Potential for utilization of entomopathogenic fungus, *Beauveria bassiana,* for control of banana weevil, *Cosmopolites sordidus* (Germar)

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A Thesis submitted in partial fulfillment for the degree of Master of Science in Horticulture in the Jomo Kenyatta University of Agriculture and Technology

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DECLARATION

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

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DEDICATION

I give glory to God my heavenly Father for granting me health, grace, wisdom and favor to accomplish this work. I dedicate this thesis with love to my late father Amos Omukoko and to all my heroes who inspired me to treasure education.

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LIST OF ABBREVIATIONS AND SYMBOLS

ANOVA	Analysis of variance
EAHB	East African Highland banana
IITA	International Institute of Tropical Agriculture
IIE	International Institute of Entomology
INIBAP	International Network for the Improvement of Banana and Plantain
MMUST	Masinde Muliro University of Science and Technology
IPM	Integrated pest management
SDA	Sabouraud dextrose agar
Spp	Species
cm	Centimeter
h	hour
cv.	Cultivar
°C	Degree centigrade
g	Gram
Kg	Kilogram
L	Litre
mm	Milimeter
min	Minute
RH	Relative Humidity
GLM	General Linear Model
SNK	Student-Newman-Keuls

ABSTRACT

Banana production in Kenya has been on the decline due to among other factors, pests and disease of which banana weevil is major. Although, entomopathogenic fungi such as *Beauveria bassiana* and *Metarhizium anisopliae* have been used successfully to control various agricultural and pasture pests, lack of effective application system limits their wider application.This study therefore seeks to evaluate the potential of utilizing the fungus to control the banana weevil using infected weevils to disseminate the entomopathogen, *Beauveria bassiana* for control of the banana weevil (*Cosmopolites sordidus)*. Tests were carried out in the laboratory with ten isolates of *B. bassiana* ; ICIPE 273, M 313, M 207, KE 300, M 221, ICIPE 50, M 573, M 618, M 470 and ICIPE 279. Pathogenicity studies were carried out and all the ten isolates of *B. bassiana* tested were found to be pathogenic to adult *Cosmopolites sordidus* causing mortalities of between 20 - 50% when a standard concentration of 1×10^8 was used 40 days post exposure. Isolate ICIPE 273 was the most pathogenic killing 50% of adults, followed by M 313 36%, M 207 30%. The other isolates KE 300, M 221, ICIPE 50, M 573, M 618, M 470 and ICIPE 279 killed less than 20% with ICIPE 279 being the least pathogenic to the adult *C. sordidus*. A virulence test of the best three isolates of *Beauveria bassiana* (ICIPE 273, M 313 and M207) at three concentrations (1×10^8, 3×10^8 and 1×10^9) was carried out in the laboratory. At higher fungal concentrations of 3×10^8 and 1×10^9 adult mortality for all the three isolates was between 35% - 70%. The LC_{50} values were 5.34×10^6, 4.22×10^8 and 8.89×10^8 conidia/ml for ICIPE 273, M 313 and M 207 strains respectively. Lethal time LT_{50} was 31, 34 and 51 days for ICIPE 273, M 313 and M 207

strains respectively. Incubation of dead weevils in a moist environment led to development of mycelia on the surface starting from intersegmental junctions, confirming that the mortality was caused by fungus.In laboratory, the rate of transmission from two infected banana weevils to non infected was between 24% - 26 % for the three isolates (ICIPE 273, M 313 and M 207) for 40days. The current studies show that *Beauveria bassiana* has potential to be used as a biological control agent for the management of *Cosmopolites sordidus*.

CHAPTER ONE

1.0 INTRODUCTION

Bananas constitute a major staple food crop for millions of people in developing countries providing energy as well as important vitamins and minerals. Majority of producers are small-scale farmers growing the crop either for home consumption or for local markets as a source of income for rural people (Karamura, 1998). This crop is also considered as a key component of sustainable agricultural systems in densely populated rainfall zones. On steep slopes, bananas reduce soil erosion and are a principal source of mulch for maintaining and improving soil fertility (Akello, 2008).

Banana production in Kenya has been declining due to environmental stresses, declining soil fertility, poor crop management as well as lack of clean planting material (Seshu *et al.,* 1998). Pest and disease pressures have also increased, reducing the life span of banana orchards (ISAAA, 1996). Diseases include black Sigatoka caused by the fungus *Mycosphaerella fijiensis* and fusarium wilt caused by *Fusarium oxysporum* f.sp. *cubense.*

Out of about 200 insect pests reported to attack banana, the most important is the banana weevil, *Cosmopolites sordidus* (Germar) (Godonou *et al.,* 2000). The other pests of economic importance are the parasitic nematodes (*Radopholus similis, Pratylenchus spp. and Helicotylenchus multicinctus*). Both the weevil and nematode infestation interfere with nutrient uptake and transport resulting in slow growth, reduced fruit filling and

susceptibility to wind lodging hence the need to device control measures for sustainable banana production (Gold *et al.,* 2003).

The yield losses associated with the banana weevil which is the only weevil known to attack banana and plantain range from 40% to 100% (Seshu *et al.,* 1998). Yield loss is due to larvae which tunnel extensively into the rhizome and pseudostem disrupting nutrient uptake in the plant. The destruction of the corm tissue by the larvae, sometimes accentuated by secondary attacks by other insects and micro-organisms leading to an increased risk of toppling (Godonou *et al.,* 2000)

Weevil feeding on rhizome surfaces may detach roots while internal damage may affect root initiation. Direct attack on roots seems unlikely (CABI, 2005). Infested farms show general decline in plant vigour, reduced bunch size, snapping and toppling (Nankinga and Moore, 2000). Banana weevil attack in newly planted banana stands can lead to poor crop establishment. In established fields, weevil damage can result in plant loss due to snapping and toppling, lower bunch weight, mat die-out and shortened plantation life (Gold *et al.,* 2003).

Eggs of banana weevils tend to be oviposited in pseudostem, and secondarily on the rhizome but rarely placed on roots. Larvae can move from mother to daughter plants (Khan and Gangapersad, 2001).

Current research suggests that there is no single control strategy for complete control of banana weevil. Chemical and cultural control methods, which are most frequently used for banana weevil control are not completely effective under subsistence farm level (Seshu *et al.,* 1998). The most commonly used control methods include the use of clean planting material, crop sanitation, agronomic methods to improve plant vigor and tolerance to weevil attack, treatment with neem extract and trapping. Application of these methods has material and labour implications and adoption by resource poor subsistence farmers is often limited (Nankinga *et al.,* 1998).

The indiscriminate use of chemicals has resulted in the development of resistance in insect pests against the insecticides, adverse ecological events affecting beneficial fauna and accumulation of residues in the environment (Gold and Messiaen, 2000). There is need therefore to develop safer and cheaper control alternatives that can be used to complement existing ones (Nankinga *et al.,* 1998).

Entomopathogenic fungi have been used successfully to control various agricultural and pasture pests. *Beauveria bassiana* and *Metarhizium anisopliae* have gained considerable attention as biological control agents for weevils and other agricultural pests (Kaaya and Hassan, 2000). *Beauveria bassiana* has been successfully used as a biopesticide in corn to suppress the European corn borer (Moschetti, 1999). Some isolates of *B. bassiana* were highly virulent to the banana weevil suggesting potential for their utilization in it's management. However, lack of an effective delivery system for entomopathogenic fungi

limit their wide application hence the need to develop an economically feasible mass-production and delivery system for their use as bio-pesticides (Gold *et al.,* 2003).

Several application methods have been used for application of *Beauveria bassiana* to the adult banana weevil with mortality rates of 19% - 82% recorded. These methods include direct spraying on the insect, spraying of the soil and immersion of pseudostem traps in spore suspension, as well as dry fungal spores on solid substrate such as rice or cracked maize (Nankinga *et al.,* 1998).

Application methods influence the infectivity of *Beauveria bassiana*. Spraying weevils with spore suspensions resulted in a higher mortality of 56 -62% than exposing weevils to pseudostem traps, which gave 10-19% after four weeks (Nankinga *et al.,* 1998). Exposing weevils to dry maize and rice based formulations resulted in nearly 100% mortality within three weeks (Gold *et al.,* 2003). Investigations were carried out in Uganda to evaluate the efficacy of maize, soil-based and oil formulations of isolates of *B. bassiana* for the control of the banana weevil. Application of maize formulation proved most effective, reducing the weevil populations by 63-72% within eight weeks after a single application. The soil based formulation was intermediate while the oil formulation was least effective (Nankinga and Moore, 2000).

Entomopathogenic fungi are sensitive to sunlight, high temperatures, soil amendments, organic material and moisture levels. *Beauveria bassiana* grows well between 19-30 ^{o}C,

it is adversely affected by temperatures above 37 °C and fungal establishment is reduced following application when temperatures are high (Gold *et al.,* 2003). This limits the use of sprays as well as dry fungal substrates since the fungi loses its persistence under unfavorable conditions of sunlight, temperatures and soil (Nankinga *et al.,* 1998).

Weevils require moisture and are very susceptible to desiccation hence burrow in the soil or find refuge in leaf sheaths, plant tissues or crop residues (Okech and Gold, 1996). It is more feasible to apply entomopathogens against the adults hence *Beauveria bassiana* is most commonly used to target adult weevils and it often requires repeated applications as a biopesticide (Gold *et al*., 2003).

1.1 Statement of the problem

Chemical and cultural methods used for the control of banana weevil are not completely effective under subsistence farm level. Cultural control methods have high costs, labour and material requirements hence adoption by resource poor subsistence farmers is limited. Chemicals have resulted in the development of resistance in insect pests, as well as residues in the environment. There is need therefore to develop safer and cheaper biologically based pest control alternatives to complement existing ones. No biopesticides so far has been recommended against the banana weevil, however recent laboratory and field evaluations of *Beauveria bassiana* have shown it's potential for use as a biopesticide but it lacks an efficient delivery system to target the hiding weevils.

Very little work has been done on the behavior of *Beauveria*- infected weevils and their potential to assist in transmitting the fungus to weevil population in the field.

1.2 Justification of the Study

The banana weevil has been recognized as a major constraint to banana production. Recent research has documented that *Beauveria bassiana* can control the banana weevil. The three main methods of delivering *B.bassiana* in the fields were through the application of the fungus to banana planting material, pseudostem and corm traps, and on soil around the banana plants with high rates of mortality recorded of up to 97%. The exposure of *Cosmopolites sordidus* adult to dead infected weevils was studied in Uganda and it resulted in mortality rates of between 64 - 98%, whereas exposure to live infected weevils had mortality rates of 24 - 82%. Infected weevils as they move have an advantage of targeting weevils where they hide, while the chlamydospores produced after death of the host, can maintain the fungus in a viable state within the host cadaver which can then be used for further infection to healthy weevils. This information is important in providing a method for field delivery of this fungus for effective management of banana weevils. This study sought to investigate the behaviour and potential of *Beauveria*-infected weevils in the transmission of the fungus to non-infected ones in the laboratory.

1.3 Objectives

1.3.1 Overall Objective

The overall objective of the study was to evaluate the potential of *Beauveria*-infected banana weevils in the dissemination of the fungus and management of banana weevil.

1.3.2 Specific objectives

The specific objectives of the study were:

1. To assess pathogenicity of 10 isolates of *Beauveria bassiana* to the banana weevil
2. To determine the dose responses of three promising isolates of *Beauveria bassiana* to the banana weevil
3. To determine the dissemination capacity of infected adult banana weevil to non-infected adult stages in the laboratory.

1.4 Hypotheses

The following null hypotheses were tested:

- The *Beauveria bassiana* isolates do not differ in their pathogenicity to the banana weevil.
- The response of banana weevil to *Beauveria bassiana* is not dose dependant.
- Infected adult banana weevils can not transmit the fungus to non-infected adult stages in the laboratory.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Banana weevil

2.1.1 General description

The banana weevil, *Cosmopolites sordidus* (Germar) belongs to the order Coleoptera, family Curculionidae (Booth *et al.,*1990; Stover and Simmonds, 1986) and is a major insect pest in all the banana growing areas of the world especially to the highland cooking bananas and plantains (Budenberg *et al.*,1993; Kaaya *et al.,*1993; Okech and Gold, 1996). The banana weevil originated in South-East Asia and has spread to all banana and plantain production regions in the tropics and subtropics and is the only species known to attack bananas (CABI, 2005; Gold *et al*., 2003). In East Africa distribution of the weevil is influenced by altitude and temperature and it is usually rare or absent at altitudes above 1500 m (Okech and Gold 1996; Gold *et al.,* 2002a). Salient features of the weevil include narrow host range**,** nocturnal activity and high susceptibility to desiccation (Gold *et al*., 1998; Gold *et al*., 2003).

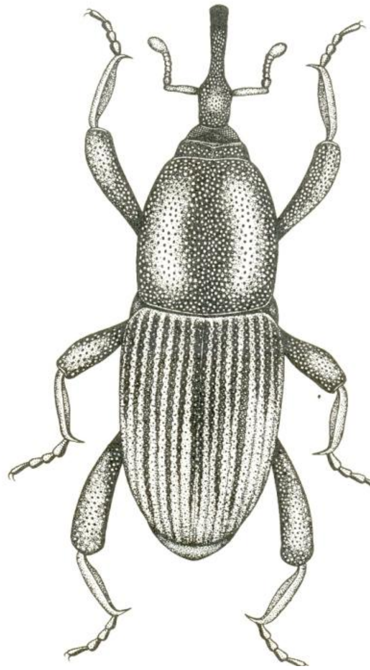

Figure 1: Adult banana weevil (Source Gold *et al.,* 1998).

2.1.2 Biology and life cycle

The banana weevil displays a classical 'k' selected life cycle with a long lifespan,low fecundity(< 2 eggs per female per week) and a slow population growth with a high mortality at the larval stages but many adults may live upto four years (Gold *et al.,* 1998; Gold *et al.,* 2003; CABI, 2005). Dissemination is primarily through passive movement of infected material containing eggs, larvae, pupae or the adults (Nankinga *et al*., 1994). Adults are free living, have functional wings but they rarely fly. They commonly crawl over the soil surface and vegetation feigning death when disturbed (Gold *et al*., 1998). The adults are most often found in the leaf sheaths, at the base of the banana mat or associated with crop debris and may survive for extended periods without feeding but die within 72 hours when maintained on dry substrates (Gold and Messiaen, 2000; Khan

and Gangapersad, 2001). Preference for moist environments may explain why weevil densities are higher in mulches and also during the rainy season (Seshu *et al*., 1998; CABI, 2005). Rainfall is believed to increase adult activity, with more females found in fields during the rainy season reflecting sexual differences in behavioral patterns (Gold *et al*., 1998; Gold *et al*., 2003).

The banana weevils are attracted to their host by plant volatiles; especially those emanating from damaged corms (Budenberg *et al.,* 1993). Males produce an aggregation pheromone that is used for host finding and female attraction (Karamura*,* 1998). The banana weevil undergoes complete metamorphosis. Egg, larvae, pupa and adult stages occur within the banana plant (Okech and Gold, 1996). Density dependent factors appear to influence oviposition and environmental factors may impede weevils from reaching their ovipositional potential. Eggs are laid throughout the year at a rate varying with temperature. The eggs are laid singly mostly in the leaf sheaths near the base of the pseudostem and the corm of banana plant (Okech and Gold, 1996; Gold *et al*., 2003). Under field conditions, egg density is greatest on flowering plants (CABI, 2005), although the weevil may oviposit on all stages of the banana plant ranging from young suckers to mature banana plants, freshly cut rhizomes and crop residues which may receive as many as 200 eggs from a single stump (Gold *et al*.,1998; CABI, 2005).

Eggs hatch in six to eight days and the young larvae tunnel into the rhizome (Khan and Gangapersad, 2001; Gold *et al*., 2003). The emerging larvae after 3-7 days preferentially

feed in the rhizome, but will also attack the true stem and occasionally the pseudostem. The larvae pass through five to eight instars and complete development in 23-33 days spending between three and five days in each instar (Okech and Gold, 1996; CABI, 2005).

Temperature is the most critical factor determining developmental rates. Other factors include: relative humidity, cultivar, age of plant, food quality and population density (Gold *et al*., 1998). Egg development does not occur below 12°C and this threshold may explain why the banana weevil is rarely encountered above 1500m above sea level. Overall the egg to adult period lasts for 6-8 weeks (Okech and Gold, 1996; Gold *et al*., 1998).

Adults are fully hardened in 5-7 days (CABI, 2005), and the banana weevil continues to breed throughout the year, provided that food supply is plenty. However oviposition rate is reduced during the cool season (Okech and Gold, 1996). Sexual maturity of females has been attained 5-20 days after emergence and for males at 18-31 days (Gold *et al*., 1998; CABI, 2005). The emerging adults are nocturnal, living freely in soil, feeding and ovipositing at night but clustering and mating throughout the day (Khan and Gangapersad, 2001; CABI, 2005).

2.1.3 Host range and pest status

The banana weevil primarily affects bananas and plantains. However, in scarcity of primary hosts, the pest may switch to alternative hosts such as Manila hemp (*Musa*

textiles (Nee)), sugarcane (*Saccharum officinarum* L.), yam (*Dioscorea batatas* (Dene)) and Irish potato (*Solanum tuberosum* L.) (Okech and Gold, 1996; Akello, 2008).

This pest causes extensive damage in all the economically important cultivars, especially in plantains (*Musa* spp., AAB genome), East African highland cooking bananas and ensete (Okech and Gold, 1996).

The banana weevil is a major constraint in all banana-growing regions in Africa, America, Southeast Asia, the Indian sub-continent, Australia and Pacific islands (Pena *et al.,* 1991; Okech and Gold, 1996). Differences have been found to exist among weevil densities among farms in a single watershed, suggesting that management may play an important role in determining weevil population levels. The economic importance of the banana weevil being a pest depends, in part, upon genome group, management, locations and farms (CABI, 2005).

Population build-up is slow and weevil problems become increasingly important in ratoon crop. For example, yield losses were estimated at 5% in the initial plantcrop, 9% in the first ratoon, 17% in the second ratoon and 44% in the third ratoon (Gold *et al.,* 1998; Gold *et al.* 2003). In general, yield losses associated with the banana weevil range from 50% to 100% in Africa (Gold *et al.,* 2003), Asia and Latin America (Pena *et al.,* 1991; Seshu *et al.* 1998).

2.1.4 Weevil damage and symptoms

The infestation by *Cosmopolites sordidus* begins at the base of the dying outermost leaf-sheath and in injured tissues at the lower part of the pseudostem. The developing larvae make several longitudinal tunnels in the surface tissue until they are able to penetrate to adjacent inner leaf-sheaths, and then bore into the pseudostem base and rhizome (Khan and Gangapersad, 2001).

Larval tunnels may run for the entire length of fallen pseudostems (Fogain and Price, 1991; CABI, 2005) and larvae feeding within the corm and pseudostem cause damage to the banana plants (Gold *et al.,* 1998). This interferes with root initiation as well as sap flow within the plant, kills existing roots, limits nutrient uptake, reduces plant vigour, delays flowering and increases infection by other pests and diseases (Braimah and Emden, 1999; Gold and Messiaen, 2000). Interior larval damage is likely to affect nutrient transport and stem growth while peripheral damage may adversely affect root development (Fogain and Price, 1991; Valmayor *et al.,* 1994). Rotting occurs through fungal decay in thoroughly riddled corms which are reduced to a blackened mass of tissue and the leaves die prematurely (CABI, 2005). The boring, if severe, so weakens the plant that it is easily knocked or blown over by wind (Bridge and Gowen, 1991; Gold *et al.* 1998).

Infested plants have dull yellow green and floppy foliage. Young infested suckers often wither and fail to develop because of larval feeding and tunnelling between the lateral

roots and the corm, have a curled roll of unopened leaves or growing part of the plant (Afreh-Nuamah, 1991; CABI, 2005).

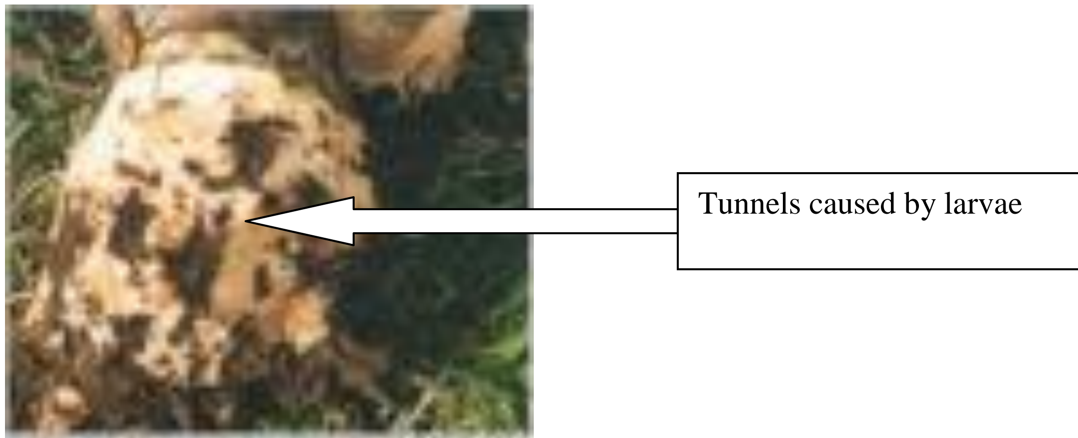

Figure 2: Banana weevil attack on the corm (Source: Gold *et al.,* 1998)

2.2 Management of banana weevil

Management of banana weevil is likely to vary among different farming systems (Gold and Messiaen, 2000), but generally an integrated pest management (IPM) approach has been promoted as the best option for controlling all pests. This includes cultural practices, chemical insecticides, host plant resistance and biological control agents (Gold *et al.,* 2003).

Cultural control is valuable in preventing the establishment of the weevil and is the only means currently available by which resource-limited, small-scale farmers can reduce established populations. Good husbandry practices such as weeding and removing the debris, manuring and mulching produce vigorous banana plants, which have improved weevil tolerance. The use of clean planting stock, sanitation and crop rotation are

cultural methods universally advocated to reduce the pest status of the banana weevil (Seshu *et al.,* 1998; CABI, 2005).

Chemical control which is normally applied close to the base of the plant is the most widespread method for controlling the weevil in large scale production (Nankinga *et al.,* 1994). However there have been reports of resistance to insecticides such as carbofuran which had initially offered good control (Kermarrec *et al.,* 1991). Although insecticides are fast acting and effective, other factors such as high costs and environmental pollution limit their utilization as the sole control method (Allard *et al.,* 1991; Nankinga *et al.,* 1994).

Host plant resistance offers safe and long-term control. It may be integrated with biological control to form an integrated pest management strategy for the control of banana weevil (Ortiz *et al*., 1995). Under field conditions *Musa* genome group AAB has been found to be the most susceptible to banana weevil attack and AA clones were resistant with little damage and limited penetration of the weevil into the corm (Hasyim and Gold, 1998; Sheshu *et al.,* 1998). The highland banana (AAA-EA) is also susceptible to banana weevil attack, while Gros Michel (AAA) has demonstrated peripheral damage, but weevil penetration into the corm is limited (Gold *et al.,* 1994). Although banana weevils freely oviposite on these resistant clones, larval survival rates are lower compared to susceptible cultivars (AAA-EA), suggesting that plant resistance could have been primarily due to antibiosis(Kiggundu *et al*., 2003).

Crop improvement through conventional and non-conventional means to deliver weevil resistant clones to farmers has been initiated in Uganda and Nigeria (Gold *et al.,* 2003). The wild diploid Calcutta-4 and the clones Yangambi-Km5 and FHIA-03 have shown high level of resistance and may be exploited in breeding programs (Kiggundu *et al.,* 1998). Calcutta-4 has already been successfully used in conventional breeding in Nigeria and Uganda while male/female fertility for Yangambi-Km5 and FHIA-03 still need to be determined (Gold *et al.,* 2003).

Breeding for resistance to banana weevil has, however, not featured prominently in any breeding programme (Gold *et al.,* 2002b). This is probably because of the absence of good sources of resistance, and the lack of a simple screening method for weevil resistance. The latter would enable breeders to rapidly pinpoint resistance across the germplasm available, a very slow process due to low seed set, aneuploidy and long crop duration (Kiggundu *et al.,* 1998).

Biological control against *Cosmopolites sordidus* has included the use of parasitoids, predators or pathogens (Hasyim and Gold, 1998). The myrmicine ants, *Tetramorium guinense,* and *Pheidole megacephala* have been used successfully to control banana weevil in plantain in Cuba. The ants have been encouraged to nest in pseudostem pieces that can then be used for their dissemination (Gold and Messiaen, 2000). In western Kenya, egg predators of *Thyreocephalus interocularis* (Eppelsheim), a predator of the weevil were laid in decomposing banana pseudostems and in moist soil below the

banana mulch, but they were not effective in regulating weevil populations (Okech and Gold, 1996).

The predatory larvae of the Javanese rhagionid fly, *Chrysophilus ferruginosus*, has been found to attack *Cosmopolites sordidus* larvae in the laboratory, but not in the field. In harvested pseudostems, *Dactylosternum abdominale* reduced the multiplication of *C. sordidus* by 40-90% at different predator population densities, and *Thyreocephalus .interocularis* reduced it by 42% (Hasyim and Gold, 1998; CABI, 2005).

The potential use of entomopathogenic nematodes for biological control has been well demonstrated (Allard *et al.,* 1991). The nematode genera that are most commonly utilized for biological control include *Steinernema* and *Heterorhabditis*. These genera have a wide host range and have the ability to kill the hosts rapidly. The nematodes *Steinernema feltiae* (Filipjer), *Steinernema glaserii* (Steiner) and *Steinernema bibionis* (Bobier), for instance attacked both banana weevil adults and larvae in the field, resulting in severe mortality even though the larvae were more susceptible to entomopathogenic nematodes than adults (Pena *et al*., 1991; Akello *et al*., 2007). Entomopathogenic nematodes have also been recovered from bananas in Kenya (IMS, 2000), and have a distinct advantage over fungi as they are able to actively seek their host in response to a CO_2 gradient and thus able to find the larvae within the tunnels provided they can withstand the pH changes (Allard *et al.,* 1991).

The potential for biological control of the banana weevil using entomopathogenic fungi has been investigated (Ogenga-Latigo and Masanza, 1996). Fungi are important for control of Coleoptera, because viral and bacterial diseases are rare among beetles (Hajek and St. Leger, 1994). The fungi *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metsch.) Sorokin has been found to be of particular interest. *Beauveria bassiana* in particular has shown substantial promise for development as an effective biopesticide (Nankinga and Ogenga- Latigo, 1996), since it was more effective to the banana weevil than *Metarhizium anisopliae* with a mortality rate of between 50-90% reported both in the field and in the laboratory(Gold *et al.,* 2003).

Fungi when used as biocontrol agents have several advantages. They infect all the developmental stages of their hosts, have a broad host range and present no health hazard to man or other vertebrates as well as the natural enemy complex. However the use of fungi has challenges such as: Difficulty in obtaining enough inoculum for use in field conditions because of sensitivity of fungal spores to desiccation and UV radiation. In addition epizootics caused by fungi are influenced by environmental factors such as temperature and water availability so it is difficult to predict the success of inoculations (Deacon, 1981).

2.3 Banana plant

Bananas originated in Southeast Asia, in the jungles of Malaysia, Indonesia and Philippines. Bananas are members of the genus *Musa* (part of the family *Musaceae*),

they are considered to be derived from the wild species *Musa acuminata* (AA) and *Musa balbisiana* (BB) (Robinson, 1996).

The banana plant is a large herbaceous perennial with an underground stem known as the corm, bulb or rhizome. The corm bears eyes on its middle and upper parts which develop into suckers. The leaf consists of three parts i.e. a sheath or enlarged petiole, a petiole and a lamina or blade. Leaf sheaths of successive leaves closely encircle each other and form a cyclindrical compact structure which is the pseudostem (Swennen and Vulysteke, 2001).The inflorescence forms from the terminal bud of the corm, which develops and rises to the top of the pseudostem turning downwards to form a bunch (Stover and Simmonds, 1986).

The root system, besides its importance in water and nutrient uptake, provides anchorage to the plant. According to Stover and Simmonds (1986), the roots reach a depth of between 15 -60 cm. Due to its shallow root system, damage by weevils, nematodes, diseases may hinder the survival of the plants (Godonou *et al.,* 2000).

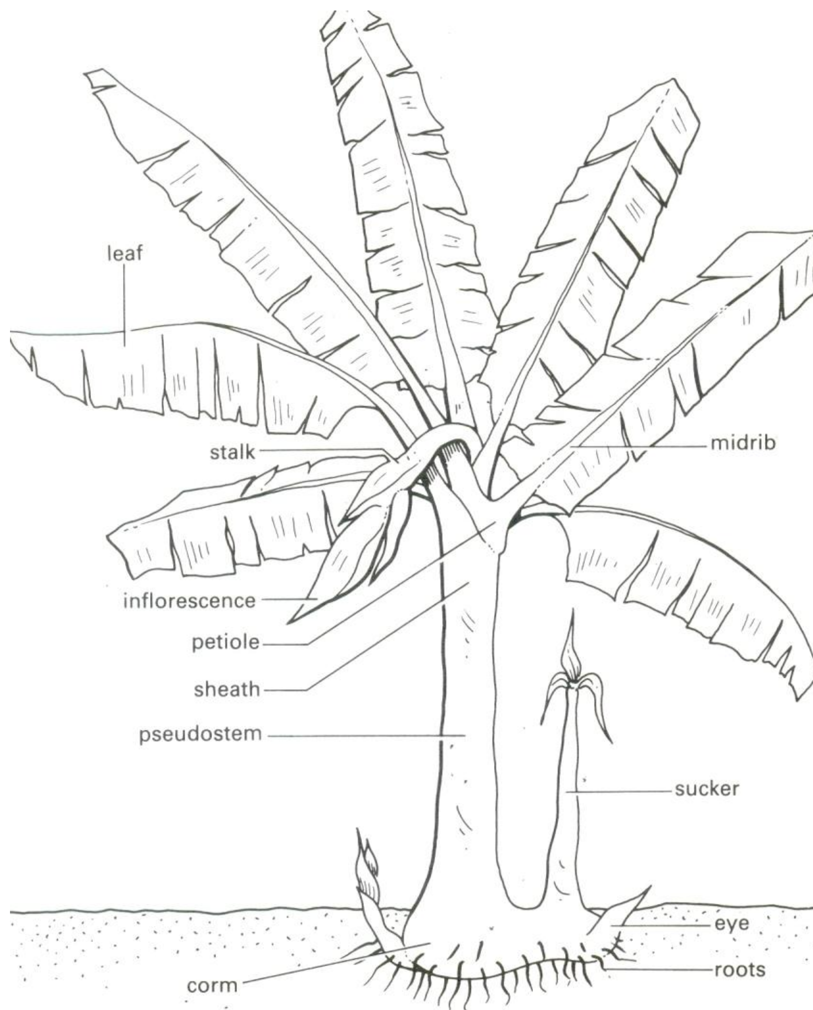

Figure 3: Structure of banana plant (Source: Montcel 1987)

2.4 *Beauveria bassiana*

Beauveria bassiana is an entomopathogenic fungus which occurs naturally in soil and plant residues as well as dead insects and is known to be virulent to over 200 different species of insects (Feng *et al*., 1994; Godonou *et al*., 2000). It can be isolated using soil-baiting techniques (Gold *et al.* 2003) and it has been known since 1835 as the cause of the white muscardine disease of silkworms and other insects (Moschetti, 1999).

Beauveria bassiana is placed in the Deuteromycotina (Fungi imperfect) within the class Hyphomycetes. The genus *Beauveria* contains several species which include: *bassiana, brongniartii, tenella, amorpha* and *velata* (Jaronski and Goettel, 1997).They produce asexually and are distinguished by the morphological and the developmental features of the spore bearing structures, the species are distinguished by the size and shape of the spores (conidia). In *Beauveria bassiana* the conidia arise as blown –out ends of the cells that grow sympodially and are swollen at the base, the conidia are small (3µm), colourless and globose (Deacon, 1981).

Figure 4: *Beauveria bassiana* isolates

As a biopesticide, *Beauveria bassiana* offers an environmentally safe control measure against soil insect pests. Dispersal and infection is by conidia, which germinate and penetrate the insect cuticle or gut wall (CABI, 2005). The spores then germinate and grow directly through the cuticle to the inner body of their host. The fungus proliferates throughout the insect's body producing toxins and draining the insect of nutrients, eventually killing it. After the insect dies, the fungus produces an antibiotic (oosporein)

that enables it to outcompete intestinal bacteria. Eventually the entire body cavity of the insect becomes filled with fungal mass (Gold and Messiaen, 2000).

The chlamydospores produced after death of the host, can maintain the fungus in a viable state within the host cadaver. These spores subsequently germinate to form emergent hyphae that sporulate on the surface of the host to produce new external infective spores (Ferron, 1978). Due to arthropod moulting and movement, the infection can be considered successful only when the fungus has reached the hypodermis (Ferron, 1978; Hajek and St. Leger, 1994). This becomes important when infected weevils are used for transmission of the fungus in that even after their death, the emergent spores can still infect healthy weevils.

Beauveria bassiana has been used in the control of *Dendrolimus* species, the Pine Moth in Pine forests in China. The fungus is locally propagated cheaply on a bran or peat substrate and is applied by air or by ground equipment as a spray or dust (Wong, 2003).

In Russia, Feng and Johnson, (1990) found *Beauveria bassiana* to be pathogenic to the Russian wheat aphid causing mortalities of up to 95% with varying concentrations of 10^4-10^7. *Beauveria bassiana* has also been found to induce high mortality in tsetse flies and evidence of transmission from infected to uninfected flies has been demonstrated (Kaaya and Okech, 1990). In ticks infected with *B. bassiana* and *M. anisopliae* there was

high mortality and reduced fecundity with a low egg viability observed by the surviving ticks (Kaaya and Hassan, 2000).

Strains of *Beauveria bassiana* have been tested against the banana weevil in Africa, America and Australia and found to cause 50-90% mortality within 5-10 days of exposure with the best strains leading to more than 90% mortality in three weeks or less (Gold *et al.,* 2003).

The method of application significantly influenced the infectivity of *Beauveria bassiana*. Exposure of *C. sordidus* adults to infected dead weevils resulted in higher mortality (64 - 98 %) than exposure to live infected weevils (26-82%), while those exposed to pseudostem traps or soil treated with water spore suspensions suffered the lowest mortality (6-19 %). When weevils were exposed to maize or rice cultures of the pathogen without use of pseudostem traps, 100% mortality occured within three weeks post-inoculation (Nankinga and Ogenga-Latigo, 1996).

In Kenya, Kaaya *et al.* (1993), found four local isolates of *B. bassiana* and one of *M. anisopliae* that were pathogenic to the third instar larvae of *C. sordidus* causing 98-100% mortality nine days post exposure to the dry fungal spores. The isolates were less pathogenic to the adult weevils.

From the literature therefore *Beauveria bassiana* has potential to control insect's pests via direct mortality. Since the fungus lacks knockdown effect, it is important to establish the behaviour of infected weevils before their death. This is important to ensure that use of infected weevils does not result in crop damage.

Horinzontal transmission occurs when a pathogen is transferred from individual to individual either through integument contact or natural body openings while vertical transmission is whereby the fungus is transferred from parent to the offsprings (Rath , 2000). Horinzontal transmission may play an important role in the management of banana weevils since they are gregarious, found in clusters, cavities and depressions in the outer sheaths of the banana close to the ground surface and also below the surface.

The exposure of *Cosmopolites sordidus* adult to dead infected weevils has been studied and it resulted in mortality rates of between 64 - 98%, whereas exposure to live infected weevils had mortality rates of 24 - 82% (Nankinga *et al.,* 1998). Studies conducted by Godonou *et al.* (2000) showed a possible dissemination of *B. bassiana* conidia from infected to non infected weevils. Details on how *B. bassiana* can be transmitted from infested individuals to non infected ones will be important in developing an effective delivery system for the pathogen (Nankinga *et al.,* 1998).

In many studies conducted, infected hosts feed less than healthy ones during the disease incubation period, resulting into decreased damage to crops, which is a benefit of using

fungal pathogens to control insects' pests (Hajek and St.Leger, 1994; Ekesi, 2001). Behavioral changes have also been observed during the period of lethal infection for example female carrot flies, *Psila rosae,* infected with *E. muscae* do not lay their eggs near carrot plants as healthy ones do reducing chances for egg survival. Ants change their normal route to avoid contact with other ants or they climb elevated locations just before dying (Hajek and St. Leger, 1994).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 General materials and methods.

3.1.1 Trapping and rearing of weevils

Adult banana weevils were obtained from naturally infested banana plants at Kenya Agriculture Research Institute, Mwea. The weevils were maintained in plastic containers at room temperature in the laboratory for one week before being used in the experiments. The covers of the containers were perforated for ventilation. Banana suckers of mutagato (EAHB) were pared and corm tissue placed in the containers for weevil feeding.

3.1.2 Fungal isolates

The following isolates of *B. bassiana* were used in the bioassays: ICIPE 273, ICIPE 279, ICIPE 50, M 573, KE 300, M 313, M 207, M 470, M 618 and M 221. They were obtained from the *icipe*'s Arthropod Germplasm Centre, Duduville, Nairobi, Kenya. The original cultures were stored at –85°C in 10% sterile glycerol. The details of the isolates are presented in Table 1.

Table 1: Source of *Beauveria bassiana* isolates used in the control of *Cosmopolites sordidus*

Isolates	Year of Isolation	Host/ Substrate	Locality/ Country
ICIPE 273	2006	Soil	Mbita/Kenya
ICIPE 279	2005	Coleopteran larvae	Kericho/Kenya
M573	2005	Soil	Mauritius
KE 300	2007	Hymenoptera	Taita hills/Kenya
M221	2005	Soil	Mauritius
M618	2005	Soil	Mauritius
M313	2005	Soil	Mauritius
M207	2005	Soil	Mauritius
ICIPE 50	1996	*Rhipicephalus appendiculatus*	Rusinga island/Kenya
M470	2005	Soil	Mauritius

3.1.3 Culturing the fungus

The isolates of *Beauveria bassiana* were sub- cultured on Sabouraud Dextrose Agar (SDA) (Appendix 1), and placed in an incubator set at 27^{0}C to allow for sporulation. For media preparation sixty five grams of SDA was dissolved in 1000 ml sterile distilled water in a conical flask, a magnetic stirrer was added and the flask placed on hot plate for the SDA to dissolve completely. The media was autoclaved at 121 °C for 20 minutes and left to cool to 50-60 °C. Antibiotic chloramphenical was added to the medium to keep off any bacterial contamination before pouring. The media was then poured into sterile 90mm petri dishes on a clean bench and left overnight to cool and solidify. A sterile inoculating loop was used to transfer fungal spores from the 3 week old culture onto the freshly prepared SDA media. The inoculated petri dishes were incubated for three weeks at 26°C and 70 % relative humidity for 21 days for sporulation to take place as in Inglis *et al.* (1996).

3.1.4 Inoculum preparation

Conidia were gently scrapped from Petri dishes and suspended in sterile 10ml sterile distilled water with Tween-20. The conidial suspension was transferred with a sterile pasteur pipette into 20 ml sterile universal bottles with glass beads. For counting the spores a dilution of x100 was prepared by removing 0.1ml using a pasteur pipette from 10ml sterile distilled water with Tween-20. The same quantity of 0.1ml was picked from the stock solution and added into the diluent. Vortexing was done for three minutes. Conidial density for each strain was then estimated by placing 0.1ml on the improved Neubauer haemocytometer and counting the spores. For the different concentrations of 1 x 10^8, 3 x 10^8, 1 x 10^9 the concentration formula was used. The spores counted from x100 dilution were multiplied by a constant 2.5 x 10^5 and x 100. This gives the spores in the standard 1 x 10^8. From the standard concentration C_1V_1 and C_2V_2 the number of spores needed for the three concentrations were then calculated.

3.1.5 Spore germination test

SDA was prepared as described above and dispensed into 90 mm diameter Petri dishes. A conidial suspension titrated $3x10^6$ conidia ml^{-1} was prepared, 0.1 ml of conidial suspension. *Beauveria bassiana* was separately inoculated and spread on SDA surface. For each of the ten isolates, three 90 mm Petri dishes containing SDA were incubated at 27 ± 2 °C for 16 h. The culture was then fixed using a few drops of lactophenol cotton blue; cover slips were placed over the area. Conidial germination was assessed by counting the total number of conidia that germinated plus those that had not germinated

in four different fields under a dissecting microscope. Conidial germination was characterized by germ tube development and these were categorized as viable conidia while the non-germinated conidia that lacked the germ tube were categorized as non-viable.

3.2 Evaluation of pathogenicity of isolates

Twenty weevils were infected by dipping them into 20 ml fungal suspension titrated 10^8 conidia ml^{-1} for 11 seconds. The excess suspension was drained and the weevils were transferred to plastic containers. Banana corms were then placed in the containers as food. Control weevils were dipped in sterile distilled water containing 0.01% Tween 20. Weevil mortality was recorded after every three days for 40 days. The experiment consisted of five replicates for ICIPE 279, M 573, ICIPE 273 and KE 300, and four replicates for ICIPE 50, M 470, M 207, M 618, M 221 and M 313.

Figure 5: Banana weevils infected with *Beauveria bassiana* and introduced into plastic containers with banana corm.

3.3 Dose response determination of best isolates

For dose-response relationships, the terms LC_{50} and LT_{50} are used as expressions of virulence. Three best isolates ICIPE 273, M 313 and M 207 from screening work were used for the dose response experiment. Conidia were gently scrapped from fungal cultures and suspended in 10 ml of 0.01% Tween-20 surfactant until all the spores had been harvested. The conidial suspension was transferred with a sterile pasteur pipette into 20 ml sterile universal bottles with glass beads to prepare the stock solution. From the stock solution, three concentrations (1×10^8, 3×10^8 and 1×10^9 conidia ml^{-1}) were prepared. A control treatment of sterile distilled water and 0.01 % Tween 20 was also prepared. The weevils were sorted in four batches of 80 adults. The four batches were arranged randomly among different treatments. For inoculation, each batch of banana weevils was placed in a petri dish and 10 ml of the appropriate conidial suspension was gently poured in immersing the banana weevils. To obtain rapid immersion, the petri dish was shaken gently for 11 seconds after which the suspensions were poured out and the infected weevils introduced to plastic containers with a piece of banana corm as a source of food. The treated banana weevils were observed after every three days for 40 days to record mortality.

3.4 Assessment of Laboratory Transmission of *Beauveria bassiana* by infected weevils

Transmission of *Beauveria bassiana* from infected banana weevils to non infected weevils was examined in an experiment in the laboratory at ICIPE. Plastic containers

with 18 non infected weevils with banana corm were kept in the laboratory for two weeks. Two infected weevils dipped at a concentration of 1x 10^9 for 11 seconds were then introduced for the three isolates ICIPE 273, M 313 and M 207. Control weevils were dipped in sterile distilled water containing 0.01% Tween 20. The experiment was terminated after 40 days but weevil mortality was checked at an interval of three days.

To confirm fungus induced mortality, dead weevils were surface-sterilized in 2% sodium hypochlorite, 70% alcohol and two rinses of sterilized water for 15 seconds before placing them in clean Petri dishes with moist sterile filter papers. Dead weevils were monitored for fungal growth for two weeks and observations recorded. Only dead weevils with fungal growth were considered to have been killed by the fungus.

3.5 Data Analysis

All data for pathogenicity was analysed with analysis of variance using the PROC GLM of SAS statistical software and the treatment means were separated using Student-Newman-Keuls (SNK) test at $p \leq 0.05$ (SAS version 9.1). Percent conidial germination was calculated as: (viable conidia/total conidia) x100. For the dose response experiment data for cumulative percentage mortality was corrected for the corresponding control mortality (Abbott, 1925). Probit analysis (PROC PROBIT) was used to estimate both the LC_{50} and LT_{50} of the isolates with 95 % confidence limits. Transmission experiments data on percentage mortality of weevils was arcsine square root transformed to

normalize data. Differences in treatment means were separated using Student-Newman-Keuls (SNK).

CHAPTER FOUR

4.0 RESULTS

4.1 Conidial germination experiment

In germination tests 81.2-92.7% of spores germinated (Table 2), for all *Beauveria bassiana* strains. There was no significant difference in percentage conidial germination among all the isolates. However there were slight differences among the isolates in germination. M 313 and ICIPE 273 gave the best germination of spores(92 %), followed by M 470, M 207, ICIPE 50 (87 % - 89 %). The rest ICIPE 279, M 573, KE 300, M 221 and M 618 had germination % that ranged between (81.3% - 86.6%).

Table 2: *Beauveria bassiana* **isolates used in the study and their percent germination**

Isolates	% Germination ± SE
M313	92.7 ± 2.1
ICIPE 273	92.2 ± 3.2
M470	89.0 ± 2.4
M207	88.0 ± 2.6
ICIPE 50	87.3 ± 2.6
ICIPE 279	86.8 ± 4.5
KE 300	86.6 ± 3.5
M573	85.4 ± 3.7
M221	82.3 ± 3.7
M618	81.3 ± 3.5
F = 1.13	P = 0.37

4.2 Pathogenicity of *C. sordidus* isolates

All the ten isolates of *Beauveria bassiana* were pathogenic to the adult *C. sordidus,* causing mortalities varying from 20-50% by 40 days post-exposure depending on the fungal isolate. ICIPE 273 was the most pathogenic killing 50% of adults followed by M 313, 36% and M 207, 30% (Figure 6). The rest KE 300, M 221, ICIPE 50, M 573, M

470, M 618 and ICIPE 279 killed less than 30%. Isolate ICIPE 279 was the least pathogenic to *C. sordidus* causing 6% mortality. Control mortality was 5%.The differences among the treatments were highly significant (P = 0.0001).

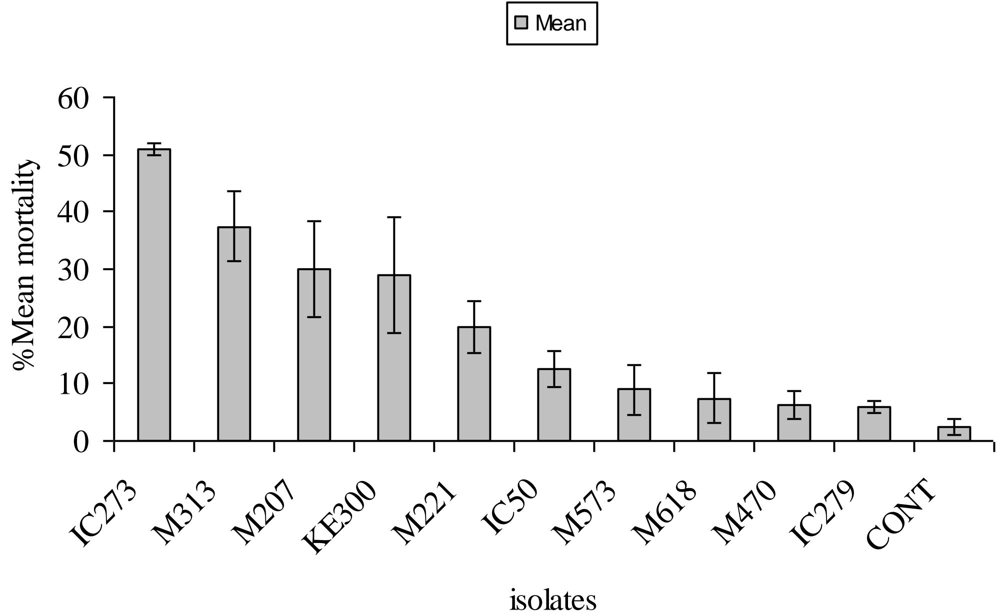

Figure 6: Mortalities in adults weevils infected with 10 ***Beauveria bassiana*** **isolates.**

Incubation of dead *B. bassiana*-treated insects in humidified chambers resulted in development of mycelia on the surface of the cadaver, starting from the intersegmental junctions of the body and legs (Figure 7).

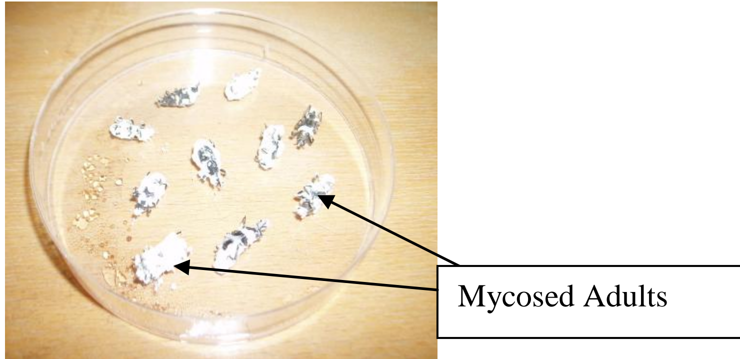

Figure 7: Adult *C. sordidus* infected with *B. bassiana*. Notice fungal growth at intersegmental junctions (arrows)

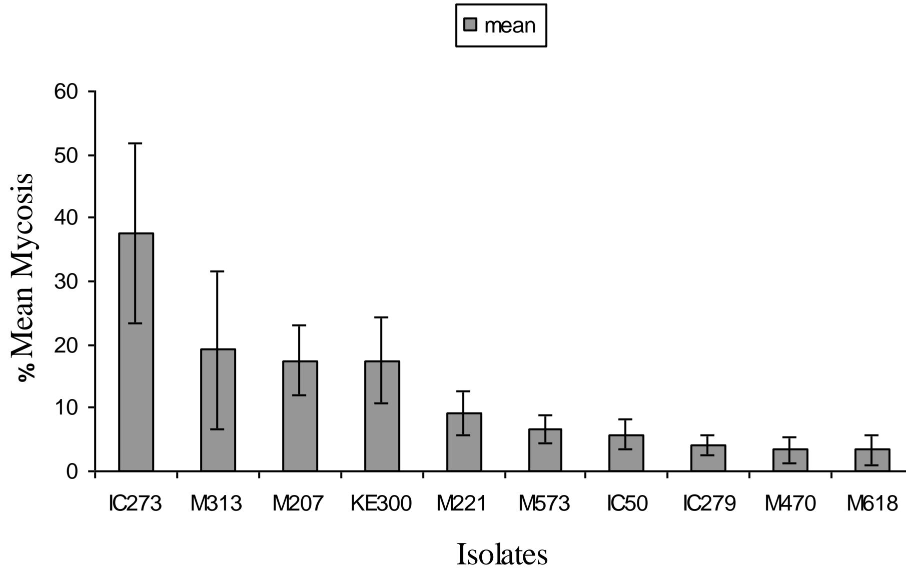

Figure 8: Percent Mean mycosis of Adult banana weevils infected with different isolates of *B. bassiana*

Figure 8 shows the various isolates with the percent mean mycoses that were observed for all the fungal isolates. There was a significant differences ($p \leq 0.05$) among the isolates for mycoses.

Table 3: Percent Mycoses on the dead Adult banana weevil

Isolate	% Body mycoses
ICIPE 273	75
M 573	82
M 313	69
M 207	65
KE 300	69
M 470	56
ICIPE 279	50
M 221	40
M 618	47
ICIPE 50	43

4.3 Percent mortalities for the different concentrations

Table 4: Percent mortality (mean ± SE) of Adult banana weevil at different spore concentrations

Isolate	Spore Concentrations		
	1×10^8	3×10^8	1×10^9
ICIPE 273	50 ± 0.9a	63.1 ± 4.0a	69.3 ± 4.1a
M 313	39.3 ± 2.5b	53.7 ± 4.2a	65.6 ± 4.6ab
M 207	30.0 ± 4.2c	36.2 ± 4.4b	55.6 ± 2.9b
CONTROL	3.7 ± 1.2d	3.1 ± 1.3c	3.7 ± 1.2c

Means followed by similar letter in each column are not significantly different at P =0.05

4.4 Lethal concentration (LC_{50})

LC_{50} is the concentration of a given insect pathogen required to kill 50 % of a large population of test insect. All the three isolates were significantly different since there was no overlap of fiducial limits. ICIPE 273 was the best of the three strains because of its very high level of virulence towards, the banana weevil as indicated by low LC_{50} values (Table 5).

Table 5: Lethal concentrations of three *Beauveria bassiana* strains applied against the adult banana weevil

Strains	LC_{50} (conidia/ml)	95 % FL limits
IC273	5.34×10^6	$4.54 \times 10^6 - 6.24 \times 10^6$
M313	4.22×10^8	$3.45 \times 10^8 - 4.95 \times 10^8$
M207	8.89×10^8	$8.05 \times 10^8 - 9.96 \times 10^8$

None overlapping FL indicate significant differences among Isolates

4.5 Lethal Time (LT_{50})

LT_{50} is defined as the time period required to kill 50% of a large population of the test insect population. This term is often used as a quantitative expression of the virulence of fungi. The LT_{50} values for the three strains of *B.bassiana* that proved to be most virulent are presented in Table 6.

Table 6: Lethal time LT_{50} for banana weevils inoculated with three strains of *Beauveria bassiana*

Strain	LT_{50} (days)	95%FL limits
IC273	31.25	30.67-31.87
M313	34.74	33.84 -35.65
M207	51.07	49.31-53.03

None overlapping FL indicate significant differences among Isolates

All these three isolates were significantly different since there was no overlap of fiducial limits as shown in Table 6. Isolate ICIPE 273 produced the shortest LT_{50} 31days while the longest LT_{50} was for M207 that took 51days.

4.5 Transmission in the laboratory

There was a significant difference ($p \leq 0.05$) between the fungal isolates and the control ($P = 0.0001$) in the mortality (Figure 9), while there was no significant difference among the fungal isolates for mycosis.

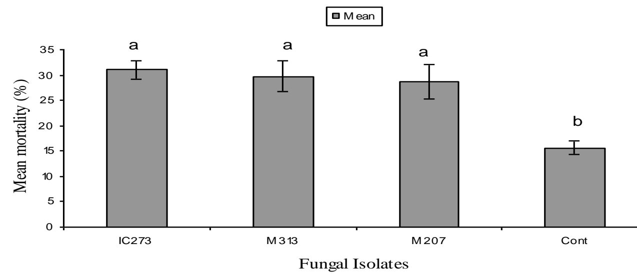

Figure 9. Adult banana weevil mortality in the laboratory. Mean (±) of bars per fungal isolate with similar letters are not significantly different (P= 0.05, SNK test).

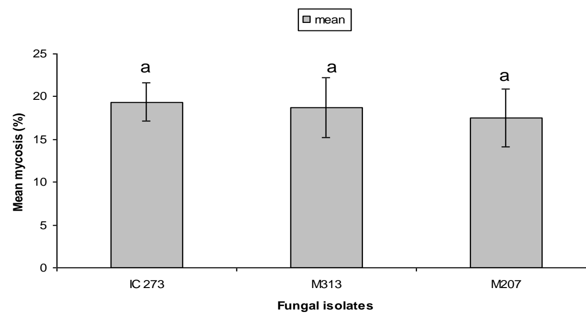

Figure 10. Mean mycosis on adult banana weevil in the laboratory. There was no significant difference among the fungal isolates (P= 0.05) as SNK test.

CHAPTER FIVE

5.0 DISCUSSION

Pathogenicity of fungal isolates against banana weevils is influenced by culturing method, dose concentrations, substrate, formulation and method of application (Gold *et al.,* 2002a). Laboratory infectivity studies indicate that *Beauveria bassiana* isolate ICIPE 273 isolated from soil in Mbita, Kenya gave better results among the tested isolates since it gave 50% mortality when a standard concentration of 1 x 10^8 for 11 seconds was used for screening work. This is acceptable for entomopathogens when used as biological control agents since they target reducing the pest population to 50% and above. The level of effectiveness obtained in our study compares favourably with those of Nankinga *et al*. (1994), Pena *et al.* (1991) and Godonou *et al.* (2000). For all the tested isolates dead banana weevils kept in humidified chambers developed surface mycelia confirming the mortality was induced by fungus.

The lethal time $LT_{50}$of banana weevil subjected to conidial suspensions of *B. bassiana* are in the same range as Kaaya *et al.* (1993) and Khan and Gangapersad (2001). The LC_{50} results compare favourably with Khan and Gangapersad (2001) who reported median lethal concentration of between 4.57 x 10^7 and 4.92 x10^8 spores/ml. for banana weevils when subjected to *B. bassiana*, *M. anisopliae* and *M. flavovirida.* The response on banana weevil to *B. bassiana* is dose dependent, large quatities of conidia have to be used to provide sufficient inoculum in order to initiate an epizootic condition and control the host population successfully. Dose mortality relationships have shown that

mortalities in insects infected with *B. bassiana* increased directly with spore concentration (Kaaya, 1989). It is important for the chosen fungus for biological control to have low LC_{50} and LT_{50} since environmental conditions are not always favourable for growth of entomopathogenic fungus (Khan and Gangapersad, 2001).

Transmission of infection from infected to non infected weevils in the laboratory gave (24%-26%) mortalities which compare favourably with Schoeman *et al.* (1998) who obtained mortalities of between 24.4% - 26.83% when he infected 15 banana weevils and introduced them to a container with 15 uninfected weevils in the laboratory for 37 days in South Africa.

Laboratory results indicated that weevils can transfer the pathogen from infected to uninfected individuals. Entomopathogenic fungi have the potential to grow, multiply and persist on the insect they kill, thus infected individuals can move away from the infection point thus carrying the pathogen throughout the pest habitat leading to an epizootic situation (Ferron, 1981).

The banana weevil is difficult to control due to its sedentary life in that it is mostly found hidden in soil or banana leaf sheath with the immature stages occurring within the host plant (Gold *et al.,* 2003). Infection is related to dosage and the initial number of infective conidia applied to the banana weevils for transmission would be a determinant factor in the spread of the disease and the eventual death of the banana weevils. When a dose of 1

$x10^9$ was used in the laboratory it was able to cause epizootics as demonstrated in laboratory transmission experiments. Isolates of *B. bassiana* which are more virulent are needed to cause high rates of transmission and infection (Schoeman *et al.,* 1998).

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The aim of this project was to test the potential of using entomopathogenic fungus *Beauveria bassiana* for biological control of banana weevils. From the results, there is potential for utilization of the fungus in the management of banana weevils. The conclusions are:

1) All isolates of *Beauveria bassiana* tested were pathogenic to banana weevils causing mortalities of 20% to 50% with the best isolates being ICIPE 273, M 313 and M 207. The rest, KE 300, M 221, ICIPE 50, M 470, M 618, M 573 and ICIPE 279 killed less than 20% of the banana weevils with ICIPE 279 being the least pathogenic to the adult banana weevil.

2) There was dose response for the three isolates, optimal *B. bassiana* dose that caused the highest mortality for the banana weevils was 1 x 10^9conidia/ml for 11 seconds for ICIPE 273, M 313 and M 207.

3) Dissemination of the fungus in the laboratory by infected banana weevils to non infected weevils occurred and caused mortalities of up to 26%.

6.2 Recommendations

1) The optimal concentration of *Beauveria bassiana* at 1 x 10^9 conidia/ml for 11 seconds attained the best results in causing weevil mortality and should be used for further trials.

2) Additional research needs to be conducted on the timing that is the interval of introducing and method of introducing the infected banana weevils, to see if transmission will give better results. The information obtained from such a study may provide means of understanding ways of using infected banana weevils as a biopesticide to transmit the fungus.

7.0 REFERENCES

Abbot, W.S. 1925. A method of computing the effectivess of an insecticide. Journal of Economic Entomology 18:265-267

Afreh–Nuamah, K. 1991 population dynamics of *Cosmopolites sordidus* in relation to sources of Planting material and cropping history of Kade, Ghana pp 68-74 *in* Biological and Integrated Control of Highland Banana and Plantain Pests and Diseases. Proceedings of a Research Coordination Meeting, 12-14 November 1991, Cotonou, Benin (C.S. Gold & B. Gemmill, eds.). IITA, Ibadan, Nigeria.

Akello, J., Dubois, T., Gold, C.S., Coyne, D., Nakavuma, J. and Paparu, P. 2007. *Beauveria bassiana* (Balsamo) Vuillemin as an endophyte in tissue culture banana(*Musa* spp.) Journal of Invertebrate Pathology, 96 (1):34 - 42

Akello, J. 2008. *Beauveria bassiana* Balsamo (Vuillemin) as an endophyte to control the Banana weevil**,** *Cosmopolites sordidus* (Germar) MSc Thesis ,Department of Crop science , Faculty of Agriculture , Makerere University , Uganda 123pp

Allard, B. G., Nankinga, C. and Latigo, O. M. 1991. The potential of indigenous Fungal pathogens as a component of Integrated management of banana weevil in Uganda in G.S. Gold and B. Gemill(eds) Biological and integrated control of highland banana and plantain, pests and diseases of a research coordination meeting. Cotonou Benin 12-14 November 1991.

Booth, R. G., Cox M. L., and Madge, R. B. 1990. International Institute of Entomology guides to insects of importance to man, Coleoptera pp 188- 192

Braimah, H. and Emden van H. F. 1999. Evidence for the presence of Chemicals attractive to the banana weevil *Cosmopolites sordidus* in dead banana weevils. Bullentin of Entomological Research, 89:485-491

Bridge J. and Gowen, S. R. 1991.Visual assessment of plant parasitic nematode and weevil damage on banana and plantain pp 147- 154 in Biological and Integrated Control of Highland Banana and Plantain Pests and Diseases. Proceedings of a Research Coordination Meeting, 12-14 November 1991, Cotonou, Benin (C.S. Gold & B. Gemmill, eds.). IITA, Ibadan, Nigeria.

Budenberg, W. J., Ndiege, I. O, Karago F.W. and Hansson, B.S.S. 1993. Behavioural and electrophysiological responses of the banana weevil *Cosmopolites sordidus* to host plant volatiles. Journal of Chemical Ecology ,19: 67-277.

CABI, 2005. Crop protection compendium CD-ROM Edition. Nosworthy way,Wallingford, United Kingdom.

Deacon, W. J .1981. Microbial control of plant pests and diseases pp 31- 42 (Burges H.D. ed) London Academic press

Ekesi, S. 2001. Pathogenicity and antifeedant activity of entomopathogenic hyphomycetes to the cowpea leaf beetle, *Ootheca mutabilis* Shalberg. Insect Science and Applied Journal, 21: 55-60.

Feng, G. and Johnson B. J. 1990. Relative Virulence of six isolates of *Beauveria bassiana* on *Diuraphinoxia* (Homoptera: Aphididae) Environmental Entomology , 19 (3):785-790

Feng, M.G., Poprawski, T.J. and Khachatourians, G.G. 1994. Production, formulation and application of the entomopathogenic fungus *Beauveria bassiana* for insect control: current status. Biocontrol Science and Technology , 4: 30-34.

Ferron, P. 1978. Biological control of the insect pests by entomogenous fungi. Annual Reviews of Entomology, 23: 409-442

Ferron P. 1981. Pest Control by fungi *Beauveria* and *Metarhizium*. Pp. 465–482 *in* Microbial Control of Pests and Plant Diseases 1970-1980 (H.D. Burges, ed.). Academic Press, London.

Fogain R. and N. S. Price. 1991. Varietal screening of some *Musa* cultivars for susceptibility to the banana weevil, *Cosmopolites sordidus* (Coleoptera: Curculionidae). Pp. 49-56 in Biological and Integrated Control of Highland Banana and Plantain Pests and Diseases. Proceedings of a Research Coordination Meeting, 12-14 November 1991, Cotonou, Benin (C.S. Gold & B. Gemmill, eds.). IITA, Ibadan, Nigeria.

Godonou, I., Green K. R., Oduro K. A., Lomer C. J. and Afreh-Nuamah 2000. Field evaluation of selected formulations of *Beauveria bassiana* for the management of banana weevil (*Cosmopolites sordidus*) on Plantain (Musa spp., AAB Group) Biocontrol Science and Technology, 10: 779-788

Gold, C. S., P. R. Speijer, E. B. Karamura & N. D. Rukazambuga. 1994. Assessment of banana weevils in East African highland banana systems and strategies for control. Pp. 170-190 *in* Proceedings of Banana Nematode/Borer Weevil

Conference. Kuala Lumpur, 18-22 April 1994 (R.V. Valmayor,R.G. Davide, J.M. Stanton, N.L. Treverrow & V.N. Roa, eds.). INIBAP, Los Baños, Philippines.

Gold, C. S., Rukazambuga , N.D., Karamura, E.B., Nemeye, P. and Night, G. 1998. Recent advances in banana weevil biology, population dynamics and pest status with emphasis on East Africa in Gold, C.S., Karamura, E. B. and Sikora, R. A. (eds). Mobilizing IPM for sustainable banana production in Africa (Proceedings of a workshop on banana IPM held in Nelspruit, South Africa, 23-28, November, 1998). INIBAP, Montpellier, France, pp35. Available: www.inibap.org. Accessed: 11/05/06.

Gold, C. S. and Messiaen, S. 2000. The banana weevil *Cosmopolites sordidus* in: *Musa* pest fact sheet no. 4. Internet. Available: www.inibap.org. Accessed: 4/05/06

Gold C. S, Tushemereirwe W.K and Nankinga C. M 2002a. Overview of *Beauveria bassiana* for the control of banana weevil, Proceedings of the promusa Sigatoka workinggroup).Available:http://www.promusa.org/publications/promusa111.pdf. Accessed: 4/05/06

Gold C. S, Okech, S. H., Nankinga C. M., Karamura, E. B., Tushemereirwe W. K. and Niere, B. 2002b.IPM strategies against banana weevil infestation in Eastern Africa: The way forward. In Integrated Pest management conference proceedings, Pp 39 - 49

Gold, C. S., Nankinga, C., Niere, B. and Godonou, I. 2003. IPM of banana weevil in Africa with emphasis on microbial control. In*:* Neuenschwander, P.,

Borgemeister, C. and Langewaid, J. (eds) Biological Control in IPM Systems in Africa. CAB International, Wallingford, Pp 243-257.

Hasyim, A. and Gold, C.S 1998. Potential of classical biological control for banana weevil, *Cosmopolites sordidus* Germar, with natural enemies from Asia *In:* Gold, C.S., Karamura, E.B. and Sikora, R.A. (eds). Mobilizing IPM for sustainable banana production in Africa, Proceedings of a workshop on banana IPM held in Nelspruit, South Africa, 23-28, November, 1998, INIBAP, Montpellier, France, pp 59

Hajek, A. E. and St. Leger R. J, 1994. Interactions between Fungal Pathogens and insect hosts. Annual Reviews Entomology ,39:293-322

Inglis, G. D., Johnson, D. L. and Goettel, M. S. 1996. Bioassay Method Used to test the efficacy of Beauveria bassiana against Grasshoppers'Journal of Invertebrate Pathology vol 67,pp 312-315

Integrated Management Systems, 2000. Biocontrol News and Information Vol. 21 No. 3

ISAAA, 1996. International Service for the Acquisition of Agri-biotech Applications (ISAAA) Annual Report. 1996. Available: http:// www.isaaa.org. Accessed: 4/05/06

Jaronski, S. T. and Goettel, M. S. 1997.Development of *Beauveria bassiana* for control Grasshoppers and Locusts. Memoirs of the Entomological society of Canada, 171:225 - 237

Kaaya, G. P. 1989. *Glossina morsitans morsitans* : Mortalities caused in adults by experimental infection with entomopathogenic fungi.Acta Tropica, 46:107 - 114

Kaaya, G. P. and Okech, M. A. 1990. Horizontal Transmission of mycotic infection in adult Tsetse , *Glossina morsitans morsitans* .Entomophaga 35 (4), 589- 600

Kaaya, G. P, Seshu-Reddy K. V, Kokwaro, E. D and Munyinyi D. M. 1993. Pathogenicity of *Beauveria bassiana, Metarhizium anisopliae* and *Serratia marcescens* to banana Weevil *Cosmopolites sordidus.* Biocontrol science and Technology Journal, 3,177-187

Kaaya G. P. and Hassan S. 2000. Entomogenous fungi as promising biopesticides for tick control. Experimental and Applied Acarology**,** 24**:**913–926.

Karamura, E. B. 1998. Importance of Banana in Sub-Saharan Africa Pp 5-11In*:* Gold, C.S., Karamura, E.B. and Sikora, R.A. (eds). Mobilizing IPM for sustainable banana production in Africa, Proceedings of a workshop on banana IPM held in Nelspruit, South Africa, 23-28, November, 1998, INIBAP, Montpellier, France. Available: www.inibap.org. Accessed: 4/05/06.

Kermarrec, A., Sirjusingh, C., Mauleon, H. Pavis, C. and. Sarah, J. L 1991. Biological control of weevils and whitegrubs in the Caribbean: a review. Pp. 155-170 *in* Biological and Integrated Control of Highland Banana and Plantain Pests and Diseases. Proceedings of a Research Coordination Meeting, 12-14 November 1991, Cotonou, Benin (C.S. Gold & B. Gemmill, eds.). IITA, Ibadan, Nigeria.

Khan, A. and Gangapersad, 2001. Comparison of the effectiveness of three entomopathogenic fungi in the management of banana borer weevil, *Cosmopolites sordidus* (Gemar) in International Pest Control Journal, 43(5):208-213

Kiggundu, A., Vuylsteke, D. and Gold, C. 1998. Recent advances in host plant resistance to banana weevil, In: Gold, C.S., Karamura, E.B. and Sikora, R.A. (eds). Mobilizing IPM for sustainable banana production in Africa (Proceedings of a workshop on banana IPM held in Nelspruit, South Africa, 23-28, November, 1998, INIBAP, Montpellier, France pp 87 Available: www.inibap.org. Accessed: 13/05/06.

Kiggundu, A., Gold C.S., Labauschagne M.T., Vuylsteke, D. and Louw, S. 2003. Levels of host plant resistance to banana weevil, *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae) in Ugandan *Musa* germplasm. Euphytica, 133:267-277

Montcel, H. T. 1987. Plantain bananas, The Tropical Agriculturalist, Macmillan Malaysia pp 8

Moschetti R. 1999. IPM of Alaska. Available: http:// www.ipmofalaska.com. Accessed: 28/05/06.

Nankinga, C. M., Ogenga-Latigo,W. M., Allard, B. G.and Ogwang, J. 1994. Potential of *Beauveria bassiana* for control of banana weevil In Uganda.African Crop Science Conference proceedings 1:300-302

Nankinga C. M. and W. M. Ogenga-Latigo. 1996. Effect of method of application on the effectiveness of *Beauveria bassiana* against the banana weevil, *Cosmopolites sordidus*. African Journal of Plant Protection 6:12-21

Nankinga, C.M., Moore, D., Bridge, P. and Gowen, S.1998. Recent advances in microbial control of banana weevil pp 73- 83 *In*: Frison, Gold, C.S., Karamura, E.B. and Sikora, R.H. (Eds.), Mobilizing IPM for sustainable banana production in Africa. INIBAP, Montpellier, France

Nankinga, C. M and Moore D. 2000. Reduction of banana weevil populations using different formulations of the entomopathogenic fungus *Beauveria bassiana.* Biocontrol Science and Technology Journal, 10(2):645–657

Ogenga-Latigo, M. W. and Masanza, M. 1996. Comparative control of the banana weevil, *Cosmopolites sordidus,* by the fungal pathogen, *Beauveria bassiana,* and some insecticides when used in combination with pseudostem trapping. African Crop Science Journal, 4(4):483-489.

Okech, S. H. O. and Gold, C. S. 1996. Relationship of the banana weevil with its host plant and soil fertility *in* The African Highland Initiative Technical Report series 2.

Ortiz, R., Vuylsteke, D., Dumpe, B. and Ferris, R.S. 1995. Banana weevil resistance and rhizome hardness in *Musa* germplasm. Euphytica, 869: 5-102

Pena, J. E., Duncan R. and Martin R.1991. Biological control of *Cosmopolites sordidus* in Florida pp 124-139 *in* G. S. Gold and B. Gemill (eds) Biological and

integrated control of highland banana and plantain, pests and diseases of a research coordination meeting. Cotonou Benin 12-14 November 1991.

Rath, C. A. 2000. The use of entomopathogenic fungi for control of termites. Biocontrol science and technology 10, 563- 581

Robinson, J.C. 1996. Bananas and Plantains. pp. 238-246. CAB International, Wallingford.

Schoeman, P. S., Schoeman, M. H. and Dochez, C. 1998. Novel techniques for the control of the banana weevil, *Cosmopolites sordidus*, in South Africa. *In*: Frison, E. A.,Gold, C. S., Karamura, E. B. and Sikora, R.H. (Eds.), Mobilizing IPM for sustainable banana production in Africa. INIBAP, Montpellier, France. pp. 259-264

Seshu, R.V., Ngode, L., Ssenyonga J.W., Wabule, M., Onyango, M., Adede T.O. and Ngoze, S. 1998. Management of pests and diseases of banana in Kenya *In* Gold, C.S., Karamura, E.B. and Sikora, R.A. (eds). Mobilizing IPM for sustainable banana production in Africa, Proceedings of a workshop on banana IPM held in Nelspruit, South Africa, 23-28, November 1998 INIBAP, Montpellier, France, pp 215. Available: www.inibap.org Accessed: 13/05/06.

Stover, R. H. and Simmonds, N. W. 1986. Bananas (3rd Edition). Longman Group, UK. 330pp

Swennen, R., and Vulysteke, D. 2001.Banana.,pp 530- 552, In Raemaekers, R. H., ed. Crop production in Tropical Africa.Directorate General for International co-operation (DGIC).Brussels,Belgium.

Valmayor, R.V., Davide, R.G., Stanton, N.L, Treverrow, N.L and Rao, V.N 1994. INIBAP Network for Asia and the Pacific, Proceedings of a conference on Nematodes and Weevils affecting Bananas in Asia and the Pacific Selangor, Malaysia 18-22, April 1994.

Wong, 2003. Fungi as bioherbicides, bioinsecticides and biofungicide. Available: http://www.botany.hawaii.edu/faculty/wong/BOT135/Syllabus2003 Accessed: 27/05/06

8.0 APPENDICES

Appendix 1.Sabourand Dextrose Agar Oxoid Limited England

Mycological peptone	10g/litre
Dextrose	40g/litre
Agar	15g/litre

Appendix 1I

Layout of the experiment in the Laboratory

IC273	M470	IC50	M313
IC279	CONT	M207	M470
M573	IC273	IC279	CONT
KE300	IC50	IC273	M207
M221	IC279	M573	IC50
M618	M207	M221	IC279
M313	KE300	M618	IC273
M207	M573	KE300	M221
IC50	M313	M470	M573
M470	M618	CONT	KE300
CONT	M221	M313	M618

Appendix III ANOVA tables

PATHOGENICITY MORTALITY

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	10	11242.91	1124.29	9.46	<.0001
Error	37	4398.75	118.88		
Corrected Total	47	15641.66			

PATHOGENICITY MYCOSES

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	9	6278.75	697.63	2.47	0.020
Error	50	14145.83	282.91		
Corrected Total	59	20424.58			

MORTALITY 1 x 10^8

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	2	8830.94	4415.47	23.59	<.0001
Error	165	30881.21	187.15		
Corrected Total	167	39712.16			

MORTALITY 3 x 10^8

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	3	16753.12	5584.37	50.95	<.0001
Error	28	3068.75	109.59		
Corrected Total	31	19821.87			

MORTALITY 1 x 10^9

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	2	9447.76	4723.88	9.13	0.0002
Error	165	85409.20	517.63		
Corrected Total	167	94856.97			

TRANSMISSION IN LABORATORY

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treatment	14	2277.17	162.65	25.04	<.0001
Error	15	97.43	6.49		
Corrected Total	29	2374.60			