EFFECT OF SOIL AMENDMENTS ON THE EFFICACY OF *Beauveria bassiana* FOR CONTROL OF THE BANANA WEEVIL, *Cosmopolites sordidus* (GERMAR).

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DECLARATION

I declare that this is my original work and that it has not been presented for examination in any other university.

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DEDICATION

This thesis is dedicated to my late father (Mr. Izidoro Kyibikyirekwa), my mother (Mrs. Poronia Kyibikyirekwa), who endeavoured to give our family a good education irrespective of their limited resources. Then, to my brothers (Vicent, Magiyo, Robert, and Koradi), sisters (Betty, Sarah, Risidi, Jane and Rebecca) for their encouragement and last, but not least, to my dear wife (Jackline Magara) and our daughters (Sonia, Roline and Biola) for all their moral support and tolerance during the challenging times of my course work, research and thesis writing.

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Abstract

For effective use of the entomopathogen *Beauveria bassiana* in the management of the banana weevil, high inoculum levels and appropriate formulations are mandatory. Equally important is the development of a suitable formulation that is persistent within the target environment, in this case the field soil ecosystem. Evaluations of *Beauveria bassiana* were carried out from Kawanda, Uganda on conidial yield from selected substrates, efficacy of different formulations against the banana weevil, and, efficacy and persistence of the fungus under organic and inorganic soil amendments. The substrates evaluated were cracked maize, maize bran, "machicha", bagasse, cotton husks, maize bran + bagasse, maize bran + cotton husks, and bagasse + spent yeast and they were formulated with clay or loam soils. The soil amendments evaluated were un-amended soils, coffee husks, decomposed cowdung manure and inorganic fertilizers. The results indicated that cracked maize grains were the most ideal for *B. bassiana* conidia production while the dried formulation of the same media was also the most effective against the banana weevil under laboratory conditions. The formulation gradually lost its efficacy as storage period increased. Overall, the soil amendments significantly reduced the efficacy and persistence of the entomopathogenic fungus over time.

Laboratory evaluation of the substrates was based on conidia counts using the improved haemocytometer. Cracked maize grains were the best substrate with 3.2 $x10^9$ conidia per gram, even though it was not significantly different from that of maize bran (3.1 $x10^9$). Evaluation of the *B. bassiana* formulations against the banana weevil

under laboratory conditions resulted in cracked maize followed by cracked maize formulated with clay soil as the most effective with over 80% weevil mortality in 30 days. Following storage under laboratory conditions for 90 days, recorded weevil mortality after exposure to the cracked maize grains formulation was > 85% in 30 days, but this declined to 20% following 180 days of storage.

In the pots, soil: amendment ratios were 100%, 50%, 33.3%, 25% and 0% (eg. un amended soil, coffee husks alone or cow dung alone to give the 100%, 1 part of soil: 1part of amendment to give the 50% by volume, etc.). One hundred grams (100g) of cracked maize *B. bassiana* formulation were applied in each pot at the same time. Ten weevils of the same age, mixed sex (1:1ratio) were released in each pot for 30 days, and this was repeated every other 30 days. Weevil mortality was monitored from pots and from *B. bassiana* culture picked from pots and tested in the laboratory at 5-day intervals. Moisture content, temperature, soil invertebrates, substrate colonisers and decomposers, and other amendment characteristics were monitored from pots during the course of the experiments. Results indicated that *Beauveria* applied in un amended soils had the highest efficacy of 55% weevil mortality for the first release and 27% after 90 days. However, this result was not significantly different ($P>0.05$), for the first release, for all the soil amendments. Significant differences were observed in the pots after 60 days. By 90 days after application, the *B. bassiana* formulation had significantly lost efficacy and persistence in all the treatments in the pots.

Similar experiments were carried out in the field with the same amendments. The organic amendments were scaled down to one level each mimicking the farmers' practices, and the same rate of the inorganic fertilizer was used as in the pot experiment. Furadan (carbo furan 5%ww) was introduced as a check for *B. bassiana* in the field. 200g of *B. bassiana* cracked maize formulation or 60g of the chemical were applied on each experimental mat. The weevil mortality results for *B. bassiana* formulation had the same trend as in pots; there was faster reduction in efficacy and persistence of the fungus applied in the amended soils. Furadan exhibited a high knock down effect against the weevil for the first release; however, it had also gradually lost its efficacy by 90 days.

In both pot and field experiments, *B. bassiana* cracked maize samples picked and tested in the laboratory against the banana weevil resulted in significantly higher weevil mortality records than recorded under pot or field conditions. The latter points to the fact that the banana weevil might be able to escape some of the entomopathogenic effects under the pot or field conditions. The faster decline in efficacy and persistence of *B. bassiana* in the amended soils could be attributed to the significantly high moisture content, fungal substrate colonisers and decomposers that contributed to fast degradation of the maize-based substrate. It could also be due to the high Carbon (C), Nitrogen (N), Phosphorous (P) and Potassium (K) levels that supposedly favoured high microbial load in the amended soils leading to the reduction in efficacy and persistence of the entomopathogen.

CHAPTER 1
INTRODUCTION

1.1. Importance of Bananas

The banana belongs to the family *Musaceae* that comprise two genera; *Musa* and *Ensete* (Purseglove, 1985). Bananas play a major role in the nutrition, well being and cultural life of millions of people in the World (Gowen, 1995). The dietary importance of bananas and plantains is probably greatest in Africa (Simmonds, 1966). In East Africa, bananas provide the cheapest source of carbohydrates (Mukasa and Thomas, 1970), and a major dietary component of Bantu people in the region. The East African highland bananas (*Musa* AAA-EA) are the principal food crop in Uganda supporting both rural and urban populations (Gold *et al.*, 1991). About seven million Ugandans are known to depend on bananas (Karamura, 1992). Uganda leads Africa in production with about 10.5 million Metric tons (FAO, 2001). Banana is considered a key component of sustainable agricultural systems in densely populated high rainfall zones (Gold *et al.*, 1991). On steep slopes, it reduces soil erosion and is a principal source of mulch for maintaining and improving soil fertility (INIBAP, 1986).

1.2. Constraints to Banana production

Banana production and yields have been declining since the 1970s. Previously, banana plantations lasted over 50 years, but recently they start to deteriorate after only four years (Karamura, 1992). Banana production in Uganda has dropped from 15-20 tonnes of bananas per acre in the 1970s (Mukasa and Thomas, 1970),

compared to the current production of only 6 tonnes per acre (FAO, 2001). The latter is just about half of the former production levels and the trends are similar in Tanzania, Rwanda and Burundi (FAO, 2001).

A wide range of factors limits banana production in Uganda. Rubaihayo and Gold (1993), recognise the following constraints; pests and diseases, soil nutrient deficiencies, poor management, social economic factors, and post-harvest handling problems. Banana pests are considered the most important constraint to banana production. The most important of these are the banana weevil, *Cosmopolites sordidus* (Germar - Coleoptera: Curculionidae) and the root nematodes. The other banana weevil species include; *Temnoschchoita nigroplagiata* (Qued), *T. erudita* (Duvid), and *T. basipennis* (Duvid) (Harris, 1947). The banana weevil is an important insect pest on highland banana and plantain in Africa (Gold *et al.* 2001). *Cosmopolites sordidus* has been implicated as a primary factor contributing to the decline and disappearance of East African Highland cooking banana (*Musa spp.*, genome group AA-EA) from its traditional growing areas in Central Uganda (God *et al.*, 1996b; cited by Gold *et al.*, 2001) and Western Tanzania (Mbwana and Rukazambuga, 1999). It has been noted as the most devastating arthropod pest of bananas in subsistence farms in Uganda and other countries as well (Whalley, 1957, Feakin, 1971).

The banana weevil damage is often severe in newly planted fields where heavy attack can kill a high percentage of suckers and lead to crop failure (Ambrose, 1984). The destructive larvae tunnel and feed on the rhizome, weakening the plant, reducing

bunch weight and in some case leading to snapping of the rhizome at the ground level before the bunch is ready for harvest (Seshu *et al.*, 1998). On the other hand, the destructive banana root nematodes include *Radopholus similis* (Cobb), *Pratylenchus spp*, and *Meloidogyne spp* that are known to attack the plants leading to toppling and bunch weight reduction (Kashaija *et al.*, 1994, Masanza, 2003). Many times, the symptoms due to weevils or nematodes are confusing and most of the field workers and farmers are not able to distinguish weevil from nematode damage (Bridge and Gowen, 1991).

Several banana weevil control measures exist under the three components of IPM; cultural, chemical and biological control measures. Cultural control practices offer the first line of control for resource poor farmers. These practices include; establishing new plantations in banana weevil free sites, paring planting material to remove weevil eggs, trapping with pseudostems, treatment of planting material with hot water at 55^0C for 27 minutes (Chalker, 1987), mulching and crop sanitation (Seshu *et al.*, 1991, Masanza, 2003). Some farmers use insecticides that are applied to the bases of the plant to control the weevil (Stover and Simmonds, 1987). The chemical insecticides currently used in Uganda include Furadan (Carbofuran 5% w/w) and Dursban (Chlorpyrifons) (Tushemereirwe, pers. comm).

Fungal pathogens are able to infect and kill a wide range of insects and have been used to control various insects in countries such as USA, China and Brazil (Roberts, 1989). In Uganda and Ghana, *B. bassiana* and *Metarrhizium anisopliae* have been

isolated, and their efficacy and pathegonicity tested against the banana weevil (Nankinga, 1994, 1999, Godonou, 1999). Strains of the entomopathogen *B. bassiana*, isolated from *C. sordidus*, other insect hosts and galleria-bait in soils of banana stands, have been shown to infect and kill the banana weevil under laboratory and field conditions (Batista F. *et al.*, 1987, Kaaya *et al.*, 1993, Nankinga, 1999, Nankinga and Ogenga -Latigo, 1996, Nankinga and Moore, 2000). Laboratory pathogenicity tests using different strains of *B. bassiana* in Uganda (Nankinga, 1994) and in West Africa (Traore, 1995, Godonou, 1999) produced 50-100% mortality in banana weevil adults in 14 days. In Kenya, Kaaya *et al.* (1993) found that four local isolates of *B. bassiana* were pathogenic to the third instar of *C. sordidus* causing 98-100% mortality, nine days post exposure to the dry fungal spores. Godonou (1999) attained mortalities between 53-76 % in 28 days for weevils exposed to the fungal conidia under field conditions. It is evident from the forgoing that *B. bassiana* is highly infective albeit more under laboratory than under field conditions.

In preliminary studies done by Nankinga (1999) efficacy and persistence of the fungus was observed to be influenced by soil amendments such as wood ash, cow urine, tobacco and pepper that are known to affect the soil conditions. Such amendments alter the soil's physical, chemical and biological characteristics thus rendering the applied fungus less effective against the target pest. Else where, Rosin *et al.*, (1996) indicated that under sterile conditions, high rates of manure were beneficial to *B. bassiana* survival, since the latter lives saprophitically in the soil. However, they could not predict what would happen under field conditions. It is therefore not certain

whether these findings are applicable under the Uganda local field conditions whereby farmers apply various manures in order to maintain fertility in their plantations.

The present study evaluated *B. bassiana* conidial production from selected substrates, the most effective formulation against the banana weevil under laboratory conditions, and the efficacy and persistence of *B. bassiana* cracked maize formulation under different soil amendments.

1.3. Objectives and hypotheses of the study

Conidial yield from *B. bassiana* depends on the type substrate onto which it is cultured (Lomer and Lomer, 1997), and the efficacy and persistence of the fungus depends on the way it is formulated and the environment into which it is applied (Navon and Asher, 2000). Soil amendments influence soil's physical, chemical and biological characteristics, which in turn may affect the efficacy and persistence of the fungus in the applied environment (Studdert *et al.*, 1990). Various authors (Rosin *et al.*, 1996; Inglis *et al.*, 1998; Nankinga (1999) have implicated organic soil amendments in the degradative effects of the entomopathogen. However, limited information is available on this aspect as most of the studies have been done in sterile environments, and are still limited in scope. The objectives of this study were therefore to: -

- Determine conidial yield of *B. bassiana* cultured on selected substrates,
- Evaluate the efficacy of different *B. bassiana* formulations under laboratory conditions,
- Evaluate the efficacy and persistence of *B. bassiana* on cracked maize applied in un amended soils, those amended with decomposed cow dung manure, coffee husks or inorganic fertilizers.

CHAPTER 2

LITERATURE REVIEW

2.1. 0. Biology and Life History of *C. sordidus*.

The adult banana weevil belongs to the order Coleoptera, family Curculionidae and species *C. sordidus*. The adult weevil is black, measures up to 14 mm long and 4mm wide (Lescot, 1988). Adult weevils deposit eggs singly in holes in rhizomes at ground level between the basal leaf scars (Ngeze, 1994). The females lay 50-100 eggs in its lifetime and the eggs are 2 mm long; oval, white, and are laid singly. They hatch within 5-8 days. Larvae range from 11-15mm in length, legless, creamy white, brownish head, with developed mandibles and a characteristic curved body (Froggat 1925, Beccaria, 1967). The larvae go through six instar stages lasting 30-40 days. They burrow inside rhizomes with their mandibles, leaving a network of tunnels. Larvae can tunnel up to 1m of the pseudo stem if the stem remains in the ground (Gowen, 1995). The larvae pupate within the host plant and this stage lasts between 5-10 days (Harris, 1947, Bakyarire and Ogenga-Latigo, 1992).

The pupae measure up to 12mm long and are 6mm wide. They are white immediately after pupation, but darkens taking on brown colouration in the later stages as the insect develops into an adult (Whalley, 1958, Seshu, 1986). *Cosmopolites sordidus* exhibits a typical k-selected life cycle; long life span, low fecundity and high survival (Gold *et al.*, 2001). The adult can live up to 2-4 years (Frogget, 1925, Gold *et al.*, 1999, Nankinga, 1999). In Uganda the complete life cycle lasts between 53 and 72

days (Bakyerire and Ogenga Latigo, 1992, Masanza, 2003). However, Gold and Messiaens (2000) reported that under tropical conditions the egg to adult period could last for 35-49 days.

The adult weevils live in the soil, feeding on rotten materials of banana and plantain and visiting the plant for oviposition (Cuille, 1950; cited by Godonou, 1999). They are sluggish, nocturnally active and seldom observed, while the immature stages may be deep within the banana corm (Gold *et al.*, 2001). The adults have been widely reported to favour crop residues and moist environments, including in or under newly cut or rotting pseudo stems, decaying stalks, cut or damaged corms, moist trash and burrowed under the soil surface (Weddell, 1945; Swaine, 1952; Jones, 1986; Pavis 1988; Treverror *et al*, 1992; Silva and Fancelli, 1998; Aranzazu *et al*, 2000; cited by Gold *et al.*, 2001). This behaviour makes adult weevil an easy target for the entomopathogenic fungus that is normally applied under moist pseudo stem traps or under the mulch around the mat.

2.2. 0. Overview of the Control of *C. sordidus*

The banana weevil, *C. sordidus* is the most notorious of the arthropod insect pests of the banana and needs to be controlled (Stover and Simmonds, 1987). Unfortunately, current research results suggest that no single control strategy will provide complete control for the weevil in the foreseeable future (Gold *et al.*, 2001). In order to control the weevil, a broad IPM approach is being sought. There are various components of

this approach and these are broadly categorised as cultural, chemical, host plant resistance and biological control methods. Culturally based practices provide the first line of defence against the weevil attack and are widely used by most banana farming communities in East Africa. These include trapping with pseudo stems, use of clean planting material and good crop husbandry to eliminate breeding sites (Feakin, 1971).

Trapping of weevils by rhizomes and pseudostems is normally used in monitoring, but can also be used in as control method (Price, 1993; cited by Masanza, 2003). In a control programme, traps are placed in banana stools against plants, and the weevils attracted to them are collected and destroyed (Hord and Flippin, 1956; cited by Masanza, 2003). Its full potential can be realized if used in combination with chemical and biological insecticides (Schmitt *et al*, 1992; cited by Masanza, 2003). In East Africa, trapping reduced pest populations (Ogenga –latigo and Bakyalire, 1993). However, during surveys, trapping appeared to be tedious and labour intensive, with variable efficiency ranging from 2-25% of marked weevils recaptured (Gold *et a*l, 1998).

Introduction of banana weevil into new stands is through infested planting material containing eggs and larvae (Gowen, 1995). Clean planting material can be realised by getting suckers from weevil free fields, paring of corms and hot-water treatment to eliminate eggs and eliminate eggs and larvae. The use of cleaning planting materials is jeopardised by weevil infestations that may be existing in neighbouring fields. The hot water treatment method is known to kill only about 1/3 of the weevil larvae (Gold

and Messiaens, 2000). Crop husbandry practices such as desuckering, mulching, pruning, clean weeding and manure application increase crop vigour and hence tolerance against the banana weevil (MacNutt, 1974; cited by Masanza, 2003).

The search for host plant tolerance and resistance is a priority especially in the context of the small-scale/ resource constrained farmer (INIBAP, 1988; cited by Masanza, 2003). In Uganda, resistance to attack by the pest has been demonstrated by the beer types (AAA) despite high weevil incidence in the plantations (Gold and Bagabe, 1997). The latter reported that the weevils attacking the beer bananas preferred crop residues to standing plants, suggesting possible break down of repellents in tissue of beer banana on decomposition. Considering that most of the bananas in Uganda are the cooking types, this mechanism may not be significant in curtailing the weevil problem.

The recent upsurge of the weevil problem has necessitated the use chemical control methods (Stover and Simmonds, 1987). This option has been considered more effective than other methods (Mitchell, 1980; cited by Masanza, 2003). The use of chemical pesticides such as dieldrin 0.5% that was considered economical and effective has been limited by development of resistance by the weevils (Gold *et al.*, 1999). In Uganda, the high cost and scarcity of chemical pesticides, together with the potential dangers to the consumers, limit their use by the dominant resource-poor small-scale farmers (Masanza, 2003). As a result, farmers tend to apply inadequate doses, a situation that renders them ineffective and enhances probability of pests

developing resistance to the chemicals (Nankinga, 1994). The latter, together with the labour requirements of cultural controls, favour the use of IPM, focused around biological control. The entomopathogen, *B. bassiana* has exhibited great potential in this aspect and may offer best promise for the control of the banana weevil (Gold *et al.*, 2001).

2.2.1. Biological control of *C. sordidus*

Biological control is the use of parasitoid, predator, pathogen, antagonist, or competitor populations to suppress a pest population, making it less abundant and thus less damaging than it would otherwise be (Driesche and Bellows, 1996). However, in general terms, the biological control of the banana weevil has been achieved by predator, parasitoids, nematodes and entomopathogens (fungi, bacteria, viruses). A number of insect natural enemies in the orders Hemiptera, Dermaptera, Diptera, Coleoptera and Hymenoptera have been cited in the control o the banana weevil (Schmitt, 1993). Amphibians, platyhelminths and fungi have also been cited as natural enemies against the weevil. In East Africa, three species of Dermaptera and three of Coleoptera were observed preying on immature weevil stages (eggs and larvae) in the rhizomes and pseudostems (Kopenhofer, 1993b).

The use of entomopathogens, in particular, has high overall comparative advantages over other control measures (Tanada and Kaya, 1993). For instance, this control strategy has the advantage of not only solving the pest problem, but also of protecting the environment, including the health of the people (Herren, 1988). This favours the

use of our indigenous entomopathogen; *B. bassiana*, that has been found to be effective against the banana weevil (Nankinga, 1994, 1999, Gold *et al.*, 2001).

2.2.1.1. Use of Arthropod predators

There are a number of arthropods which have been reported to prey on the banana weevil. These are categorised into egg, larvae, pupae and adult predators. Among the egg and larvae predators is *Plaesius javanus* (Coleoptera: Histeridae) a beetle that eats eggs and larvae (Jepson, 1914; cited by Moznette, 1920). The beetle successfully controlled the weevil in Fiji. The failure for this beetle to control the weevil in Africa was possibly due to the insufficient number of released vigorous beetles (Harris, 1947). Hargreaves (1940) was the first to suggest that ants might have potential as biological control agents of the banana weevil in Africa. The ants; *Tetramorium guineense*, *T. bicaninatum* and *Pheidole megacephala* Fabricius, have been demonstrated to contribute to the banana weevil control in Cuba (Hasyin and Gold, 1998). In Uganda, Abera (pers.comm.) has observed *Pheidole* spp. removing eggs and larvae from pseudo stems in laboratory studies. However, most of the literature is unclear about the real potential of these arthropod predators in the control of the banana weevil, particularly in Uganda.

2.2.1.2. Use of Entomopathogenic nematodes

Entomopathogenic nematodes have been successfully used as biological control agents of the banana weevil. Nematodes have been widely used because of their wide host range, ability to kill host rapidly, and lack of adverse side effects on the environment (Schmitt, 1993). The entomogenous nematodes that have been used to

control the weevil include species that fall in genera of *Steinernema* and *Heterorhabditis*, and were able to attack both the larvae and adult stages of the banana weevil (Schmitt, 1993). The effectiveness of these nematodes against the banana weevil has been demonstrated in Australia, the Caribbean, Florida, and Brazil (Driesche and Bellows, 1996). In these studies, it was shown that entomogenous nematodes cause high mortality of the weevil, but with great variability among nematode strains and species. In contrast, the nematodes were found to be less self-perpetuating in the environment and very sensitive to draught, high temperatures and ultra violet light, leading to their limited efficacy (Parnitzki, 1992). There seems to be no records of tests done on nematodes against the weevil in Africa, and moreover, the cited inefficiencies make them more unlikely candidates for the management of the banana weevil, particularly in Uganda.

2.2.1.3. Use of Endophytes

Endophytes are micoorganisms that inhabit plant tissues for at least one period of their life cycle (Carrol, 1991). A wide variety of endophytic fungi have been isolated from many plants and plant tissues and some act as antagonists to pests and diseases (Carroll, 1991). In Uganda, work done by Griesbach (1999) established that *Fusarium* spp., *Acremonium* spp. and *Geotrichium* spp. spore suspensions cause 80-100% mortality in banana weevil eggs. He was also able to successfully inoculate tissue culture plants with the endophytes. However, preliminary pot trials on the effects of the inoculated endophytes on weevil damage in tissue culture plants

produced promising but inconsistent results. More studies are underway to establish the potential of the endophytes on the other developmental stages of the weevil.

2.2.1.4. Use of Entomopathogenic fungi

Various species and strains of entomopathogenic fungi have been isolated from *C. sordidus* and other insect hosts, and have been shown to infect and kill the banana weevil under laboratory and some preliminary field studies (Batista *et al.*, 1987, Kaaya *et al.*, 1993, Nankinga, 1994, Traore, 1995, Nankinga and Ogenga -Latigo, 1996, Nankinga, 1999). The most common of these are *Beauveria bassiana* and *Meterhizium anisopliae* (Nankinga, 1994). These are essentially important for controlling cryptic insects, including the banana weevil, which are not accessible to arthropodal natural enemies (Gold *et al.*, 2001).

The most effective strains of *B. bassiana* are capable of causing high mortality in the laboratory at lower spore concentrations and in shorter periods of time. Laboratory infectivity tests conducted using different strains of *B. bassiana* in Uganda (Nankinga, 1994) and in West Africa (Traore, 1995, Godonou, 1999) gave good results of up to 100% weevil mortality. In Kenya, Kaaya *et al* (1993) tested exotic and local isolates and found that four local isolates of *B. bassiana* were pathogenic to the third instar of *C. sordidus* causing 98-100% mortality, 9 days post exposure to the dry fungal spores. Godonou (1999) showed weevil mortalities between 53.4 and 75.5 % caused by *B. bassiana* isolate IM1330194 in the weevils exposed to the fungal conidia under laboratory conditions in 28 days, however, percentage mortality decreased with time,

for most of his treatments. Overall, mortality caused to *C. sordidus* suggests that a number of the local *B. bassiana* isolates have potential as biological control agents against the banana weevil (Nankinga, 1994, 1999).

In Africa, the development of microbial pesticides is still in infancy stage and yet the banana weevil menace is steadily proliferating. Although a number of strains have shown promise in the laboratory and in preliminary field studies, efficient and economically viable mass production and delivery systems still need to be developed, while the performance of microbial control agents against banana weevil under different agro-ecological conditions is not well understood (Gold *et al.*, 2001). It is therefore necessary to carry out further studies on ideal substrates for production and formulation of *B. bassiana* and evaluate the effects of different soil amendments commonly applied in banana fields, on the fungus.

2.3.0. Factors that influence the efficacy and persistence of Entomopathogenic fungi

The mode of parasitism and survival in nature of most entomopathogenic fungi is influenced by biotic and abiotic factors (Carruthers and Hural, 1990; Gottwald and Tedders, 1984; Lingg & Donaldson, 1981). Also, for successful development of entomopathogenic fungi as a viable component in pest control systems, economic production, effective formulation, appropriate delivery to the target, adequate persistence and compatibility with other pest management practices, are needed to ensure wide spread acceptance. Environmental factors such as light, temperature

moisture, and soil type, natural and artificial antagonists, either directly or indirectly, affects both persistence and survival of various fungi in nature (Robert and Campbell, 1977, McCoy, *et. al.*, 1988). Other influential factors cited include dosage applied, timing of application, coverage, antimicrobiosis, target pest, compatibility, host resistance, age and condition of the pest and soil management practices (Driesche and Bellows, 1996). Therefore, for effective control of the banana weevil using the entomopathogen *B. bassiana*, the detrimental effects arising from such factors should be clearly quantified and the possible remedies outlined.

2.3.1. Light

Sunlight consists of UV radiation that is damaging to biological organisms and is known to damage and kill entomopathogens (Zimmermann, 1982, Roberts, 1983). Natural sunlight in the range of 290 to 400nm has been reported to affect persistence of entomopathogenic fungi occurring on foliage (Fuxa, 1987). Exposure of *B. bassina* to sunlight for two hours significantly reduced the viability of the spores (Muller Kogler, 1970). In general, the half-life of spores of most entomopathogenic fungi exposed to simulated sun light range from about one to four hours (Ignoffo *et al.*, 1997). Farques *et* al., (1988) concluded that spore viability was reduced 10,000-fold after seven days in full sun compared to 100 times during a cloudy seven-day period. Furthermore, sun light may directly affect chromosomal DNA within the cell or indirectly inactivate the entomopathogenic fungi via the production of reactive radicals. Sunlight has been reported to inactivate *B. bassiana* applied in fields for the control of Colorado potato beetle (Anderson *et al.*, 1988). Generally *B. bassiana* germination was significantly

reduced when exposed to sunlight. Plant shading has been found to be important in preventing inactivation by sunlight (Carruthers *et al.*, 1988). In pot and field experiments for this specific study, plant canopy and mulch are anticipated to minimize the effects of light on the applied *B. bassiana*.

2.3.2. Temperature and moisture

Temperature and moisture are influential in the survival of fungal etomopathogens in all habitats and their effects are intimately related. The relationship between soil water potential, temperature and persistence of conidia in different soils has been demonstrated by Studdert *et al.*, (1990) working with *B. bassiana*. In their study, it was established that persistence of *B. bassiana* conidia increased with decrease in soil temperature, and also decreased as the soil became wetter; persistence was highest at soil temperatures of 10°C at water potentials of -10 bars, and lowest at 40°C, at 50°C conidia did not survive.

Survival of *B. bassiana* and *M. anisopliae* spores in the field decreased as soil moisture increased (Lingg & Donaldson, 1981, Studdert *et al.*, 1990); for instance, higher mycosis of Diaprepes larvae was observed in dry soils (<2%), as compared to saturated soils (>18%). Rainfall increases the soil moisture and may also influence the rate of degradation of the fungus in the field (Nankinga, 1999).

On the other hand, exposure to extremes of high temperature has been reported to be detrimental to many fungal pathogens (Clark and Medelin (1965). Entomopathogenic fungi require optimum temperatures for growth, development, pathogenicity and survival that range between 10 to 30°C (Roberts and Campbell, 1977, McCoy *et al.*, 1988), with 20-30° C being the most favourable (Driesche and Bellows, 1996). However, the optimum temperatures for entomopathogenic fungal survival also differ among taxa, for example, spore of entomophthorales appear to be more sensitive than conidia of most deuteromycetes (Lingg and Donaldson, 1981). Steinhaus (1960), Clark and Medelin (1965) noted a decline in viability of *B. bassiana* at higher storage temperatures of over 35°C. Preliminary experiments by Nankinga (1999) in Uganda indicated that *B. bassiana* was adversely affected by high temperatures above 37° C, which was reflected by delayed germination in addition to death of conidia.

The geographic location of the entomopathogens and storage media also seem to influence their infectivity. For example, Yip *et al.*, (1992), found that conidia germination for *M. anisopliae* range from 2 to 37°C among isolates collected from different temperate regions. McCammen and Rath (1994; cited by Godonou, 1999) also identified strains of *M. anisopliae* that are infectious at 5°C and persist for two years in the soil. Further more, high relative humidity or free water are generally required by entomopathogenic fungi for germination of infective propagules and the formation of reproductive structures outside the host (Yip *et al.*, (1992). Entomopathogenic fungi may tolerate temperature extremes if there is more moisture in the air or when condensation readily occurs and water loss is minimized (Pingel

and Lewis, 1997). Moisture conditions favouring epizootics of entomophthorales generally require 8 to 10 hours of relative humidity, higher than 90% with prolonged periods of dew or fog occurring over a period of two to three days (Pingel and Lewis, 1997). Therefore, the entomopathogen should be applied in conditions that are favourable for fungal survival, sporulation and subsequent weevil infection.

2.3.3. Production technique and Substrates

Several cheap mass production technologies for *B. bassiana* have been developed and these include liquid fermentation, solid substrates and diphasic methods (Gold *et al.*, 2001, Godonou, 1999). The liquid fermentation method in submerged culture was developed along time ago but was abandoned because of the difficulties of storing of the infective spores (Ferron, 1991; cited by Godonou, 1999). Liquid media for mass production of *B. bassiana* was later developed (Ferron, 1981; cited by Godonou, 1999). On the other hand, solid substrates such as rice or maize have been frequently used (Mendoca, 1992, Nankinga, 1994, Godonou, 1999), but on a small scale. Nankinga (1999) found cracked maize grains to be the best solid substrate as it was associated with high sporulation, low contamination, high viability and weevil mortality. In the production of the fungus, a substrate that results in high conidial yield is most desirable.

The diphasic method for mass production of *B. bassiana* was first used in the former Soviet Union (Ferron, 1981; cited by Godonou, 1999). The first stage involved the

production of mycelia on a liquid carbohydrate rich source in a farmentor. The second phase was a surface-culture on nutrient medium in trays for sporulation from where the spores are later harvested. The diphasic method is currently being used for mass production of the fungus on solid substrates especially for research purposes in Uganda and Ghana (Nankinga, 1994, 1999, Godonou, 1999).

2.3.4. Formulation

Formulation refers to the resultant composition when a pesticide is mixed with an ingredient (s). Although any material added to a microbial-control agent makes a formulated product, formulation generally refers the form in which the microbial – control agent is applied (Bateman, 1997). One of the limitations in the development of fungi for insect control is the lack of readily available formulation technology (Goettel and Roberts, 1992). It is through formulation that improved shelf life, persistence, efficacy and field targeting can be achieved. The entomopathogenic fungi are living organisms and need to be formulated prior to application. Along this line, protectants such as attapulginite, montmorillonite clays (Silvie, 1983), alginate and polyethylene glycol and alternative sugars such as sucrose, glucose and dextrose (Roberts *et al.*, 1987) have been used successfully to improve survival of entomopathogenic fungi. Also, oil based formulations have been reported to greatly improve the effectiveness of insect pathogenic fungi (Kooyman and Abdalla, 1998). However, such protectants make the formulation more costly and put it out of reach of resource-poor farmers. Rosin *et al.*, (1996) and Studdert *et al.*, (1990) demonstrated that coating of conidia

with clay reduces damage by decomposing organisms and thus prolongs the life of *B. bassina* conidia in soil. A formulation that prolongs the life of the infective spores under field conditions is very crucial. Since clay soil is locally available in Uganda, it may be necessary to evaluate its potential in this respect. The efficacy and persistence of the cracked maize formulation has not yet been fully evaluated under the different soil amendments normally applied under various banana management practices.

2.3.5. Antagonists

Antagonists are biological agents or their products with the potential to interfere with life processes of the entomopathogen thus leading to its inability to cause disease (James and Kenneth, 1989) to the target pest. The biological and physical characteristics at play in the different environments are influential in both infectivity and survival of the entomopathogenic fungus and may act as antagonists at various levels.

2.3.5.1. Natural antagonists

Naturally occurring microbes and their secondary metabolites function as antagonists under certain soil conditions, influencing both infectivity and survival of entomopathogenic fungi (Lingg and Donaldson 1981). Studies with *B. bassiana* suggest that inhibition in natural soils is caused by fungistasis, rather result of the microbial competition (Shields *et al.*, 1981). This effect is related to some of the well-known and documented antagonistic microflora like *Penicillium utricae* and

Aspergillus clavatus, which are mojor factors in the survival of conidia in non sterile soils (Inglis *et al.*, 1998, Roberto *et al.*, 1992). Lingg and Donaldson (1981) working with *B. bassiana* in sandy-loamy soil showed that the addition of both carbon and /or nitrogen to the soil is not a good idea in view of fungistasis. Traore (1995) found higher rates of infected weevils when *B. bassiana* was applied to sterile soil than when applied to non-sterile soil, further suggesting the antagonistic action of other soil organisms. Thus, soil antagonism may affect conidia in the soil, prevent attachment to insects, or prevent development of infection by inhibiting germination and penetration (Roberto *et al.*, 1992).

According to Nankinga (1999), the rotting of maize culture by saprophytic fungi and mites under highly moist and rotting pseudo stem traps can influence viability of fungus and can lead to reduced efficacy of fungus in the field. Attack by micro-organisms increased as soil moisture content increased with the maize culture showing the fastest contamination within 2-3 weeks. The persistence of maize and soil formulations of *B. bassiana* was reduced due to attack by saprophytic fungi, bacteria and mites and this was predicted to worsen in soils with high moisture contents, a situation that occurs during the wet season. Therefore, there is need to effectively formulate *B. bassiana* so as to counteract the inhibitory effects of the natural antagonists.

2.3.5.1. Artificial antagonists

An array of artificial antagonists exists within different soils that can influence survival and susceptibility of the host to infection. Entomopathogenic fungi, in general, tolerate a wide range of hydrogen ion concentrations ranging from a pH5 to 10 with an optimum at approximately pH7 (Robert and Campbell, 1977). Copper and sulphur, which are usually introduced in fertilization, will affect overall survival of the entomopathogen. Many in vitro studies have demonstrated that some pesticides restrict or prevent entomopathogenic fungi growth, sporulation, and germination (Majchrowicz and Poprawski, 1993). These compounds interfere with fungal epizootics by reducing the available host population or inhibit spore germination and vegetative development of the fungi. In Uganda, the use of inorganic fertilizers such as NPK, is gaining prominence under the government's plan for modernization of agriculture (PMA). It is therefore desirable for *B. bassiana* to be effective under field conditions that may be using any of the commonly used inorganic implements for banana management or pest control.

2.3.6. Effect of soil amendments.

Beauveria bassiana will be used in banana fields under various environmental conditions and banana management practices, such as application of organic manure or various types of mulch. Various manures like cow dung, poultry refuse, elephant grass, maize stover mulch, coffee husks (Ngeze, 1994), and mixtures of ash, urine and insecticidal plants are usually applied in banana fields to improve on soil fertility or for pest control (Nankinga, 1999). The practices in turn influence the pH,

temperature, moisture, soil fauna and flora which directly or indirectly influence the efficacy and persistence of the entomopathogenic fungus in the soil (Lingg and Donaldson, 1981).

High organic soils have high populations of bacteria, fungi and actinomycetes which show antagonistic activity against entomopathogenic fungi (Lingg and Donaldson, 1981). Particularly, *B. bassiana* is known to be invaded by numerous microorganisms including saprophytic fungi and bacteria, which cause biodegradation of the fungus in about 3 weeks (Lingg and Donaldson, 1981). This process is facilitated by amending the soils with carbon, nitrogen sources, or a combination of carbon and nitrogen that reduce *B. bassiana* conidial survival (Lingg and Donaldson, 1981). Biodegradation is a major factor in the survival of conidia in non-sterile soil, as it may decrease the persistence (Lingg and Donaldson, 1981) and efficacy of *B. bassiana* against insect pests (Pereira *et al*; cited by Inglis *et al*, 1998). However, studies done by Rosin *et al.*, (1996) had indicated that under sterile conditions, high rates of manure were beneficial to *B. bassiana* survival, since the latter is a saprophyte, but they could not predict what would happen under field conditions. Thus we are not certain whether these findings are applicable under our local conditions. In the case of the banana weevil control, it is desirable for *B. bassiana* to be infective in field conditions that may be using soil amendments for banana management or weevil control.

2.3.7. Literature summary

The Literature indicates that *C. sordidus* is a serious field insect pest of bananas due to the damage it causes to the plant. Microbial biological control of the banana weevil using *B. bassiana* is portrayed as one of the most promising components of the IPM strategy. However, *B. bassiana* has optimum operational range of conditions. Farmers' agronomic practices can alter the soil's physical, chemical or biological conditions thus rendering the entomopathogen more or less effective against the weevil. Considering that *B. bassiana* has been found to be more effective under laboratory than field conditions, there is need to evaluate the most promising formulation further in pot and field conditions under different soil amendments.

Further more, the survival of the fungal spores depend on the combined effects of several soil factors such as temperature, soil moisture, biota and organic matter. The success of any microbial control agent depends on its ability to persist and remain active in the environment of the target pest. However, *B. bassiana* will be used in banana fields under various environmental conditions, some of which may be detrimental to its survival. Therefore, it is necessary to evaluate the efficacy of the cracked maize *B. bassiana* formulation and investigate the trends in the soil characteristics, and determine how they influence the rate of degradation of the fungus under pot and field conditions.

CHAPTER 3

GENERAL MATERIALS AND METHODS

3.1.0. Experimental site description

The research was undertaken at the Kawanda Agricultural Research Institute (KARI) (0°25'N, 32°32'E, 1190 m above sea level), located 13 km north of Kampala, Uganda, with 12-hour day length throughout the year. The site has two rainy seasons (March-May and September-December), with mean annual precipitation of 1190 mm, and average daily temperatures of 16°C minimum and 29 °C maximum, with mean relative humidity of 76%. The soil is an isohyperthermic Kandiudalfic Eutrodox (USDA taxonomy) with high water holding capacity, medium acidity (pH 5.9-6.3), low organic matter content, deficient in nitrogen and phosphorus (McIntyre *et al.*, 2001, E. Sendiwayo, pers. comm.).

The study involved laboratory, pot and field experiments. The laboratory experiments were carried out from the insect pathology laboratory at Kawanda whereas out door pot experiments were set in the open outside the insect pathology laboratory at KARI. The field experiments were carried out in a newly established banana plantation at the institute. One local, susceptible and easily available East African cooking banana cultivar (cv Mpologoma; genome AAA-EA) was used in the pot and field experiments.

3.2.0. Banana weevils rearing

The initial batch of banana weevils was trapped from KARI and farmers' banana plantations in Masaka using the split pseudo stem traps method (Mitchel, 1980; cited

by Gold *et al.*, 2001). The weevils were reared from metallic drums on fresh banana corms under a shade as described by Nankinga (1999). The adult weevils were introduced to pared banana corms to oviposit eggs for seven days and there after the banana corms were maintained in metallic drums for 60 days to allow development of eggs to adults. The drums were covered with papyrus mats to avoid desiccation. The 60-day old corms were poured down in the shade and split pseudostem traps used to capture the newly emerging adult weevils.

The weevils were sexed on the basis of punctuations (pits) patterns on the rostrum after Longoria (1968; cited and used by Rukazambuga, 1996) and angle of inclination of 9^{th} abdominal segment after Roth and Wills (1963; cited and used by Rukazambuga, 1996). In the females, the punctuations on the rostrum were found towards the head end and they did not extend beyond the middle of the rostrum length, while in the males the punctuations extended beyond the middle of the length of the rostrum towards the tip of the mouth. On the other hand, the 9^{th} abdominal segment of males was sharply curved, while in the females the bend was not sharp.

3.3. 0. Determination of the effects of soil and environmental characteristics on the efficacy and persistence of *B. bassiana*.

3.3.1. Moisture content (MC), temperature and other soil characteristics

The MC and temperatures were recorded at 10-day intervals. The MC was taken by weighing 20g of soil or soil-amendment combination that was colleted from the upper half of the pots, weighed in Petri dish and dried in an oven at 100°c for 24 hours. The MC was calculated using the formula shown in appendix (a). The temperatures were taken using Centigrade clinical thermometers that were inserted in the pots, at 2.5cm soil depth below the mulch. Other amendment characteristics (N, P, K, organic matter, pH, sand, silt and clay) were determined from the soils analytical laboratory at Kawanda following the method described by Okalebu *et al.*, (2002). The analysis of these characteristics was done at 30-day intervals.

3.3.2. Micro - and macro -fauna

The dominant micro-organisms that attacked the maize based *B. bassiana* formulation were collected and taken to the laboratory for further subculturing and later identified using a handbook and identification keys as given by Kuhwant *et al.*, (1991). The macro-fauna were identified from a representative sample of 500cc from each experimental unit obtained at destructive sampling. This was achieved by spreading the sample on a white background and hand sorted for the different macro-fauna visible to the naked eye, that were latter counted and identified to order level.

3. 4. 0. Meteorological data

The pot experiments were conducted between July 2002 and April 2003, whereas the field experiments were done March-August, 2003. The following figure (Figure 1) shows the trends in the rainfall pattern for July 2002-August 2003; the period that covered the experimental periods. The data was obtained from the meteorological station at KARI. As seen from the figure, the pot experiments were conducted during a period of scanty rainfall and this necessitated regular watering of the plants. The field experiment was conducted during heavier rains and this could have affected the performance of the fungus. Heavy rains are associated with high soil moisture levels that are conducive to a wide range of biota that can compete with the fungus or lead to quick deterioration of the substrate onto which is cultured.

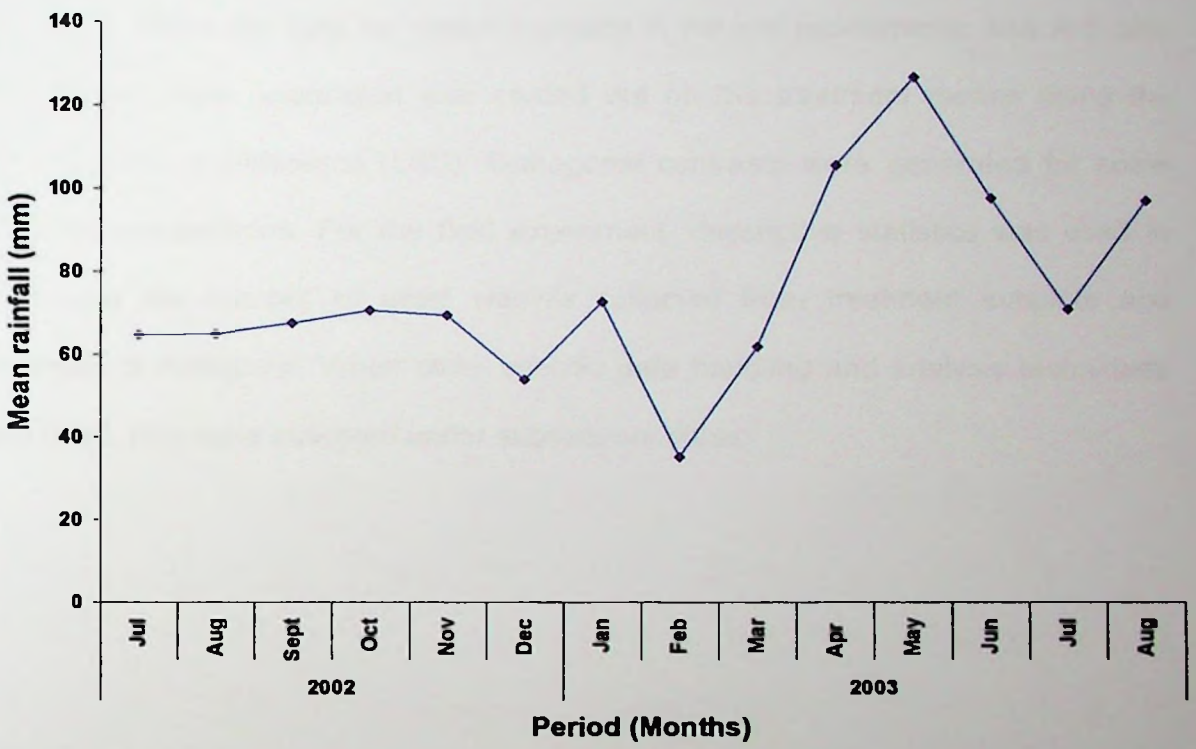


Figure 1: Rainfall distribution for July 2002-August 2003 at Kawanda Agricultural Research Institute (KARI), Uganda.

Source: KARI meteorological station data.

3.5. 0. Data analyses

The data for conidial yield from the substrates, weevil mortality from the formulations were subjected to statistical analysis using the ANOVA of SAS package (SAS Institute Inc. 1990). While the data for weevil mortality in the pot experiments was Arc Sine transformed. Mean separation was carried out on the treatment means using the Least Significant Difference (LSD). Orthogonal contrasts were generated for some treatment comparisons. For the field experiment, descriptive statistics was used to summarise the number of dead weevils collected from treatment subplots and presented in histogram. When other specific data handling and analysis techniques were used, they were indicated under subsequent tittles.

CHAPTER 4

EVALUATION OF SUBSTRATES FOR PRODUCTION OF *Beauveria bassiana* AND EFFICACY OF DIFFERENT FORRMULATIONS AGAINST THE BANANA WEEVIL

4.1. 0. Introduction

Beuaveria bassiana occurs naturally in *C. sordidus* affected areas and survives as parasite or as saprophyte in absence of the host (Charles, 1941). The saprophytic nature of the fungus suggests that the fungus can readily establish on a variety of organic substrates. Indeed, both nutritive solid substrates such as cracked maize, maize bran, rice and non nutritive ones such as vermiculite, sponge, cloth, solid substrates have been reported to variably support *B. bassiana* (Jenkins and Goettel, 1997). Conidia are the infective propagules against the weevil and high inoculum levels are required for effective pest control. This will require that appropriate substrates be identified and used for large-scale production of *B. bassiana*. A substrate that supports high conidia yield should be cheap, available and easy to culture. It is therefore necessary to evaluate the conidia yield of *B. bassiana* cultured on selected substrates.

Equally important is the development of a suitable formulation that is persistent with the target environment, in this case the field soil ecosystem. This necessitates that the active ingredient be formulated or mixed with other materials (Lomer and Lomer, 1997). This formulation enables *B. bassiana* to withstand the biotic and abiotic stress within the environment; the soil antagonists, ultraviolet light, temperature and humidity extremes commonly encountered in the field. Photo inactivation is one of the major

environmental factors affecting efficacy and thus persistence of entomogenous fungi. Formulation has been reported to greatly enhance the efficacy and persistence of entomopathogens under field conditions (Navon and Ascher, 2000). Besides, appropriate formulations ensure safer and improved effectiveness of the entomopathogen (Lomer and Lomer, 1997), and appropriate formulation continues to be a challenge in success use of entomopathogenic fungi (Paau, 1998). Similarly for *B. bassiana* to be effective in the applied environment, it must be formulated with the right materials. Elsewhere, *B. bassiana* has been formulated with a number of materials, but with varying performance against the target insect (Lomer and Lomer, 1997).

Limited information is available on the effectiveness of *B. bassiana* formulation under banana farming systems. Thus the specific objectives of this study were to;

1) Evaluate selected substrates for *B. bassiana* conidia production,

2) Evaluate efficacy of selected *B. bassiana* formulations and the infectivity of cracked maize *B. bassiana* formulation over time.

4.2. 0. Materials and methods

4.2.1. Assessment of conidia yield on selected substrates

4.2.1.1. Source of substrate used

The highly infective strain of *B. bassiana* (code G41), characterised by high pathenogenicity to *C. sordidus*, superior growth and sporulation (Nankinga, 1999) was used in the study. It was cultured in Kawanda Agricultural Research Institute (KARI) Insect pathology laboratory. Eight substrates: Cracked maize, maize bran, bagasse, "machicha"[2], cotton husks, maize bran + bagasse, maize bran + cotton husks and bagasse + spent yeast were assessed in the study. Where combinations were made, they were in the ratio of 1:1 by volume. The substrates and their source are as indicated in Table 1. The substrates were cultured following the modified diphasic method (Nankinga, 1999), which entails preparation of sucrose yeast to initiate fungal mycelial growth that is eventually inoculated and incubated with the solid substrate.

Table 1: The substrates evaluated for conidial yield and their source

Substrate	Source
Cracked maize grains	Reliable maize mill, Kawempe
Maize bran	Reliable maize mill, Kawempe
Bagasse[1]	Lugazi sugar works
"*Machicha*"[2]	Kawanda *malwa* (local brew) pub
Cotton husks	Kawempe ginnery
Spent yeast	Uganda Breweries Ltd, Luzira

[1] Bagasse is a dry fibrous material left behind after the liquid sucrose sugar has been extracted from the sugar canes.

[2] "*Machicha*" is spent millet and yeast residue obtained after a local potent gin ("*malwa*") has been extracted.

4.2.1.2. Preparation and inoculation of sucrose yeast media

Spent yeast in liquid form was collected from Uganda Breweries Luzira, and processed into dry yeast granules from the laboratory at KARI. The spent yeast was dispensed into one-litre bottles, but filled to three quarter mark to avoid overflow during autoclaving. The Bottles were there after covered with aluminium foil and autoclaved at 121°C for 30 minutes to sterilize the yeast. The bottles were cooled for about 12 hours and the supernatant liquid decanted off the yeast. The remaining layer of the slurry yeast was filtered through a double layer of white tissue paper (size 190x200mm, 14/15gsm, made in UAE) to remove excess water. Lamps of yeast were removed from the bottles and dried on plastic trays in open sunshine. The resultant hard coffee-brown granules were ground to a powder and stored in sealed plastic bottles under laboratory conditions.

One litre of the sucrose-yeast media was prepared by mixing 20g each of the dried ground yeast and sugar (sucrose), with 1000 mls of tap water. The mixture was blended in a mechanical blender for 2-3 minutes to clear the lamps of yeast. 50 mls of the Sucrose yeast media were dispensed into 150 ml jar glass bottles, plunged with aluminium foil, and then autoclaved at 121°C for 30 minutes. The bottles were then cooled at room temperature for about 12 hours. One inoculating loop-full of spores (about 6 x 10^7 spores) was scooped from the *B. bassiana* maintained on silica gel in the fridge at 4°C, and dispensed into 50 mls of the sterilized sucrose-yeast media. The bottles were shaken and placed on the laboratory bench for incubation for 3-4 days; there after, the bottles were shaken twice daily.

4.2.1.3. Preparation and inoculation of substrates.

The "machicha" was washed to remove excess alcohol and starch, and then dried before use. The cracked maize grains were also washed to remove the loose starch between the broken grains, dust and bad grains. The bagasse was also washed to remove excess sugar. The substrates were then soaked in water for about six hours by adding 2 parts of tap water to one part of the substrate and drained.

Two hundred grams of the substrate were placed in plastic bags (35 x 16 cm) and autoclaved at 121°C for 30 minutes. They were left to cool for about 12 hours, and then inoculated with four day old *B. bassiana* sucrose media prepared as mentioned in section 4.2.1.2. The inoculated bags were placed in plastic trays and kept in wooden cupboard for incubation under laboratory conditions. Every after three days, the bags were shaken and messaged. Once the fungus had sporulated over the whole surface of the substrate (for 5-7 days), the bags were opened up and contents poured onto clean paper in tray for drying (for 6-7 days), depending on the environmental conditions. The dried *B. bassiana* formulation was packaged in airtight plastic bags and ready for use. This diphasic process takes between 21 to 25 days (see appendix d).

4.2.1.4. Determination of conidial yield on substrates

The amount of conidia produced in each gram of substrate was determined using the improved bright line Neuber Hemacytometer counting chamber (0.100mm deep, Bausser, made in USA), following the method as described by Lomer and Lomer

997). One gram of fungal substrate was weighed using a sensitive balance (type M 300, made in England), and placed into a bottle vial, and mixed with 100 ml of stilled water. Two drops of liquid soap (Mukwano type; locally manufactured etergent) were added to facilitate dispensing of the conidia and minimise clustering, nd the solution was left to settle for 10 minutes, and shaken vigorously. One ml of olution was measured off and mixed with 9 mls of distilled water (= 10^{-1} dilution). One rop was introduced into the counting chamber using a dropper. Spores were counted om the 5 diagonal big squares, in 2 grids. This was repeated three times for each ubstrate. The amount of conidia per unit gram of the substrate was calculated using ie formula shown in the appendix (b). The conidia counts were analysed using NOVA models of SAS package (SAS institute Inc. 1990). Mean separation was arried out on the treatments using the least significant different (LSD).

.2.2. Assessment of infectivity of the different *B. bassiana* ormulations

leven *B. bassiana* formulations were evaluated under laboratory conditions; maize an alone, maize bran + loam soil, maize bran + clay soil, "Machicha" ("bussa") one, "machicha" + loam soil, "machicha" + clay soil, Cracked maize alone, cracked aize + loam soil, cracked maze + clay soil, Loam soil alone, clay soil alone, and ntrol (nothing added).

The loam soil was collected from the banana field at KARI with the physical characteristics of estimate levels of sand (52%), silts (28-50%), clay (7-28%), and high water holding capacity (23%). The clay soil was the grey type, mined from Kawanda water-logged swamp, with particle size of approximately 0.002 mm (Prasad and Power, 1997).

The *B. bassiana* grown on cracked maize substrate was used as the standard because of its high conidial yield, as determined under section 4.2.1. For cracked maize, one gram was used in each replicate and mixed with one gram of either clay or loam soil (1:1 ratio). The amounts of other substrates used depended on the amount of conidia determined in a unit gram, but they were also in the ratio of 1:1(substrate: formulation combination). The formulated substrates were weighed into plastic petri dishes and replicated three times.

The banana weevils used were of same age (0-4 weeks old) obtained from the stock reared on corms in metallic drums as described under the section 3.2 of General Materials and Methods. The weevils were quickly rinsed in distilled water to remove any surface contamination. Ten weevils of mixed sex (1:1 ratio) were introduced into each plastic petri dish with the formulations, and then left over night to walk on the fungus and ensure complete body coverage with fungal conidia. There after, the weevils were transferred into petri dishes lined with moistened tissue paper, but covered with the top lid to prevent weevil escape. Each treatment was replicated three times and kept on the laboratory bench at an average of 26°C and 79% R.H. The

treatments were assigned in a completely randomized design (CRD) and replicated thrice. The number of dead weevils was recorded at five-day intervals for a period of 30 days. Dead weevils were removed, and put into moistened tissue paper where they were observed for any fungal growth. Only weevils that showed mycosis were subjected to analysis using the ANOVA models of SAS package (SAS Institute Inc. 1990). Mean separation was carried out on the treatments using the Least Significant Difference (LSD).

4.2.3. Infectivity of *B. bassiana* on cracked maize over time

An important characteristic of a fungal pathogen is its ability to remain viable and infective after a period of storage (Pereira and Roberts, 1990). Part of the *B. bassiana* applied in the pots was therefore kept in the laboratory shelves at 26°C, 79% R.H and with 11.5% moisture content (M.C). Several 100g lots of the fungus packed in autoclavable bags, folded carefully and kept in a plastic bucket, placed on top of the cupboard in the laboratory, to mimic the farmer's storage conditions. The samples were routinely monitored for infectivity against *C. sordidus*, starting immediate after drying, but before storage. At 30-day intervals, 2g samples were picked at random from the bags and weighed into Petri dishes. Six samples were picked at each sampling time. Ten weevils of mixed sex and age (2-4 weeks old) were introduced into each dish and left to stand over night. The weevils were then removed and put into other sets of Petri dishes with moistened tissue paper where they were observed for mortality at 5-day intervals for 30 days. Dead weevils were removed, placed into

clean moistened tissue and observed for mycosis. The weevils that showed mycosis were subjected to analysis using the ANOVA of SAS package (SAS Institute Inc. 1990). Mean separation was carried out on the treatments using the Least Significant Difference (LSD).

4.3.0. Results

Results of conidia yield are presented in Table 2. Both cracked maize and maize bran produced significantly higher conidia than the other substrates. Cracked maize yielded the highest amount of conidia per unit gram of substrate ($3.2x10^9$ conidia per gram), while cotton husks had the lowest ($2.6 x10^8$ conidia per gram). However, the conidial yield from cracked maize and maize bran was not significantly different ($P>0.05$) from each other. It was generally observed that *Beauveria* on cracked maize sporulated more profusely than on other substrates.

Data for the weevil mortality caused by different *B. bassiana* formulations is presented in Table 3, and the cumulative mortality in Figure 2. Again, weevil mortality was significantly different ($p\leq0.05$) as observed between the different formulations. Cracked maize-based *B. bassiana* formulations caused the highest weevil mortality (90%), and while maize bran + loam soil, caused the lowest. Weevil mortality progressively increased with number of days with the lowest registered at 5 days, and highest at 30 days (Figure 2). It was only cracked maize that caused 50 % mortality at 15 days (Figure 2). It was also observed that soil formulations of "machicha" and maize bran reduced its infectivity except perhaps the clay based formulation of cracked maize (Table 3).

Results for infectivity of *B. bassiana* over time are presented in Table 4 and Figure 3. For the first three consecutive months (90days), *B. bassiana* caused weevil mortality of over 85% in 30 days, this was not significantly different ($P>0.05$) from each other. There after, the fungus started loosing its efficacy and by 180 days, it could cause mortality of only 20%. The freshly prepared cracked maize formulation was characteristically white, however, as storage duration increased, the formulation lost its colour due to attack by mites (Plate 1(a) and 1 (b). The mites were able to penetrate through the folds of the paper bags. The dead cadavers also expressed a lot of *Beauveria* sporulation on their surfaces (Plate 2).

Table 2: *Beauveria bassiana* conidia yield on selected substrates

Substrate	Mean conidia count/gram of substrate ($x10^8$)
Cracked maize	32.7
Maize bran	30.7
Maize bran + bagasse	6.5
" Machicha"	5.0
Bagasse	3.8
Cotton husks + Maize bran	3.7
Bagasse + spent yeast	2.9
Cotton husks	2.6
LSD (P=0.05)	**3.6**
CV (%)	**18.8**

Table 3: Mean accumulated mortality attained in weevils exposed to different *B. bassiana* formulations

Formulation	Mean weevil mortality* (%)
Cracked maize + clay	90.0
Cracked maize alone	80.0
Cracked maize + loam	70.0
" Machicha"	63.3
"Machicha" + clay	46.7
Maize bran + clay	30.0
"Machicha" + loam	26.7
Maize bran alone	13.3
Maize bran + loam	3.3
LSD (P=0.05)	**2.8**
CV (%)	**34.9**

*Weevil mortality assessed for a period of 30 days.

Table 4: Infectivity of cracked maize *B. bassiana* formulation stored at room temperature for 180 days

Storage Period (Days)	Mean Weevil Mortality (%)*
Before storage	100.0
30	98.3
60	100.0
90	86.7
120	58.3
150	60.0
180	20.0
	11.03
LSD (P=0.05)	
CV (%)	**12.58**

* Weevil assessment for each period done at 5-day intervals for 30 days.

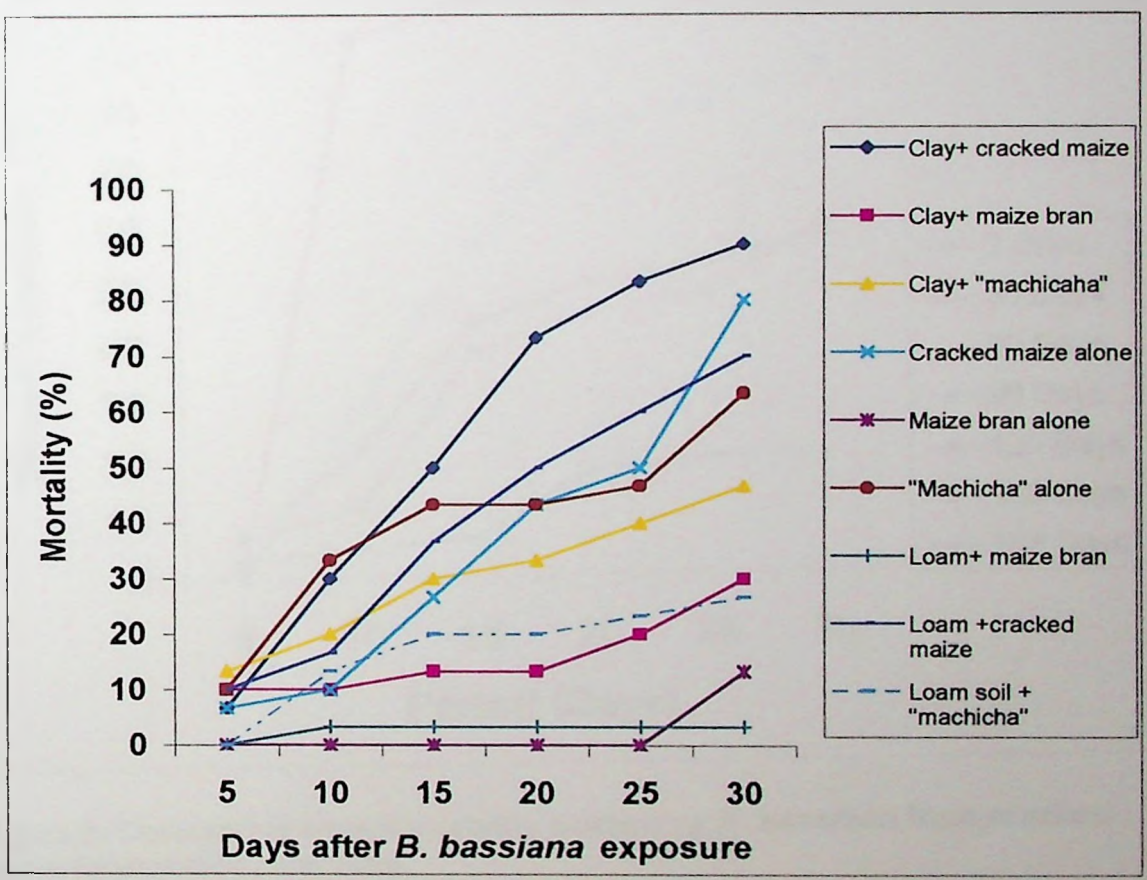


Figure 2: Cumulative weevil mortality caused by different *B. bassiana* formulations over time.

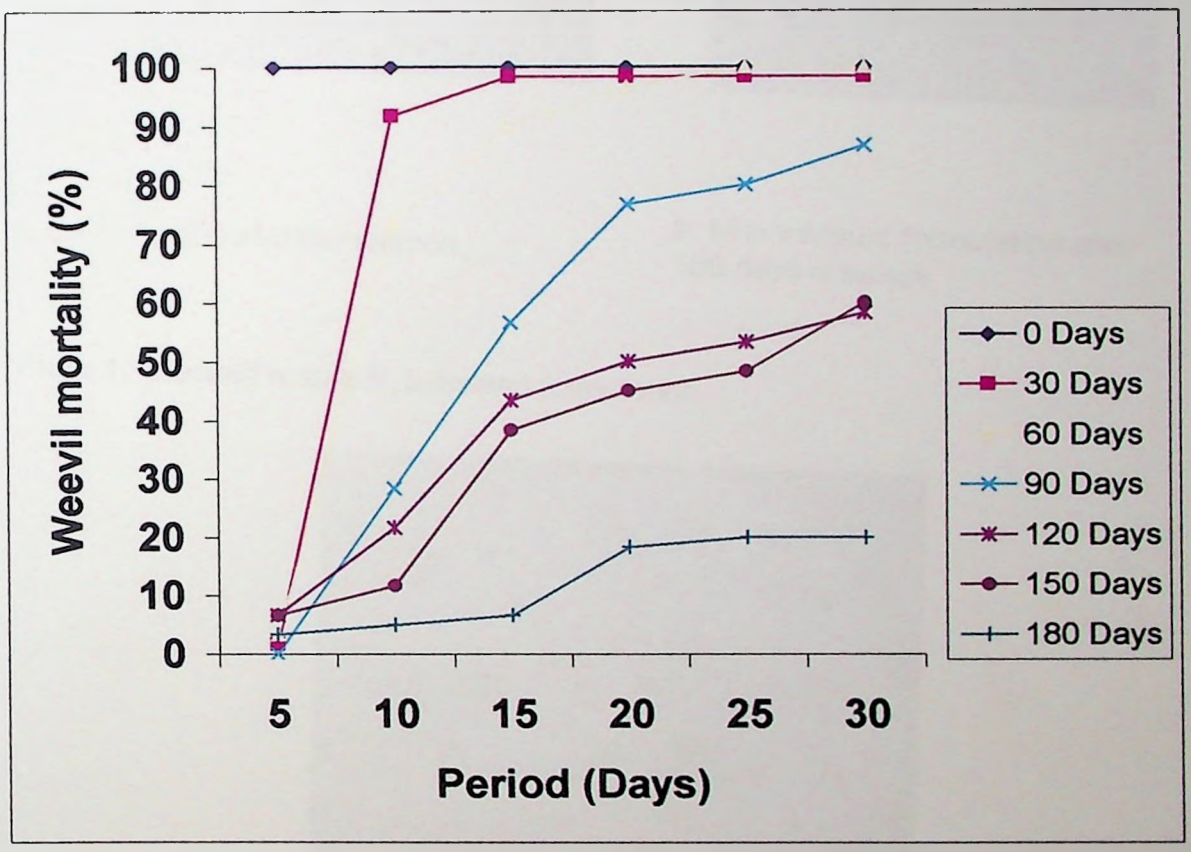
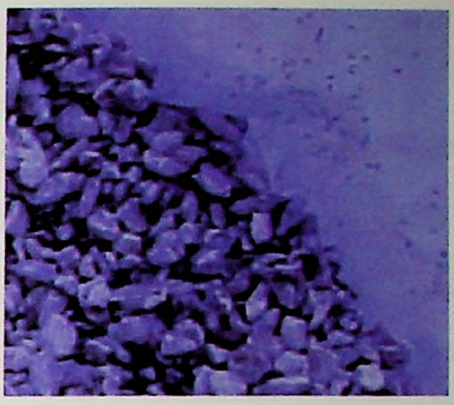


Figure 3: Cumulative weevil mortality caused by *B. bassiana* from cracked maize formulation over time.

a. Newly cultured formulation.



b. Mite infested formulation after 180 days of storage

Plate 1: Cracked maize *B. bassiana* formulation

Plate 2: Dead, B. bassiana infected weevils from the fresh cracked maize formulation.

4.4. Discussion

The results above portray variability in *B. bassiana* conidia production on the different substrates evaluated; cracked maize was the best substrate. As compared to other substrates, cracked maize is readily prepared and hence experiences less chances of substrate caking during *B. bassiana* culturing and incubation. Godonou (1999), working with Rice achieved a lower yield of $5x10^8$-$6x10^8$ conidia per gram. Whereas Feron and Antia-London *et al.*, (1992; cited by Godonou, 1999) working with rice achieved a higher yield of $6x10^9$ and $7.7x10^9$ conidia per gram respectively. *B. bassiana* can grow saprophitically and requires a carbon source for its growth. The fungal substrates that provide sufficient quantities of carbon will most likely support fungal growth and sporulation. In the present study, cotton husks, "machicha" and bagasse are insufficient, and thus did not facilitate its establishment. It is therefore most likely that *B. bassiana* conidia yield is influenced by both the substrate and culturing condition. Cotton husks, bagasse, and "machicha" were comparatively deficient in conidia yield and therefore are not good candidates for the fungus production.

Results further demonstrated the role of formulation in increasing infectivity of *B. bassiana*. Clay seemed to have some synergistic effect in all the formulations as indicated by higher mortality in most of the formulations where it was incorporated. Contrastingly, loam-based formulations exhibited reduced weevil infectivity. Not withstanding its recorded high conidial yield, maize bran was not as effective against the weevil. This could be explained by its surface configuration; the particle nature of

the maize bran hides away some of the conidia and is thus may not readily be exposed to the weevils, leading to the recorded low mortalities. This is in contrast to the maize grains that have bigger surface area, whereby the conidia are fully exposed to the weevils. However, this is an area for further investigation. The influences of clay, although not positively quantified, appear to be indirect. Clay is known to be adhesive and absorb water (Prasad and Power, 1997), and it is most likely that it reduced the moisture content of the *B. bassiana* substrates, and hence increased their infectivity (Fagues *et al.*, 1983, Studdert *et al.*, 1990). Indeed, earlier studies established that entomopathogenic conidia maintained at low moisture content, are highly pathogenic (Lingg & Donaldson, 1981). The relatively high mortality in the clay based formulations is testimony to this fact. Elsewhere, clay based formulations are being advocated for pest management (Fagues *et al.*, 1983, Studdert *et al.*, 1990).

The laboratory infectivity studies indicated that *B. bassiana* conidia remained effective for 150 days (>50% mortality). It is known that the spores of entomopathogenic fungi can remain viable for two or more years depending on the storage conditions (Smith, 1990; cited by Nankinga, 1994). Nankinga (1994) showed that *B. bassiana* conidia could remain viable in the soil under laboratory conditions for at least two years. In this study, mites (Arachnida) had infested the formulated fungus in the bags by 120 days of storage (Plate 1(a) and this led to the fast degradation of the maize substrate, and subsequently degradation of the fungus.

Basing on these results, it is recommended that the fungus should not be stored longer than 150 days on a carbohydrate source under laboratory conditions (26^0C, 79% RH). Apparently, this is a limited shelf life, which has also been pointed out in earlier studies as one of the limitations of using pathogens in pest control (Tanada and Kaya, 1993). The freshly prepared fungus has been demonstrated to be able to infect and kill the banana weevil under the laboratory conditions. This study has shown that there is a need to improve the production system of the fungus with emphasis on proper drying, packaging and storage methods.

CHAPTER 5

EFFICACY AND PERSISTENCE OF *Beauveria bassiana* APPLIED IN POTS UNDER DIFFERENT SOIL AMENDMENTS

5.1.0. Introduction

Banana farmers normally add organic or inorganic amendments in banana fields to boost soil nutrient level for increased banana productivity. The amendments can affect the chemical (pH, nutrient levels) physical (temperature, moisture) and biological (antagonists and decomposers) properties of the soil (Lopez, 1998). *Beauveria bassiana* is known to be associated with natural habitats of high organic matter (Mietkiewsk *et al.*, 1997) and can persist in such soils for long periods (Benz, 1997; cited by Nankinga, 1999). However, both survival and persistence of conidia may be reduced by biotic and abiotic factors caused by addition of amendments in agricultural fields (Lingg and Donaldson, 1981, Roberts and Campbell, 1977). Faye *et al.*, (1996) established that soil containing fresh cow manure was detrimental to *B. bassiana,* while high rates of compost were beneficial, under sterile laboratory conditions, yet urea had no significant effect on the fungus.

Lingg and Donaldson (1981) also observed that soil amendments beneficial to conidia survival in sterile soil were detrimental in non-sterile soils because of stimulation of microflora. Studies done by Rosin *et al.*, (1996) indicated that under sterile conditions, high rates of manure were beneficial to *B. bassiana* survival, since the latter is a saprophyte. However, they could not predict what would happen under field

conditions. Nankinga (1999) noted the variation in growth and infectivity of *B. bassiana* applied to soil incorporated with ash or tobacco, urine and ash based mixtures used by farmers to control the banana weevil in Uganda. These results suggested that *B. bassiana* would exhibit varying levels of efficacy and persistence under different soil conditions.

Farmer's implement a wide range of banana agronomic management practices including maintaining different plant densities, application of mulches and various organic and inorganic amendments, and chemical pesticides. These practices can affect the soil's pH, temperature, moisture content, and biological macro and micro fauna, which in turn may affect the performance of *B. bassiana*.

This study aimed at evaluating the effect of different soil amendments in form of coffee husks, decomposed cow dung manure, or inorganic fertilizers on the efficacy and persistence of *B. bassiana*.

5.2.0. Materials and methods

An outdoor pot experiment was set up at Kawanda Agricultural Research Institute to evaluate the efficacy and persistence of *B. bassiana* in soil amended with organic or inorganic amendments. The strain of the fungus (code G41), used under laboratory tests and known to have high pathenogenicity to *C. sordidus*, superior growth and sporulation characteristics, was used. The fungus was cultured on cracked maize

substrate using the diphasic method as described in Chapter 4 and applied as a dried formulation.

The *B. bassiana* formulation was applied to ten treatments: (1) loam soil without amendments; (2) pure decomposed cow dung manure; (3) pure coffee husks; (4) loam soil mixed with coffee husks at a ratio of 1:1; (5) loam soil mixed with coffee husks at a ratio of 1:2; (6) loam soil mixed with coffee husks at a ratio of 1:3; (7) loam soil mixed with cow dung manure at a ratio of 1:1; (8) loam soil mixed with cow dung manure at a ratio of 1:2; (9) loam soil mixed with cow dung manure at a ratio of 1:3; (10) inorganic fertilizers. The ratios; soil: amendments were by volume using a 1.25 litre capacity plastic bowl as the standard measure; however, the recommended weights for the inorganic fertilizers were adopted.

Freshly hulled coffee husks were purchased from a commercial coffee processor in Matugga Township, near KARI. The cow dung manure was 3 months old, completely decomposed and obtained from a local farmer's kraal near the research institute. The artificial fertilizer used was an NPK composite, applied at a rate of 69g of NPK (19:19:19), 28g of Urea (Urea 46%), 31g of KNO_3 (13% Total N: 13% No_3: 33%K), 3g of $MgSO_4$, applied per pot (D. Bwamiki, pers. comm. 2002). All amendments were incorporated into the soil.

Single pared banana suckers of local cooking East African Highland banana cultivar "Mpologoma" (cv Mpologoma, AAA-EA) were planted in 6-litre capacity pots each

containing a specific treatment discussed above. The buckets were perforated at the bottom with holes to facilitate water drainage. Muslin cloth was placed in the bucket at the bottom and used to cover the top to limit weevil escape and invasion. These suckers were allowed to grow and establish for 44 days after which 100g (about 3.2 x 10^9 conidia/gram) of the maize-based formulation of *B. bassiana* was applied on top soil (Plate 3) and mulched with about 50g of dried banana leaf. Four experimental units were prepared for each amendment and release (see Plate 4), the treatments (plants in pots) were assigned in a completely randomized design and replicated thrice. Ten adult weevils were released on each pot within experimental unit at the interval of 30 days. For each release, the pots were monitored every 5 days for dead weevils infected with *B. bassiana* for a period of 30 days. At the 30th day of monitoring, 2 g samples of *B. bassiana* maize based formulation were also collected from each of the pots in a given release. Ten weevils were introduced into each of the petri dishes containing the 2g sample and monitored for mortality and *Beauveria* infection at 5-day intervals for another 30 days. The dead weevils were tested for infectivity in the laboratory by introducing them in moistened tissue paper and observed for mycosis due to *B. bassiana*. This was repeated after 60, 90 and 120 days. The pots were maintained outdoors from July 2002 to April 2003. During dry periods, we provided routine watering of the plants as necessary.

During the course of the experiment, the soil moisture content (MC), quality characteristics (NPK, OM, pH, C), surface temperature, soil macro-fauna, fungal substrate colonisers or decomposers, were all taken as described in general materials

and methods (Chapter 4). Corm weevil damage assessment was done 42 days after weevil release. This was done on the 2 cross sections of the corm (upper and lower), and inner and outer cortex using the technique described by Gold *et al.*, (1994a) and modified by Rukazambuga (1996). In the method, the upper section was made at the crown and the lower at 5 cm below the crown. The cut section was divided into 8 equal parts. The inner central cylinder and outer (cortex) section were assessed for the area damaged by the weevil larvae. The damage was estimated by consolidating the damaged area in the 8 portions of the outer and inner sections of the corm. The inner damage (ID) and outer percentage damage (OD) were averaged to obtain the total damage per plant.

The weevil mortality and corm weevil damage data were subjected to statistical analysis using the ANOVA of SAS package (SAS Institute Inc. 1990) and Arc Sine transformed. Mean separation was carried out on the treatments using both Least Significant Difference (LSD) and orthogonal contrasts. Temperature, moisture content and macro-fauna were analysed using ANOVA of the same package and means separated using standard errors (SE) and presented graphically. OM, N, P, K were analysed using the ANOVA and means separated by Turkeys' Studentized Range (HSD). The pH was computed using simple means.

Plate 3: *Beauveria bassiana* cracked maize formulation immediately after application in a pot.

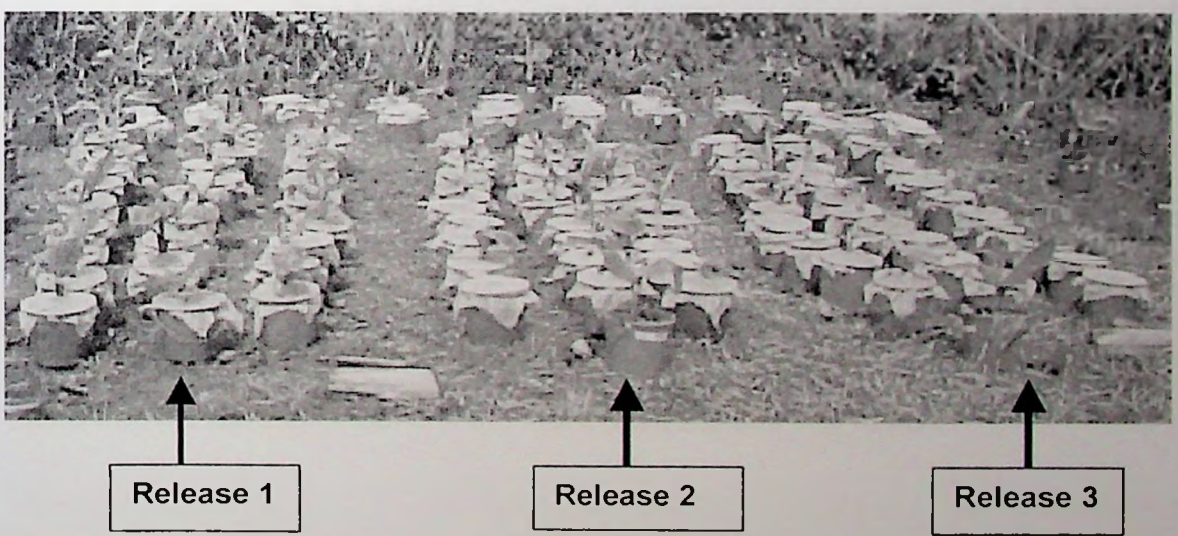


Plate 4: Out door arrangement of pots according to the different weevil release periods. The fourth release batch is not shown in the plate.

5.3.0. Results

Results for weevil mortality from *B. bassiana* applied in pots under different soil amendments are presented in Table 5a and 5b. The highest mortality (31-55%) was obtained for weevils released in pots immediately after *B. bassiana* application and steadily decreased thereafter with lowest mortality rates (5-31%) for weevils released 90 days after applying *B. bassiana*. *Beauveria bassiana* applied to soils without amendments consistently caused higher mortality than other treatments for all release periods, and was significantly different for the 60-day release period (Table 5a and 5b). Also, within the same amendment and across the various release periods, significant differences in weevil mortalities were mostly realised after the 60-day release period. The observed resumption in weevil mortality for the 60 and 90-day periods in some of the treatments (Tables 5a and 6a respectively) was attributed to the drier conditions prevailing during that sample period of Dec.2002-Jan.2003 (Figure 1). Most of the *B. bassiana* infected weevils were recovered from the dry moist banana sheaths attached onto the plant and from the soil around the potted plants (Plate 5). The majority of the dead *B. bassiana* infected weevils were discovered from the banana sheaths (Plate 5) and in pseudostem traps. A few other dead weevils were also found in the moist dried banana leaf mulch.

For the samples picked from pots and tested in the laboratory, highest banana weevil mortality (59-81%) was obtained for samples taken after 30 days and declined thereafter (Table 6a). Samples collected 120 days after treatment, caused the lowest mortality of 15-26%. The trend for un amended soil was not very evident for the 30,

and 60 exposure periods, even though it had the highest mortality records for the 90 and 120 day periods (Table 6a).

Comparisons of the two weevil mortality data sets (Tables 5a and 6a), indicated obvious differences in mortality for the pot and the laboratory, with latter being higher in most cases. Contrast comparisons indicate significant differences between unamended soils and the rest of the amendments (Table 5b, Table 6b).

The mean corm weevil damage results for the four sample periods are shown in Table 7a. For all the treatments, more damage was recorded on the lower than than upper sections of the corm. Also, the plants in soil amndementments indicated significantly higher corm damage than in un amended soils, with plants in coffee husks based amendments expressing the highest damage (Table 7a and 7b).

The results for the soil moisture content (MC) are shown in figure 4 and Figure 5, soil macro fauna in Figure 6 and soil temperature in Figure 7, whereas the soil quality characteristics are shown in Table 8. Soils with coffee husks or cow dung manure had significantly higher levels of moisture content (MC) than un amended soil (Figures 4 and 5). The trend was the same for organic matter. Furthermore, coffee husks based amendments had the highest number of the macrofauna, and unamended soils showed the lowest. Coffee husks based amendments and decomposed cow dung manure had significantly high organic matter; 6-19% and 6-10% respectively (Table 8).

The macro fauna recorded from the samples were essentially of the following orders; Isoptera (ants), Nematoda and Annelida (worms), Milliapoda (centipedes and millipedes), Archinida (spiders) and Collembolans (spring tails; parainsects) and Gastroppoda (snails and slugs). *Aspergillus flavus* and *Penicillium crysogenum* were the dominant fungal colonisers, with the latter being more prevalent (Plate 6). These two fungi were observed in all treatments from 28 days after treatment. However, the rate of substrate colonisation and abundance of the colonisers was observed to be more in amended than un amended soils.

Temperature variation from the different amendments was not significantly different (Figure 7). Temperatures varied between 20-34^{o}c in the amendments throughout the study period.

Table 5: Mean weevil mortality (%) due to *B. bassiana* applied in different soil amendments over time

Amendment + *B.bassiana*	Weevil release period (Days)			
	0	30	60	90
Un amended soil	55.1 (66.7)	45.0 (50.0)	36.7(23.0)	26.7(31.0)
Coffee husks alone	38.9 (40.0)	18.0 (10.0)	14.9 (6.7)	12.2 (3.3)
Cow dung manure alone	34.2 (33.3)	26.1 (20.0)	15.3 (6.7)	12.2 (3.3)
Soil + Coffee husks (1:1)	32.2 (30.0)	20.7 (13.3)	12.2 (3.3)	18.0 (10.0)
Soil + Coffee husks (1:2)	31.0 (26.7)	13.3 (19.1)	15.3 (3.3)	25.4 (10.0)
Soil + Coffee husks (1:3)	39.1 (40.0)	28.8 (23.3)	26.1 (20.0)	14.9 (6.7)
Soil + Manure (1:1)	46.9 (53.3)	22.3 (16.7)	12.2 (3.3)	18.0 (10.0)
Soil + Manure (1:2)	36.9 (38.7)	43.0 (46.7)	9.1 (0.0)	21.2 (13.3)
Soil + Manure (1:3)	38.2 (40.0)	28.8 (33.3)	18.0 (10.0)	20.7 (13.3)
Inorganic fertilizers in soil	41.2 (43.3)	25.2 (20.0)	20.0 (26.1)	12.2 (3.3)
LSD (P=0.05)	**21.17**	**23.26**	**10.99**	**13.46**
CV (%)*	**31.57**	**48.56**	**34.59**	**42.51**

Note. The data in Table 5 above has been Arc Sine transfomed and the actual means are indicated in the parenthes. The indicated LSD and CV are for the transformed data. Zero (0) period is the starting period.

Table 6: Mean Weevil mortality (%)caused by *B. bassiana* formulation picked from pots after 30, 60, 90 and 120 days of exposure in the pots

Amendment + *B.bassiana*	*B.bassiana* Exposure period (Days)			
	30	60	90	120
Un amended soil	64.7 (80.0)	43.1 (46.7)	35.2 (33.3)	26.1 (20.3)
Coffee husks alone	64.8 (80.0)	23.0 (16.6)	30.3 (26.7)	21.2 (13.3)
Cow dung Manure alone	69.8 (86.7)	15.3 (6.6)	12.2 (3.3)	18.0 (10.0)
Soil + Coffee husks (1:1)	63.1 (76.7)	39.2 (43.3)	24.2 (20.0)	15.3 (10.0)
Soil + Coffee husks (1:2)	67.0 (83.3)	48.9 (56.7)	20.3 (13.3)	21.2 (13.3)
Soil + Coffee husks (1:3)	63.1 (76.7)	41.1 (10.0)	31.1 (30.0)	23.9 (16.7)
Soil + Manure (1:1)	59.0 (73.3)	48.9 (56.7)	9.1 (0.0)	26.1 (20.0)
Soil + Manure (1:2)	80.9 (100.0)	38.9 (40.0)	9.1 (0.0)	14.9 (6.7)
Soil + Manure (1:3)	65.0 (80.0)	41.1 (43.3)	14.9 (6.7)	26.7 (20.0)
Inorganic fertilizers in soil	68.9 (86.7)	30.3 (30.0)	18.3 (10.0)	26.1 (20.0)
LSD (P=0.05)	**21.21**	**33.62**	**18.66**	**11.27**
CV (%)*	**18.69**	**56.96**	**53.59**	**30.18**

Note. The data in Table 6 above has been Arc Sine transfomed and the actual means are indicated in the parenthes. The indicated LSD and CV are for the transformed data.

Table 7: Corm weevil damage (%) from plants in pots with *B. bassiana* applied under different soil amendments

Amendment + *B. bassiana*	Mean corm damage (%) Upper damage	Lower damage
Un amended soil	1.21 (1.2)	3.5(3.4)
Coffee husks alone	11.7 (15.0)	15.1 (18.5)
Manure alone	5.6 (5.1)	9.1 (7.9)
Soil + Coffee husks (1:1)	10.0 (13.0)	13.6 (17.8)
Soil +Coffee husks (1:2)	10.8 (13.1)	12.4 (15.4)
Soil +Coffee husks (1:3)	8.8 (8.7)	14.9 (18.0)
Soil + Manure (1:1)	6.2 (6.2)	14.2 (14.0)
Soil + Manure (1:2)	5.7 (4.4)	11.2 (11.3)
Soil + Manure (1:3)	5.3 (4.8)	8.4 (5.8)
Inorganic fertilizers	4.5 (4.7)	8.5 (10.5)
LSD (P=0.05)	5.45	6.99
CV (%)*	56.74	45.35

Note. The data in Table 7 above has been Arc Sine transfomed and the actual means are indicated in the parenthes. The indicated LSD and CV are for the transformed data.

Plate 5: Dead, *B. bassiana* infected banana weevils located from banana sheaths in the pot trial on the efficacy of the fungus under different soil amendments.

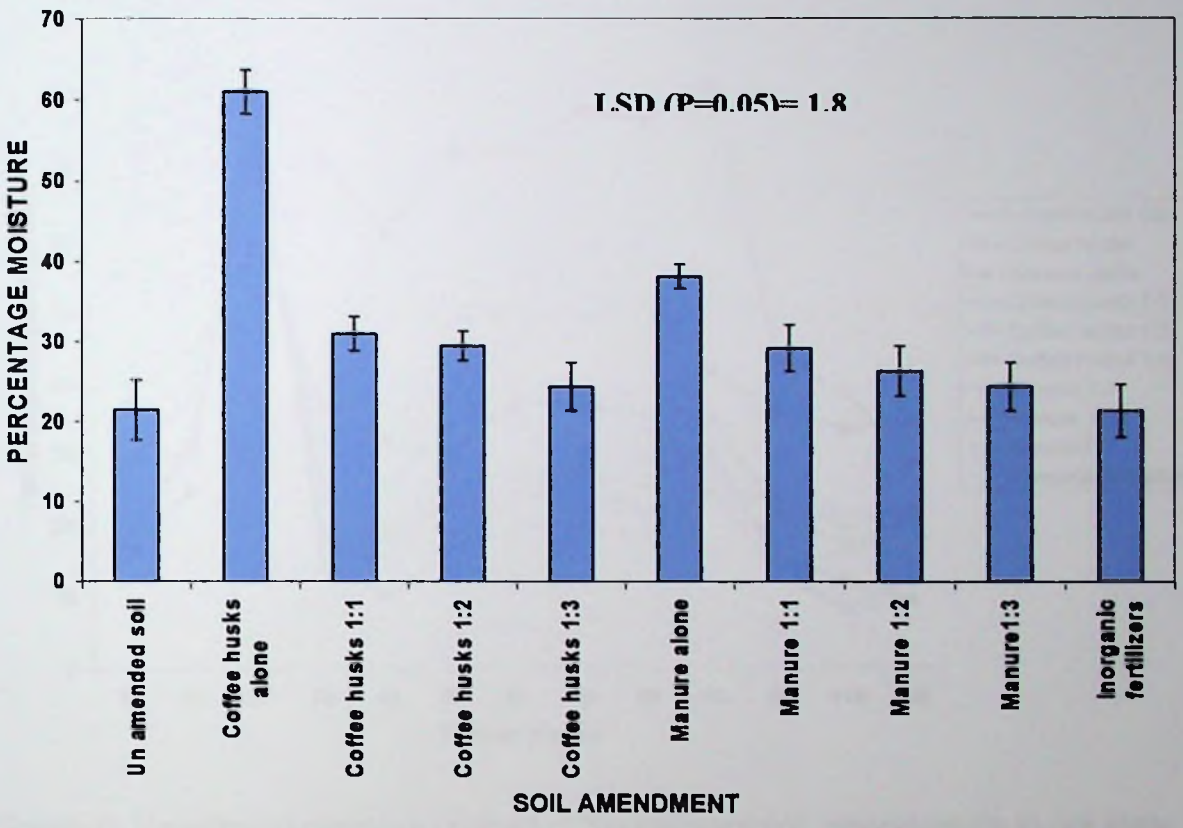


Figure 4: The mean moisture content in the different soil amendments in pot trial on efficacy of *B. bassiana* against banana weevil.

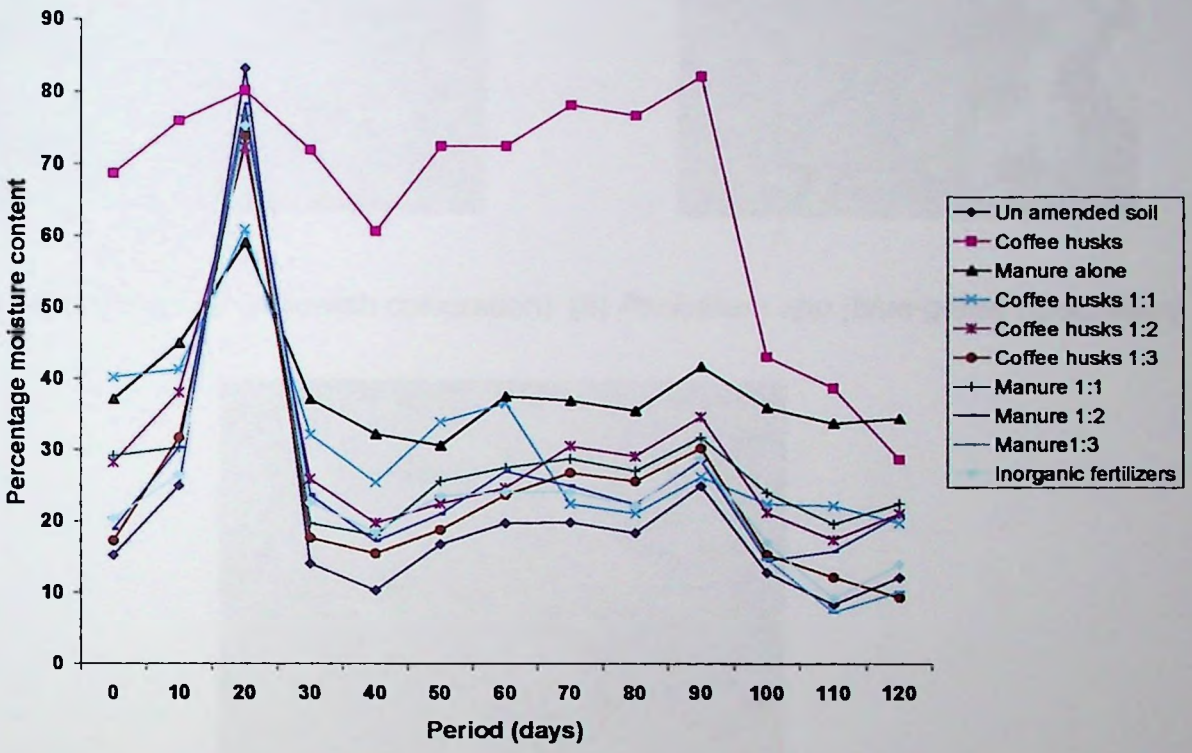
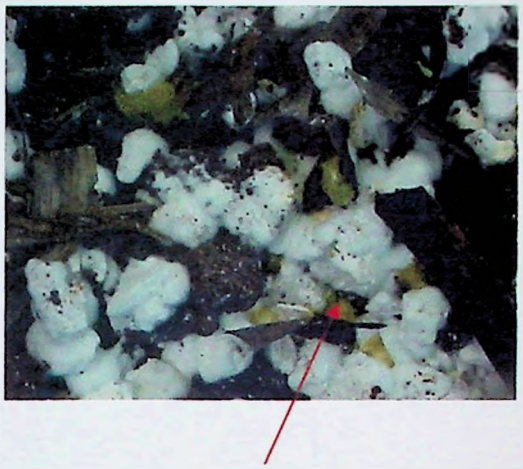
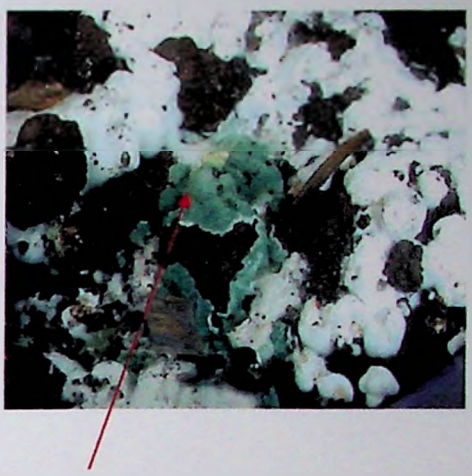
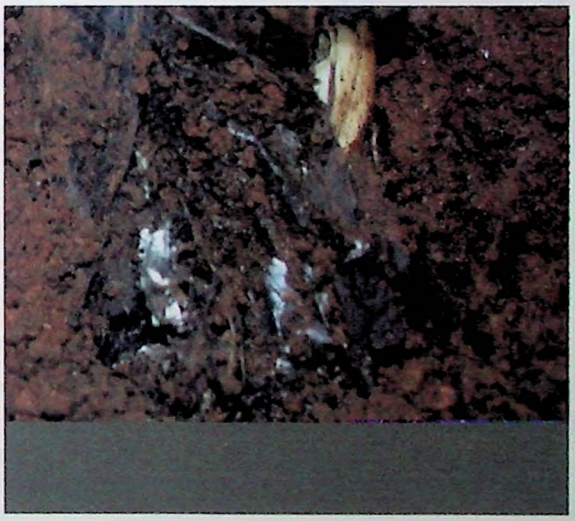


Figure 5: Variation in moisture content of the different soil amendments in pot trials on efficacy of *B. bassiana* against banana weevil





(i) *Aspergillus spp* (yellowish colouration) **(ii)** *Penicillium spp (blue-green colouration)*



(iii) Completely decomposed *B. bassiana* substrate after 90 days in the pot with coffee husks

Plate 6: Fungal Substrate colonisers and decomposers that attacked *B. bassiana* substrate leading to complete deterioration.

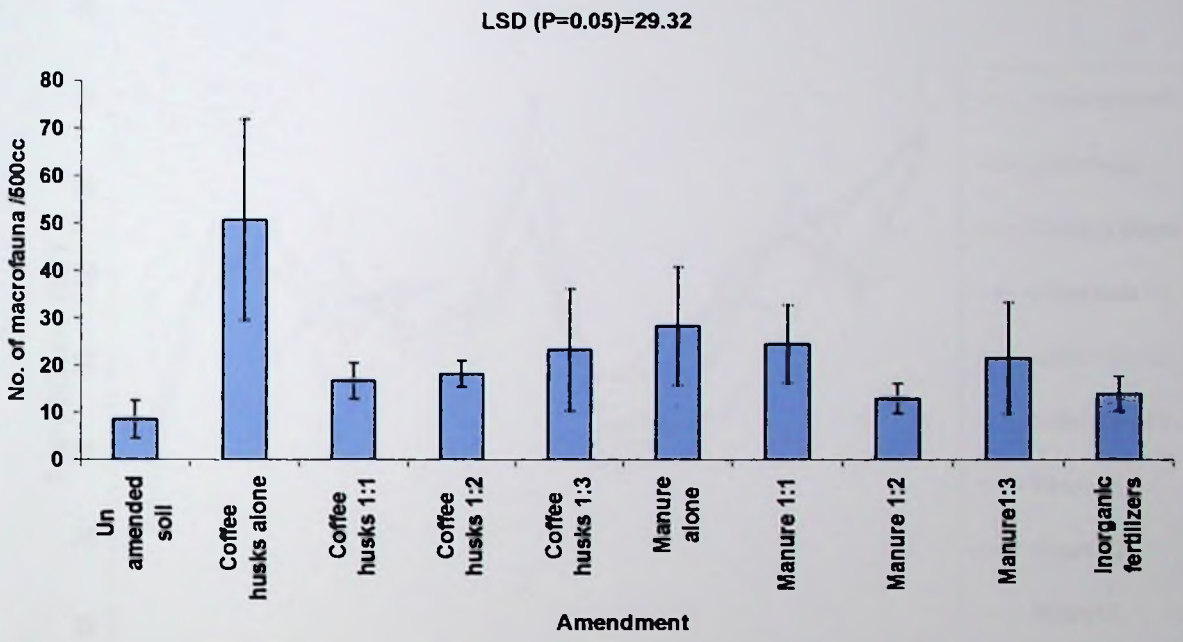


Figure 6: Abundance of Soil macro fauna from the different soil amendments in pot trial on efficacy of *B. bassiana* against banana weevil. The soil: amendment ratios were by volume (V/V).

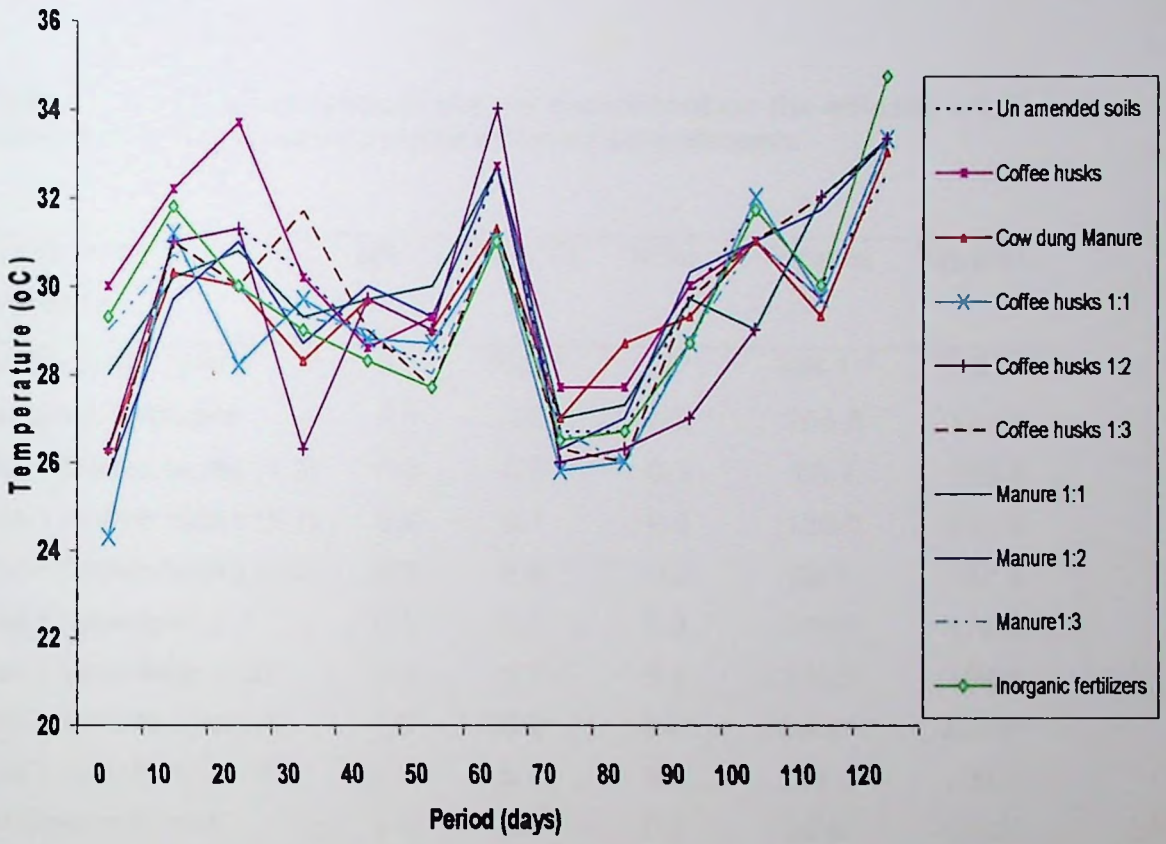


Figure 7: Temperature variation in the different amendments in the pot trial on the efficacy of *B. bassiana* under different soil amendments. The soil: amendment ratios were by volume (V:V).

Table 8: Soil characteristics in the pot experiment on the efficacy and persistence of *B. bassiana* under different amendments

Treatment + *B. bassiana*	pH	OM (%)	N(%)	P(ppm)	K(ppm)
Coffee husks alone	6.7	19.9**	1.4**	488.1**	718.8**
Inorganic fertilizers	5.1	3.6	0.2	254.8	182.5
Soil + Coffee husks (1:2)	6.5	6.8	0.3	96.1	189.5
Soil + Coffee husks (1:1)	6.9	8.1	0.3	126.0	331.6
Soil + Coffee husks (1:3)	6.2	6.3	0.3	69.0	187.4
Soil + Cow dung 1:1	6.6	6.5	0.3	378.0	170.8
Soil + Cow dung (1:2)	6.5	5.7	0.2	213.6	184.4
Soil + Cow dung alone	7.3	10.2*	0.4*	929.9***	377.6*
Soil + Cow dung (1:3)	6.4	5.9	0.3	217.7	105.8
Un amended soils	4.9	4.2	0.2	39.6*	22.3

NB. Comparisons significant at 0.05 levels by Turkey's Studentized Range test (HSD) in a given column are indicated by same asterisk. The soil: amendment ratios were by volume (V:V).

5.4.0. Discussion

The present study demonstrated that overall, soil amendments reduce the efficacy and persistence of *B. bassiana*. The degradative effects of the soil amendments take time to be witnessed and that explains the lack of significant differences in weevil mortality for the first two releases. Among the major influential effects are the high moisture levels that are known to promote substrate attack by other fungal colonisers or decomposers (Lingg and Donaldson 1981 and Studdert *et al.*, (1990), and the rate of fungal culture degradation was consistently high in amendments with high MC levels and during wet conditions.

Nankinga (1999) observed similarly that most of the dead infected weevils were found in the banana sheaths. Most banana weevils are known to live and hide mainly in leaf sheath or in the soil at the base of the mat (Gold *et al.*, 1999d), and when infected by the entomopathogen, they are likely to die from the same locations.

Samples tested in the laboratory showed reduction in *B. bassiana* efficacy over time under pot conditions. It is evident that B. *bassiana* was more infective under laboratory than under pot (semi field conditions). Such differences in mortality rate have also been reported from field trials using *B. bassiana* against grasshoppers (Goettel and Inglis, 1995; cited by Inglis *et al.*, 1998). Rosin *et al.*, (1996) showed that under sterile conditions, high rates of manure were beneficial to *B. bassiana* conidial survival as it provided the carbohydrate requirement. Also, Pena *et al.*, (1993) and Traore (1995) found higher rates of infected banana weevils when *B. bassiana* was

applied to sterilized soil than when applied to non-sterile soil under laboratory conditions, suggesting the antagonistic action of other soil organisms. The conditions under field conditions are different (not sterile) and the presence of soil fauna may affect fungal efficacy and persistence. In related studies, Nankinga (1999) suggested that microbial degradation of entomopathogens was more pronounced in soils with high organic matter that exhibited high moisture content.

The recorded soil parameters could also be used to explain the trends in weevil mortality. Some of the recorded soil macro fauna and micro biota are known fungus eaters and antagonists respectively. For instance some arthropods like Collembollans and mites reportedly feed on fungal spore heads (Burges and Raw, 1967). The presence of high organic matter is also known to double populations of soil arthropods such as collembollans and mites, which reportedly feed on spore heads (Burges and Raw, 1967). *Penicillium* spp and *Aspergillus* spp recorded in this study are among the well known fungal antagonists that may affect the *B. bassiana* infective units even when they have already contacted the pest (Roberto *et al.*, 1992). Overall, several soil antagonists are known to adversely affect the entomopathogen by preventing attachment to the pest and prevention of development of infection. The macro-and micro-biota may have the potential to reduce the efficacy and persistence of the applied fungus as they grow and colonise the substrate onto which *B. bassiana* is cultured and disseminated in the soil.

It is possible that high soil moisture led to the fast degradation of the maize-based *B. bassiana* formulation. Lingg and Donaldson (1981) and Studdert *et al.*, (1990) reported decreased survival of *B. bassiana* as moisture content increases. The recorded moisture content in amendments with coffee husks or decomposed cow dung manure was obviously above the optimum range of 10-30% required for *B. bassiana* survival in the environment. The moisture content in un amended soil and that amended with inorganic fertilizers was not significantly different from each other. This is an indication that the inorganic fertilizers did not affect the water holding capacity of the soil and moisture content in this treatment and subsequent weevil mortalities in the two treatments did not differ.

In all cases, the recorded temperatures never went above the 37°c mark that is reported to be detrimental to *B. bassiana* (Nankinga, 1999, Driesche and Bellows, 1996). Therefore, temperature is not considered to be influential in the observed weevil mortality trends in these experiments. However, the combined effects of such temperatures and soil moisture are known to be detrimental to survival of entomopathogens. Also, according to Roberts and Campbell (1997), the pH levels recorded in this study are within the optimum pH ranges (pH 4-7) for field fungal survival, therefore pH did not as well affect the mortality trends. There was no specific measure for light, however, as mentioned before, the plant canopy is presumed to have protected the fungus from the destructive effects of ultra violet light.

Plants in unamended soil had the least mean corm weevil damage. This is an indication that the amendments could have contributed to reduction in *B. bassiana* efficacy and persistence and thus more of the weevils were able to survive, lay eggs that later hatched into the destructive weevil larvae stages. It was also observed that more damage was recorded on the lower sections of the plants than the upper sections indicating the weevils' preference of the lower, deeper sections of the plant where they can easily escape entomopathogen's effects.

It is worthwhile noting that there were high Coefficients of Variation (CV) for results in tables 5a, 6a and 7a, even after transformation of the data. That was probably as a result of wide varying conditions in the pots; indeed some reseachers prefer to call these semi field experiments (Godonou, 1999), that are inherent with high variability. In addition, the behaviour of the banana weevil has been reported to be very un predictable (Gold *et al.*, 2001), and the weevil populations are normally clusterred. Thus, it was very unlikely that the trend in the different units in a given treatment would be very precise. Also, the recovery rate for the released weevils was less than 100%, which is a common trend with weevil release and recapture studies, resulting in the underestimation of the actual mortalities under pot or field situations (Simon Gowen, pers. Comm.).

In conclusion, the fore going studies suggest that soil amendments in form of coffee husks, cow dung or inorganic fertilizers may reduce the efficacy and persistence of

the entomopathogen *B. bassiana* in the applied environment resulting into more weevil survival and plant damage. The possible factors responsible for this reduced efficacy are the high moisture levels, organic matter, soil macro and micro fauna that are inherent in such environments.

CHAPTER 6

FIELD EFFICACY AND PERSISTENCE OF *Beuaveria bassiana* APPLIED TO SOIL AMENDED WITH COFFEE HUSKS, DECOMPOSED COW DUNG MANURE AND INORGANIC FERTIIZERS.

6.1.0. Introduction

Organic and inorganic soil amendments have been used by farmers since time immemorial to maintain soil fertility and boost productivity of crops, including bananas. Organic amendments such as plant residues, animal and house hold wastes benefit the soil and plants and have been proved effective (Romero, 2000). For example, Zake, *et al.*, 1993; (cited in Romero, 2000) evaluated the types of cover for bananas in Uganda and found out that coffee husks, gramineous grains and corn stalks gave better results than live cover. In Uganda farmers are known to apply cow, goat and chicken manure, crop residues and at times inorganic fertilizers, in banana fields to maintain or improve fertility. These soil amendments can change the soil physical, chemical and biological characteristics that in turn can influence the persistence of fungal entomopathogens applied in the management of soil dwelling insects (Lingg and Donaldson, 1981, Faye *et al.*, 1996, Rosin *et al.*, 1996, Nankinga, 1999). An understanding of the efficacy and persistence of *B. bassiana* when applied with commonly used amendments in banana fields will provide useful information on the performance of the fungus under varying banana fields' environment.

Therefore the present study aimed at evaluating the field efficacy and persistence of *B. bassiana* applied under different organic or inorganic soil amendments.

6.2.0. Materials and methods

Studies in this chapter were a follow up on the findings of the pot experiments in chapter 5. It was observed that *B. bassiana* applied in organic or inorganic soil amendments gradually lost its efficacy and persistence against the banana weevil compared to that applied in unamended soils. A simple field study was therefore conducted to evaluate the efficacy and persistence of cracked maize *B. bassiana* formulation applied in un amended soils, those amended with decomposed cow dung manure, coffee husks or inorganic fertilizers. A commercial insecticide, Furadan (5% ww carbo furan) was included as a local check.

6. 2.1. The experimental field

The experiment was conducted at Kawanda Agricultural Research Institute (KARI) during the first rains, from February-June, 2003. The experiment was conducted in a newly established banana plantation measuring 0.5 hectares (Plate 7). The plantation had been established in March 2002, in an area, which was previously under fallow for about two years. During planting, a spade full of farmyard manure (90 days old) was dispensed into each of the dug holes (2x2ft) a day before placement of the suckers. The plants were established at a spacing of 2.5 x 2.5m. Overall the field had 262 mats of the local cooking East African highland banana cultivar ("mpologoma",

AAA-EA), and each mat was maintained with an average of two plants per mat throughout the trial time. There were several gaps in the field due to failure of plant establishment probably due to drier conditions that prevailed soon after the planting in April 2001. The field was mulched with elephant grass once after sucker emergence from the soil, but all these had disintegrated and only a few stems were still visible scattered in the plantation (Plate 7).

The following treatments were evaluated in the field; (1) un amended soil + *B. bassiana*, (2) Coffee husks + *B. bassiana*, (3) decomposed cow dung manure + *B. bassiana*, (4) inorganic fertilizers + *B. bassiana*, and (5) un amended soil + Furadan. The field was divided into five blocks that were assigned to each treatment. Each block was sub divided into four plots each with at least nine experimental mats to represent the replicates. The treatments were purposefully allocated to plots to minimise on interplot interference. In order to reduce further on the interference, trenches measuring 1 ft deep by 1 ft were dug between blocks and amendments. Pseudo-stem traps were placed in the trenches to trap weevils and minimize weevil movement across the different plots and treatments.

6.2.2. Amendment and *B. bassiana* application

The different soil amendments were applied and mixed in the top 10cm layer of the soil and spread over 45cm around the mats, 30 days prior to *B. bassiana* application. For coffee husks and decomposed cow dung, one basin full (measuring 10,000 cm^3) was used. The coffee husks and the artificial fertilisers were obtained from

commercial dealers, the cow dung from a local kraal, as described in chapter 5. The amendments were applied on all the plants in the plot. The recommended NPK (Nitrogen, Phosphorous, Potassium) inorganic fertilizer was applied at the rate of 69g of NPK, 28g of Urea, 31g of KNO_3, 3g of $MgSO_4$, per mat as in pot experiments (Chapter 5). The fertilizers were incorporated in the soil using a hand forked hoe.

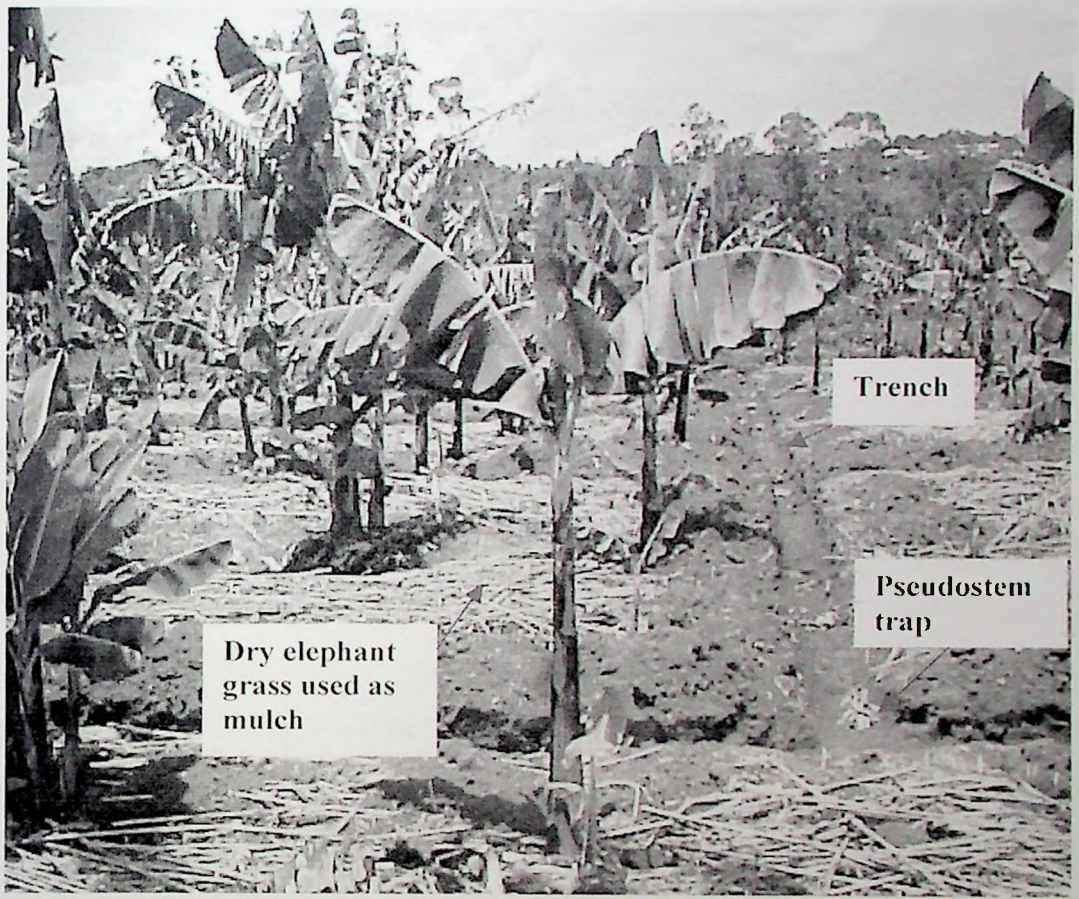


Plate 7: A section of the banana plantation used for the field trial on the efficacy of *B. bassiana* under different soil amendments.

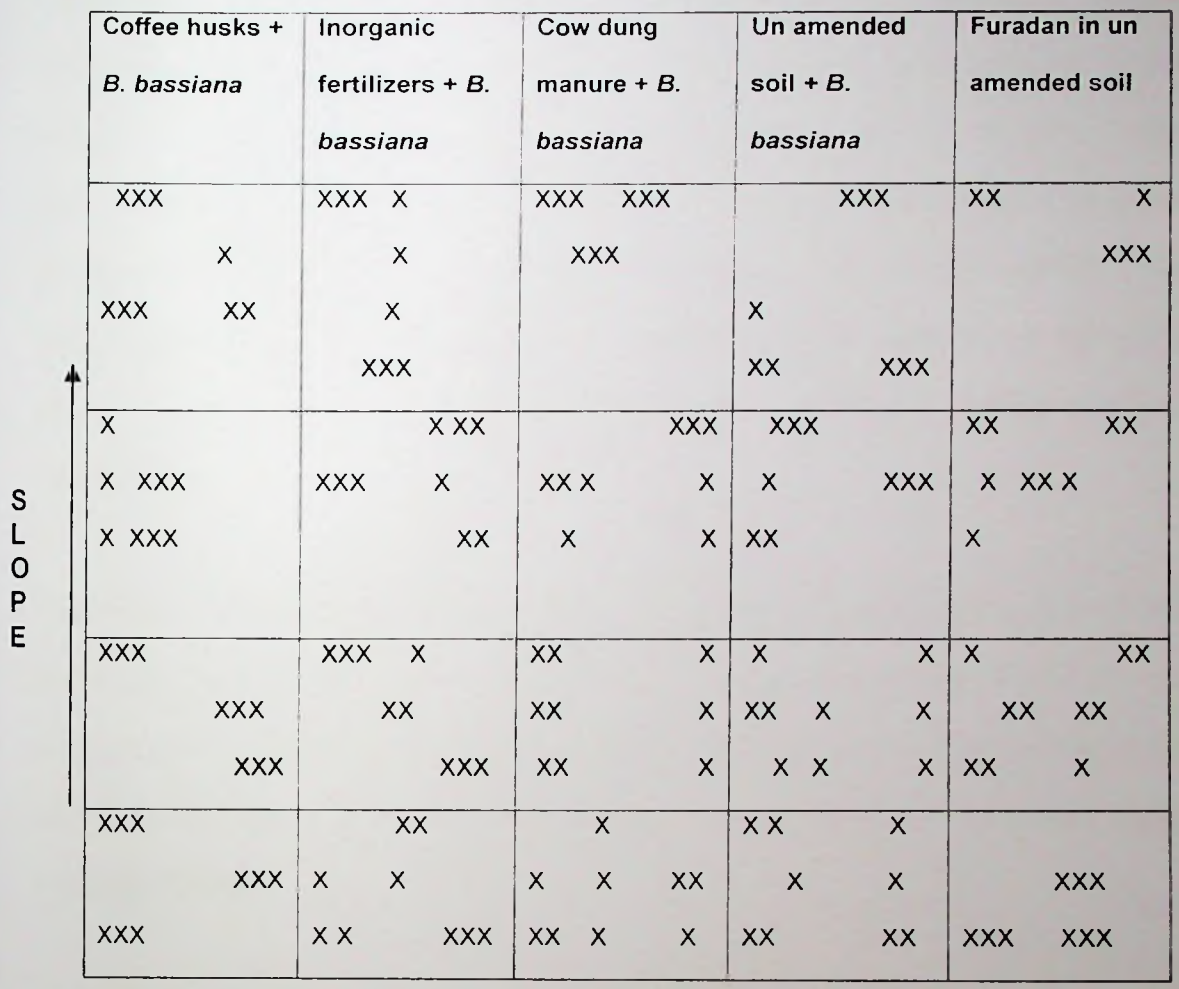


Figure 8: Field plan (not to scale)

In the figure above, x; represents an experimental mat, and this shows the 9 experimental mats and positions occupied in each sub plot. The guard rows and non-experimental plants are not shown in the plan. On average three non-experimental plants existed in each sub plot.

6.2.3. Banana weevil releases and monitoring

The banana weevils were released immediately after the fungal or chemical application. Ten weevils in sex ratio of 1:1 (female: male) were released in three batches on each experimental mat at 30-day intervals as follows; at start (0 days), after 30 days and after 60 days of fungus or chemical application. The weevils released in each plot were marked with specific marks indicating plot, treatment and release period as follows; the numbers of scratches on the thorax, left elytra, right elytra to represent the sub plot, amendment and release period (replicate) respectively.

Two weeks old maize-based formulated *B. bassiana* was applied at a rate of 200g/ mat in two splits; 100g applied 14 days prior to weevil release, the other half on the same day of the first weevil release. Furadan was applied at a rate of 60g around each experimental mat before weevil release and incorporated in the soil using a hand-forked hoe.

Weevil mortality was monitored at 10-day intervals by searching for dead weevils from mulch, plant sheaths and the pseudo stem traps laid in the trenches. Live weevils were recorded and left on respective sites while dead weevils were picked and incubated in moistened tissue paper under laboratory conditions to confirm cause of mortality. If the weevil died of *Beauveria* infection this would be evidenced by white characteristic fungal growth on the cadavers. Every after 30 days, 2g of *B. bassiana* culture or soil with Furadan granules were picked from the mats and evaluated for

infectivity against the weevil in the laboratory following the procedure described in Chapter 5. Ten weevils were introduced into each of the petri dishes containing the 2g sample and monitored for mortality and *Beauveria* infection at 5-day intervals for another 30 days. The dead weevils were introduced in moistened tissue paper and observed for mycosis due to *B. bassiana*.

6.2.4. Effect of soil and environmental characteristics on the efficacy and persistence of *B. bassiana*

Additionally, soil parameters namely; soil quality, temperature, moisture content, micro and macro fauna were monitored and determined following the procedure in Chapter 3. The soil qualities were determined from Kawanda soils analytical laboratory following Okalebu *et al.*, (2002) method. The soil temperatures were determined using clinical thermometers inserted into the soil at a depth of 2.5 cm, whereas the moisture content was determined using the oven method at 120^0C for 24 hours, as described Chapter 5. The macro fauna were determined by hand sorting a sample from a white background, counted and identified to order level. The dominant fungal colonisers or decomposers were identified as they appeared on the substrates in the various treatments.

6.2.5. Data analysis

Descriptive statistics was employed to summarise the number of dead weevils collected from each of the four treatment subplots that was computed out of the 90

released per plot and presented in histogram. Means were also calculated on the averages of dead weevils evaluated under laboratory conditions by computing percentage means (out of ten) for the deed weevils. The total number of macro fauna from a sample in each of the four plots of a given treatment was added and simple means also computed. Simple means were also computed for the soil moisture content (M.C), organic matter (O.M), Nitrogen (N), Phosphorous (P), Potassium (K) and pH.

6. 3. 0. Results

Banana weevil mortality from *B. bassiana* is presented in Figures 9 and 10. Weevil mortality caused by *B. bassiana* was influenced by the release period and the type of soil amendment. When weevils were released at the onset of the treatment; after fungus and Furadan application, weevil mortality was highest in the Furadan treatment. Nevertheless, the difference in weevil mortality between the amendments was not significant. On the other hand, when weevils were released 30 days after the fungus and Furadan application, significant differences in weevil mortality were observed between the different amendments. Mortality was still highest in Furadan, followed by *B. bassiana* applied in un amended soil, then cow dung, inorganic ferilizer and least in coffee husks. Furadan consistently caused highest mortality, as compared to *B. bassiana*, except after 60 days when both recorded very low weevil mortality (Figure 9). There was a steady reduction in weevil mortality in all the treatments over time. Nearly the same weevil mortality of about 18 dead weevils per

plot was achieved for *B. bassiana* applied in un amended soils, coffee husks or cow dung manure in the weevils released immediately after treatment application, however, in weevils released 60 days after treatment application, *B. bassiana* in the un amended soils caused higher mortality (10 weevils per plot) compared to that in the amendments.

When weevils were exposed to *B. bassiana* and Furadan samples picked from the field, the field picked samples caused consistently higher mortality than those in the field (Figure 10). Samples picked after 30 days, caused more than 90% mortality, while soil samples from Furadan treatment caused only 70%. At 90 days after treatment application in un amended soil, *B. bassiana* was still able to cause 40% weevil mortality, whereas Furadan caused no mortality to the banana weevils.

As in pot experiments, most of the dead and fungus-infected weevils were recovered from the banana sheaths in the experimental plants. Other dead weevils were recovered from the pseudo-stem traps laid in the trenches. However, a number of dead and infected weevils were also recovered from the non-experimental plants, which were never treated with the fungus. For example for weevils released immediately after treatment application, 3 dead weevils were recorded from the coffee husks treatment and two in the un amended soils under non treated mats. There were no dead weevils in the pseudo stem traps placed in the trenches bordering the Furadan plots, suggesting that weevils probably died at banana mats of release and treatment application.

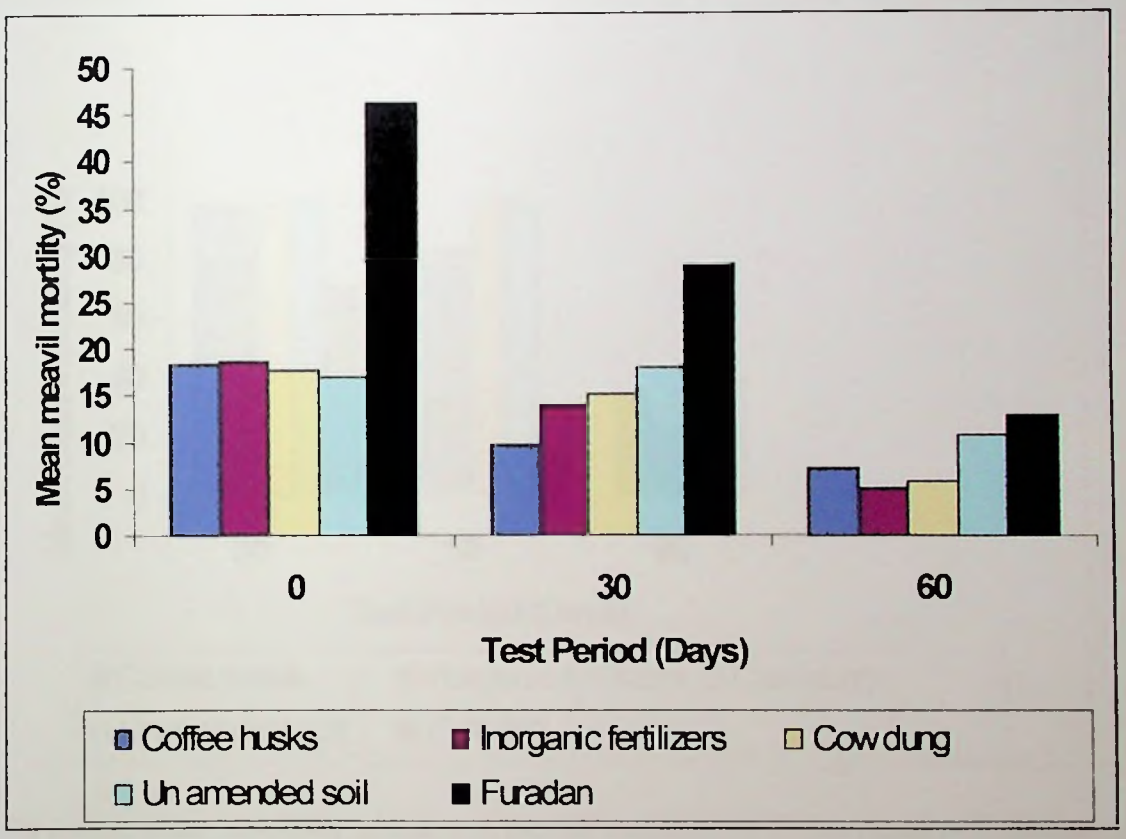


Figure 9: Mean mortality of banana weevils released per plot into the field on the first day, 30 days, and 60 days after *B. bassiana* or Furadan application.

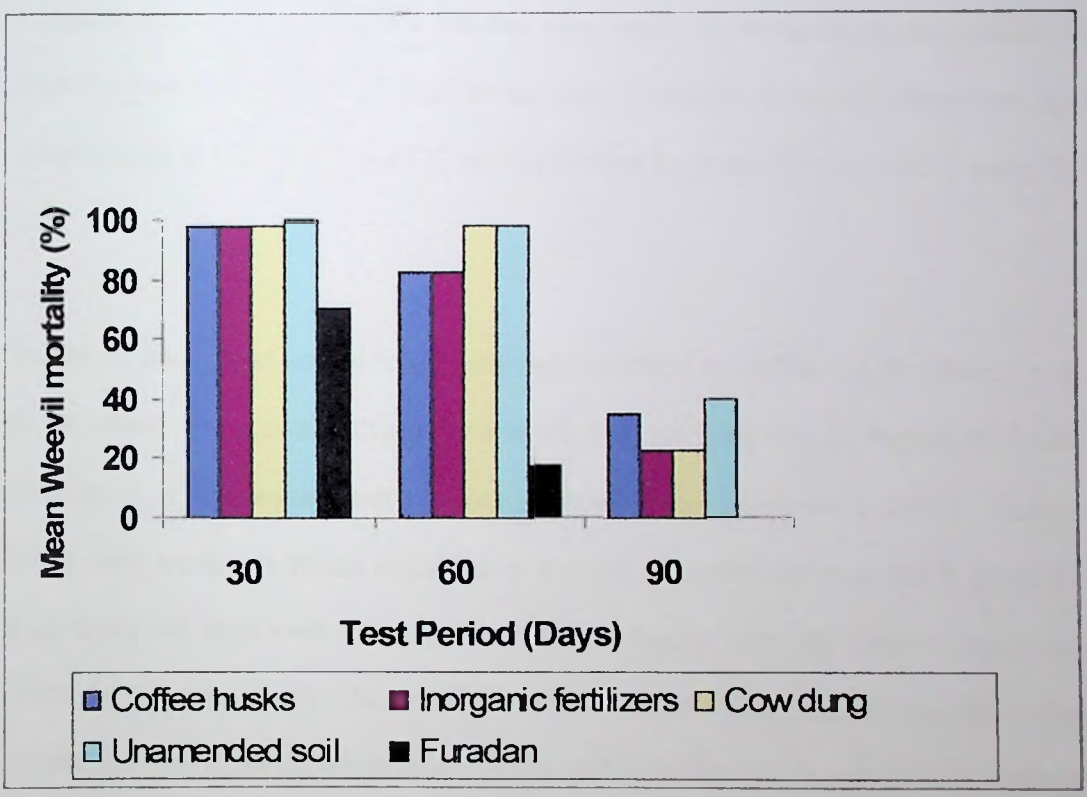


Figure 10: Mean mortality of banana weevils exposed to *B. bassiana* or Furadan picked from field after 30, 60, and 90 days.

Results for the soil moisture content, temperature and soil macro fauna are shown in Figure 11, Figure 12 and Figure 13 respectively, whereas the O. M, N, P, K, pH are presented in Table 9. For all the sampling occasions, soils with coffee husks had the highest moisture content (20-33%), while the un amended soil had the least (13-23%) as seen from Figure 11. Moisture content records from soils with inorganic fertilizers, Furadan and unamended soil did not vary much on most sampling periods as seen from the trend in Figure 11. Soil temperature records were not influenced by the soil amendment; a range of 20-25^{0}C was recorded for most of the sample periods (Figure 12).

Figure 13 show that soil macro fauna were highest in coffee husks (45/cc) and lowest in Furadan). Fungal substrate colonisers and decomposers; *Aspergillus flavus* and *Penicillium crysogenum,* were identified from all the treatments with *B. bassiana* and these were similar to those recorded in the pot experiments (chapter 5, plate 6). It was also observed that Furadan killed soil macro fauna such as; coleopterans (beetles), Annelids (earthworms), Isopterans (white, black and red ants) and Reptilians (snakes), Arachnida (spiders and mites), and Gastropods (snails), and some of these are visible in Plate 9. Live fauna reappeared in the Furadan treatment 90 days after application, when the chemical had presumably lost its efficacy (Figure 13).

Soil chemical characteristics are shown in Table 9. The pH ranged of 4-7. Nitrogen and organic matter content were higher in the soils amended with coffee husks (3.2% and 10.6% respectively), and lowest in the un amended soils. Soils amended with

inorganic fertilizers exhibited high Phosphorous (387.7ppm), while the un amended soils recorded the least amounts (15.5ppm). Coffee husks recorded higher amounts of Potassium (519.5 ppm), and un amended soils the least.

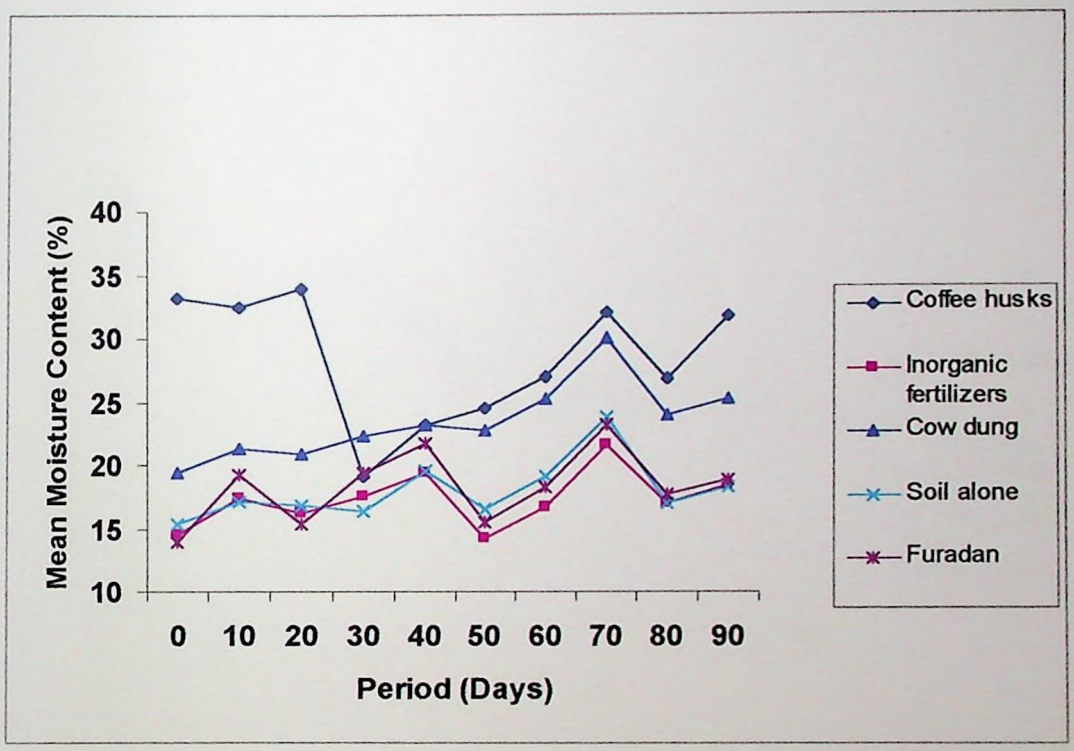


Figure 11: Variation in moisture content of the different treatments in field trial on efficacy of *B. bassiana* against the banana weevil.

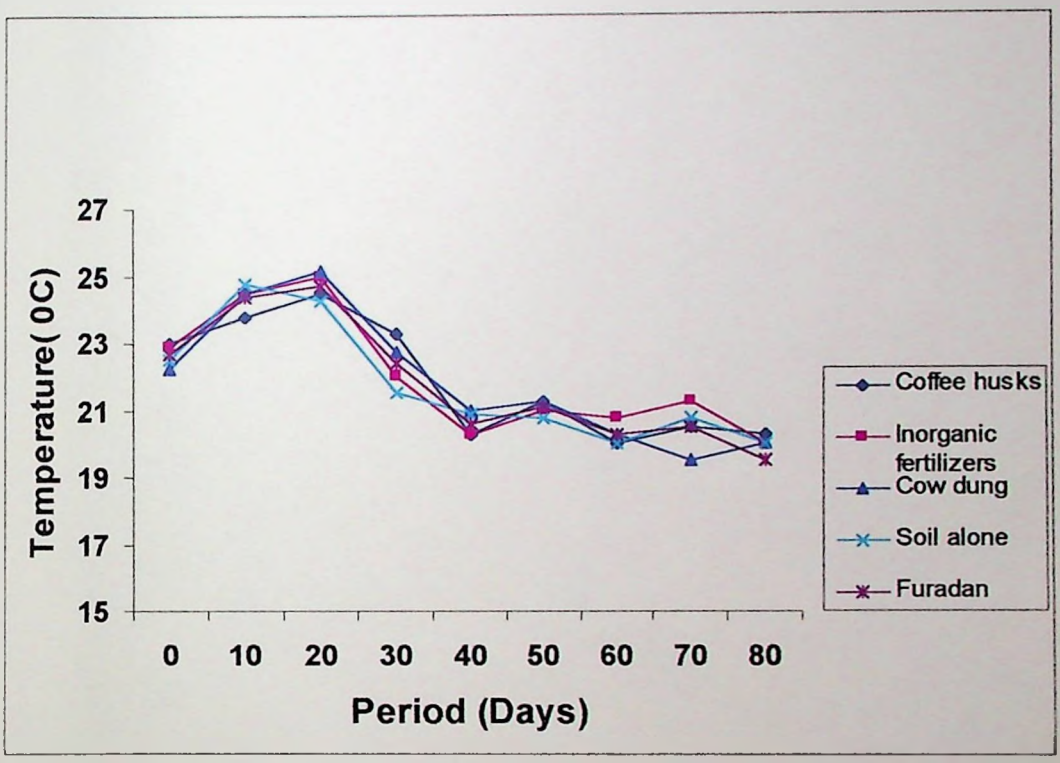


Figure 12. Variation in temperature of the different treatments in field trial on efficacy of *B. bassiana* against the banana weevil.

Plate 8: Earthworms and millipedes found dead in the Furadan treatment under a banana mat in the field trial.

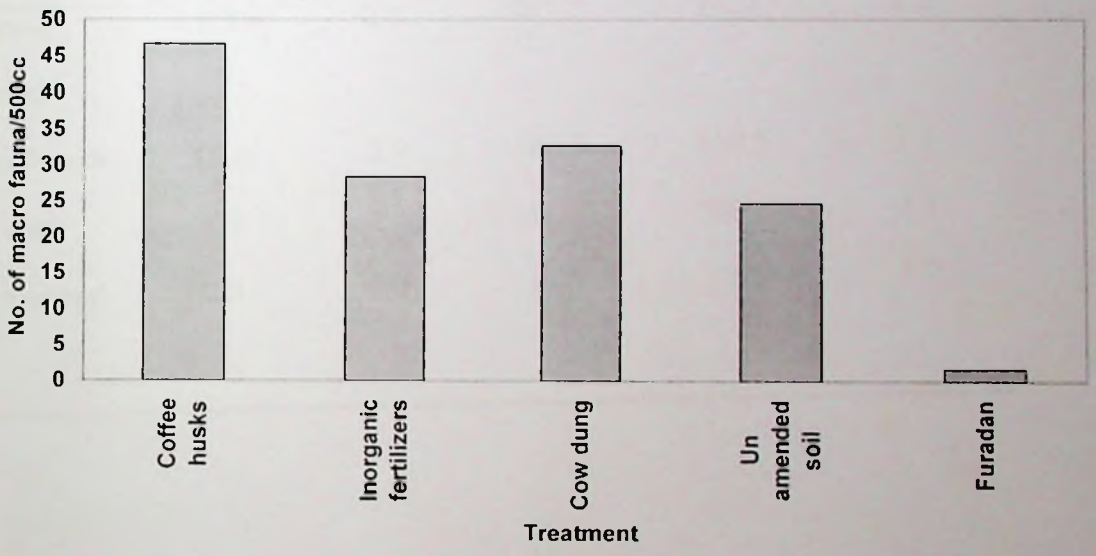


Figure 13: Abundance of soil macro-fauna from the different treatments in field trials on efficacy of *B. bassiana* against banana weevil.

Table 9: Soil chemical characteristics as influenced by selected soil amendments in the field for evaluation of efficacy and persistence of *B. bassiana*.

Treatment	O. M (%)	N (%)	P (ppm)	K (ppm)	pH
Inorganic fertilizers	4.7	2.5	387.7	81.9	5.8
Coffee husks	10.6	3.2	185.3	519.5	7.1
Cow dung	4.4	2.1	160.0	186.3	4.1
Un amended soil	3.9	1.8	15.5	12.2	5.2

6.4. 0. Discussion

As in the pot experiment, consistent reduction in efficacy and persistence of *B. bassiana* applied in the soil amendments was evident. These results confirm earlier studies, which showed that soil amendments have a degradative effect on *B. bassiana*, which was witnessed by the lower weevil mortality records. These results are in agreement with Pena *et al.*, 1993, Traore, 1995, who established that soil amendments such as organic amendments could degrade *B. bassiana.* The detrimental effects of the soil amendments are most likely to be indirect. High moisture levels and higher microbial load were associated with amended soils; these have been reported to reduce efficacy of entomopathogens (Lingg and Donaldson, 1981, Faye *et al.*, 1996). Furadan killed more banana weevils than *B. bassiana* for the first 60 days after application, but showed more negative effects on the environmental non target organisms. Chemicals have been reported to be successful in banana weevil control, but most chemicals have fallen out of favour due to high levels of mammalian toxicity, environmental concerns and / or development of resistance (Gold *et al.*, 2001).

The loss in efficacy and persistence of *B. bassiana* could be explained by the same factors recorded in Chapter 5. The moisture content, invertebrate reservoir, substrate colonisers and decomposers, which were higher in the amendments than the un amended soils, could have facilitated the degradation of the cracked maize based *B. bassiana* formulation. The moisture content is believed to be the most important factor at play as it supports the proliferation of a wide diversity of biota. This is deduced from

the observed relationship between moisture content and weevil mortality in the different amendments. The amendments exhibiting high moisture levels also recording lower weevil mortalities and vice versa. Burges and Raw, 1967 demonstrated that soil macro and micro fauna are associated with moist soils with high organic content. The high moisture content could have led to fast deterioration of the cracked maize substrate where the fungus was cultured and this could have intensified with time.

In the field experiment it was observed that some weevils from the non-experimental plants died from *B. bassiana*. This is an indication that the marked and released weevils were moving across the plots from plant to plant and in effect became dissemination agents for the fungus. This phenomenon would be of significant importance in *B. bassiana* transmission among weevil population in the field. Earlier studies conducted in Uganda, demonstrated that *B. bassiana* can be transmitted from infected to healthy weevils (Nankinga, 1999). In this study, even though not clearly quantified, affirms the fact that there is field spread of the entomopathogen. The latter further strengthens the choice for the entomopathogen in the management of the banana weevil.

The samples tested in laboratory revealed that *B. bassiana* applied in un amended soil was more effective than the chemical pesticide after 90 days in the field. This, suggests that although Furadan has a high knock down effect, weevil invasion that may occur 90 days post Furadan application, may survive the chemical's effects.

These results strenghten the need to use *B. bassiana* for the long term management of the banana weevil. Thus, the lower efficacy of the fungus could in the long run be compesated for by this comparatively longer persistence factor recorded under the field conditions .

This result has further demonstrated the degradative effects of soil amendments on the efficacy and persistence of cracked maize *B. bassiana* formulation. However, this was a simple field experiment and therefore more large-scale field trials are here by suggested.

CHAPTER 7

GENERAL DISCUSSION, CONCLUSSIONS AND RECCOMENDATIONS

The present study is a follow up of previous studies (laboratory and field) conducted in Uganda. The study has examined the conidial yield of *B. bassiana* on selected substrates; Cracked maize, maize bran, bagasse, "machicha", cotton husks, maize bran + bagasse, maize bran + cotton husks and bagasse + spent yeast. Cracked maize was further confirmed as the ideal substrate for *B. bassiana* production; it supported the highest conidial yield, and offered the best handling and culturing characteristics. These results are consistent with Nakinga's (1994) findings, which established maize as a good substrate for *B. bassiana* culturing and exhibited high sporulation ability. More research is required in the aspects of conidia harvesting and storage, that can enable conidia to remain viable and infective for long periods.

Weevil infectivity studies have established that cracked maize formulation caused the highest mortality in the laboratory. Elsewhere, severe epizootics have been reported to occur when both the host (insect) and the inoculum (entomopathogen) are available in sufficient quantities, and that the environment must be favorable. It is therefore expected that substrates that support high microbial conidia, in this case cracked maize, will cause the highest weevil mortality. Using *B. bassiana* as cracked maize based formulation for long-term efficacy and persistence in the field remains a challenge. *Beauveria bassiana* formulations with clay soil caused higher weevil mortality suggesting possible synergistic effects. Follow up studies will be necessary to establish the killing mechanisms involved in the *B. bassiana* clay based

formulations. Equally important are studies to establish the efficacy and persistence of clay based formulations under the complex and variable field situations. Considering the higher weevil mortalities from the cracked maize formulation, under laboratory conditions, *B. bassiana* remains a promising candidate for long term management of the banana weevil, if what is recorded under laboratory conditions could be transformed into the banana field situation. Overall, these results suggest further research into formulations that are more persistent and can withstand extreme environmental degradation. Eventually, the use of *B. bassiana* as a microbial control agent will substantially reduce the necessity for chemical pesticides necessary to control the weevil.

It is a common practice by banana farmers in Uganda to replenish fertility in their plantation through incorporation of organic mainly manures and at times inorganic fertilizers. The present study evaluated the efficacy and persistence of *B. bassiana* under soils amended with coffee husks, decomposed cow dung manure and inorganic fertilizers. The major finding was that *B. bassiana* efficacy and persistence was highest in the un amended soil as compared to soils amended with either organic or inorganic amendments. The reduced weevil mortality was attributed to the degradation of maize substrate due to attack by soil micro fauna. This is a limitation in the use of this fungus considering the fact that farmers apply these amendments to improve banana productivity. However, there could be a way out of this scenario; spatial separation of the applied amendment and the *B. bassiana* formulation is suggested. A suggestion on how this limitation can be overcome is to employ spatial

separation while applying the amendment and the *B. bassia*
field. The amendments could be applied one meter away a
plants can access the nutrients, and the fungus applied clo
banana weevils have intensified activity especially at nigh
farm further studies can test the different application method
lures like pheromones or kairomones could be evaluated
weevils in soils not amended with the organic or inorganic
amendments have not been applied), where they would be
entomopathogen.

Furadan, which was used in the filed as a check, exhibited h
However, gradual loss in efficacy was very evident. By 90
performing better than Furadan; this trend further strengthe
can accrue from long-term use of this biological control agent

For both the fungus and the chemical treatments, the low
under field conditions could as well be attributed to se
infected and dead weevils are known to hide in several
banana sheaths, mulch or even soil debris, a trend encoun
studies (Simon Gowen, pers. Comm.).

Considering the highlighted disadvantages associated w
pesticides (though not confirmatory), *B. bassiana* seems to

long-term management of *C. sordidus*, since it has less effect on non target organisms. However, this was a simple field experiment to give a quick prediction on the field performance of the entomopathogen under the selected soil amendments, and also to provide insights on methodologies for subsequent field trials, therefore, large scale field evaluations are here by suggested. The evaluation of the entomopathogen performance against the weevil under different agro ecological conditions is also suggested.

In conclusion, cracked maize substrate has further been demonstrated as the ideal substrate for *Beauveria bassiana* conidia production. The dried formulation was the most effective under laboratory conditions. From the pot and field experiments, it was established that soil amendments in form of coffee husks, decomposed cow dung manure or inorganic fertilizers reduced the efficacy and persistence of the maize based *B. bassiana* formulation that was witnessed by lower weevil mortality records. The latter was attributed to the significantly high moisture content and soil biota that were associated with the amended soils that might have led to quick degradation of the maize substrate, leading to reduction in efficacy and persistence of the entomopathogen. For effective control of the banana weevil using the fungus, it is suggested that application be done at an interval of at least three months (90 days).

Further studies will address the performance of the different *B. bassiana* formulations against the banana weevil under different agro ecological conditions and wider banana management practices.

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Appendices

1. Formula used for the computing the percentage soil moisture content (MC);

$MC = X_1\text{-}X_2/X_1 \times 100,$

Where; X_1= original fresh weight (=20g),

X_2= Dry weight after oven drying.

2. Formula for computing the conidial yield per unit gram of substrate;

The formula $C= A \times 5 \times 10^4$ was used to compute the spore suspension; where C is the concentration of spores/ml in the diluted quantity and A is the average spore counts from the 2 grids (grids1+2/2), was used. The concentration of spores in the original solution before dilution was calculated as follows; $S= C \times 10^n$ where n is the number of dilutions. This implies that $S= A \times 5 \times 10^4 \times 10^n$, where n is the number of dilutions. Finally, the amount of conidia per unit gram of substrate was computed as S x 100 mls, since one gram of substrate was dissolved in 100mls of water.

3. Mean number of functional leaves per plant at destructive sampling

Treatment + *B. bassiana*	**MEAN NO. LEAVES**
Un amended soils	4.3
Inorganic fertilizers	4.2
Soil + Cow dung (1:2)	4.0
Cow dung alone	3.3
Soil + Cow dung (1:3)	3.3
Soil + Coffee husks (1:1)	3.2
Soil + Coffee husks (1:2)	2.8
Coffee husks alone	2.8
Soil + Cow dung (1:1)	2.7
Soil + Coffee husks (1:3)	2.9
LSD (P= 0.05)	**0.56**
CV (%)	**32.9**

The number of functional leaves still on the plants at sampling time could be an indication of fungal efficacy and persistence. From the table above, it is evident that plants grown in un amended soils and those within inorganic fertilizers had significantly higher number of leaves strengthening further the fact that organic amendments are detrimental to the fungus. For the first 30 days (not shown in table), most of the plants still had 4-5 leaves, indicating that the fungus was still able to protect the plants. But by 90 days, the number of functional leaves had reduced tremendously to around two in soils amended with coffee husks or cow dung.

4. Critical stages in *Beauveria bassiana* production in the laboratory using the diphasic process, on cracked maize substrate.

STAGES	REQUIREMENTS	DURATION (DAYS)
1. Sucrose-Yeast preparation; Weighing and autoclaving the substrates.	20g sugar, 20g yeast, 1000mls of water; for 100bags of 200g each.	1
2. Inoculate the sucrose-yeast with *B. bassiana*.	2mls of *B. bassiana* spore suspension per bottle.	
3. Incubation of the combination in 2 above.	Incubation chamber.	4-5
4. Cracked maize preparation; soaking, weighing and autoclaving. Autoclave at 18psi, 120^0c for 30 minutes, then cool.	Autoclavable bags, weighing scale; 200g maize/bag.	1
5. Inoculate cracked maize with *B. bassiana* in the sucrose-yeast in 2 above.	Sterile environment.	1
6. Incubation period.	Incubation chamber.	5-7
7. Opening of the bags with *B .bassiana culture.*		2
8. Pour out the fungus into drying trays.	Trays, sterile paper.	1
9. Spread the fungus in trays for drying.	As above	6-7
10. *B. bassiana* weight standardisation and packaging in 100g or 200g units.	Weighing scale and bags.	1

Note: The whole production process takes between 21 to 25 days.

5. Table 5a. Analysis of Variance to partition soil amendment treatments by using contrasts for weevil Mortality records in pots.

Source of variation	df	MS	F-value	Pr>F
Treatment	9	391.10	3.93	0.005
Coffee husks alone Vs other coffee husks	1	80.46	0.81	0.379
Coffee husks alone Vs coffee husks 1:3	1	11.02	0.11	0.743
Coffee husks 1:1 Vs 1:3	1	284.85	2.86	0.106
Cow dung manure alone Vs other manures	1	61.82	0.62	0.440
Cow dung manure alone Vs manure 1:3	1	11.02	0.11	0.742
Cow dung manure 1:1 Vs 1:3	1	337.50	3.39	0.080
Un amended soil Vs the rest	1	1645.12	16.53	0.006
Un amended soil Vs coffee husks alone	1	1369.14	13.76	0.001
Un amended soil Vs manure alone	1	1999.78	20.09	0.0002
Un amended soil Vs Inorganic fertilizer	1	186.63	1.88	0.186

6. Table 6a: Analysis of Variance to partition soil amendment treatments by using contrasts for weevil mortality records in samples picked from pots and tested in the laboratory.

Source of variation	df	MS	F-value	Pr>F
Treatment	9	469.40	2.29	0.059
Coffee husks alone Vs other coffee husks	1	149.33	0.73	0.403
Coffee husks alone Vs coffee husks 1:3	1	125.51	0.61	0.443
Coffee husks 1:1 Vs 1:3	1	176.97	0.86	0.364
Cow dung manure alone Vs other manures	1	22.94	0.11	0.741
Cow dung manure alone Vs manure 1:3	1	39.21	0.05	0.819
Cow dung manure 1:1 Vs 1:3	1	117.62	0.57	0.458
Un amended soil Vs the rest	1	1206.25	5.89	0.025
Un amended soil Vs coffee husks alone	1	292.27	1.43	0.246
Un amended soil Vs manure alone	1	2376.53	11.60	0.003
Un amended soil Vs Inorganic fertilizer	1	613.14	2.99	0.099

7. **Table 7:** Analysis of Variance to partition soil amendment treatments by using contrasts for lower corm weevil damage.

Source of variation	df	MS	F-value	Pr>F
Treatment	9	124.26	4.05	0.004
Coffee husks alone Vs other coffee husks	1	1.54	0.05	o.825
Coffee husks alone Vs coffee husks 1:3	1	0.49	0.02	0.901
Coffee husks 1:1 Vs 1:3	1	8.82	0.29	0.598
Cow dung manure alone Vs other manures	1	99.74	3.25	0.084
Cow dung manure alone Vs manure 1:3	1	55.43	1.81	0.194
Cow dung manure 1:1 Vs 1:3	1	0.14	0.00	0.771
Un amended soil Vs the rest	1	702.01	22.89	0.001
Un amended soil Vs coffee husks alone	1	934.71	30.48	0.0001
Un amended soil Vs manure alone	1	451.17	14.71	0.001
Un amended soil Vs Inorganic fertilizer	1	192.87	6.29	0.021