination of 84% and 80% from these two suspensions were however higher than those from the other concentrations in the 1st trial. Treatment C3 of 7.1 x 10^7 spores/ml in the 2nd

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experiment had highest germination % for seed treated in mycelia mat for *T. pseudokoningii.*

4.9.3 Percentage germination of maize seeds treated with *B. subtilis*

The percentage germination of maize seeds at 7 days with *B. subtilis* treatment, benomyl performed better than the different concentrations of *B. subtilis* that were tested. C2 (1×10^8 cfu/ml) was the most effective level of *B. subtilis* application with 60.33% which is significantly lower to benomyl of 70% germination. Also at 7 DAP, C1 (1×10^9 cfu/ml) of 49.66% and C3 (1×10^7 cfu/ml) of 39.66% were significantly different from each other, even lower in performance to control 50%. At 14 days after planting, there was no significant difference in C1, C2 and C3 and they were all lower than the control which makes them ineffective. C2 gave the better percentage germination after benomyl which was observed to best at this hour. At 21 and 28 DAP, it was noticed that the germination values are now constant and benomyl still maintained its effectiveness, followed by C2. The least effective concentration was C4 (1×10^6 cfu/ml). There was no significant effect of C1and C3. In all, it's noted that benomyl; the synthetic fungicide was more effective than the use of *B. subtilis* in the *in vivo* study (Table 4.10).

Benomyl was 60% significantly higher than the rest of the treatments at 7 DAP, followed in efficacy are C1 and C2. For the 21 and 28 DAP, C2, 64.66% was significant as the benomyl treatment, 64% (Table 4.10). It was noted in this trial that the other concentration of *B. subtilis* did well compared with the first trial where the treatments effect was worse than the control. At the end of the observation, C2 64.66% and benomyl 64% are not significant different from each other. C1, C3 and C4 were significantly better than the control which has the pathogen, *F. verticillioides* in the soil.

Generally, germination percentage of maize in soil samples treated with *B. subtilis* had very poor vigour, the germination percentage was lower. It is only the 200 ml that performed well in the two experiments but lower than the chemical control and control itself. The effect of *B. subtilis* in the study has really compromised the other reports by researchers, of its ability to act as an antagonist against plant pathogens. *B. subtilis* was noted to be a weak antagonist, so its use in this study was discontinued

Table 4.9: Percentage germination of maize seeds treated with *T. harzianum* at different spore concentrations

1st trial	Days after planting			
T. harzianum	7	14	21	28
C1	52.66c	60.66b	70.00c	75.00b
C2	64.00ab	72.00ab	78.00b	84.00a
C3	54.00bc	62.00b	69.00d	69.00cd
C4	50.00c	58.00b	64.00d	64.00d
Benomyl	70.00a	76.66a	86.00a	86.00a
Control	50.00c	68.00b	66.66cd	70.66bc
Mean	56.77	66.22	72.27	74.77
S.E (0.05)	10.926	14.133	5.779	5.825
2nd Trial				
C1	55.00b	58.00b	62.00b	63.66a
C2	63.00a	65.00a	65.00a	65.00a
C3	53.00b	56.00b	58.00c	58.00b
C4	45.00c	48.00c	48.00d	48.00c
Benomyl	60.00a	65.00a	64.00ab	64.00a
Control	40.00d	40.00d	40.00e	40.00d
Mean	52.66	55.33	56.16	56.44
S. E	3.773	3.081	2.515	2.334

C1 = 1.8 x 10^{10}spores/ml; C2 = 1.7 x 10^8spores/ml;
C3 = 2.3 x 10^7spores/ml; C4 = 7.8 x 10^7spores/ml
Means of 3 replicates (cm). Means in the same column followed by the same letter are not significantly different at 0.05 level.

For the *Trichoderma* species, the effect of 1g of *T. pseudokoningii* and *T. harzianum* on germination percentage into 300 ml of 7.1 x 10^{10} spores/ml and 200 ml of 1.7 x 10^{10} spores/ml were significantly different from the other concentrations, gave better germination of the maize seeds planted in *F.verticillioides* infested soil treated with the antagonists.

4.9.4 Percentage (%) germination of maize seeds treated with *P. fluorescens*

The percentage germination of maize seeds at 7 days with *P. fluorescens* treatment compared with benomyl, showed benomyl performed better than the different concentrations of *P. fluorescens* that were tested. Though, C2 (1 x 10^8 cfu/ml) was the most effective level of *P. fluorescens* application with 65.33%. This was significantly different from benomyl that had 70% germination. Also at 7 DAP, C1 (1 x 10^9 cfu/ml) had 51% and C3 (1 x 10^7 cfu/ml) 49.33% germination were observed not to be different from each other, but better in performance than the control which gave 50% germination. At 14 days after planting, C2 (had 73.33%) gave poor percentage germination compared with benomyl (76.66%) which was observed to best at this day. It was observed that all the plants started to increase in the vigour of the plants in them. There was no significant difference in C1, C3 and C4 and their values were all lower than the control which makes them ineffective.

At 21 and 28 DAP, it was noticed that the germination percentages were now constant and benomyl still maintained its effectiveness, followed by C2. The least effective concentration was C4 (1 x 10^6 cfu/ml). Statistically, there was no significant differences in the effect of C1, C3 and C4, but the plant were seen not to be strong seedlings as such, compared to the control and benomyl. This suggests that C2 can be a good alternative or replacement for the use of benomyl. C1, C3 and C4 are all potential control inoculum concentration provided the pathogen's inoculum level is not high in the soil or not found in the soil as of the time of application. In all, it is noted that benomyl; the synthetic fungicide was more effective than the use of *P. fluorescens* in the study (Table 4.11).

In the 2nd trial, benomyl was 60% germination of the maize seeds significantly higher than the rest of the treatments at 7 DAP, followed by C2 and C1 in efficacy. At the 21 and 28 DAP, C2 (with 72.66% and 73.33%) was significantly different from the benomyl treatment, with 64% (Table 4.11). It was noted in this trial, that the other concentrations of *P. fluorescens* did not do well compared with the first trial where the

Table 4.10: Percentage germination of maize seeds treated with *B. subtilis* at different concentrations

1st trial	Days after planting			
B. subtilis	Germination	percentage	(%)	
	7	14	21	28
C1	49.66c	55.66cd	60.33c	60.33c
C2	60.33b	68.33b	72.33b	72.33b
C3	44.33cd	50.00d	60.00c	60.00c
C4	39.66d	49.00d	54.33d	54.33d
Benomyl	70.00a	76.66a	86.00a	86.00a
Control	50.00c	58.00c	70.66b	70.66b
Mean	52.33	59.60	67.27	67.27
S.E (0.05)	7.016	7.406	5.451	5.45
2nd Trial				
C1	48.66b	54.66b	58.66b	58.66b
C2	53.00b	61.33a	64.66a	64.66a
C3	42.33c	48.00c	54.66bc	54.66bc
C4	42.33c	48.00c	52.33c	52.33c
Benomyl	60.00a	65.00a	64.00a	64.00a
Control	40.00c	40.00d	40.00d	40.00d
Mean	47.72	52.83	55.71	55.71
S. E (0.05)	5.483	6.523	5.303	5.303

C1 =1 x 10^9 cfu/ml; C2 = 1 x 10 cfu^8/ml;
C3 = 1 x 10^7 cfu/ml; C4 = 1 x 10^6 cfu/ml
Means of 3 replicates (%). Means in the same column followed by the same letter are not significantly different at 0.05 level.

treatments effect was worse than the control. At the end of the observation, C2 (73.33%) and benomyl (64%) are significantly different from each other. C1, C3 and C4 were significantly better than the control which has the pathogen, *F. verticillioides* only in the soil.

The performance of *P. fluorescens* at C2 was the highest i.e. it has the highest germination percentage in all but was not significantly different from C1. Statistically to summarise the effect of the antagonists' inoculum on the germination of maize seeds planted in infested soil (Table 4.12). In the first trial, *T. harzianum*'s C2 and benomyl are not significantly different from each other, but different from the control, C1, C3 and C4. C1 of this antagonist did not act significantly different from control; control was not also different from C3. This suggests that for *T. harzianum* to be effective as an antagonist, nothing more than C1 should be used and nothing less than C3 should be used. But to have an efficient control as observed in the benomyl, C1 should be used.

T. pseudokoningii at its C3 gave the best germination percentage which was not significantly different from the C1 of it. The least effective of the concentrations of *T. pseudokoningii* inoculum was C4. From this, it is certain that for efficient control, to have good germination of maize on infested soils, C3 should be used, and concentration above C1 should not be used.

P. fluorescens inoculum at C2 was the best for the control of the pathogen causing seedling blight of maize seeds. C2 was as effective as benomyl in this trial against *F. verticillioides*. For the *P. fluorescens*, all the concentrations used were effective, but dilutions less than C4 should not be used in this kind of control, due to its insensitivity in *in vivo* state. *B. subtilis* was a weak bacterium against this pathogen in vivo. However, C3 concentration can still be used. Benomyl was the most effective control, but for its residual effect on the environment, as a control measure, the use of environmentally friendly control alternatives like *T. harzianum, P. fluorescens* and *T. pseudokoningii* should be used.

Table 4.11: Percentage (%) germination of maize seeds treated with *P. fluorescens* of different inoculum concentrations

1st trial *P. fluorescens*	Percentage 7	germination 14	(%) 21	28
C1	51.00b	56.66b	63.33cd	63.33ab
C2	65.33a	73.33a	78.00b	80.00a
C3	49.33b	55.00b	65.00cd	65.00ab
C4	44.33b	54.00b	59.33d	59.33ab
Benomyl	70.00a	76.66a	86.00a	86.00a
Control	50.00b	58.00b	66.66c	50.67b
Mean	54.99	62.27	69.71	67.38
S.E (0.05)	8.184	9.243	6.119	27.512
2nd Trial				
C1	52.66b	58.33b	65.00b	65.00b
C2	60.66a	68.00a	72.66a	73.33a
C3	50.66bc	57.66bc	61.33cd	61.33cd
C4	44.33cd	50.00c	59.33d	59.33d
Benomyl	60.00a	65.00ab	64.00bc	64.00bc
Control	40.00d	40.00d	40.00e	40.00e
Mean	51.38	56.49	60.38	60.49
S.E (0.05)	6.345	7.674	3.328	2.994

C1 = 1 x 10^{10} cfu/ml; C2 = 1 x 10^9 cfu/ml;

C3 = 1 x 10^8 cfu/ml; C4 = 1 x 10^7 cfu/ml

Means of 3 replicates (%). Means in the same column followed by the same letter are not significantly different at 0.05 level (LSD).

Table 4.12: The effect of the antagonists' inoculum on maize seed germination (expressed in %)

1st trial

Treatments	*T. harzianum*	*T. pseudokoningii*	*P. fluorescens*	*B. subtilis*
1st Trial				
C1	75.00b	74.00ab	63.33ab	60.33c
C2	84.00a	70.00d	80.00a	72.33b
C3	69.00cd	75.00a	65.00ab	60.00c
C4	64.00d	60.00e	59.33ab	54.33d
Benomyl	86.00a	73.00bc	86.00a	86.00a
Control	70.66bc	72.00c	50.67b	70.66b
Mean	74.77	70.66	67.38	67.26
S.E(0.05)	5.825	1.452	27.512	5.451
2nd Trial				
C1	63.66a	75.00b	65.00b	58.66b
C2	65.00a	75.00b	73.33a	64.66a
C3	58.00b	80.00a	61.33cd	54.66bc
C4	48.00c	60.00e	59.33d	52.33c
Benomyl	64.00a	73.00c	64.00bc	64.00a
Control	40.00d	72.00d	40.00e	40.00d
Mean	56.44	72.50	60.49	55.71
S. E	2.334	0.726	2.994	5.303

C1, C2, C3 and C4 are based on individual concentrations
Means of 3 replicates (%). Means in the same column followed by the same letter are not significantly different at 0.05 level.

4.10 Effect of botanicals on the target pathogen (*F. verticillioides*)

4. 10.1a Effect of dry *T. diversifolia* on *F. verticillioides*

At 48 hours of incubation of the pathogen on the different concentrations of *T. diversifolia* incorporated PDA, the pathogen, *F. verticillioides* grew almost at the same rate with the control. The mycelia at this time had no depression in the growth and morphological features. The mycelia were fluffy and densely populated. This was the case for all dry samples of *T. diversifolia* incorporated in the PDA at 10.0%. The 10.0% performed best at this time (48 hours) as it had mycelial extension of 2.80 cm which was better than 5.0% that had extension of 3.10 cm. When the culture was 72 hours old, the 10.0% and 5.0% concentration had no effect in their growth of 4.0cm and 3.90 cm respectively. The lowest concentration (2.5%) of dry *T. diversifolia* incorporated in the agar throughout the experiment showed that it had potentials to act as growth stimulator and not inhibitor for *F. verticillioides*.

The dry sample of *T. diversifolia* of 25.0% and 15.0% in the PDA at 48 hours till 168 hours of incubation showed that the 25.0% at 48 hours inhibited the growth of *F. verticillioides*. The 25.0% gave mycelia extension of 2.60 cm which was fluffier as the incubation period increased; it gave 5.4cm at 96 hours and 7.80 cm at 168 hours. Dry *T. diversifolia* of 15.0% in the growth media was more efficient against the pathogen's growth; it was 2.70 cm at 48 hours and 3.70 cm at 72 hours. But, it was observed that the mycelia extension was retarded at 96 hours with 5.20 cm. The 15.0% of *T. diversifolia* incorporated into the agar was most effective in inhibiting the pathogen's growth compared with the 25.0% of the *T. diversifolia* incorporated agar. The plates were observed not to be as fluffy when compared with others.

The dry *T. diversifolia* incorporated at 30.0%, 20.0% and 5.0% concentrations into the growth media showed that at the early incubation time (48 hours), the growth of *F. verticillioides* was greatly affected, the mycelia extension of the pathogen was 2.80 cm at 30.0% and 20.0% concentration, but this was not the case with the 5% *T. diversifolia* in the growth media as it had 3.80 cm, 5.00 cm and 5.80 cm at 48, 72 and 96 hours of incubation respectively. The fast growth rate in this 5.0% concentration was reduced at 120 hours of incubation, 6.26 cm and 7.20 cm at 168 hours. The higher concentration of 30 and 20.0% from 120 hours incubation became fluffier and showed the light pinkish colour of the pathogen. This indicated that the dry *T. diversifolia* is not a good botanical in the control of *F. verticillioides invitro* as the highest and the

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lowest concentrations of the botanical really showed its inability to control the pathogen, it acted as stimulator instead.

The different concentrations of the dry *T. diversifolia* at 48 hours of incubation with *F. verticillioides* compared with benomyl showed that benomyl can significantly control the pathogen. It was noted that 15% of *T. diversifolia* in the growth media was not significantly different from the effect of benomyl on the pathogen. The 30.0%, 25.0%, 20.0%, 15.0%, 10.0% and 5.0% concentration of *T. diversifolia* incorporated into growth media were not significantly different from each other at the end of the experiment. The incorporation of 5.0%, 2.5% concentration in the growth media and control were not significantly different from each other which implied that the minute volume of whatever concentration of *T. diversifolia* will be ineffective against *F. verticillioides* at 48 hours of incubation (Table 4.13).

At 72 hours, benomyl was significantly effective against the *F. verticillioides*, but 15.0% of *T. diversifolia* was more effective than benomyl and significantly different from each other. Just as it was observed at 48 hours of incubation, 30.0%, 25.0%, 20.0%, 15.0%, 10.0%, and 5.0% were not significantly different from each other, but were effective in inhibition than the 5.0%, which were not significantly different from each other and not different from the control plates. The 96 hours of incubation showed Benomyl to be most effective against *F. verticillioides*. At this hour, 30.0%, 25.0%, 20.0%, 15.0%, 10.0% and 5.0% were not significantly different in efficacy at 96 hours of incubation. It was observed that the mycelia mass in 30.0% concentration was very fluffy and well formed than the control plates.

Benomyl maintained its ability to inhibit the growth of *F. verticillioides* throughout the period of incubation. At 120 hours of incubation, *F. verticillioides'* growth in concentrations 30.0%, 10.0% and control had no difference in its effect on the pathogen. Also, 25.0%, 20.0%, 10.0% were not significantly different from each other, but they were all significantly different and weaker in action compared with the other concentrations and better than the control. It shows that smaller volumes of *T. diversifolia* incorporated into the media were more effective than the higher concentrations of the extract. But, 15.0% was not significantly different from 5%, which means both can serve as good alternatives to the use of synthetic fungicide, benomyl. There were no significant differences at 144 and 168 hours of incubation. So, 168 hours of incubation showed highest concentration of 30.0% to be growth promoter for the *F. verticillioides*, as the mycelial extension was more at this concentration than

the control. At $P \leq 0.05$, 30.0%, 20.0%, 10.0% were not different significantly from control. The 5.0% and 25.0% inhibited the growth of *F. verticillioides* but not as much as in benomyl treatment. The 15.0% is the minimum inhibitory concentration of *T. diversifolia* against *F. verticillioides.*

4.10.1b Effect of dry *Physalis angulata* on *F. verticillioides'* growth

There was no clear zone of inhibition/suppression of the growth of *F.verticillioides* on agar incorporated with the different concentrations of *P. angulata* extract. The 10.0% extract of *P. angulata* incorporated into the agar had 2.90 cm of growth of the pathogen after 48 hours of incubation. The 20.0% had the same growth measurement. Measurement of 3.00 cm was taken on the average of three replicates of poisoned agar. The other concentrations were not different from each other in action against the pathogen except for 5.0% concentration that was not significantly different from benomyl. Benomyl was significant and highly efficient against the pathogen, it was pinkish and the mycelia were suppressed.

By 7 days of incubation, the mycelia of the pathogen had extended to 7.40 cm on agar incorporated with 30.0% *P. angulata* extract. The mycelial mass was fluffy with pinkish white coloration. The underside of the plate was brownish, which may be due to the colour of the extract or reaction of the phytotoxic substances being secreted by the pathogen into the medium to counteract the effect of the poison (Plate 4.14).

The lower concentrations of *P. angulata* were more effective against the pathogen at the early incubation period/time. As from 120 hours of incubation, the higher concentration suppressed the growth of *F. verticillioides,* 20.0% concentration had significant effect on the pathogen compared with the other concentrations. At 144 and 168 hours of incubation, there were no significant differences in the effect of 30.0%, 20.0% and 5.0% concentrations. It showed that these concentrations were the most effective and the minimum inhibitory concentration was 5.0%. The growth diameter of mycelia of the pathogen in *P. angulata* incorporated media was 7.80 cm with white pinkish fluffy mycelia, but they were not as dense as those grown in the highest concentration at 7 days after inoculation. There was no decrease in fluffiness of the pathogen mycelia mass as the concentrations of the diluted plant extract (Plate 4.14). It was noted that the mycelia extension and the growth rate of the pathogen was even
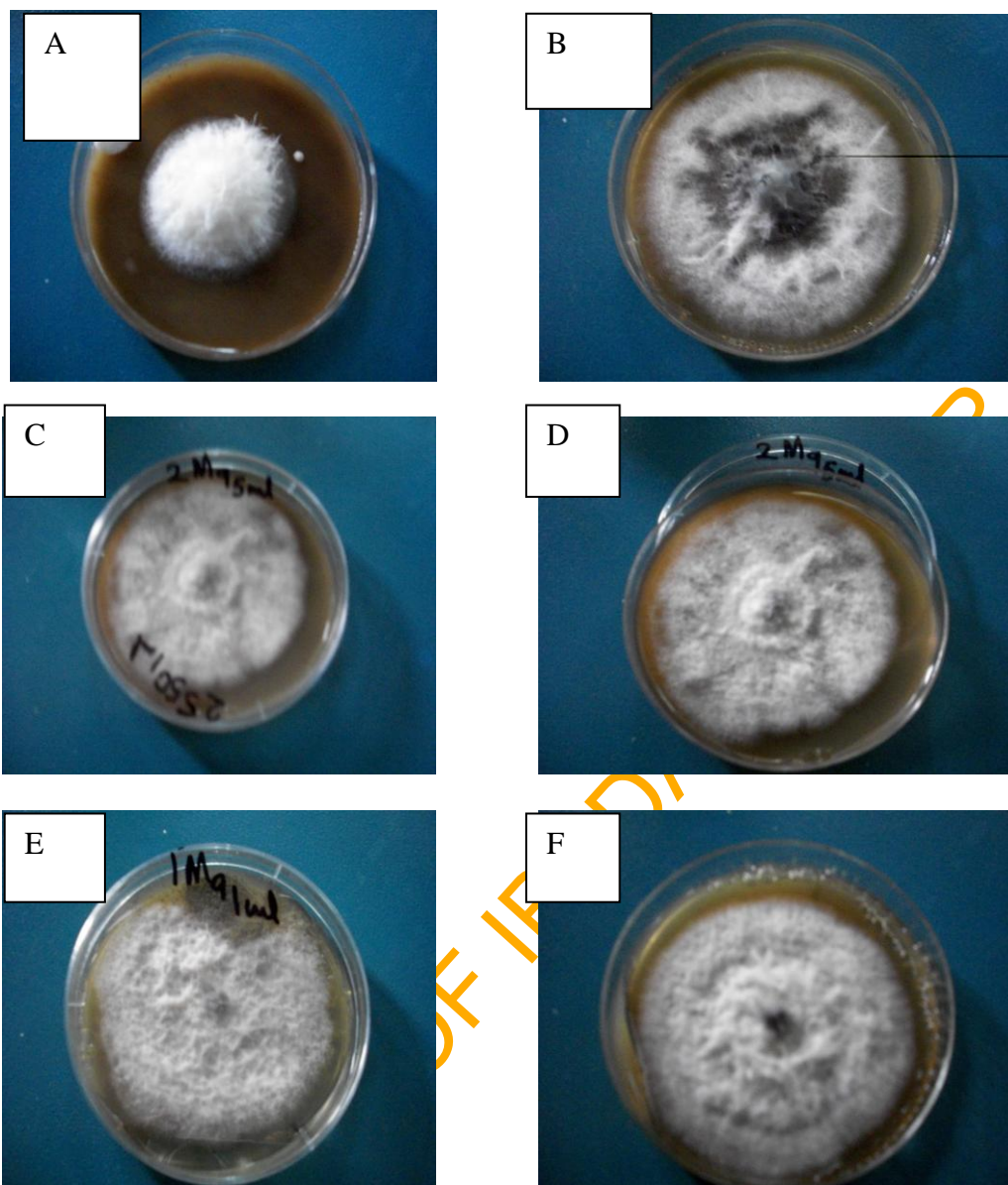
Plate 4.13: Effect of the different concentrations of botanicals incorporated into the growth media.

(A). *S. occidentalis* at 30.0% incorporation, 3 days after incubation

(B). *S. occidentalis* of 15.0% incorporated into growth media, 7 days after incubation.

(C and D). *M. jalapa* of 25.0% incorporated into the media, 5 and 7 days after incubation.

(E). *M. jalapa* of 2.5% in the growth media at 7 days after incubation

(F). *T. diversifolia* of 30.0%, 3 days after incubation.

Table 4.13: Mycelial extension of *F. verticillioides* (cm) due to the effect of different concentrations of dry *T. diversifolia* extracts incorporated into the growth media

Growth of *F. verticillioides* at time of incubation

Concentration (%)	48	72	96	120	144	168
30.0	2.80c	4.06b	5.50cd	8.00a	8.50a	8.77a
25.0	2.90c	4.10b	5.40cd	7.00cde	7.50cde	8.00cd
20.0	2.80c	4.20b	5.50cd	7.30bcde	7.80bcde	8.30abc
15.0	2.60cd	3.70d	5.20d	6.80ef	7.30ef	7.80c
10.0	2.80c	4.00b	5.30cd	7.50abcd	8.00abcd	8.50ab
5.00	3.80ab	5.00a	5.80bc	6.26f	6.76f	7.20d
Benomyl	2.00d	2.80c	3.20e	4.00g	4.50g	5.00e
Control	4.20a	5.50a	6.70a	7.60ab	8.10abc	8.60a
Mean	2.98	4.17	5.32	6.80	7.30	7.71
SE (0.05)	3.349	2.762	2.516	3.097	3.097	2.714

Figures in each column followed by the same letter(s) do not differ significantly from each other by DMRT at P < 0.05. Benomyl concentration = 1.7×10^{-4}

Table 4.14: Mycelial extension of *F. verticillioides* (cm) due to the effect of different concentrations of dry *P. angulata* extracts incorporated into the growth media

Concentrations (%)	48	72	96	120	144	168
30.0	2.60b	3.60c	5.20c	6.40b	6.90c	7.40c
25.0	2.70b	3.70bc	5.50bc	7.00a	7.50b	8.00b
20.0	2.70b	3.90bc	5.40bc	5.43c	5.90d	6.43d
15.0	2.70b	3.70bc	5.70bc	7.10a	7.60b	8.10b
10.0	2.90b	4.10b	6.10ab	7.40a	7.90ab	8.40ab
5.0	2.60b	3.90bcd	5.16c	7.20a	7.70ab	8.20ab
Benomyl	2.00c	2.80d	3.20d	4.00d	4.50e	5.00e
Control	4.20a	5.50a	6.70ab	7.60a	8.10ab	8.60a
Mean	2.80	3.90	5.37	6.51	7.01	7.51
SE (0.05)	2.260	2.000	3.254	2.775	2.775	2.775

Numbers in each column followed by the same letter(s) do not differ significantly from each other by DMRT at $P < 0.05$

higher on the poisoned plate than that of the control (Table 4.14). The plant extract had stimulatory effect on the pathogen.

4.10.1c Effect of dry *M. jalapa* on *F. verticillioides*

Fungal growth on the highest extract concentration, 30.0% at 48 hours after inoculation measured 30 mm (3.00 cm) of the pathogen's radial growth, 29 mm (2.90 cm) of the pathogen diameter on poisoned agar with 3 ml and the control. Seven days after inoculation, the 5.0% and 10.0% had mycelia extension of 85 mm, the whitish colour of the mycelia changed to dark-pink colour which can be attributed to the ageing (long incubation period) of the pathogen. The underside of the plate was brownish (Plate 4.13c & d); this may be due to ageing and effect of the botanicals on the pathogen. The mycelia at 15.0% concentration was a little more coloured than those on the higher concentrations, mycelia were fluffy and dense. The underside of the plate was yellowish. A wider mycelia extension was observed at 10.0%; the underside of plate was brownish with dark colours from the centre of the inoculated point. Poisoned agar of 15.0% recorded 84 mm (8.40 cm) with distinct light pink colour and more fluffy mycelia. The control (without extract) recorded 77 mm (7.70 cm) mycelia growth.

Generally *M. jalapa* had poor growth on it at first. The fluffiness of the mycelia increased with increase in the time of incubation. It was observed that the bottom of the plate i.e. the reverse side of the plate was dark brown in colour. There was a smell from the plate whenever it was observed. *M. jalapa*'s 5.0% concentration tested was not significantly different in ineffectiveness against *F. verticillioides*, whereas the various concentrations were similar in action against the pathogen, at 48 and 72 hours of incubation. The 30.0%, 25.0%, 20.0% and 15.0% concentrations at 96 hours are significant in action compared with others, 5.0% and 10.0% are not significant different from each other but different from control and the lower concentrations. At 120, 144 and 168 hours, 20.0% (Table 4.15) was the most effective and 5% or 25.0% may serve as the minimum inhibitory concentration for the control of *F. verticillioides* of maize.

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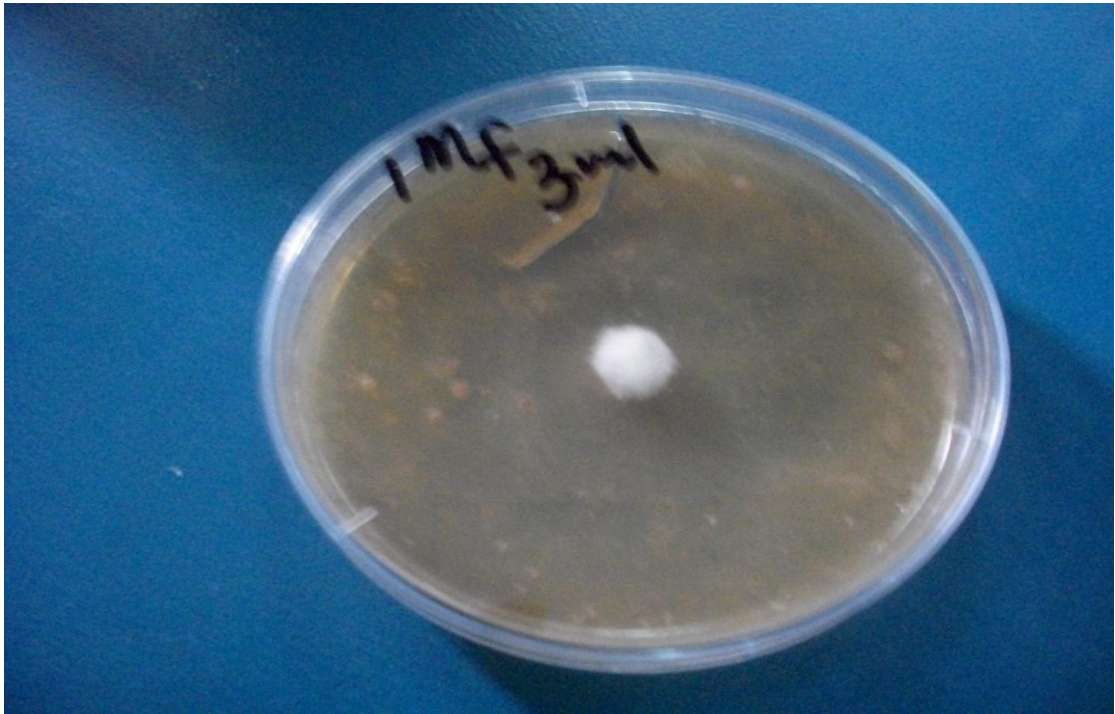
Plate 4.14: *F. verticillioides*' growth at 3 days after inoculation on PDA incorporated with 15% of *M. jalapa*

Table 4.15: Mycelial extension of *F. verticillioides* (cm) due to the effect of different concentrations of dry *M. jalapa* extracts incorporated into the growth media

Concentrations (%)	Time	Of	Incubation	(hrs)		
	48	72	96	120	144	168
30.00	2.73b	3.60c	5.20e	6.40b	6.90b	7.40b
25.00	2.70b	3.70c	5.50de	7.00ab	7.50ab	8.00ab
20.00	2.70b	3.90c	5.40e	5.30c	5.80c	6.30c
15.00	2.70b	3.70c	5.70cde	7.10a	7.60a	8.10a
10.00	2.90b	4.10c	6.10cb	7.40a	7.90a	8.40a
5.00	2.90b	3.90c	5.40e	7.20a	7.70a	8.20a
Benomyl (1.7×10^{-4})	2.00c	2.80d	3.20f	4.00d	4.50d	5.00d
Control	4.20a	5.60a	6.70a	7.60a	8.10a	8.60a
Mean	2.82	3.91	5.40	6.50	7.00	7.50
SE	2.479	2.449	2.403	2.867	2.867	2.867

Numbers in each column followed by the same letter(s) do not differ significantly from each other by DMRT @ P = 0.05. Benomyl (1.7×10^{-4}) %

4. 10.1d Effect of dry *S. occidentalis* on *F. verticillioides*

Seven days after inoculation, the 30.0% and 20.0% had *F. verticillioides* mycelial extension of 7.00 cm. The white pinkish colour of the mycelia changed to faint-pink colour which can be attributed to the ageing (long incubation period) of the pathogen and the probable effect of toxic substance(s) in the extract. The underside of the plate was brownish (Plate 4.13). The mycelia at 15.0% of the extract was little more coloured than that of the higher concentrations, mycelia had its usual pinkish white colour, it was not fluffy and not dense. The underside of the plate was light brown. A wider mycelial extension was observed at 15.0% extract incorporated in the agar plate, the underside of plate was brownish with dark colours from the centre of the inoculated point. Control (without extract) recorded 77 mm with distinct light pink colour of mycelia which was fluffier.

For *S. occidentalis* concentrations, there was no significant difference on their individual effect on *F. verticillioides*, though better than the control but not as effective as benomyl at 48 hours. There was no significant difference at 72 hours, 96 hours of incubation recorded significant effect on *F. verticillioides* at 30.0%, this was not significantly different from 20.0 and 5.0% (Table 4.16). The minimum inhibitory concentration of *S. occidentalis* was 5.0% that gave 7.40 cm of *F. verticillioides*. Different concentrations of diluted extract from the leaves of *S. occidentalis* were incorporated as poison into the growth medium of the pathogen. Fungal growth on the highest concentration, 30.0% at 48 hours after inoculation measured 30 mm of the pathogen, while it was 2.90 cm of the pathogen diameter on the control plate.

Table 4.16: Mycelial extension of *F. verticillioides* (cm) due to the effect of different concentrations of dry *S. occidentalis* extracts incorporated into the growth media

Concentrations (%)	48	72	96	120	144	168
30.00	2.60b	3.60bc	4.60e	6.30cb	6.80cb	7.30bc
25.00	2.90b	4.10bc	5.10cde	6.67b	7.16b	7.66b
20.00	2.70b	3.50c	5.30cd	6.50b	7.00b	7.50bc
15.00	2.80b	3.70bc	5.10cde	5.80c	6.33c	6.86c
10.00	2.80b	4.20b	5.40bcd	6.70b	7.20b	7.70b
5.00	2.70b	4.00bc	5.70bc	6.4cb	6.90bc	7.40bc
2.50	2.80b	3.90bc	5.20cde	6.20bc	6.70bc	7.20bc
Benomyl (1.7×10^{-4})	2.00c	2.80d	3.20f	4.00d	4.50d	5.00d
Control	4.20a	5.50a	6.70a	7.60a	8.10a	8.60a
Mean	2.83	3.92	5.14	6.24	6.74	7.25
SE	2.815	2.645	2.748	2.873	2.886	2.899

Numbers in each column followed by the same letter(s) do not differ significantly from each other by DMRT @ P = 0.05

4. 10.2 Effect of wet botanicals on *F. verticillioides*

A). *T. diversifolia*

It was observed that the mycelia on higher concentrations of the plant extract into the growth media were not as fluffy as the lower concentrations of the plant extract on the *F. verticillioides*. When comparing the effect of the wet with the dry plant samples used for the extraction, it was noted that for the dry plant used, their higher concentrations acted as growth stimulators for the mycelia to flourish in, whereas the wet samples acted as mycelia growth inhibitors. It was noted that the wet was most effective as inhibitor at 15.0% and 25.0% concentration especially to control the pathogen and air-dried plant extracts acted as growth stimulator at higher concentration, it was however able to inhibit the pathogen at lower concentrations. From this it can be inferred that wet (fresh) *T. diversifolia* is more effective in the control of the pathogen. But, for the purpose of this study, dry *T. diversifolia* was used for the reason of proper documentation of the actual concentration used.

It was observed that the mycelia extension of *F. verticillioides* due to benomyl (1.7×10^{-4}) % incorporation into the media was 1.80 cm. Higher concentrations of *T. diversifolia*, 30.0%, 25.0%, 15.0%, 10.0% and 5.0% were not significantly different from each other but better than the control plates at 48hrs of incubation. There was no significant difference in all the treatments compared with the control plate at 72 hours. The 120, 144 and 168 hours incubation showed no statistical difference from one another, but each period recorded increase in the mycelia extension (Table 4.17). The three periods observed can be summarized thus: 5.0% was not significant in inhibiting the growth of *F. verticillioides*, 30.0%, 20.0%, 10.0% and 5.0% inhibited the pathogen's growth significantly to 7.10 cm and 7.00 cm.

Table 4.17: Effect of the different concentrations of wet *T. diversifolia* extracts on *F. verticillioides*

Concentrations (%)	Time	Of	Incubation	(hrs)		
	48	72	96	120	144	168
30.00	2.80b	3.70b	5.20ab	6.10bc	6.60bc	7.10bc
25.00	2.90b	3.80b	5.10ab	6.00c	6.50c	7.00c
20.00	2.80b	3.60b	5.40ab	6.00c	6.50c	7.03c
15.00	2.70b	3.60b	5.00ab	6.60abc	7.10abc	7.60abc
10.00	2.80b	3.80b	5.20ab	6.70ab	7.20ab	7.70ab
5.00	2.90b	3.60b	4.80b	6.40abc	6.90abc	7.40abc
2.50	3.00ab	4.10b	5.30ab	6.80a	7.30a	7.80a
Benomyl (1.7×10^{-4})	1.80c	2.60c	3.00c	3.80d	4.30d	4.80d
Control	3.20a	4.80a	5.20a	6.90a	7.60a	7.90a
Mean	2.30	3.73	4.91	6.87	6.66	7.15
SE (0.05)	0.72	0.74	0.72	0.66	0.72	0.68

Numbers in each column followed by the same letter(s) do not differ significantly from each other by DMRT @ P = 0.05

b). *S. occidentalis*

The wet leaves and stem sample of *S. occidentalis* used in the *in vitro* study for the control of *F. verticillioides* showed that at 48 hours of incubation that the highest concentration, 30.0% was bioactive against the pathogen which compared well with the synthetic fungicide used, meaning that it can be a better alternative to the use of fungicides. The 15.0% incorporated into the medium was bioactive as it was able to control the mycelia growth of the pathogen but not as much as the highest concentration 30.0%, here the mycelia growth was really scanty. It was observed in this study that the higher the concentration the more bioactive the effect of the concentration against the pathogen. The 72 hours of incubation followed the same trend as observed at 48 hours. Throughout the incubation times, benomyl significantly inhibited the growth of *F. verticillioides* which gave purplish/pinkish colour in the plates. The mycelia extension of *F. verticillioides* was not significantly different from each other at 96 hours of incubation in all the concentrations except for 2.5% in the media. The 2.5% concentration was the weakest in culture as the inhibitory effect on the pathogen was not different from the control. As the mycelia growth of these two concentrations were like the control plates and was so throughout the incubation periods (Table 4.18).

At 120,144 and 168 hours of incubation, agar plates with concentrations 30.0%, 25.0%, 20.0% and 15.0% had inhibitory effect on the pathogen. Though not as effective as the benomyl treatment, but it had inhibition of the organism, it had 20% reduction of the pathogen's growth (at 168 hours, control, and 8.50 cm minus 6.60cm divided by 8.50 cm multiplied by 100). The 2.5% are not effective in inhibiting the pathogen's growth. It was noted that the higher the concentration and volume of *S.occidentalis* the better the inhibitory action on the pathogen. Though the inhibitory action was not as effective as the benomyl, it can as well serve as a good alternative control measure of the pathogen, *F. verticillioides*. This wet *S.occidentalis* was noted to have minimal contaminant on the growth medium when compared with the dry sample used. The only challenge of the use of this wet sample was the quantification of the concentration used for the procedure. When the fresh samples were weighed and blending was done with the addition of little water not as much as 100ml used in the dry sample. The water in the individual plant tissues determined the quantity of the water that was added to it during processing.

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Table 4.18: Effect of the different concentrations of wet *S. occidentalis* sample extracts on *F. verticillioides* (cm)

Concentrations (%)	Time 48	Of 72	Incubation 96	(hrs) 120	144	168
30.00	2.20de	3.13ef	3.60d	5.10f	5.60ef	6.10f
25.00	2.60cd	3.60cde	4.50c	5.30ef	5.80ef	6.30ef
20.00	2.40cd	3.10ef	4.20c	4.90f	5.50f	6.00f
15.00	2.30cde	3.10ef	4.20c	5.20ef	5.70ef	6.23ef
10.00	2.50cd	3.80cd	5.00b	5.80cd	6.30cd	6.80cd
5.00	2.80bc	3.50de	5.20b	6.10c	6.60c	7.10c
2.50	2.80cb	4.40ab	6.20a	6.80b	7.30b	7.80b
Benomyl (1.7×10^{-4})	1.86e	2.63f	3.03e	3.83g	4.30g	4.83g
Control	4.10a	4.10ab	6.60a	7.40a	8.00a	8.50a
Mean	2.30	3.48	4.72	5.60	6.12	6.62
SE (0.05)	0.92	0.94	0.92	0.86	0.92	0.88

Numbers in each column followed by the same letter(s) do not differ significantly from each other by DMRT @ P = 0.05

c). *M. jalapa*

The effect fresh sample of *Mirabilis jalapa* at the highest volume concentration on *F. verticillioides* was as effective as the Benomyl at all the various times of incubation. The lowest volume and concentration was also effective against the pathogen. The growth of *F. verticillioides* was 2.20 cm in 48 hours of incubation on 30.0% extract which was not significantly different from benomyl treatment that had 2.00 cm mycelia extension. The other lower concentrations were not significantly different from each other. This trend was observed till 72 hours of incubation (Table 4.19). At 96 hours, the 30.0% extract incorporated into the medium was bioactive but, even effective but, not as much as benomyl treatment, with 3.70 cm and 3.20 cm growth respectively. It was the most effective of all the concentrations used against the pathogen.

It was observed that the higher concentrations of the extracts were more effective than the lower concentrations and had inhibitory effect on the pathogen at the various times of incubation. Benomyl inhibited the pathogen significantly throughout the period of incubation. The minimum inhibitory concentration of *M. jalapa* was the 15.0%.

d). *P. angulata*

At 48 hours of incubation, there was no visible difference in all the concentrations used against *F. verticillioides*, though they all inhibited the growth of the pathogen when compared with the control plates, but the individual concentrations used on the pathogen had no significant differences individually on the pathogen's mycelia (Table 4.20). The action of the different concentrations of the extracts acted differently at 72 hours of incubation. It was noticed that the mycelia growth of the pathogen were not as fluffy i.e. the mycelia were densely populated in some plates though mycelia extension was observed in others. The plates at 96 hours of incubation showed 25.0%, 15.0% and 2.5% concentrations to be effective against the pathogen. At the end of the incubation period, just like for the other extracts earlier used, the 30.0% concentration was the most effective in inhibiting the growth of *F. verticillioides*.

Table 4.19: Effect of the different concentrations of fresh *M. jalapa* sample on *F. verticillioides* for 168 hours of incubation (cm)

Concentrations (%)	Time 48	Of 72	Incubation 96	(hrs) 120	144	168
30.00	2.20ef	2.80e	3.70e	5.20h	5.70h	6.20g
25.00	2.60cd	3.70bcd	4.80c	6.10de	6.60de	7.06d
20.00	2.40de	3.00e	4.40d	5.40gh	5.90gh	6.40fg
15.00	2.63cd	3.70bcd	4.40d	5.60fg	6.10fg	6.60ef
10.00	2.80bc	3.80bc	5.70b	6.60c	7.10c	7.60c
5.00	2.60cd	3.60cd	5.80c	6.80bc	7.30bc	7.80bc
2.50	2.80bc	3.76bcd	4.90c	5.80ef	6.30ef	6.80de
Benomyl (1.7×10^{-4})	2.00f	2.80e	3.20f	4.00i	4.50i	5.00h
Control	4.20a	5.50a	6.70a	7.60a	8.10a	8.60a
Mean	2.69	3.63	4.84	5.90	6.40	6.89
SE (0.05)	0.66	0.66	0.66	0.70	0.68	0.71

Numbers in each column followed by the same letter(s) do not differ significantly from each other by DMRT @ P = 0.05

Table 4.20: Effect of the different concentrations of fresh *P. angulata* sample on *F. verticillioides* for 168 hours of incubation (cm)

Concentration (%)	Mycelial growth of *F. verticillioides* (cm) at incubation time (hrs)					
	48	72	96	120	144	168
30.00	2.60c	3.40de	5.00de	6.20d	6.00e	6.83e
25.00	2.70c	3.00e	4.80e	6.80bc	7.33cd	7.73bcd
20.00	2.70c	3.90cd	5.23cd	6.60c	7.66d	7.40cd
15.00	2.70c	3.06e	5.10cde	6.80bc	7.00cd	7.70bcd
10.00	2.80c	4.00bc	5.70cd	7.00b	7.00cd	7.90bc
5.00	2.80c	4.20bc	5.60b	7.00b	7.00b	7.90bc
2.50	2.90c	4.36bc	5.40bc	7.10b	7.00bc	8.00b
Benomyl	2.00d	2.80e	3.20f	4.00e	4.00f	5.20f
Control	4.20a	5.50a	6.70a	7.60a	8.00a	8.50a
Mean	2.82	3.80	5.19	6.56	7.55	7.45
SE (0.05)	0.64	0.90	0.64	0.66	0.79	0.81

Numbers in each column followed by the same letter(s) do not differ significantly from each other by DMRT @ P = 0.05

4.11 Effect of the different plant extracts treatments on *F. verticillioides*

4. 11. 1 Percentage inhibition of dry botanicals on the pathogen

a) *S. occidentalis*

The percentage inhibition of the botanical showed the rate of growth reduction by each of the four plant extracts tested (Table 4.21). It was observed that the lower the percentage inhibition, the less effective the concentration that inhibited the growth of the pathogen. At 48 hours of incubation, benomyl was 52.4% (the difference between control and the mycelia extension of the pathogen, expressed in percentage), next to it was 30.0% and 2.5% concentrations with 38.1% each and this inhibition percentage was not significantly different from 20.0%, 15.0% and 5.0% concentrations of *S. occidentalis*. The 20.0% of *S. occidentalis* incorporated into growth media had inhibition percentage of 36.36% which was not significantly different from 30.0%, 15.0%, 2.5% at 72 hours, these concentrations proved to be better alternative to the synthetic fungicide as they were able to inhibit the growth of pathogen. As expected/observed, benomyl did not tolerate the further growth of the *F. verticillioides* in culture, its mycelia was very pinkish almost turning to purple, at longer hours of incubation the *F. verticillioides,* mycelia turned dark dirty brown off purple in colour. This was not so in the case of plant extract of *S.occidentalis* used. The longer hours (120, 144 and 168 hours) of incubation showed no statistical significant difference in the concentrations, that all the concentrations of *S.occidentalis* can inhibit the growth of the pathogen. However, 15.0% had 20.17% inhibition of *F. verticillioides* at 168 hours, followed by 2.5% with 16.47% these concentrations at their minimum values more inhibitory effect on the pathogen (Table 4.21).

Table 4.21: The percentage inhibition of *F. verticillioides* by the different concentrations of dry *S. occidentalis* extracts for 168 hours of incubation.

Concentrations (%)	Percentage inhibition (%) at time of incubation (hrs)					
	48	72	96	120	144	168
30.00	38.10bc	34.53bcd	31.33b	17.10b	16.00b	15.10b
25.00	30.93c	25.46cd	23.90bcd	12.26b	11.50b	8.13b
20.00	35.73bc	36.36b	20.90bcde	14.46b	13.56b	12.80b
15.00	33.33c	32.73bcd	23.90bcd	23.66b	21.80b	20.17b
10.00	38.10bc	23.63d	19.40cde	11.83b	11.10b	10.47b
5.00	35.73bc	27.26bcd	14.90de	15.76b	14.80b	13.93b
2.50	33.33c	29.06bcd	22.40bcde	18.43b	17.30b	16.47b
Benomyl	52.40a	49.10a	52.20a	47.40a	44.40a	41.90a
Mean	37.20	32.26	26.11	20.11	18.80	17.37
S.E (0.05)	1.96	1.68	1.91	2.48	3.34	4.22

Values show the percentage growth inhibition by the corresponding treatment.

Numbers in each column followed by the same letter(s) do not differ significantly from each other by DMRT @ P = 0.05

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b) *T. diversifolia*

The 30.0%, 25.0%, 20.0%, 15.0%, 10.0% and 5.0% of the *T. diversifolia* plant extract at the initial stage 48 hours, were not significantly different from each other. The concentrations at that time appeared promising that it was able to inhibit the growth of *F. verticillioides.* The same was also observed at 72 hours of incubation with the highest inhibitory percentage as 32.7% of 15.0% concentration. This means that for dry *T. diversifolia* to be effect against *F. verticillioides*, high concentration with shorter time of expected efficacy could be adopted. The 3 ml of 50% is the minimum inhibitory concentration against the pathogen (Table 4.22). The longer time of incubation and at highest and lowest concentrations used in this study showed *T. diversifolia* stimulatory effect on the *F. verticillioides*. Instead of inhibiting its growth, the *F. verticillioides* mycelia became fluffy and every more beautiful off pink-white mycelia.

c). Dry *M. jalapa*

The dry *M. jalapa* extracts exhibited good control of *F. verticillioides*. The 30.0%, 25.0%, 20.0%, 15.0%, 10.0% and 5.0% had significant inhibitory effect on the pathogen at 48 hours of incubation, though there was no significant difference at these concentrations mentioned. At 96 hours, 30.0% *M. jalapa* was significantly high in reducing the mycelia extension of the pathogen though not as high as the effect of benomyl on the pathogen. But at this level, this concentration can still serve as alternative to synthetic fungicide, benomyl. The 30.0 and 20.0% of *M. jalapa* were significantly higher in action in inhibiting the pathogen (Table 4.23). Its efficacy can also stand the test of time, as the longer period of incubation still showed the plant extract's ability to inhibit the pathogen.

Table 4.22: The percentage inhibition of _F. verticillioides_ by the different concentrations of dry _T. diversifolia_ extracts for 168 hours of incubation

Dry _T. diversifolia_ Concentrations (%)	Inhibition percentage (%) at the time of incubation (hrs)					
	48	72	96	120	144	168
25.00	33.33bc	26.03b	17.90b	- 5.26d	- 4.93c	- 1.90b
16.67	30.93bc	25.43b	19.40b	7.90bcd	7.36bc	6.97b
15.00	33.33bc	23.63b	17.90b	3.93bcd	3.70bc	3.50b
10.00	38.10bc	32.73b	22.40b	10.53bc	9.86bc	9.30b
8.33	33.33bc	27.26b	20.90b	1.30cd	1.23bc	1.97b
5.00	26.16c	28.46b	13.40bc	9.20bcd	8.63bc	8.13b
3.33	4.76d	5.43c	7.46cd	7.90bcd	7.40bc	13.63b
Benomyl (1.7×10^{-4})	52.40a	49.10a	52.20a	47.40a	44.70a	41.90a
Mean	31.54	27.25	21.44	10.36	9.74	10.43
S. E (0.05)	2.43	1.80	1.84	2.52	3.36	4.38

Each value shows the percentage growth inhibition by the corresponding treatment.

Numbers in each column followed by the same letter(s) do not differ significantly from each other by DMRT @ P = 0.05

Table 4.23: The percentage inhibition of *F. verticillioides* by the different concentrations of dry *M. jalapa* extracts for 168 hours of incubation

Concentrations (%)	Inhibition percentage (%) at the time of incubation (hrs)					
	48	72	96	120	144	168
30.00	34.93bc	34.53b	22.40b	15.80c	14.80bc	13.93bc
25.00	35.70bc	32.73bc	17.90bcd	7.90cd	7.40c	6.97bc
20.00	35.70bc	29.06bc	19.40bc	30.26b	28.40ab	26.73ab
15.00	35.70bc	32.70bc	14.90bcd	6.56cd	6.20c	5.80bc
10.00	30.93c	25.43c	8.93de	2.63cd	2.50c	4.67bc
5.00	30.93c	29.10bc	19.40bc	5.26cd	4.96c	4.63bc
2.50	0.00d	3.60d	3.00e	0.00d	0.00c	0.00c
Benomyl (1.7×10^{-4})	52.40a	63.50a	52.20a	47.40a	44.50a	41.90a
Mean	32.03	31.33	19.76	14.47	13.59	13.07
S. E (0.05)	1.86	1.59	1.81	2.48	3.34	4.24

Values show the percentage growth inhibition by the corresponding treatment.

Numbers in each column followed by the same letter(s) do not differ significantly from each other by DMRT @ P = 0.05

4. 11. 2 Percentage inhibition of wet botanical on the pathogen

a) Fresh *S. occidentalis*

The germination percentage of wet *S.occidentalis* at 48 hours showed no significant difference in the 30.0%, 25.0%, 20.0%, 15.0%, 10.0% and 5.0%. concentrations. The *S. occidentalis* of 2.5% of its concentration of 33% inhibition of the pathogen. The 2.5% concentration of the fresh plant sample had the lowest inhibitory effect on the pathogen throughout the incubation time. It shows that the lower concentration of *S. occidentalis* fresh plant extract may not be as effective as the other concentrations. The minimum inhibitory concentration of wet *S.occidentalis* was the 15.0% concentration (Table 4.24). The effect of wet *S.occidentalis* was better than its dry plant sample as higher percentages inhibition was observed in the wet than in the dry. At 168 hours, 30.0%, 25.0%, 20.0%, 15.0%, 10.0% and 2.5% concentrations of *S. occidentalis* were not significantly different from each other but, 2.5% concentration was the least effective in inhibiting *F. verticillioides* pathogen of maize and were different from the former concentrations earlier mentioned.

b) Fresh *T. diversifolia*

The action of fresh *T. diversifolia* showed that it is better to use the fresh samples in controlling *F. verticillioides* as seen in this study than the dry plant samples. The effect on *F. verticillioides* at 48 hours in benomyl incorporated plates showed significant effect (Table 4.25). The plates throughout the period of observation showed pinkish/purple colour of the pathogen. The growth of *F. verticillioides* was so sparse on the plates with little mycelia extension. From 72 hours to when the plates were discarded, all the concentrations showed inhibitory effect on the pathogen as there were no significant differences in the treatments and the variations in the mycelia growth was minimal in all. The 15.0% was the minimum inhibitory concentration on the pathogen.

Table 4.24: The percentage inhibition of growth of *F. verticillioides* due to the different concentrations of fresh *S. occidentalis*.

Concentrations (%)	Inhibition percentage (%) at the time of incubation (hrs)					
	48	72	96	120	144	168
30.00	47.63a	49.06bc	46.26ab	32.86b	30.90ab	29.10ab
25.00	38.06ab	34.53de	32.83cd	30.26b	28.40abc	26.73ab
20.00	42.83ab	43.63bcd	37.36bc	34.23b	32.10ab	30.20ab
15.00	45.23ab	43.63bcd	37.30bc	31.56b	29.60abc	27.50ab
10.00	40.46ab	30.90e	25.36cd	23.66bcd	22.20bc	20.90ab
5.00	33.33bc	36.36de	22.40d	19.73bcd	18.50bc	14.73b
2.50	33.33bc	19.96f	7.46e	10.50cd	9.90bc	9.30b
Benomyl(1.7 x 10^{-4})	55.56a	52.10ab	54.40a	49.56a	46.86a	43.80a
Mean	42.05	38.77	32.92	29.04	27.30	25.28
S. E (0.05)	2.22	1.97	2.21	2.90	3.88	5.00

Values show the percentage growth inhibition by the corresponding treatment.

Numbers in each column followed by the same letter(s) do not differ significantly from each other by DMRT @ P = 0.05

Table 4.25: The percentage inhibition of growth of *F. verticillioides* due to the different concentrations of fresh *T. diversifolia*.

Concentrations (%)	Inhibition percentage (%) at the time of incubation (hrs)					
	48	72	96	120	144	168
30.00	33.33ab	32.73c	22.40b	19.73b	18.50b	17.40ab
25.00	30.93ab	30.90c	23.90b	21.03b	19.73b	18.60ab
20.00	33.33ab	34.53c	19.40b	21.03b	19.73b	18.20ab
15.00	35.73ab	34.53c	25.36b	13.16b	12.30b	11.60ab
10.00	33.33ab	30.90c	22.40b	11.83b	11.10b	10.47b
5.00	30.93ab	34.53c	28.36b	15.76b	14.80b	13.93ab
2.50	28.56b	25.43c	20.90b	10.53b	9.90b	9.30b
Benomyl(1.7×10^{-4})	50.76a	47.86a	51.73a	46.50a	44.00a	40.33a
Mean	35.28	33.92	26.80	19.94	18.75	17.47
S. E (0.05)	2.31	2.08	2.37	3.01	3.86	5.00

Values show the percentage growth inhibition by the corresponding treatment.

Numbers in each column followed by the same letter(s) do not differ significantly from each other by DMRT @ P = 0.05

c)   *P. angulata*

The rate of growth of *F.verticillioides* in *P. angulata* incorporated into the agar was as explained in section 4.9.2d. At 48 hours of incubation, there were significant differences in all the treatments but benomyl was significantly different from the treatments, as it was able to significantly inhibit the growth of the pathogen. The 72 hours of incubation showed ineffectiveness of concentrations less than 10.0%. The 30.0% concentration of *P. angulata* was not significantly different in action from the plates with benomyl treatment. Benomyl and 30.0% concentration of *P. angulata* both had significant effect on the *F. verticillioides* mycelia. There were no significant differences in 96, 120,144 and 168 hours statistically, as the density of the pathogen mycelia were not the same, the significance experienced here were only due to the mycelia extension action of the plant extract incorporated plates. The longer the incubation of the pathogen in 2.5% concentration of *P. angulata*, the more the growth of *F.verticillioides* as it even did better than the control plate in its fluffiness and colour. The mycelia of *F.verticillioides* were stimulated at these times in concentration low as 2.5% of *P. angulata* (Table 4.26).

d)   *M. jalapa*

The rate of growth of *F.verticillioides* in *M. jalapa* incorporated into the agar was as explained in section 4.9.2c. The concentrations less than 10.0% of *M. jalapa* incorporated into the growth agar were weak at reducing the mycelia extension of *F. verticillioides,* significantly lower in the value of inhibition percentage than the other concentrations. As the incubation period progressed, the action of the plant extract was evident on the mycelia growth of the pathogen. It was observed that 30.0% and 20.0% showed better reduction of the mycelia growth of 20.5% and these were different from what was observed for 15.0% and 2.5% concentrations of the *M. jalapa* plant with 10.5% and 6.9% inhibition respectively. The minimum inhibitory concentration of *M. jalapa* is of 15.0% (Table 4.27).

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Table 4.26: Percentage inhibition of *F. verticillioides* by fresh extract of *P. angulata*

Concentrations (%)	Inhibition percentage (%) at the time of incubation (hrs)					
	48	72	96	120	144	168
30.00	38.06bc	37.96bc	23.90bc	18.43b	19.73b	20.53ab
25.00	35.70c	45.23ab	28.36b	10.10bc	9.46bc	10.13b
20.00	35.70c	29.06de	22.40bc	13.16b	13.96bc	13.97b
15.00	35.70c	37.96bc	23.90bc	10.53bc	9.86bc	10.50b
10.00	33.33c	27.26de	22.40bc	7.90bc	7.40bc	8.13b
5.00	33.33c	23.63e	16.40c	7.90bc	7.40cd	8.13b
2.50	30.93c	32.73cd	19.40bc	6.65bc	6.16bc	6.97b
Benomyl (1.7×10^{-4})	52.36a	49.06a	52.20a	47.36a	44.43a	40.67a
Mean	42.24	35.32	26.12	15.25	14.80	14.87
S. E (0.05)	1.54	1.43	1.74	2.37	3.27	4.27

Values show the percentage growth inhibition by the corresponding treatment.

Numbers in each column followed by the same letter(s) do not differ significantly from each other by DMRT @ P = 0.05

Table 4.27: Percentage inhibition of *F. verticillioides* by fresh extract of *M. jalapa*

Concentrations (%)	Inhibition percentage (%) at the time of incubation (hrs)					
	48	72	96	120	144	168
30.00	47.60ab	49.06a	44.80a	31.56b	29.60a	27.90ab
25.00	38.06cd	32.73cd	28.33b	19.73bcde	18.50abc	17.83b
20.00	42.83bc	45.43ab	34.33b	28.93b	27.16ab	25.60ab
15.00	37.26cde	32..73cd	34.30b	26.30bc	24.70abc	23.27ab
10.00	33.33de	30.90cd	14.90c	13.16cde	12.20abc	11.60b
5.00	38.06f	34.53cd	25.40b	10.53de	9.73bc	9.30b
2.50	33.33de	30.90cd	26.90b	23.66bcd	22.20abc	20.93ab
Benomyl(1.7×10^{-4})	52.36a	49.06a	52.20a	47.36a	44.43a	41.87a
Mean	40.34	38.16	32.64	25.15	23.56	22.27
S. E (0.05)	1.58	1.43	1.74	2.38	3.27	4.19

Values show the percentage growth inhibition by the corresponding treatment.

Numbers in each column followed by the same letter(s) do not differ significantly from each other by DMRT @ P = 0.05

4.12 Reaction of QPM to ear rot and other diseases

Table 4.28, shows the mean of two season trials in 2007 and 2008 to show the bases for working with the selected varieties for the screenhouse and field trials. It was observed in the seasons that the incidence of ear rot in ART- 98-SW5-OB was the least with 0.66 which fell between 0 and 1 (less than 20% infection). Next to it were ART-98-SW4-OB, ART-98-SW6-OB and TZPB which were not significantly different from each other with 1.00 score but, recorded not less than 20% infection. Obatanpa and ACR99TZL had 1.33 scores, which were not significantly different from ART-98-SW4-OB, ART-98-SW6-OB and TZPB. The 1.33 score showed the ear rot incidence on the variety was around 30% infection. ILE-1 OB was found to be the most susceptible as 1.66 score of ear rot was observed in the trials, which means that more than 30% ear rot incidence was observed on the maize variety at harvest. This means that ILE-1-OB was susceptible to ear rot disease, while ART-98-SW5-OB was resistant to this disease and the local check ACR99TZL was moderately susceptible to ear rot.

The foliar diseases such as *Curvularia* leafspot, *Dreschlera* leaf blight were observed and scored for on the trials. ART-98-SW5 had least incidence of foliar diseases. The foliar diseases observed were scored 1.00, especially for the *Dreschlera* leaf blight. Not that there were no incidence of leafspot and the other foliar diseases, but they were insignificant small that one can ignore them on the field. But the *Drechlera* blight was most pronounced during the two planting seasons. ILE-1 OB with 3.00 was the most susceptible to the blight with about 60% incidence on the field. ACR99TZL was not significantly different in resistance from ILE-1 OB, TZPB, Obatanpa and ART-98-SW4-OB and ART-98-SW6-OB.

For the stemborer attack, ART-98-SW5-OB was susceptible to stemborer which was not significantly different from Obatanpa. These two varieties were most susceptible to the attack. ART-98-SW4-OB and ART-98-SW6-OB were resistant to stemborer. ART-98-SW5-OB was resistant to root lodging with less than 40% lodged plants. Obatanpa and ACR99TZL were most susceptible to root lodging and were not significantly different from ART-98-SW4-OB, ILE-1-OB and TZPB. The selection for the screenhouse and field trials were based on these observations.
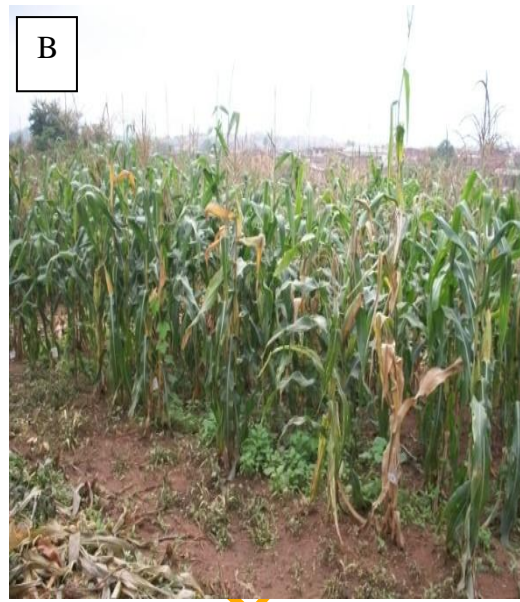
Plate 4.15: Field treatments of maize cultivars with botanicals, bioagents and chemical control.

    a.   Plot of maize plants treated with benomyl

    b.   Plot of maize plants which served as the control

    c.   Plot of maize plants with bioagents treatments

    d.   Plot of maize plants with botanicals treatments
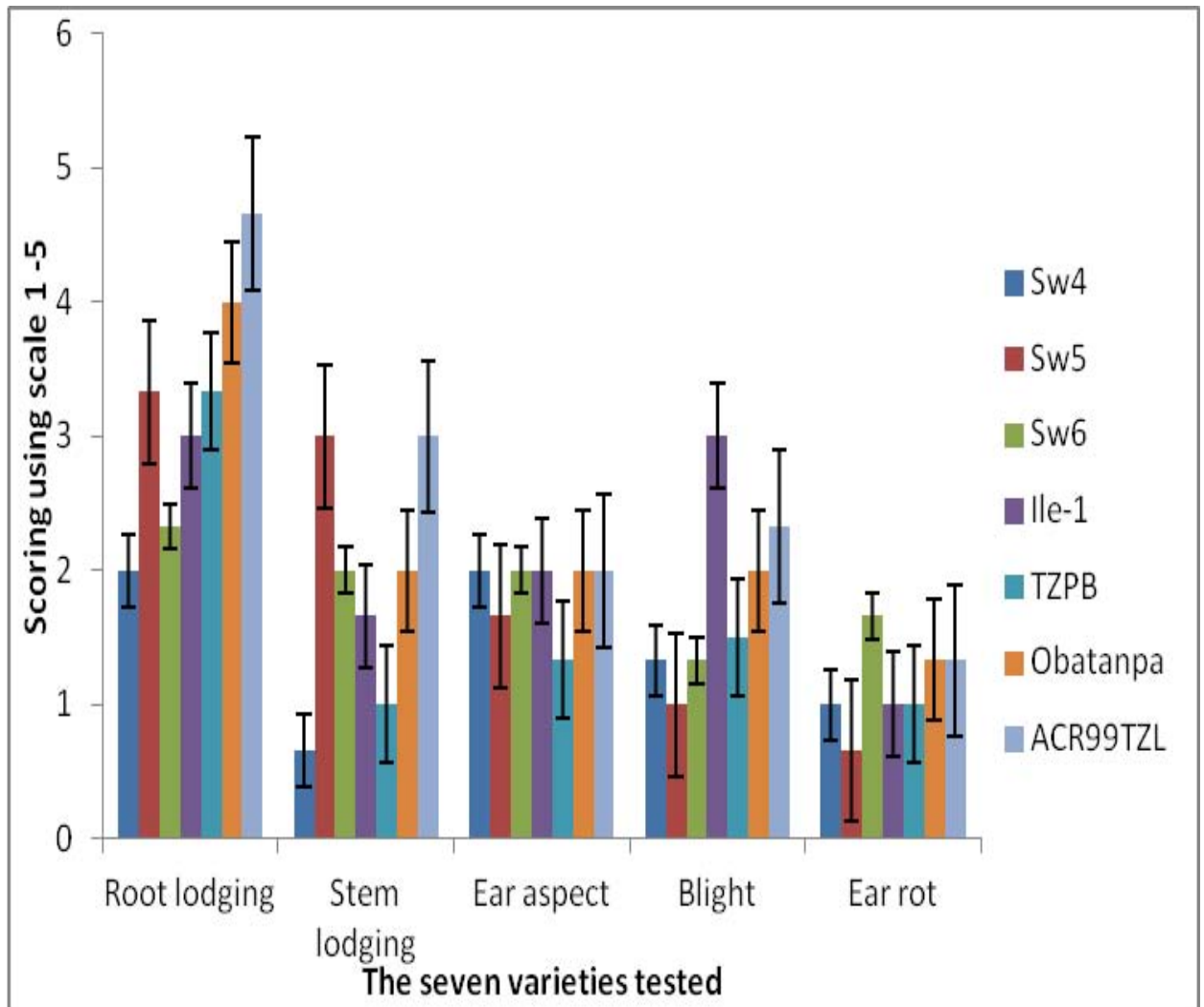
Fig. 4.2: The average response of the seven (6 QPM and 1 check) maize varieties to ear rot and other diseases incidence and severity of scale 1 - 5

Varieties of maize used as indicated in the legend: TZPB, OBATANPA, ACR99TZL, ART-98-SW4-OB = SW4, ART-98-SW5-OB = SW5, ART-98-SW6-OB = SW6, ILE 1-OB = ILE 1.

4.13 Effect of coating methods of maize seeds with botanicals and antagonists

The three coating methods used to apply the botanicals and bioagents were the cooked cassava coating method, powder coating method and water suspension. It was noticed that irrespective of the treatment added to the cooked cassava coating method, for mixing the maize seeds and botanicals/bioagents there was adhesion of the treatment to the side. But it was observed that the starch became deliquescent after some time. The powder coating method did not allow much adhesion. The water suspension really covered the whole seeds. The germination of the seeds was good, with slight variation in seedling vigour under the field conditions.

Observations were made on seedling diseases, and all the treatments were able to overcome the diseases. It was observed that the seeds treated with synthetic fungicide, benomyl gave healthier plants with dark green leaves and very few symptoms of leafspot weeks after planting. Even, the leaf blight symptoms were not prominent; it was very few on benomyl-treated maize seeds. The water suspension application was better than the control (maize seeds without any treatment) in terms of the vigour, germination and susceptibility to maize seedling diseases. It was also noted that these treated seeds were not susceptible to stemborer attack at 2, 4, and 6 weeks after planting. This may be due to the vigour developed by the plants or the presence of the eco-friendly protectants used on the seeds planted.

The three coating methods used showed control against *F.verticillioides* seedling attack and other foliar diseases. It was noted that some neighbouring maize plots in the experimental field, had rust disease on the leaves but this did not get to maize plants in the experimental plot. The absence of rust disease may be due to the foliar treatments that were done when the plants were six weeks old. The plants did not tassel at the same time despite the fact that they were the same varieties. Plants treated with the powder coating method tasseled about two, 2 days earlier than the other two methods and the control. They tasseled at about day 47. The cooked cassava and benomyl-treated plots tasseled 49 days after planting.

There were no significant differences in the number of Days to tassel with regards to the three different coating methods. Though, the cassava coating method had a delay in days to tasselling this was not significantly different from water, benomyl and control, significantly different from powder treatments which tasseled earlier than the rest (Table 4.28).

For days to silk the same trend was observed as noticed in the days to tassel (Table 4.28). Lateness to tassel did not mean that the maize plants will silk early, because it was also observed that the plants with the three coating methods and benomyl silked as they tasseled. Maize plants with powder treatment were shorter than cassava starch-treated plants. They had the least height of 117.90 cm and the tallest plants were observed in the benomyl treated plots. Benomyl and control had significantly higher plant height compared with the other coating methods, but all these were not significantly different from the cassava starch coating method.

For the growth parameters such as plant height, ear height, etc, it was observed that cassava starch coating method had better effect on the performance of the maize plants which was almost the same as was observed in the benomyl treatment. In that the plant vigour, sturdiness and height were stronger and more pronounced in the plots with cassava starch application. Powder coating method of the maize seeds planted did not perform as well as the other two methods.

Field weight of harvested maize seeds in plots treated with water suspension, Benomyl plots and control plots were not different from each other. They all yielded of 0.13 kg per row of maize. These were not significantly different from the field weight of the maize harvested in the other treatments. Cassava starch coating method had 0.12 kg per row of maize and powder application with 0.10 kg per row of maize (field weight) (Table 4.28).

The three coating methods had significant effect on the Ear aspect (the scores of 1-5, 5 = very poor). The score was given when the pile of harvested ears of each plot was spread out and the general "look" of the ears was taken into account. Ear size, uniformity of size, uniformity of color and texture, grain fill, disease and insect damage were all considered for this score). A score of 1.00 showed the ear aspect were good for benomyl treatment. This is a positive indicator that the cobs were free from rot or any other attack or infection. The ear rot disease assessed in the trials, along the rows planted showed that cassava starch coating method was the best to control ear rot disease using both botanicals and bioagents. It was as effective as the benomyl application; the powder treatment was the weakest in controlling ear rot disease of maize.

For the days to tassel, days to silk, plant height, field weight and ear rot, the powder application of the botanicals and bioagents was the least effective. Thus, in terms of the growth, yield and performance of maize plants planted with powder

Table 4.28: Effect of methods of seed treatment with *antagonists* against the maize ear rot pathogen on agronomic and yield parameters

	Coating methods				
	C	H2	Pw	Ben	Control
Days to tassel	49.11a	48.66ab	47.77b	49.00a	48.33ab
Days to silk	54.11a	53.88ab	52.77b	53.33ab	53.33ab
Plant height	120.25ab	118.50b	117.90b	123.46a	122.90a
Field weight of maize	0.12ab	0.13ab	0.10b	0.13ab	0.13ab
Ear aspect	1.11ab	1.33a	1.33a	1.00b	1.44a
Ear rot	1.22ab	1.33ab	1.44a	1.00b	1.33a
Incidence at 4 weeks	1.44a	1.33ab	1.66a	1.00b	1.00b
Incidence at 6 weeks	1.66b	2.00a	2.00a	1.00c	2.00a
Incidence at 8weeks	1.44ab	1.33b	1.66a	1.00c	1.66a
Incidence at 12weeks	1.33b	1.33b	1.33b	1.00c	1.66a
Incidence at harvest	1.33b	1.66a	1.55ab	1.00c	1.66a
Severity at 4weeks	1.22ab	1.00b	1.33a	1.00b	1.00b
Severity at 8 weeks	1.33ab	1.33ab	1.55a	1.00b	1.66a
Severity at harvest	1.33b	1.33b	1.33b	1.00c	1.66a
Blight	1.00a	1.00a	1.00a	1.50a	1.33a
Leafspot	1.00a	1.00a	1.00a	1.00a	1.00a

C = cassava starch coating, H2 = water treatment, Pw = Powder treatment, Ben = Benomyl treatment. Numbers in each row followed by the same letter(s) do not differ significantly from each other by DMRT @ P = 0.05

treatment of the botanicals and bioagents was the least effective of the three methods of coating.

For the incidence of foliar symptoms of *F. verticillioides* at 4weeks after planting benomyl appeared to be the most effective control measure. It was observed that benomyl plots were free of foliar diseases; there were no leafspot nor blight nor rust. Benomyl controlled these diseases to the rate of about 33.0%. This effective controlling action was also observed in water application of the botanicals and bioagents. Powder applications were least effective in controlling *F. verticillioides* on the maize plants at 4 weeks after planting. At 6 weeks after planting, water and powder application of the botanicals and bioagents were the least effective as they had the highest incidence of foliar symptoms of *F. verticillioides* while Cassava starch coating had fewest incidences on the maize plants.

In all, the benomyl was the most effective in combating the incidence of *F. verticillioides* on maize plants. At 6 weeks after planting, foliar application of the bioagents and botanicals was carried out, as a follow-up treatment and this brought significant change in the foliar infection by *F. verticillioides.* At 8 weeks after planting, the maize plants under water-based treatment reduced the incidence of foliar *F. verticillioides* symptoms to 1.33 on the average, cassava starch coating and powder applications of the botanicals and the bioagents with 1.44 and 1.66 respectively were not significantly different from each other. They were not as effective as the water application of the treatments. The effect of the 6 weeks follow-up foliar application of the botanicals and bioagents was significant when the maize plants were observed for incidence at 12 weeks after planting, all the three methods showed effective reduction in incidence of the disease but they were not as effective as the benomyl application.

The severity at harvest was scored on scale 1 – 5; 1 = healthy plants with infection less than 20%. The cassava starch, water suspension and powder application of the treatments had 1.33 score each, which was not as effective as the fungicide treated maize plots with 1.00 score and the control was 1.66. The maize cobs harvested in the control plots were not as healthy as the cobs harvested from benomyl fungicide-treatment plots. The severity of *F. verticillioides* ear rot symptoms at harvest no significant difference in the three coating methods used. In that, the three methods can be used to control the disease. Benomyl had significant difference in its effect when compared with the coating methods and control. The severity was lowest when the

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plants were treated with benomyl and the coating methods were all also better than the control.

It was observed that the plots of maize treated with *T. pseudokoningii* had tall plant stands of 120.00 cm tall. The *T. harzianum* treated plants had the shortest height of 117.00 cm all the treatments. The ear aspect of the plots treated with *T. harzianum* and *P. fluorescens* were not as healthy as the other treatments. The ear rot disease score after the husk has been removed showed that benomyl with 1.00 score had little ear rot problem on ears harvested from the treated plots. The control and *T. harzianum* plots were the worst of the all the cobs harvested. *P. fluorescens* had healthy cobs which were rated 1.22. The benomyl treated plants had cobs that were the best of them all as the ear/cobs were very clean.

There were no significant differences in the plant height of maize treated with *T. pseudokoningii*, benomyl and control. They were taller than *T. harzianum-* and *P. fluorescens*-treated maize plots. The height of *T. harzianum-* treated plants *(*117.97 cm) was the shortest of them all. The statistical analysis showed that there were no differences in the maize plants treated with *T. pseudokoningii, T. harzianum* and *P. fluorescens,* used to coat the seeds before planting. For the ear aspect of maize plants treated with *T. pseudokoningii* and benomyl were not significantly different as the ears were healthy. Benomyl-treated maize plants showed no ear rot disease symptoms on the cobs at harvest and this was not significantly different from *T. pseudokoningii* and *P. fluorescens* (Table 4.29).

T. pseudokoningii was the most effective of all the bioagents used in this study in controlling the incidence of Fusarium ear rot pathogen of maize; this was second best to benomyl used in this study. The incidence of *F. verticillioides* at 6 weeks showed that the efficacy of the botanicals and bioagents were reduced and foliar application with the bioagents and botanicals was done on the maize plants. The incidence at 8weeks showed that the follow up foliar application was effective in controlling the foliar diseases of maize plants and reduced the contamination that the cobs were likely to experience in the field (Table 4.30). At 8 weeks *T. harzianum* was effective in the reduction of the incidence of the disease by 33 %. Benomyl at this time too was still highly effective in inhibiting the disease at 50%; *P. fluorescens* was the weakest bioagent at this time. As from 8 to 12 weeks after planting till harvest, there were visible changes in the plants' response to disease. *T. pseudokoningii* showed its effectiveness against the disease at 12 weeks and at harvest it was still the most

Table 4.29: Effect of methods of seed treatment with antagonists against the ear rot pathogen of maize

Treatments	Days tassel	Plant height	Ear aspect	Ear rot	Field weight (kg)
Tp	47.88a	120.63ab	1.00b	1.22ab	0.16
Th	48.77a	117.97b	1.33a	1.55a	0.09
Pf	48.88a	118.04b	1.44a	1.22ab	0.14
Ben	49.00a	123.46a	1.00b	1.00b	0.17
Control	48.33a	122.90a	1.00b	1.50a	0.13

Tp = *T. pseudokoningii*, Th = *T. harzianum*, Pf = *P. fluorescens*, ben = benomyl Numbers in each column followed by the same letter(s) do not differ significantly from each other by DMRT @ P = 0.05

efficient bioagent used. Next to *T. pseudokoningii* in effectiveness was *P. fluorescens*. *T. harzianum* which was effective at the beginning slowed down. This may be due to the slow growth of the organism. Even though, *P. fluorescens* was also slow growing it was still able to inhibit the growth of the pathogen (Table 4.30).

In terms of severity, all the bioagents used reduced the incidence of blight disease of maize plant. The benomyl was not as effective as the bioagents and it was even worse against blight disease compared with the control. There was no significant difference in the leafspot disease of the varieties of maize plants (Table 4.30). At harvest, the severity of Fusarium ear rot disease of maize was greatly reduced by *T. pseudokoningii*.

When considering the effect of the bioagents used with the different formulations, the height of the maize plants treated with *T. pseudokoningii* was 120.6 cm, the highest, which was not significantly different from the other bioagents used. There was no significant difference in the days to tassel for all the treatments. The ear rot study showed *T. pseudokoningii* to be effective in controlling this disease. *T. psuedokoningii'*s effectiveness was not significantly different from the effect of benomyl on the plants (Table 4.30). The incidence of Fusarium ear rot disease was 1.22 at 4 WAP on plants treated with *T. pseudokoningii.* The height of plants treated with *T. pseudokoningii* and *T. harzianum* were not significantly different from control with a score of 2.00, *P. fluorescens* was the only organism that minimized the symptoms of the disease. The height of benomyl-treated plants with a score of 1.00 was significantly different from others in inhibiting growth of maize. The follow-up spray of the treatments favoured plants as *T. harzianum* with a score of 1.33 had a better effect on the plants. This was not significantly different from *T. pseudokoningii* at 8 WAP. *P. fluorescens* was the weakest with 1.44 at 12 weeks. The effects of *T. pseudokoningii* and benomyl were not significantly different from each other. At harvest, the incidence of Fusarium ear rot was reduced by *T. pseudokoningii o*f all the bioagents used. Benomyl was still very effective.

Table 4.30: Effect of methods of seed treatment with antagonistic bioagents on incidence of *Fusarium* ear rot pathogen of maize

Incidence at weeks after planting

	4 weeks	6 weeks	8 weeks	12 weeks	At Harvest
Tp	1.22b	2.00a	1.44ab	1.00c	1.33b
Th	1.66a	2.00a	1.33b	1.33b	1.66a
Pf	1.55a	1.66b	1.66a	1.66a	1.55ab
Ben	1.00b	1.00c	1.00c	1.00c	1.00c
Control	1.00b	2.00a	2.00a	2.00a	2.00a

Tp = *T. pseudokoningii*, Th = *T.harzianum*, Pf = *P. fluorescens*, ben = benomyl.

Numbers in each column followed by the same letter(s) do not differ significantly from each other by DMRT @ P = 0.05. Basis of scoring is as explained in the materials and methods.

Table 4.31: Effect of methods of seed treatment with antagonistic bioagents on severity of *Fusarium* ear rot pathogen of maize

Severity at weeks after planting

	4 weeks	8 weeks	Blight	Leafspot	At Harvest
Tp	1.00b	1.33ab	1.00a	1.00a	1.00c
Th	1.00b	1.33ab	1.00a	1.00a	1.33b
Pf	1.55a	1.55a	1.00a	1.00a	1.66a
Ben	1.00b	1.00b	1.50a	1.00a	1.00c
Control	1.00b	1.66a	1.33a	1.00a	1.66a

Tp = *T. pseudokoningii*, Th = *T.harzianum*, Pf = *P. fluorescens*, ben = benomyl.

Numbers in each column followed by the same letter(s) do not differ significantly from each other by DMRT @ P = 0.05. Basis of scoring is as explained in the materials and methods.

4.14 Effect of methods of seed treatment with antagonistic bioagents on incidence and severity of *Fusarium* ear rot pathogen of maize

Studies on the effects of the three methods of seed treatment used in controlling the ear rot pathogen showed that cassava starch coating increased days to tassel. The maize seeds planted with the coating method tasseled 49 days after planting. This was not significantly different from the water suspension treatment. The plants developing from under powder application of the bioagents tasseled earlier rest at 47 days after planting. Effect of the treatments (coating methods) on the days to silk stage was different from the days to tassel.

The heights of the plants when treated with powder and water suspension methods were not significantly different from each other but were significantly shorter than plants treated with cassava starch coating. From the growth parameters, it was observed that the cassava coating had taller plants and longer days to tassel and silk when compared with the powder application which had shorter plants and tasseled and silked earlier. The plants developing from benomyl application were not significantly different from those from cassava starch.

The harvested ears of the plants on the three coating methods plots gave slight differences in their field yields. The cassava starch-coated maize plots had a mean of 0.14 kg of ears per row maize plants. Water suspension and powder treated plants had the field weight of 0.13 kg yield, which suggest that the shortness in height and early tasselling and silking had an effect on the yield of the plants. The fungicide-treated plants had the highest yield of 0.19 kg. The yield from powder-treated plants, water suspension and cassava coating methods were not significantly different from each other. Cassava starch coating had a score of 1.77 from scale 1-5 rating of ear aspect. The cobs here were healthier and clean of contamination. Cassava coating treated plants had the least incidence of ear rot of all the coating methods used. It was not significantly different from others.

Four weeks after the maize seeds were treated with the various formulations and it was observed that water treatment with botanicals and bioagents were the most effective in the reduction of the incidence of Fusarium rot as it was able to combat the attack on the maize plants. The result of this was evident at 8 weeks after planting with water suspension having the least score of disease. So also the plants at 12 WAP showed no significant difference in the incidence and severity also at harvest (Table 4.31).

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Table 4.31: Effect of methods of seed treatment with antagonistic bioagents on incidence and severity of *Fusarium* ear rot pathogen and growth of maize

	C	H2	Pw	Benlate	control
Plant height	119.20abc	116.67bc	115.80c	120.03ab	120.86a
Field weight	0.14b	0.13b	0.13b	0.19a	0.09c
Plant.aspect	1.66a	1.55a	1.77a	1.00b	2.00a
Ear aspect	1.77a	2.00a	2.11a	1.00b	2.00a
Incidence at 2 weeks	1.44a	1.00b	1.00b	1.00b	1.33ab
Incidence at 4 weeks	2.00a	1.11b	1.33b	1.00b	1.33b
Incidence at 6 weeks	2.00a	1.33bc	1.66ab	1.00c	1.66ab
Incidence at 8 weeks	1.77a	1.33ab	1.66a	1.00b	1.33b
Incidence at 12 weeks	1.22a	1.22a	1.33a	1.00a	1.00a
Incidence at harvest	1.44a	1.00b	1.00b	1.00b	1.00b
Severity at 4 weeks	1.77a	1.00b	1.66a	1.00b	1.00b
Severity at 8 weeks	1.66a	1.00b	1.66a	1.00b	1.00b
Blight	1.00b	1.57ab	1.14ab	1.00b	1.00b
Leafspot	1.00a	1.14a	1.42a	1.00a	1.00a

C = cassava starch coating, H2 = water treatment, Pw = Powder treatment, Ben = Benomyl treatment.

Numbers in each row followed by the same letter(s) do not differ significantly from each other by DMRT @ P = 0.05

4.15 Effect of seed treatment with botanicals on incidence and severity of *Fusarium* ear rot pathogen of maize

Results on the effect of the treatments with the botanicals on the plant height showed that those treated with *M. jalapa* had an average height of 118.54 cm. Those with benomyl treatment had an average height of 120.03 cm and the control had 120.86 cm. *S. occidentalis* treated plants had the best plant aspect of 1.11 in all the botanicals used. *M. jalapa* and *T. diversifolia* treated plants had their plants aspect to be 2.11 and 1.77 respectively. The control plants had better appearance than the plants treated with *M. jalapa*. Benomyl treated plants had 1.00, with very good ears. The *S.occidentalis*-treated plants and benomyl-treated plants were not significantly different from each other. The other botanicals, *M. jalapa* and *T. diversifolia* recorded 2.11 and 1.77 respectively which was not significantly different from the control. Benomyl-treated plants had the highest field weight of 0.19 kg followed by *S.occidentalis* 0.16 kg which were not significantly different from the *T. diversifolia* (Table 4.32). *T. diversifolia* was not effective in controlling the ear rot disease of maize, but it had significant effect on the yield when compared with control. *S. occidentalis* and benomyl were most effective in reducing ear rot incidence. They had a score of 1.00 each. The moisture content of the maize grains was highest in *T. diversifolia* treated plants which made the grains more susceptible to rot. *S. occidentalis* had lowest ear rot disease symptoms of all the botanicals used at 4 weeks after planting. Treated plants 6, 8 and 12 WAP all had lower incidence of the symptoms compared with the other botanicals used. It was observed *in vitro* that *M. jalapa* was effective against *Fusarium verticillioides*. But, it was observed that the potency against *F. verticillioides* on the field was low and unnoticed. Even *T. diversifolia* performed better than *M. jalapa* on the field. *S. occidentalis,* irrespective of the kind of treatment method employed was as efficient as the fungicide, benomyl. All the botanicals used reduced the foliar diseases of the maize plants in significant and non-significant manners (Table 4.32).

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Table 4.32: Effect of methods of seed treatment with botanicals on incidence and severity of *Fusarium* ear rot pathogen of maize

	M. jalapa	*S.occidentalis*	*T. diversifolia*	Benlate	Control
Plant height	118.54ab	117.06ab	116.06b	120.03a	120.86a
Plant aspect	2.11a	1.11b	1.77a	1.00b	2.00a
Field weight	0.10c	0.16b	0.14b	0.19a	0.09c
Ear aspect	2.11a	1.77a	2.00a	1.00b	2.00a
Ear rot	1.11a	1.00a	1.22a	1.00a	1.33a
Moisture content	11.60ab	11.84ab	12.24a	11.66ab	11.43b
Incid at 4 weeks	1.66a	1.33ab	1.44ab	1.00b	1.33ab
Incid at 6 weeks	1.88a	1.33bc	1.77a	1.00c	1.66ab
Incid at 8 weeks	1.77ab	1.11c	1.88a	1.00c	1.33bc
Incid at 12 weeks	1.33a	1.11a	1.33a	1.00a	1.00a
Incid at harv	1.33a	1.00b	1.11ab	1.00b	1.00b
Severity at 4 weeks	1.66a	1.22b	1.55a	1.00b	1.00b
Severity at 8 weeks	1.66a	1.00b	1.66a	1.00b	1.00b
Severity at harv	1.00a	1.66a	2.00a	1.00a	
Blight	1.33ab	1.00b	1.22ab	1.00a	1.66a
Curvularia	1.44a	1.00a	1.11ab	1.00a	1.00a

Incid = incidence, harv = harvest. Numbers in each row followed by the same letter(s) do not differ significantly from each other by DMRT @ P = 0.05

The effect of the seed treatments on days to tassel showed that using cassava starch to coat maize seeds was the most effective of all treatments, Days to tassel for the cassava coating was shortest followed by water suspension. These two treatments were not significantly different from those of maize plants that were treated with benlate. The days to silk was also shorter in cassava starch coating and the water suspension. They were not also significantly different from the control. The days to tassel and silk were shorter for maize seeds coated with cassava starch and water suspension. The ear height of the plants (i.e. the distance from the base of the plant to the node which bears the top ear) with water suspension-treated plants was the shortest, significantly shorter than benlate treated plants but not significantly different in height from the others, including the control.

Benomyl-treated seeds yielded the highest and this was also significantly different from the other coating methods used and the control. The yield of the cassava starch coated plants was higher and appeared promising. The incidence of ear rot on the benomyl-treated plants at 2 weeks after planting was not significantly different from the plants treated with cassava starch coating. These treatments did not reduce the seedling incidence of Fusarium rot, as such (Table 4.33). In this case, water suspension and powder application really controlled the seedling rot. So also were plants treated with cassava coating that earlier proved effective against the rot. Benlate still maintained its potency. It was observed that at 6 and 8 weeks after planting, the incidence of seedling rot worsened as the plants grew. This gave the idea for a follow-up spray that was done at 6 weeks after planting. The incidence of disease at harvest of the cobs showed plants growing from cassava coated seeds to have less ear rot attack on the cobs.

The severity of seedling diseases at 4 weeks after planting showed plants with cassava starch coating to have less infection than the rest which were not significantly different from the benlate-treated plants. The severity at 8 weeks showed there was no significant difference in the coating methods used.

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Table 4.33: Effect of seed treatment on growth and yield parameters

	C	H2	Pw	ben	Control
Days to tassle	48.88b	49.22b	50.00ab	49.66ab	51.00a
Days silk	53.88b	53.55b	55.00ab	54.66ab	56.00a
Ear height	51.08ab	47.88b	50.26ab	53.10a	50.76ab
Field weight(kg)	0.12b	0.13b	0.08c	0.17a	0.13b
Seedling rot incidence at 2 weeks	1.77ab	1.00c	1.66b	2.00a	1.00c
Incidence at 2 weeks	1.11b	1.66a	1.22b	1.00b	1.00b
Incidence at 4 weeks	1.44a	1.77a	1.66a	1.00b	2.00a
Incidence at 6 weeks	1.66a	1.88a	2.00a	1.00c	2.00a
Incidence at 8 weeks	1.44b	1.88a	1.66ab	1.00c	2.00a
Incidence at 12 weeks	1.33b	1.77a	1.33b	1.00c	2.00a
Incidence at harvest	1.33b	1.66a	1.55ab	1.00c	2.00a
Severity at 4 weeks	1.22bc	1.33b	1.33b	1.00c	2.00a
Severity at 8 weeks	1.33a	1.33a	1.55a	1.00b	2.00a
Severity at harvest	1.33b	1.66a	1.33b	1.00c	2.00a

C = cassava starch coating, H2 = water treatment, Pw = Powder treatment, Ben = Benomyl treatment. Numbers in each row followed by the same letter(s) do not differ significantly from each other by DMRT @ P = 0.05

4.16 The effect of seed treatment with bioagents on the growth and yield of maize, incidence and severity of Fusarium ear rot of maize

When the effect of the different seeds treatments (coating methods), the use of bioagents, and synthetic fungicide, benomyl. The days to tassel of the bioagents-treated maize plants had no significant different in the number of days for it to tassel. However, the *T. pseudokoningii-, P. fluorescens-* and benlate-treated plants irrespective of the coating methods tassled at about the same time while the control plants stayed longer for them to tassel. The same trend was observed in the days to tasseled silk but *T. pseudokoningii* earlier than the remaining treatments. The ear height for *T. harzianum* was shortest in all the treatments while the *T. pseudokoningii, P. fluorescens* and benomyl were not significantly different from each other. For the field weight, benlate had the highest yield followed by *T. pseudokoningii.* The yields of *T. harzianum* and *P. fluorescens* were not significantly different from each other (Table 4.33).

Table 4.33: Effect of microbial agents seed treatments on growth and yield parameters

effect of the treatment (che, org and bot) on growth, yield and Ear rot incidence

	Days to tassel	Days silk	Ear height	Field weight	Ear rot incidence at 2weeks	Incidence at 4weeks
Th	50.00ab	55.00ab	48.33b	0.10c	1.66b	1.77a
Tp	49.00b	53.33b	50.61ab	0.13b	1.66b	1.55a
Pf	49.11b	54.11ab	49.80ab	0.10c	1.11c	1.55a
Ben	49.11b	54.66ab	53.10a	0.17a	2.00a	1.00b
Control	51.00a	56.00a	50.76ab	0.13b	1.00c	1.00b

Tp = *T. pseudokoningii*, Th = *T. harzianum*, Pf = *P. fluorescens*, ben = benomyl, Inciden = incidence, harv = harvest. Numbers in each column followed by the same letter(s) do not differ significantly from each other by DMRT @ P = 0.05

Table 4.34: Effect of seed treatment on disease development

	Inciden at 6 weeks	Inciden at 8 weeks	Inciden at 12weeks	Inciden at harvest	Severit at 4 weeks	Severit at 4 weeks	Severit at 8weeks	Severit at harv
Th	1.77ab	1.44b	1.33b	1.66a	1.00c	1.00c	1.33a	1.33b
Tp	2.11a	1.88a	1.44ab	1.33b	1.33b	1.33b	1.33a	1.33b
Pf	1.66b	1.66ab	1.66a	1.55ab	1.55b	1.55b	1.55a	1.66a
Ben	1.00c	1.00c	1.00c	1.00c	1.00c	1.00c	1.00b	1.00c
Control	2.00ab	1.00c	1.00c	1.00c	2.00a	2.00a	1.00b	1.00c

Tp = *T. pseudokoningii*, Th = *T. harzianum*, Pf = *P. fluorescens*, ben = benomyl, Inciden = incidence, harv = harvest, severit = severity. Numbers in each column followed by the same letter(s) do not differ significantly from each other by DMRT @ P = 0.05

CHAPTER FIVE

5.0 DISCUSSION

Four microorganisms *Trichoderma harzianum, T. pseudokoningii, Pseudomonas fluorescens* and *Bacillus subtilis* were tested *in vitro* for their antagonistic action on maize ear rot pathogen, *F. verticillioides*. Results obtained from the *in vitro* study showed that *T. pseudokoningii* could be used effectively against the seedborne and soilborne pathogen, *F.verticillioides*. When the pathogen was paired on PDA with *T. pseudokoningii*, almost total control of the pathogen was achieved. It was able to stop further growth of the pathogen by the third day after pairing. The fast mycelial growth of *T. pseudokoningii* and *T.harzianum* irrespective of pairing method with *F.verticillioides*, showed the inhibitory ability of the two *Trichoderma* species on the pathogen.

The *Trichoderma* species used in this study possessed the ability to grow aggressively to colonize and occupy the Petri plates within a short time. This gave the pathogen little or no space to grow as reported by Sharma and Sankara (1988). Prescott *et al*. (2002) stated that a good antagonist should be able to compete well for space and nutrients. *T. pseudokoningii* exhibited competition and mycoparasitism on *F. verticillioides* due to its ability to compete well for space and nutrient. The zone of inhibition of growth of *F.verticillioides* caused by *T. harzianum* irrespective of the pairing methods, showed a probable antibiosis-mode of inhibition.

The pronounced growth of the antagonist (*T. harzianum*) in the presence of the pathogen suggested that the antagonist received from the pathogen one or more growth stimulating metabolite(s) in the course of interaction. This suggests that some organisms are effective as antagonists only in the presence of other organisms thereby acting synergistically upon the pathogen in question. Ironically, this was a case of a pathogen stimulating the growth of an antagonist targeted against it. The success of *T. harzianum* against the pathogen was due to the fact that the antagonist grew fast enough to colonize more space than the pathogen, hence the latter's diametric growth was greatly affected in spite of its high colonizing ability.

The action of *T. harzianum* against *F.verticillioides* showed the antagonist to be a stronger competitor for food and space but to a stage that antibiosis was not involved

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in the mode of action because; there was no clear zone of inhibition. This means that antibiosis is not just the production of inhibition zones as it is often seen. Mohammed and Amusa (2003) observed that *T. harzianum* grew over all the pathogens tested in their study. Mechanism for *Trichoderma* species antagonism suggested by other workers were antibiosis (Fravel, 1988), mycoparasitism (Elad *et al*., 1983) and rhizosphere competence (Howell, 2003) and all these modes of antagonism might be responsible for the observed hyperparasitsm.

The antagonistic potentials of the two *Trichoderma* species against *F. verticillioides* were shown in all the three pairing methods. This suggests the ability of each of the *Trichoderma* species to inhibit the mycelial growth of *F. verticillioides* irrespective of time of application. This tells us that if *F. verticillioides* occurs on a maize field before the two *Trichoderma* species were introduced; either of the two can still suppress it to some extent. Strains of *T.pseudokoningii* were successful in inhibiting growth of *F.verticillioides in vitro* in a similar manner and were also able to check occurrence of the pathogen significantly within maize (*Zea mays*) stem in the field, irrespective of pairing methods (Sobowale *et al.,* 2007). The significance of mycelial suppression of *F. verticillioides* by *T. pseudokoningii* over that of *T. harzianum* suggests *T. pseudokoningii*'s better competitive ability than *T. harzianum* against *F. verticillioides. T. pseudokoningii* appeared to be more promising than *T. harzianum* in checking the occurrence of the pathogen on maize fields.

Inoculating antagonist before pathogen had a significant advantage and preference over the other two pairing methods in aiding effective growth inhibition of the pathogen. This means that inoculating antagonists before pathogen is better than the other two pairing methods. In inhibition of *F. verticillioides* growth, it is better for the antagonists to be already established on the field before the pathogen comes in. The highly significant interaction between the *Trichoderma* species and pairing methods showed that mycelia suppression of F. *verticillioides* by either *T. pseudokoningii* or *T. harzianum*. For a more competitive exclusion of *F. verticillioides* from maize plant, it is better for *Trichoderma* species to be on the field before the occurrence of the pathogen.

The growth of *T. pseudokoningii* on some parts of the mycelial mass of the pathogen and the hyphal distortions of the pathogen suggested hyperparasitism. Minor morphological disturbances and hyphal distortions of one fungus by another is a form of hyperparasitism. The hyperparasitic action of the antagonist against the pathogen

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agreed with the work of Adekunle *et al*.(2001) and Peluola (2005) which concluded that hyperparasitism also encompasses overgrowth of one fungus by another. The fading of the colour of the pathogen when *T. pseudokoningii* gradually grew over its mycelial mass might mean gradual extermination of the pathogen and thus gradual death. This is an indication of hyperparasitism. It means that hyperparasitism of the pathogen, even when it was inoculated before the antagonist could mean that the pathogen will pose little or no problem even if it arrives on the phylloplane of maize plant well before the antagonists. The growth of the antagonist might mean competition for space as its mode of action against the pathogen.

The inability of the pathogen to grow in certain spaces not yet colonized by *T. pseudokoningii* might be due to the yellowish-green metabolite produced by the antagonist which suggests antibiosis as a mode of action which includes mycoparasitism and competition for space and substrate. *T. pseudokoningii* antagonistic effect against *F.verticillioides* may be due to the fact that the antagonist was able to colonize as much space as possible before the arrival of the pathogen. Competition for space was involved in its mode of action against *F.verticillioides*. *T. pseudokoningii* exhibited hyperparasitism on this pathogen by growing faster and stopping growth of the pathogen by the 3rd day. This observation was also noted by Peluola (2005) when *T. pseudokoningii* was used against seedborne pathogens of cowpea. This present study suggests the versatile and broad spectrum activity of *Trichoderma* species. Many researchers reported the potential values of *Trichoderma spp* as bioagents for the protection of various seed and soil borne diseases of crops (Latunde-Dada, 1993; Adekunle, 1998; Adekunle *et al*., 2001; Howell, 2003; Bankole and Adebanjo, 2004; Peluola, 2005).

The effectiveness of the two *Trichoderma* spp used against *F. verticillioides* in this study may be due to their fast growth and high sporulating ability. Competition for space, food and hyperparasitism are their mode of actions against the pathogen.

Results obtained from the *in vitro* study showed that *P. fluorescens* could be used effectively against this seed, air and soil-borne pathogen of maize. *P. fluorescens* when paired with *F.verticillioides* achieved a remarkable and almost total inhibition of the pathogen. Though it was not a fast-growing organism, it was able to stop further growth of the pathogen at a distance by the third day after pairing. It is conjectured that its inhibitory effect may be due to a metabolite secreted by the bacterium into the

medium that brought about the change in colour of the whitish fluffy mycelia of *F.verticillioides* to purple. From the end of the first week of observation to the second week, *P. fluorescens* maintained its inhibitory action on the growth of the pathogen. It was not determined if the release of the metabolite was due to the presence of the antagonist or it was a normal secretion of metabolites by the target pathogen in reaction to the presence of the antagonist. It was the ability of *P. fluorescens* to produce the metabolite that enhanced its usefulness as a biocontrol agent since it caused inhibition of growth of the target pathogen at a distance from their site of application.

In this study, it was observed that the effect of *P. fluorescens* was not noticed early but it exhibited a sustainable and steady action on the pathogen. *P. fluorescens* possesses the potential to control the disease, but its usefulness/ efficacy will be better felt when the disease is likely to follow the disease progress curve.

Species of *Pseudomonas* are known to produce siderophore (Pseudobactiri) which may deprive the pathogen of iron (Upadhayay and Rai, 1988), which effectively controlled *Pythium aphanidermatum, Verticillium dahlia* and *Alternaria spp* as cited by Ajibade (2002). Cultures of *Pseudomonas sp* inhibited the hyphal growth and the production of fruiting bodies (acervuli) by the fungi. The siderophore-mediated antagonism was found to be the prominent mechanism of antagonism in the biocontrol agent *P. fluorescens* against many pathogens of plant (Prescott *et al*., 2002).

Prescott *et al.* (2002) reported that a strain of *P. fluorescens* carries the gene of *Bacillus thuringiensis* toxin. It produces an intracellular protein toxin crystal (the parasporal body) during sporulation which can act as a microbial insecticide for specific insect groups. It is weakly toxic to insect pests such as the cabbage loop and the European corn borer (Prescott *et al.*, 2002).

The inhibition of mycelial growth of *F.verticillioides* by *Pseudomonas fluorescens* showed the effectiveness of the antagonistic microorganism as biocontrol agent. The antibiotic properties produced by a strain of *P. fluorescens* that the secondary metabolites produce antibiotics phenazine (PHE) 2, 4-diacetyl phloroglucinol (PHL) and siderophore phyoverdin (PYO). These are produced in King's B medium and Succinic acid media (Bourah and Kumar, 2002). These antibiotics were said to be effective antifungal and antibacterial *in vitro* and *in vivo* and increased plantlet growth.

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The combination of *P. fluorescens* and *T. harzianum* was found to be highly effective in controlling the wilt of chickpea in the presence or absence of root-knot nematode leading to 31-57% increase in yield (Tingay *et al*., 2002). A strain of *P. fluorescens* reduced the incidence of fireblight of pear and apple caused by *Erwinia amylovora (*Johnson *et al*., 2002). The proven ability of *P. fluorescens* to provide diffusible substance toxic to the fungus supports the hypothesis and recommends *P. fluorescens* as an important bioagent that can be used against *F.verticillioides*.

B.subtilis was the least effective as an antagonistic organism in the control of *F.verticillioides* because of its inability to inhibit the growth of *F.verticillioides* significantly. This is not in line with the observation of Aigbe (1990) that *B. subtilis* controlled *Sclerotium rolfsii*. Peluola (2005) reported that the recovery of *Colletotrichum destructivum* mycelia was less than 20% of the plate in the subcultured plate of *B. subtilis* while high heat treatment with *B. subtilis* inhibited the growth of *Colletotrichum capsici* and *C. destructi*vum by 19.0% and 19.4% respectively. The role of *B. subtilis* in inhibiting plant pathogens had been reported. Mohammed and Amusa (2003) reported that *B. subtilis* inhibited the mycelial growth of a range of seedborne fungal pathogens with zone of inhibition ranging from 35.5 to 57.8%. *B. subtilis* possesses a lytic factor in its wall. Young *et al*. (1974) reported at least five antibiotics produced by *B. subtilis* which are subtillin, bacitracin, bacilli, subtenolin and bacilomycin. These antibiotics might be responsible for inhibition observed in this study.

B. subtilis is generally known to inhibit the growth of other microbes by antibiosis. The restriction observed on the growth of *F.verticillioides* in this *in vitro* study may be due to the colonization of the growth substrate by *B. subtilis. B. subtilis* grew faster than the *P. fluorescens.* The antibiotics secreted by *B. subtilis* into the growth medium stopped the hyphal growth of *F.verticillioides.* The effect of the antibiotics was not noticed in the *B. subtilis* used in this study.

The observation from this study could be as suggested by Onyeka (1997) that *B. subtilis* produces antibiotics in response to the presence of selected organisms and more so that the culture medium composition used in the study does not support the synthesis of antibiotics, as bacteria perform best on nutrient agar.

The organisms that adapted to the same habitat as the pathogen are usually preferred to foreign ones those from other habitats as antagonists. This is so because the foreign antagonists in most cases are not easy to manage in their new ecosystem. This suggests that the environment plays an important role in the choice of antagonists. It is better for any organism to operate in its adapted habitat as an antagonist where it is most likely that the pathogen and the antagonists are sensitive to the same environmental conditions. In case the antagonist (bioagent) broke down to become pathogenic it might be a weaker pathogen than the pathogen itself since it is epiphytic in nature and does not penetrate into the tissues of the host plant.

The mechanisms of antagonism exhibited by almost, if not, all the antagonists against the pathogen agreed with the findings of Leslie *et al.* (2005) which stated that some antagonists may be effective biocontrol agents against one pathogen by more than one mode of action and this invariably broadens the antagonist's capability for biocontrol. The overall behaviour of the antagonists against *F.verticillioides* pathogen agrees with the report of Leslie and Summerell (2005) on the possibility of an antagonist controlling more than one pathogen on a plant. They suggested that the mode of action of such an antagonist may be non-specific and may be better than one with a specific mode of action.

T. pseudokoningii is effective in the midst or in the abundance of the pathogen due to its ability to colonize the growth media which both it and the pathogen depend on and also its ability to overgrow the pathogen thereby depriving the pathogen of oxygen and all other things essential for the organism's growth. *P. fluorescens* was observed to be as effective antagonist as it was observed in the first trial. The color change due to the effect of this antagonist on the pathogen was also observed. The second trial was a confirmation of all that were observed in the first trial.

Statistical analysis showed that any of the four antagonists is a good antagonist against the pathogen. The fact that inoculating the antagonist before the pathogen being better than the other two types of inoculation agreed with the work of Sobowale (1994) who believed that there were no biocontrol agents that have high enough competitive ability to displace an already established pathogen. The time lag between the inoculation of the antagonist and the inoculation of the pathogen might have contributed to the success recorded with the antagonists against the pathogen. This agreed with the emphasis on the importance of time-lapse between the arrival of the

antagonist and later pathogen on the phylloplane. This allows adequate increase in sporulation and subsequent colonization of the plane by the antagonist before the arrival of the pathogen.

The data on the growth of *F.verticillioides* 48 hours after inoculation on leaf extracts of the botanicals tested showed that there were no significant differences in the treatments with *P. angulata, T. diversifolia, M. jalapa* and *S. occidentalis* at different concentrations. The growth of the pathogen (*F.verticillioides)* was the same on agar incorporated with the extracts. The results obtained when the incubation period was 72 hours, was not significantly different from that obtained at the 48 hours, no significant difference was recorded in either the treatments or the concentrations.

At 96 hours after inoculation on the growth medium incorporated with plant extracts, there was a significant difference in the concentration effect. The leaf extract of M*irabilis jalapa* at a concentration of 25% inhibited the growth of *F.verticillioides* when compared with the control which had the highest growth measurement, its normal growth. It could be deduced from this that the highest concentration of *M. jalapa* water extract from the leaves had good inhibitory effect on the pathogen, *F. verticillioides*.

At 96 hours, 25% *M. jalapa* was significant in reducing the mycelial extension of the pathogen though not as high as the effect of benomyl on the pathogen. But at this level, this concentration can still serve as alternative to synthetic fungicide, benomyl. The 30.0 and 20.0% of *M. jalapa* were significantly higher in action in inhibiting the pathogen. Its efficacy can also stand the test of time, as the longer period of incubation still showed the plant extract's ability to inhibit the pathogen

The S*enna occidentalis* was able to inhibit the growth of *F.verticillioides* at a lowest concentration and highest concentration; the highest concentration did better against the pathogen. *Senna occidentalis* leaf extracts have demonstrated an anti-inflammatory, hypotensive, smooth-muscle relaxant, antispasmodic, weak uterine stimulant, vasoconstrictor, and antioxidant activities in laboratory animals (Tona *et al*., 2004). *S. occidentalis* and benomyl were most effective in reducing ear rot incidence. They had a score of 1.00 each. , which makes both of them highly effective against the

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disease. This indicates that *S.occidentalis* is a better replacement for benomyl commonly used.

Tithonia diversifolia could not inhibit the growth of *F.verticillioides* as much when compared with other plant extracts. It however reduced the growth of *F.verticillioides* at the highest concentration, 25%. *T. diversifolia* has been reported for its improving effect on soil fertility in Kenya (Nziguheba *et al*., 2002), and as supplement to phosphorus in maize (Thor Smestad *et al*., 2002). This implies that high concentrations of Mexican sunflower leaf extract will be best for inhibition purposes as a result of the high concentration of phosphorous in its tissue. This suggests that *T. diversifolia* is likely to be better used in yield and plant growth enhancement.

The longer time of incubation and at highest and lowest concentrations used in this study showed *T. diversifolia* stimulatory effect on the *F.verticillioides*. Instead of inhibiting its growth, the *F. verticillioides* mycelia became fluffy and more beautiful off pink-white mycelia. The reason for this is not farfetched. *T. diversifolia* produces a substance which acts as growth stimulator for the pathogen. The dry *T. diversifolia* is not good in controlling *F. verticillioides*, the plant extract at lower concentrations may be better for the inhibition.

In the present study, Quality Protein Maize (QPM) varieties, ART-98-SW4-OB; ART-98-SW5-OB; ART-98-SW6-OB, ILE 1-OB; TZPB; OBATANPA ; and ACR99TZL(a local check), used to study their resistance and susceptibility to *Fusarium* ear rot disease incidence, effect on seed germination, seedling vigour, field emergence and grain yield. Varieties ART-98-SW5-OB, ILE 1 – OB and ACR99TZL were observed to be resistant, susceptible and moderately resistance to the disease respectively. These three varieties were used for the rest of the experiment to check the seed coating methods and effects of the bioagents and botanicals. Under field conditions as seed treatment and the application of the inoculum and botanical at 6 week old of the plants to evaluate field emergence, ear rot disease and grain yield for two years.

The results varied with cassava starch coating, water suspension and powder treatments. The water suspension of *P. fluorescens* was the most effective of the different formulations that had *P. fluorescens,* in reducing the incidence of *F.*

verticillioides and increased the seed germination and seedling vigour compared with the chemical control using benomyl. Similar results have been reported in sorghum by Raju *et al.*, 1999 and in rice and also through the use of pure culture of *P. fluorescens* (Praveen *et al*., 2000; Nayaka *et al.,* 2008). Several researchers have reported that an application of fluorescent *Pseudomonas* to seed (Callan *et al*., 1990), soil (Hebbar *et al)* and foliage (Gnanamanickam and Mew, 1992; Clarkeson and Lucas, 1993; Praveen *et al*., 2000) also controlled several plant diseases. Lower disease incidence and resultant yield increase in seeds treated with microbial agents might be attributed to rapid multiplication of antagonists in the soil and its colonisation in the roots of seedlings, thereby preventing the establishment of the pathogens in the rhizosphere. Its effect on the phylloplane was also observed cause of its ability to control the foliar diseases. This proves that the cassava coating method of applying the bioagents can serve as better alternative to the use of benlate to control this fungal problem. The cassava coated maize plots had 0.14 kg per row. Water suspension and powder treatments had the field weight of 0.13 kg yield, which suggest that the shortness and early tasselling and silking have effect on the yield of the plants.

The present results revealed that *P. fluorescens* significantly controlled ear rot disease and also improved field emergence and grain yield in maize grains. Possibly both rhizosphere (to help the root systems) and phyllosphere populations of *P. fluorescens* helped to control disease. Both direct inhibition of the pathogen to systemically induced resistance in maize plants could be involved in control. Similar observations were also made on rice plants (Albouvette *et al*., 1992) in the case of *P. fluorescens*. Increase in yield owing to *P. fluorescens* has been reported in several crops (Gamilel and Katan, 1991; Vidjasekaran and Muthamilan, 1995; Niranjan *et al*., 2004; Srinivas *et al*., 2005).

Pseudomonas fluorescens is known to produce several plant growth regulators such as gibberellins, cytokinins and indole acetic acid (Lifshitz *et al*., 1987; Dubeikoysky *et al*., 1993). Although effective control of ear rot disease and yield increase appear to be dependent on method of treatment used. The overall performance of *P. fluorescens* under field conditions was consistent during the study. It showed a considerable reduction in disease epidemics compared with untreated seeds. This study demonstrated that *P. fluorescens* meets several attributes essential for an effective

biocontrol agent. One feature is that the biocontrol agent must colonise the substrate or plant part targeted by the pathogenic organism. Its incorporation in the soil, plant environment and the presence on the phylloplanes reduced or minimised the opportunity the spores of *F.verticillioides* who have gotten to have access to the ears. The biocontrol agents on maize plants fulfilled another criterion, in that the biocontrol agent must be active under natural environmental conditions such as air, pH, moisture content and temperature requirement, so that growth of the biocontrol agents and antagonists coincide.

However, previous studies have indicated that species of *Pseudomonas* may be more effective in certain ecological niches, such as specific soil types and temperature (De Weger *et al*., 1987, 1989). These results from this present study also support earlier reports that certain strains of *P. fluorescens* inhibit *F. verticillioides* growth (Calistru *et al*., 1997). This study has shown that *P. fluorescens* is ecofriendly (it was isolated from both water and maize rhizosphere at the beginning of this study) and much more effective against *F. verticillioides* and can be used as an alternative to fungicides to control toxigenic moulds.

The activities of species of *Trichoderma* showed good action in the control of the incidence of *Fusarium* ear rot disease on the field. The incidence of *F.verticillioides* was drastically reduced with the *Trichoderma* species compared to the untreated control. The action and ability of *Trichoderma* to act as mycoparasites of hyphae and resting structures of plant pathogens have been demonstrated both in *in vitro* and natural soil (Sampere and Santamarina, 2008; Akinbode and Ikotun, 2011) .

Ojha and Chatterjee (2011) reported the direct attack and lysing of mycelium of *F.verticillioides* by an isolate of *T. harzianum*. The hyphae of *T. harzianum* grew towards and coiled around the hyphae of the pathogen, *in vitro* and in sterile soil, causing partial degradation of the *F.verticillioides* cell wall (Ojha and Chatterjee, 2011). *Trichoderma* species are good sources of various enzymes such as exo- and endo-glucanases, cellobiase, chitinase and growth stimulators (Mari Aidemark e*t al.,* 2010). This proven ability of species of *Trichoderma* to produce diffusible substances toxic to other fungi *in vitro* and even in organic substrates in soil (Mohiddin *et al.*, 2010), strengthens and suggests the importance of the native *Trichoderma* in biocontrol. Other workers have found that there are great variabilities between the

different strains of *Trichoderma* in their ability to colonize their rhizosphere. It was observed that there was an increase in susceptibility of the plants to the disease probably due to the absence of the organism in the maize plant phyllosphere; this brought the idea of spraying with the bioagents at 6 weeks after planting.

In this study, *Bacillus subtilis* had a limited antagonistic effect on the *F.verticillioides* it was tested against, as such was not used in further studies conducted on the field. Of the three microorganisms used for seed treatment *T. pseudokoningii, T. harzianum* and *P. fluorescens*, the least effective bioagents used as seed treatment against *F. verticillioides* in the study was *T. harzianum*. In the applications of the bioagents on the seeds, the powder formulation was the least effective with the *Trichoderma* species used. The water suspension in the bacterium, *P. fluorescens* was effective.

The control tasseled earlier than the treated plots, this suggests that it is possible for treatments to have effect on the tasselling time of the maize plants. This was either to delay the tasselling by the different treatments used in this study, so that the cobs would have escaped infection by the pathogen before silk initiation. Entry of *Fusarium* spp. into maize ears can occur by growth of mycelium down silks to the kernels and cob (rachis) from spores germinating on the silks (Reid *et al*., 2002). For *F. verticillioides*, colonization of corn does not occur until after the onset of silk senescence (Reid *et al*., 2002). The delay to tassel means delay to silk, which help the silk to escape the *F. verticillioides* spores in a common planting season where the other maize planted would have accommodated the spores.

Infected seedlings are usually stunted, wilting, chlorotic, and have pale green or purple leaves and poor roots (CIMMYT, 2007). Symptoms of *Fusarium* stalk rot in mature plants are difficult to distinguish from those of other stalk rots, but the internal tissues of affected stalks are usually reddish-brown and rotted. The discolouration may also be seen on the surface of the stalks near nodes. Stalks are weak and lodge easily. There was no significant difference in the leafspot disease of the plants, this may be probably due to the fact the maize varieties used were resistant to the disease At harvest, the severity of Fusarium ear rot disease of maize was greatly reduced by *T. pseudokoningii* which can serve as a good alternative to the synthetic fungicide, benomyl used.

The main objectives of this study were achieved. There was the successful isolation and identification of antagonistic microorganisms from the soil which were tested *in vitro* and *in vivo*. Four plant extracts (*S. occidentalis, P. angulata, M. jalapa* and *T. diversifolia*) were assessed and the efficacy of the antagonists treatments were compared with the different concentrations of the plant extracts. For these concentrations, the effect of extract was also investigated. As a result of this study, *P. fluorescens, T. pseudokoningii* and *S. occidentalis* can be used successfully to prevent maize ear rot caused by *F.verticillioides* as observed *in vitro* studies. Mycelia suspension was assessed and the efficacy of the antagonist treatments was compared to a chemical fungicide, benomyl. Seed treatment application method was studied along with the time of its application.

CHAPTER SIX

CONCLUSION

As a result of this study, the four microbial agents: *Trichoderma pseudokoningii*, *T. harzianum*, *Pseudomonas fluorescens* and *Bacillus subtilis* bioassayed in laboratory, *P. fluorescens* had the highest inhibition percentage of 51.7%, *T. pseudokoningii* 44.7%, *T. harzianum* 35.1% and least effective on *F. verticillioides*' growth was *B. subtilis.* And on the field as protectants against *F. verticillioides, P. fluorescens* at 1.0 x 10^9 cfu/ml significantly inhibited mycelial growth by 51.8% followed by *T. pseudokoningii* at 7.1 x 10^8 spores/ml by 44.7%; which indicated its potential to effectively control *F. verticillioides* in the field.

Aqueous extracts of four botanicals: *Tithonia diversifolia*, *Mirabilis jalapa*, *Senna occidentalis* and *Physalis angulata* evaluated in laboratory and on the field for fungicidal activities against *F. verticillioides* showed *Senna occidentalis* at 15.0% concentration caused the most significant mycelial growth inhibition of 20.1% followed by *M. jalapa* 16.7% at 30.0% concentration. The inhibitory property of *S. occidentalis* produced 3.1% yield increase compared with the control. Conversely, *T. diversifolia* stimulated rather than inhibit mycelial growth of *F. verticillioides* by 1.9% at 30.0% concentration resulting in yield reduction by 1.4%. The effect of the botanicals on the pathogen varied from no effect, stimulatory to inhibitory. The highest protection from ear rot in maize seeds treated with botanical extracts was from *Sienna occidentalis* at all concentrations.

The three coating methods: Cooked-cassava Starch Slurry (CSS), Water Suspension (WS) and Powder Treatment (PT) used and were compared with a carbamate (benomyl) at 0.5 g/l. The most effective coating method was CSS with significantly lowest incidence of ear-rot of 15.0% followed by WS 20.0% and PT 30.0%. The coating of the seeds with cooked cassava starch and dry ground botanicals gave good germination and plant vigour. *T. pseudokoningii* as microbial agent had highest effect (18.0%) on maize germination followed by 15.3% *P. fluorescens*, 13.3%, *T. harzianum* and 1.6% for *B. subtilis*. *S. occidentalis* and *M. jalapa* as botanical had significant effect of 11.1% and 8.3% respectively on maize germination

compared with control, while *T. diversifolia* had 3.0% and *P. angulata* 0.9% different from the control.

The seeds treatment was a good mode of application of the bioagents to control ear rot. Seed treatment with *T. pseudokoningii* prevented *F.verticillioides* from infecting maize ear and increased yield significantly. *T. pseudokoningii* can be used to control ear rot disease. Also, it is better to introduce the antagonist early or at the same time the seeds are planted so that the antagonists can fight the pathogen before it will develop in the stalk that will most likely transmit it to the ear, since *F.verticillioides* survives in the debris and is a systemic pathogen.

Six Quality Protein Maize (QPM) varieties: ART/98/SW5/OB, ART/98/SW4/OB, ART/98/SW6/OB, OBATANPA, ILE-1-OB, TZPB and ACR/99/TZL (local check) evaluated in the field and screenhouse for resistance to *F.verticillioides* in two planting seasons revealed variety ART/98/SW5 with 15% ear-rot; 13% seedling blight and 18% lodging after tasseling was rated highly resistant. The other five varieties had greater than 30.0% lodging after tasselling; greater than 20% ear-rot and seedling blight, rated moderately susceptible. The QPM varieties and the check reacted differently to *F.verticillioides* infection. ILE-1-OB was most susceptible, ART-98-SW5-OB was most resistant and ACR/99/TZL was moderately susceptible to *F.verticillioides*. ART-98-SW5 is therefore recommended for breeding programmes.

The use of resistant variety-ART/98/SW5, aqueous extract of *Senna occidentalis* at 15.0% concentration and *Trichoderma pseudokoningii* at 7.1×10^8 spores/ml provided best controls of *Fusarium verticillioides* of ear-rot disease of Quality Protein Maize. These controls could be incorporated into integrated management of the pathogen.

In summary, the effect of botanical extracts on the pathogen studied varied from no effect to stimulatory and inhibitory with the water extract. Highest protection from seed and seedling blight and ear rot were obtained when maize seeds were treated prior to planting with the aqueous extract of *Senna occidentalis* at 30.0% under artificial and natural conditions. *S. occidentalis* coated seeds gave highest germination, protection and increase yield significantly than the other botanicals used.

Seed dressing with *T. pseudokoningii* protected the plant from seedling blight and ear rot infection of maize and increase yield significantly. It is concluded that botanicals or microbial biocontrol agents not only controlled ear rot but also increased yield of QPM significantly from the present study. It is recommended for use by the peasant farmers. But, it is noteworthy that bioagents are better at controlling the pathogen than the botanicals. Hence, they are recommended to farmers for use. With the use of bioagents and botanicals, fewer resources would be spent than using exorbitant non-environmentally friendly synthetic fungicides in the control of this disease. It is recommended that husk cover of maize ear needs to be worked upon by the breeders. Husk infection is the only way the pathogen can use to infect the ear after the root, stalk have been protected by seed treatment.

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