EVALUATION OF ENTOMOPATHOGENIC FUNGUS *Metarhizium anisopliae* var. *anisopliae* FOR INTEGRATED MANAGEMENT OF TERMITES (Isoptera: Termitidae) IN MAIZE IN SIAYA COUNTY.

BY

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DECLARATION

DECLARATION BY THE CANDIDATE

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I dedicate this thesis to my beloved family for their invaluable input and inspirations.

ABSTRACT

Maize ***(****Zea mays* L**)** is one of the major staple food crops in Africa contributing significantly to food security; however its production has been declining in the recent past. Termites are one of major yield reducers accounting for 50-100% losses in maize. Chlorinated hydrocarbon insecticides used for their control have been restricted thus, the need for an alternative control measures. Recent studies have demonstrated the efficacy of the entomopathogenic fungus *Metarhizium anisopliae* for controlling termites, but little is yet known about practical and sustainable application of this bio control agent. This study was therefore conducted to evaluate different application rates of *M. anisopliae* and the effect of maize-based intercropping system on performance of fungus for integrated termite management in maize field and secondly to assess persistence of *M. anisopliae* in the soil. The fungi granules were mass produced in the laboratory at ICIPE, Nairobi. Three application rates of the fungus granules at 40.0 kg/ha, 60.0 kg/ha 80.0 kg/ha plus untreated control were evaluated in the field under three cropping systems; maize monocrop, maize + soybean intercrop and maize +common bean intercrop. Treatments were replicated three times in a RCBD. Head count of lodged maize due to termite attack was recorded weekly; temperature and relative humidity at the base of maize plant were recorded using hygrothermometer and yields per plot were taken at harvest. To assess persistence, four soil samples were picked at randomly per plot at planting and at harvest and fungus persistence test done in the laboratory. The number of colony forming units per gram of fresh soil was measured after seven days. Data were subjected to ANOVA analysis and means were compared by Tukeys' ($P \leq 0.05$) using Genstat software. Application of *M. anisopliae* of 60.0 kg/ha and 80.0 kg/ha significantly reduced maize lodging and increased maize yield. Maize yield was high in intercrop plots treated with *M. anisopliae;* application of 40.0 kg/ha of *M. anisopliae* in maize + soyabean intercrop increased maize yield to level comparable to 80.0 kg/ha of *M. anisopliae* in maize monocrop treatments. Spore density at harvest in all the treatments in the three cropping system differed significantly ($P \leq 0.05$) with application of *M. anisopliae* at 60.0 kg/ha and 80.0 kg/ha in maize intercrop having high conidia persistence compared to maize monocrop. The study demonstrates that application of *M. anisopliae* protects maize against termite attack and the use of legume intercrops enhances the efficacy and persistence of the fungus in the field. It is recommended that application of 60.0 kg/ha *M. anisoplie* granules in maize monocrop and 40.0 kg/ha *M. anisoplie* granules in maize soybean intercrop are effective for termite management.

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LIST OF ABBREVIATIONS AND ACRONYMS

ACDS	African Centre for Disaster Studies
ATC	Agricultural Training Centre
CFU	Colony Forming Units
CRA	Commission on Revenue Allocation
DAP	Diammonium Phosphate
ECAMAW	Eastern and Central Africa Maize and Wheat research Network
EPF	Entomopathogenic fungi
FAO	Food and Agriculture Organization
ICIPE	International Centre of Insect Physiology and Ecology
IPCC	Predicted Impact of Climate Change
KARI	Kenya Agricultural Research Institute
KNBS	Kenya National Bureau of Statistics
MOA	Ministry of Agriculture
POPs	Persistent Organic Pollutants
RCBD	Randomized Complete Block Design
TM	Trade Mark
UNEP	United Nations Environment Programme
VAR	Variety
UV	Ultra Violet

CHAPTER ONE

INTRODUCTION

1.0. Background information

Maize ***(****Zea mays***)** is one of the major staple food crops in Africa contributing significantly to the agricultural sector in food production (ECAMAW, 2005). For many people, it is the main staple food accounting for about 40 per cent of daily calories especially in the southern and eastern region of the continent as evidenced by the annual consumption level of 81kg per capita in the region and 103 kg per capita in Kenya (Pingali, 2001). This translates to between 30 and 34 million bags (2.7 to 3.1 million metric tonnes) of annual maize consumption in Kenya. Maize is also important in Kenya's crop production patterns, accounting for roughly 28% of gross farm output from the small-scale farming sector which makes up to 70% of the total production and over 80% of the total maize area (Kibaara, 2005; GOK, 2008; Olwande, 2012).

In Siaya County, like most parts of western Kenya maize is a major food crop constituting up to 80% of daily meal, especially the rural population (Kodhek, 2005; MOA, 2007). The county has a high agricultural potential and receives bimodal rainfall, it is among the counties that are expected to produce enough maize to feed its people and surplus to feed people in other parts of the country and for export. Maize production in the county however, has continued to decline over time due to both abiotic and biotic factors which act synchronously and losses caused to such staple crops directly impinge on the livelihoods of local population, especially the rural poor (Kodhek, 2005).

Recently, Siaya County has been experiencing prolonged dry spells like many other parts of the counties as a result of climate change (ACDS, 2006). As a result of these prolonged dry spells, there has been an increase of pest pressure on maize crop in the field. For instance, pests like termites which initially were known to play an integral role in both environment and human life and not a threat to maize production in the county, are now increasingly reported to cause huge losses comparable to loss caused by pests such as larger stem borer, army worms and striga weed (MOA, 2007).

Termite destruction on maize crop in the field is usually localized and varies from field to field in the county; however damage is more severe in the dry months compared to the wet months with losses of up to 60% recorded. This therefore sounds alarm for maize production in the county whose major part of arable land is under maize cultivation both in the long and short rain seasons. Also, the county is located within the lake region with major part of the year being hot and humid; these are ideal conditions for most termite species build-up. The conditions coupled with prolonged dry spells predicted in Africa as a result of climate change (ACDS, 2006) means increase in case of termite outbreak hence increased damage to maize crop in the field and like most parts of the country, the county's food security is pegged on availability of maize. Therefore, any challenge to the crop's production poses a challenge to the county's food security.

1.1. Management of Termite.

A number of techniques have been reported to be used to manage termite densities within a field crop in the county; although the technique used is usually based on the level of termite destruction and termite species (Gitonga, 1992). For example the use of plant extracts for both mound building and non-mound building species; destruction of the colony for majorly mound building species and cultural practices for non-mount building species like deep ploughing and frequent tillage. However, results are often unsatisfactory and the techniques are labour-intensive (Abdulrahman, 1990; Gitonga, 1992; Gethi *et al.*, 1995). Another common option is the application of chlorinated hydrocarbons such as aldrin, dieldrin, chlordane and heptachlor which are sufficiently effective and inexpensive (Wightman, 1991). However, the relatively high human toxicity and the unacceptable environmental consequences of the widespread use of cyclodienes have resulted in severe restrictions being placed on their use in both developed and developing countries (UNEP, 2000). As a result, most manufacturers voluntarily discontinued the production of these insecticides. This voluntary withdrawal of the insecticides from the market has left most maize farmers in the county exposed to great losses due to termite destruction, thus the need for alternative control measure that is environment and human friendly to be used for combating termite menace in maize fields in the county.

Elsewhere, a number of other insecticides (e.g. chlorpyrifos, isofenphos, permethrin) are marketed for termite control (UNEP, 2000), but are not as effective as the cyclodienes and need to be applied more frequently. For example, in South Africa, during seasons of high termite pressure, neither seed treatments nor multiple applications of insecticides to maize prevented severe levels of lodging. Some of these new insecticides are also phytotoxic; therefore their adoption in the county has been very limited, thus the needs for an alternative control technique.

Also, studies around the globe have shown that microbial organism have wide application in control of termite with less harmful effect to both animal and the environment (Goble, 2009). For instance Rath (2000) showed t entomopathogenic fungi to be suitable bio-control agents for termite with no harmful effect to environment, animal and non-target arthropods. One of the pioneering studies at ICIPE has indicated the effectiveness of an Entomopathogenic fungus, *M. anisopliae* Isolate ICIPE 30 to control termite in pastures, nursery trees and mounds in Kenya (ICIPE, 1997). Although, there exists no evidence on the use of microbial organism to control termites in Siaya county, studies in Gulu, Uganda and Machakos, Kenya by Sekamatte, (2001) and Maniania *et al*, (2002) respectively showed the effectiveness of *M. anisopliae* to control termites in maize cropping system. The present study therefore was set to evaluate the use of *M. anisopliae* with either maize + common bean intercrops or maize + soyabean intercrop as integrated management of termites in maize fields in Siaya County.

1.1.1. Entomopathogenic fungus; *Metarhizium anisopliae* var. *anisopliae*

In 1883, Metchinikoff initiated mass culturing of *M. anisopliae* and carried out the first experiment with two beetle pests; ever since *M. anisopliae* (Metschnikoff) has been widely exploited as an entomopathogen in bio-control attempts. It is known to attack over 200 insect species. *Metarhizium anisopliae* acts as a twofold bio-control agent; contact and repellent (Rath, 2000; Mburu *et al.,* 2009). In contact *M. anisopliae* spore attach to insect pest body parts and initiate infection leading to death (Rath, 2000; Krutmuang and Mekchay, 2005). As repellent *M. anisopliae* produces volatile compounds which repels termite within the applied sites (Mburu *et al.,* 2009; Mburu *et al.,* 2010).

Termites have an inherent potential to detect specific repellent signals from potentially infective fungi and therefore avoid any contact with the fungi, this is a key part of their adaptive survival (Wright *et al.,* 2005; Mburu *et al.,* 2009; Mburu *et al.,* 2010). This type of avoidance behaviour has been exploited to manage termites residing in difficult to reach locations, such as underground nests (Milner, 2003; Mburu *et al.,* 2009; Hussain *et al*., 2010).

For instance, in an experiment to control termite in maize fields using *M. anisopliae* Maniania *et al*. (2002) applied *M. anisopliae* granules in hills at planting and reported significant decline in maize lodging and increased grain yield in plots treated with *M. anisopliae* granules. These authors attributed reduction in maize lodging and subsequent increase in maize yield to repellent action of *M. anisopliae* conidia against termites in plots applied with treated fungus. There are reports also on the successful management of termites by direct blowing conidia of *M. anisopliae* strains into termite galleries, resulting in management of not only those termites which were directly hit by the conidia but all population in nest or galleries (Milner 2000). The use of *M. anisopliae* could therefore provide an opportunity for sustainable control of termites in maize fields in the county.

However, information pertaining to optimum application rates is limited yet this is critical. Varying results have been reported by different investigators with regard to efficient application rate of *M. anisopliae* to manage termite both in field and laboratory. Maniania *et al*. (2002) reported that application of 1.0 gram of *M. anisopliae* granules per maize hill significantly reduced maize lodging while Fernandes and Alves (1991) and Hussain and Tian (2013) reported 100 % mortality of termites within 10 and 5 days respectively of application of 5.0 and 2.0 grams of *M.*

anisopliae granules in the laboratory. Although literature review show no evidence of application of *M. anisopliae* fungus to control termite in Siaya county, use of different application rates of the fungi elsewhere has shown potential to control termites. The study, therefore investigated three different application rates of *M. anisopliae* granules to control termites in maize fields in Siaya County.

Metarhizium anisopliae like most entomopathogenic fungi is affected by environmental factors such as temperature, relative humidity and precipitation. Legume crops such as soybean have been reported to increase relative humidity within the canopy cover during their vegetative growth (Sprenkle, 1979). Therefore integrating *M. anisopliae* in cultural practice of maize-food legume intercrop will be exploited in the study. The use of intercrop legume is expected to have a modifying effect on the harsh environmental factors within area applied with fungus. The use of legume crops also has additional advantage of increasing of amount of organic matter in the soil which are known to decrease antagonistic activities against hypocrealean entomopathogenic fungi (Fargues and Robert, 1985; Stansly *et al.*, 1990).

1.2. Problem Statement

Estimated losses due to termites attack on maize crop vary widely; losses of 50-100% have been reported. Siaya County largely relied on broad-spectrum and persistent organochlorine insecticides. However, there has been limitations and increasing legal restrictions associated with their applications. Secondly a number of small scale farmers also use other traditional techniques such as application of plant extracts; destruction of the colony, queen removal and cultural practices e.g. frequent weeding to control termites, however results are often unsatisfactory and the techniques labour-intensive. Studies have shown the ability of *M. anisopliae* isolate ICIPE 30 to control termites on maize (Maniania *et al.,* 2002). Although these studies have produced impressive results, there has been no follow up study to assess optimum application rates, its persistence in the soil and the compatibility of the isolate with other termite control methods nearly two decades. Such information is necessary for developing integrated termite management strategies.

1.3. Justification.

Maize is one of the most important staple foods crop for most of the population in the county, where it serves majorly as a source of dietary carbohydrate. Losses caused to such staple crop directly impinge on the livelihoods of many, especially the rural poor (ECAMAW, 2005). It is thus imperative that sustainable technologies are developed to mitigate any maize production constraints such as termties attack. The most relied on broad-spectrum and persistent organochlorine insecticides have been faced out (UN, 1987). Whereas the use of traditional termite control methods, like queen removal and use of plant extracts are very labour intensive and some areas lack this knowledge.

Biological control using entomopathogenic fungi, *M. anisopliae* provides an alternative control method for termite infestation problem. Studies under laboratory condition have shown that the fungi can cause both termite mortality and repellence of up to 100% (Krutmauanga and Mekchay, 2005; Mburu *et al.,* 2009; Hussain *et al.,* 2011; Hussain and Tian, 2013). However, no adequate field trials have been done other than modified field condition in the laboratory. Thus the study seeks to establish fungus application rate, persistence and the effect of intercropping legumes with maize on performance of *M. anisopliae* as an integrated management of termite.

1.4. Objectives

1.4.1. Broad Objective

To evaluate the potential of *Metarhizium anisopliae* as a component of integrated termite management in maize (*Zea may*) cropping systems.

1.4.1.1. Specific Objectives

i. To establish the application rate of *M. anisopliae* for termite management in maize fields in Siaya County.

ii. To assess the effects of maize-based cropping systems on the performance of *M. anisopliae* in the management of termites.

iii. To establish the persistence of *M. anisopliae* in the soil in maize-based cropping systems.

1.5. Hypothesis

H_0 Varying applications rates of *M. anisopliae* applied at maize planting does not manage termite in maize fields.

$H_{0.}$Varying type of maize-based cropping system does not affect the performance of *M. anisopliae* against termite in maize fields.

H_0 *Metarhizium anisopliae* applied in the soil does not persist up to the end of maize growing season.

CHAPTER TWO

LITERATURE REVIEW

2.0. Maize (*Zea mays*)

Maize (*Zea mays*) is a monoecious plant and it is the world's leading food grain, with over 100 million people in Africa utilizing it as a staple food crop (Awour, 2003). Between the years 2002-2009 the total maize production in Kenya has ranged from 2.5 to 3.0 million metric tonnes of maize grain while its consumption is currently above 3.2 million metric tonnes per year (Gudu, 2003; Rojas, 2009). The current national mean yield is 1.5 t/ha, which is 14% less than the 2008 estimates and close to the 2004 situation (Rojas, 2009). This indicates that maize production may continue to take a downward trend in the coming years which may be a threat to the achievement of the millennium development goals of food security and the social-economic pillars of Kenya's vision 2030 considering that maize is a stable food for the Kenyan population.

Siaya County like most parts of the country too has had a noticeable challenge in maize production in the recent past as a result of both abiotic and biotic factors which act synchronously (Kodhek, 2005); this has and continues to threaten household food security and income sources for the local populations in the county who majorly rely on maize production. Infestation and damage by pests has been ranked as the second most important constraint on maize production for small scale farmers after nitrogen and phosphorous deficiency in the county (Songa *et al.,* 2002). The following insect pests (Table 1) have been documented as the most common in Siaya County. Recent studies in the county show that maize fields infested with termites have recorded decline in maize yields comparable to losses caused by larger stem borer or strigaweed (MOA, 2010) (Table 1), although these losses are reported to be varied from field to field depending on termite species present and prevailing weather conditions.

Table 1: Maize yield losses between 2007 and 2009 in tonnes per hectare, Siaya County.

Pest	2007 t/ha	2008 t/ha	2009t/ha
Larger grain borer (*Prostephanus truncates)*	1.2	1.8	3.3
African Armyworm (*Spodoptera exempta)*	0.5	0.0	0.3
Stemborers (*Busseola fusca)*	0.	1.2	1.8
Cutworms (Agrotis spp)	0	0.2	0.1
Termites (*Microtermes* spp.)	0.3	1.6	3.5
Maize aphid (*Rhopalosiphum maidis*)	1.3	0	0
Striga weed (*Striga hermonthica*)	4.9	4.9	3.0
Phosphorous deficient	7.9	8.9	8.0

Source: MOA, 2010.

Termite attack on maize is more severe during dry conditions with losses ranging between 50 and 100% reported (Logan *et al.*, 1990; Tenywa, 2008). Farmers in the Siaya County also have reported that termite damage on maize is more severe in the dry months compared to the wet months (MOA, 2010) this coincides with findings elsewhere in Africa. For instance, Sileshi *et al.* (2009) and Nyeko and Olubayo (2005) working in Zambia and Uganda respectively reported termite damage on crop is more severe during dry period. One of the impacts of climate change has been predicted to be increased risk of drought (prolonged dry period) and this will be felt more in Africa than any other part of the world (ACDS, 2006) (Fig 1). Therefore farmers in Siaya County are likely to face further maize losses due to termite as time passes by. Present statistics in the county on maize losses as a result of termite damage coupled with predicted prolonged dry weather conditions is evident that termite is a threat to the county's food security.

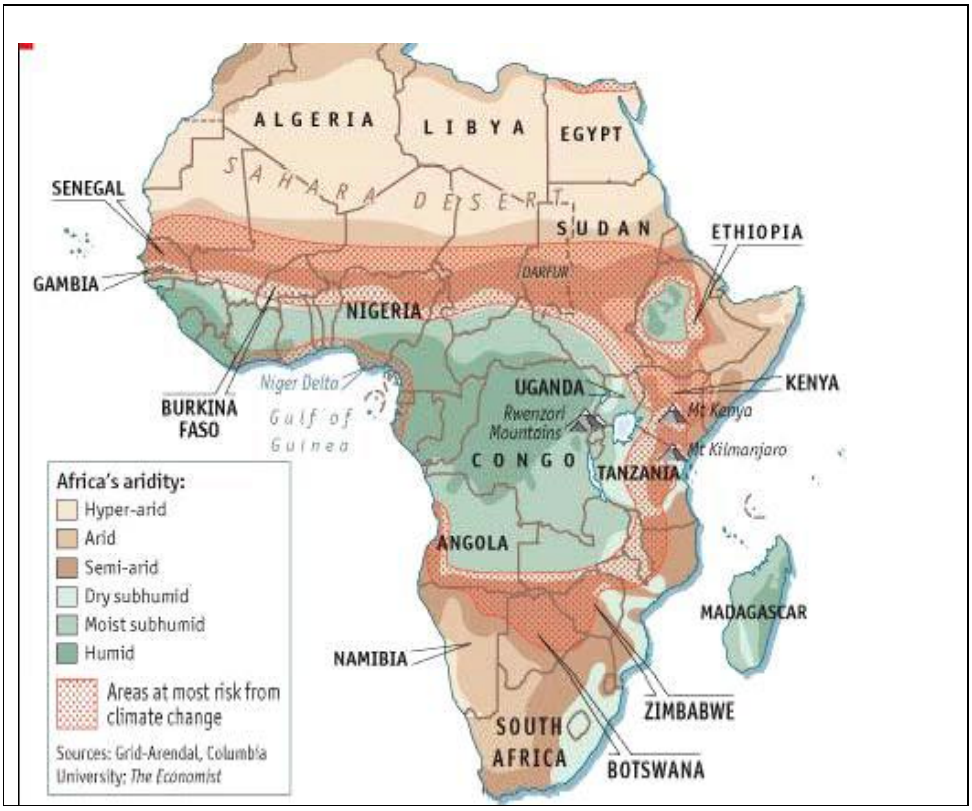


Plate 1: Areas at risk from climate change in Africa (Source: Sileshi et al., 2009).

In the last two decades, maize has yielded compelling success stories with regard to innovation and adoption of new practices and technologies that has increased smallholder maize production; like breeding for acidic tolerance varieties, striga tolerance and the control of large stem borers (Gressel *et al.*, 2004). The acceptability of these new innovations in the county like the rest of African continent has been more widespread for maize than for any other food crops. These successes provide platform for further investigation for any emerging threat to maize production like termite attack hence capping food insecurity at household level.

2.1. Termites (*Microtermes* spp., *Macrotermes* spp.)

Termites are members of the Isoptera, a relatively small order with approximately 2600 species (Engel and Krhishna, 2004; Sileshi *et al.*, 2009). They are soft-bodied insects with cryptic habits, living in family groups (colonies) commonly referred to as white-ants. Among the more advanced classes of termite the colony comprises a reproductive pair (usually a king and queen, but in some species there may be multiple or neotenic queens) and numerous sterile workers and soldiers whose tasks include foraging, nest building and maintenance, care of eggs and young, and defense (Weesner, 1960) (Fig 2).

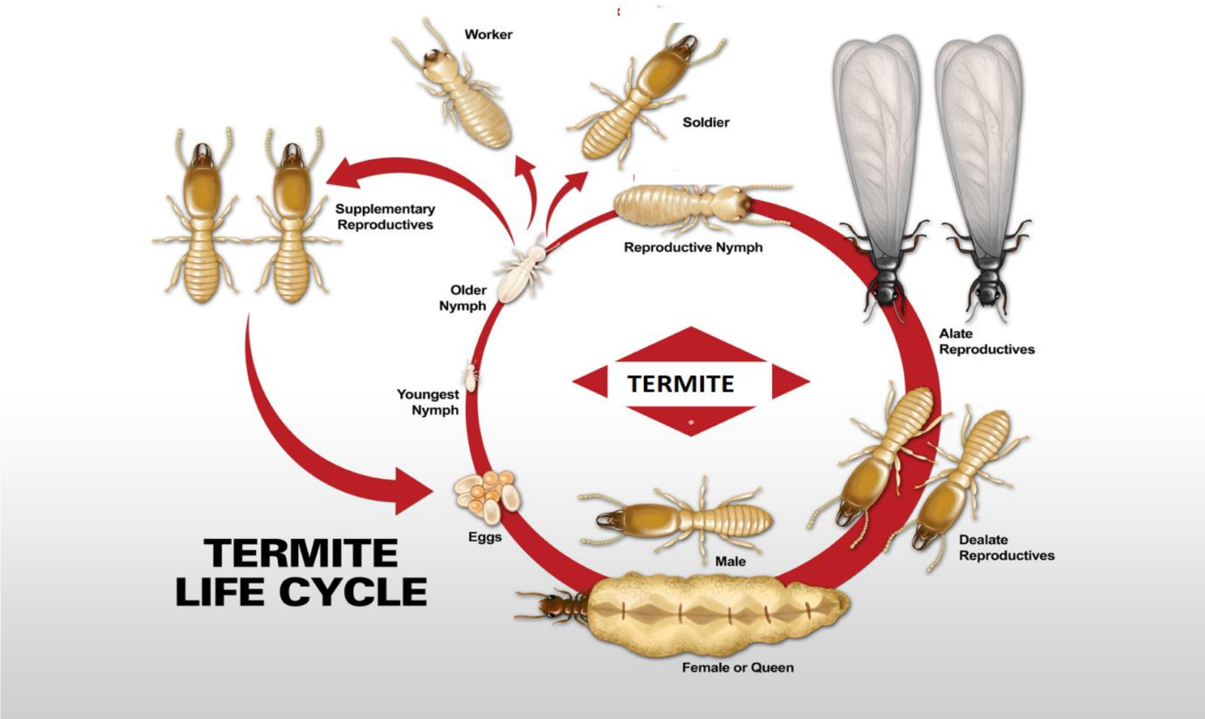


Plate 2: Termite Life Cycles. (Source: http://www.termite-life-cycle.)

Ecologically, termites are classified under three main groups: damp wood termites, dry wood termites and subterranean termites (Collins, 1989). Subterranean termites are known to concentrate their feeding activities on plant material generally in the form of wood, leaf litter and roots, by these activities they contribute to the soil profile, soil texture and redistribution of organic matter (Sileshi *et al.,* 2008). These species are common in tropical countries and are characterized by many diffused systems of galleries in the soil, or construct complex central nests in the soil, log or stump of a tree (Sileshi *et al.,* 2008).

Whereas termites pose a great danger to food security in the county and Africa as a whole, they still play an integral role in both environment and human life. Termites constitute often more than 90% of the insect biomass in the soils of tropical forests, and their biomass can reach up to 100 gm^{-2} (Eggleton *et al.,* 1996; Eggleton *et al.,* 2002), they are important for decomposition processes (Holt and Lepage, 2000). Some species are eaten a cross African communities while others cultivate edible mushrooms in the genus *Termitomyces* in the termitaria (Nyeko and Olubayo, 2005).

However, termites are also reported to cause huge economic losses both in field crops and store crops (Sileshi *et al.,* 2009). Studies in Africa show enormous economic losses caused by termites in maize crops. In Ethiopia, Sileshi *et al.* (2009) reported that losses over 60 % are incurred in crop maize as a result of termite damage, Wood *et al.* (1980) reported damage of up to 56 % per maize plot in the southern Guinea Savanna zone of Nigeria and interviews with farmers in the humid forest zones of southwestern Nigeria reported that 100 % damage due to termite occur in maize (Umeh and Ivbijaro, 1997). In East Africa (Nyeko and Olubayo (2005) and Tenywa (2008) reported losses of 50% -100%.

Most of the termite species attacking maize in Siaya belong to the subfamily Macrotermitinae of which *Macrotermes* and*Microtermes* are the prominent genera. Also, the *Macrotermes* spp and *Microtermes* spp tolerate very dry weather conditions (Eggleton, 2000) thus cause maximum destruction during dry period.

Assessment by farmers in the county rated maize as highly susceptible to termite damage followed by groundnuts and sugarcane. *Macrotermes* spp attack maturing and mature maize plants, while *Microtermes* spp. cause damage to both seedlings and mature crop by consuming and lodging the entire maize crop (Nyeko and Olubayo, 2005; Sileshi *et al.*, 2008). Lodging of mature maize plants lead to increased susceptibility to pathogens especially where the grain of lodged maize plants touches the ground. Soil fungi such as *Aspergillus* may invade grain making it unsuitable for human consumption resulting into 100% maize loss (Plate 1).

Plate 3: Macerated maize plant with maize grain exposed to soil pathogens such as Aspergillus as a result of termite attack in the field. (Source: Author, 2012)

Some termite species like *microtermes* spp. construct shallow subterranean foraging galleries radiating from the nest for distance of up to 50 m, from where they forage directly on underground part of the maize plant thus exploit potential food resources. As a result seedlings are either cut just below or above the soil surface, resulting in lowered plant populations (Pardeshi and Prusty, 2010). While other species enter and consume the entire maize plant root system, which directly kills the plant or indirectly lowers maize yield through decreased translocation of water and nutrients which might cause up to 100% yield loss.

Although maize yield losses as a result of termite attack is not well documented in the county and vary from field to field, available statistics in the county show increased losses in the present times. For instance MOA (2010) revealed that losses of 30% to 50% are incurred in maize as a result of termite damage. Initially, Gitonga (1996) had reported losses of up to 30% on maize crop with loss of up to 100% suffered during prolonged dry weather conditions. These enormous economic significant of termite, therefore compels for a management strategy (s) that sanctions their existence to below economic thresholds on maize.

2.2. Termite Management

A number of techniques are used to reduce termite densities in the crops for example; plant extracts, destruction of the termite mount, cultural practices and biological control. Chemical control is used for total eradication of the termite in agricultural fields, though it is facing legal restriction because of negative impact on the environment (UNEP, 2000).

2.2.1. Destruction of termite colony

Different methods are used in destruction of the termite mount, these included digging the nest and removing the queen; burning wood, grass, or cow dung; pouring hot water, insecticides, rodenticides, or paraffin; and flooding the nest with rainwater to kill the colony (Malaret and Ngoru 1989, Nyeko and Olubayo 2005). Although destruction of the colony has been advocated by researchers (Logan *et al.,* 1990), success has been limited because of various constraints including labour requirements and lack of knowledge about termite biology. Also, this practice is directed toward mature colonies of the mound-building species while species that do not build mounts e.g. *Microtermes* spp. is often overlooked (Shileshi *et al*., 2009) and these species therefore continue to pose great challenges to farmers. In Siaya county presence of both *Macrotermes* spp. (mount building species) and *Microtermes* spp. (non-mount building species) makes the approach unsuitable for managing termite in the county. Thus need for an alternative management method that is not labour intensive and capable to control the two species in the county.

2.2.2. Plant extracts

Various parts of plants and plant extracts are known to be either toxic or repellent to termites and are widely used in rural settings (Ahmed et *al.,* 2007). For example farmers believe planting *Euphorbia tirucalli* in crop fields or applying its branches in planting holes deters termites attack on crops (Sileshi *et al.,* 2008). Application of wood ash has been widely mentioned as one of the termite management practices (Banjo *et al.,* 2003; Sileshi *et al.,* 2008). However, most plant materials break down rapidly in the soil and do not give prolonged protection from termite attack (Logan *et al.,* 1990). In Siaya county farmers apply hot wood ash to manage termites (Gitonga, 1996). The technique is tiresome and very dangerous because of risk of fire break out as result of use of hot ash which might contain burning woods/charcoal. Also, wood ash is easily washed away by rain water or strong winds. This therefore calls for an alternative termite control strategy to be sort that is able to protect crop throughout its growth period in the field.

2.2.3 Cultural Practices

Deep ploughing or hand tillage exposes termites to desiccation and to predators, thus reducing their number in the crops (Sileshi *et al*., 2008). However, this is labour intensive, costly and only applicable at planting. Intercropping has been mentioned as an effective cultural practice used by small-scale farmers to manage pests that have specific host range. According to farmers maize intercropped with sorghum or with food legumes like soybeans (*Glycine max*), common beans (*Phaseolus vulgaris*), usually suffers less termite damage than pure maize stands. It is believed that the sorghum crops and the two legumes crops acts as reservoirs of the predatory ants which prey on termites thus reduce termite attack on maize (Sekamatte *et al.,* 2003). However, the impact of intercropping on termite damage depended largely on the legume species in the intercrop and presence of predatory ants (Sileshi *et al.,* 2005). For instance termite attack was reported to be lower in maize–soyabean than in maize–common beans intercrops (Sekamatte *et al.,* 2003). These difference in damage could be a result of variations in leaf litter, which serves as an alternative source of food for termites, thus prevent termites from achieving pest status. Alternatively these legumes through its canopy sheltering effect (density canopy modifies environment below) thus influencing the presence of fauna (predatory ants) life within canopy cover which then preyed on termite thus protecting maize against termite attack. However, because these authors relied on the natural occurring predatory ant it is impossible to transfer the strategy to another area especially where these predatory ants are not naturally occurring. Therefore substitution of predatory ant with another convenient (movable) alternative that could be integrated with the legumes intercrop could assist in termite management. In Siaya County, farmers majorly relay on deep ploughing and hand tillage as cultural of control termites in maize fields, however the practice has been reported to be labour intensive and ineffective for controlling termite species that are not associated with mount building.

2.2.4. Chemical control

Organochlorines, which are regarded as persistent organic pollutants (POPs) have been widely used for termite control, but there are serious limitations and increasing legal restrictions associated with their application and efficacy (Logan *et al*., 1990; Langewald *et al*., 2003). Therefore, with the banning of POPs, the search for alternative insecticides has increased. A number of other insecticides (e.g. chlorpyrifos, isofenphos, and permethrin) are marketed for termite control. However, these insecticides are not as effective as the cyclodienes and need to be applied more frequently. For example, in South Africa, during seasons of high termite pressure, neither seed treatments nor multiple applications of insecticides to maize prevented severe levels of lodging (Logan *et al.*, 1990). Some of them also are phytotoxic, therefore the need for an alternative termite control technique that is safe and effective. Like most farmers globally, farmers in Siaya County relied on broad-spectrum organochlorines, which has been phased-out thus a need for alternative means to manage termite menace in the county.

2.2.5. Biological control

Biological control constitutes a more environmentally acceptable alternative to traditional chemical control measures (Krutmuang and Mekchay, 2005). It includes the use of natural enemies like predators, parasites and pathogens in pest management. Biological control can be accomplished in three ways; introducing exotic natural enemy to pests of exotic origin (classical biological control), enhancing the effects of natural enemies through manipulation of the environment and augmentative application of control organisms. Natural enemy numbers may be augmented with releases of laboratory-reared organisms (microbial insecticides). Termites have a wide variety of predators; birds, mammals, reptiles and ants (Sekamatte *et al*., 2003). Although ants have been reported widely to limit termite numbers in the field, their suitability for use as biological control agents for termite management has yet to be ascertained because of variation of both termite and ant species in different locality, hence need for another termite management which is effective irrespective of the termite species thus efficacy can be ascertained. As a result the use of laboratory reared microorganism offer a viable strategy for managing termites in maize field. Studies have shown that microorganisms like entompathogenic fungus hold potential in managing insects and disease vector (ICIPE, 1997). For instance the entomopathogenic fungus; *M. anisopliae* var. *anisopliae* is well known for its ability to control insect pests for example locust (ICIPE, 1997). The fungus has been developed into commercial products for use in several countries. A few examples include: Bio-Green and Bio-Cane granules for control of soil grubs of pasture and sugar cane in Australia, Green Muscle for control of locusts in Africa, Ago Biocontrol for control of various pests of ornamental crops in South America, and BioPath for control of cockroaches in the United States.

Different strains of *M. anisopliae* are species specific, meaning that *M. anisopliae* found to infect one insect species will not necessarily infect other insect species. While this limits its use as a general pest control, it makes the fungus safer by limiting its effects on non-target organisms.

Study by Rath (2000) showed that entomopathogenic fungi; *M. anisopliae* to be suitable bio-control agents for termite with no harmful effect to environment, animal and non-target arthropods. International Centre of Insect Physiology and Ecology (ICIPE) has been conducting research on the development of entompathogens fungus and results have shown ability of *M. anisopliae* Isolate ICIPE 30 to control termite in pastures, nursery trees and mounds in Kenya (ICIPE, 1997). Studies by Sekamatte, (2001) and Maniania *et al*, (2002) in Uganda and Kenya respectively gave promising results on use of *M. anisopliae* (Isolate ICIPE 30) to control termites in maize cropping system. Although studies by these authors showed promising results on the use of *M. anisopliae* (Isolate ICIPE 30) to control termites, there is no literature showing the use of the fungus to manage termites in Siaya County despite the losses caused by termite on maize in the county. The present study was therefore undertaken to explore performance of the fungus against termite in the county

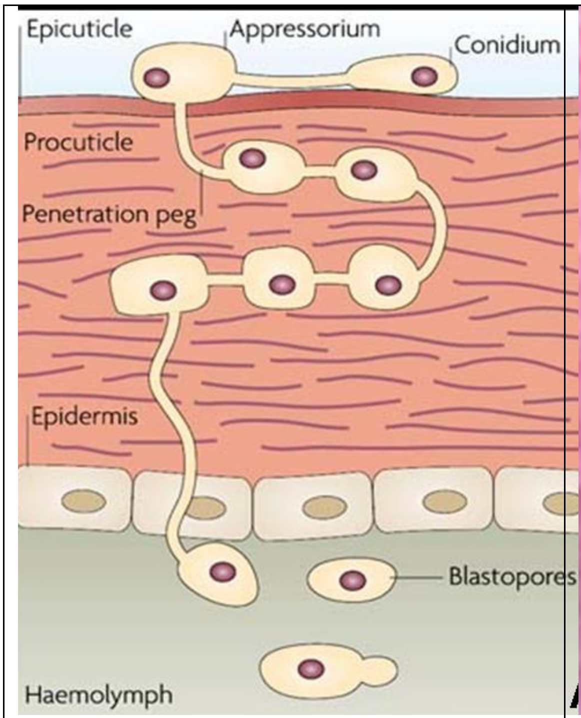
2.2.5.1. Entomopathogenic fungus; *Metarhizium anisopliae* var. *anisopliae*.

Metarhizium anisopliae is an entomopathogenic fungi belonging to the Kingdom: Fungi, Phylum: Ascomycota, Class: Sordariomycetes, Order: Hypocreales, Family: Nectriaceae; Class: Sordariomycetes; variety: *anisopliae* (Driver *et al.*, 2000; Bischoff *et al.,* 2009). *M. anisopliae* is a widely distributed soil-inhabiting fungus (Bruck, 2005; Meyling and Eilenberg, 2007; Bruck, 2009). The fungus has been widely exploited as an entomopathogenous fungus in bio-control attempts. It is known to attack over 200 species of insects belonging to orders coleoptera, dermoptera, homoptera, lepidoptera and orthoptera (Milner *et al.,* 1998; Cloyd, 1999). *M. anisopliae* is also, widely categorized as a green muscardine fungus due to the green colour of the sporulating colonies (Plate 2).

2.2.5.2. *Metarhizium anisopliae* Mode of Action and Environmental Cycling.

Metarhizium anisopliae acts as a twofold bio-control agent; contact and repellency mode (Rath, 200; Mburu *et al.,* 2009; Hussain *et al*., 2010). In contact mode *M. anisopliae* attaches to insect pest body parts and enters insects through spiracles and pores in the sense organs (Krutmuang and Mekchay, 2005; Scholte *et al.,* 2006). Once inside the insect, the fungus produces a lateral extension of hyphae, which eventually proliferate and consume the internal contents of the insect (Sun *et al.,* 2002; Krutmuang and Mekchay, 2005). Hyphal growth continues until the insect is filled with mycelia. When the internal contents have been consumed (Plate 2a), the fungus breaks through the cuticle and sporulates, which makes the insect appear "fuzzy" (Plate 2b) (Jiang *et al.,* 2002; Scholte *et al.,* 2006). *M. anisopliae* can release spores (conidia) under low humidity conditions (Milner *et al.,* 1997; Sun *et al.,* 2002). In addition, *M. anisopliae* can obtain nutrition from the lipids on the cuticle. The fungus can also produce secondary metabolites, such as destruxin, which have insecticidal properties on the host, suppressing host immune defenses and fending off potential microbial competitors (Freimoser *et al.,* 2003). Finally, after the host dies due to mycosis, the fungus will penetrate out of the integument and grow conidiophores, on which environmentally stable aerial conidia are produced. These conidia are passively disseminated into the environment and eventually infect new hosts (St. Leger, 2008).

In repellency mode *M. anisopliae* produces volatile compounds; these volatiles elicit olfactory-guided responses such as attraction or repellency (Sun *et al*., 2003; Mburu *et al.,* 2009; Hussain *et al*., 2010). Mburu *et al* (2009) showed that termite *Macrotermes michaelseni* Sjölstedt (Isoptera: Macrotermitidae) detects at a distance the presence of *M. anisopliae* through olfaction and thus avoids direct physical contact with the fungus. This inherent potential to detect specific repellent signatures from potentially infective fungi is therefore a key part of the adaptive repertoire for survival of termite (Mburu *et al.*, 2009; Mburu *et al.*, 2010). This type of avoidance behaviour is very important for management of termite residing in difficult to reach locations, such as underground nests (Milner, 2003; Hussain, 2006; Mburu *et al.*, 2009; Hussain *et al*., 2010).



B

Plate 4: Morphological symptom of infected worker termites after they were exposed with M. anisopliae var. anisopliae (A) Schematic diagram of an M. anisopliae fungus invading the host insect cuticle (B) Worker termite cadaver covered with green mycelia of M. a anisopliae (Source :Thomas & Read, 2005)

In their studies Maniania *et al*. (2002) and Sekamatte (2001), attributed the decline on maize lodging and increase in maize yields in plots treated with *M. anisopliae* due to repellence action of fungus against termites, the repellent mode of conidia was utilized to protect maize crops from deeply-soiled termite species.

2.3 Application Rates of *Metarhizium anisopliae* and Integrated Management of Termite

The use of *M. anisopliae* could therefore provide an opportunity for sustainable management of termites in agro-ecosystems; as its production is cheap and facile, does not require high input technology and can be formulated and applied in a variety of ways. The low fungus toxicity to humans compared with chemical pesticides is an advantage too. However, information pertaining to the optimum application rates is limited, yet this is critical especially in the smaller-holder farmers setting (Maniania *et al.*, 2002). Several laboratory studies have reported up to 100% mortality of termite once applied with the fungus. For instance; Fernandes and Alves (1991) achieved 100 % mortality of *Cornitermes cumulans* Kollar colonies within 10 days of application of 5 g of dust of *M. anisopliae* in the laboratory, 100% mortality of *Reticulitermes* spp. at 3 g (Ramakrishnan *et al*., 1999), 100% mortality of *Nasutitermes* spp. at 2-3 g (Milner, 2003), 100% mortality of *Heterotermes tenuis* Hagen at 2 g (Moino *et al*., 2002) and 100% mortality of *Coptotermes* spp. at 2 g within five days (Ahmed *et al.*, 2009, Hussain *et al*., 2010; Hussain and Tian, 2013). However, very limited data is available on application rate of *M. anisopliae* in control of termite in the fields. A blanket application of *M. anisoplia*e as a bio-pesticide showed minimum reduction of termite destruction on field crops (ICIPE, 1997), while Krueger *et al.* (1992) and Booth *et al.* (2000) in their studies recommended for the use of 50 kg and 60 kg granule per hectare of *M. anisopliae* in the control of *Odontotermes spp* and *Coptotermes heimi* respectively on the other hand Maniania *et al.* (2002) and Sekamatte (2001) in one of the pioneering studies in East Africa region they used approximately 30-40 kg of *M. anisopliae* (Isolate ICIPE 30) granules/ha at planting to control of termites in maize in Kenya and Uganda respectively and they reported decreased maize lodging and increased grain yield comparable to lindane treatments. However, all these authors recommended for further studies to establish optimum dosage for field application in different agro-ecological zones.

Secondly, use of *M. anisopliae* as bio-control agent against termite also faces the challenge of reduced efficacy of the inoculum in an agro-ecosystem as a result of varying environmental conditions commonly; low relative humidity and/or high temperature which adversely affected the pathogenicity and persistence of *M. anisopliae* propagules (Rath, 2000; Jaronski *et al.,* 2007; Sun *et al.,* 2008). Further studies have been recommended on improving performance of *M. anisopliae* inoculum against termite in an agro-ecosystem. The study by Hussain *et al*. (2011) on the use *M. anisopliae* to control subterranean termites in sugarcane showed an increased efficacy of the conidia when applied in combination with diesel oil comparable to thiamethoxam. However, the combined formulation of *M. anisopliae* + diesel oil adversely affected germination of sugarcane seeds by 47.43-53.40%. Therefore there is need for another strategy that is compatible with the conidia of *M. anisopliae* and still does not affect the germination of the target crops, thus these authors recommended for further studies on the improvement of these adverse effects in future studies.

Cultural practices, such as intercropping, have been reported to reduce the populations of the pest (Kyamanywa and Tukahirwa, 1988). Also, intercropping maize with food other legumes like common beans, soybean, groundnuts and peas has been reported to influences micro climate (sheltering effect) factors especially temperature, relative humidity and light intensity within cover crop canopy and the immediate environment at base of the crop (Studdert and Kaya, 1990; Ekesa *et al,* 1999). Temperature and relative humidity are very important factors which influence pathogenicity and persistence of *M. anisopliae*.

Although review of literature indicates that there is no information on the effect of intercropping on the performance of entomopathogenic fungi against termite, however cases of enhanced activity of pathogens like *Bacillus thuringiensis* (Berliner) under intercropping (maize with common bean) have been reported (Baliddawa, 1985). Ekesi *et al.* (1999) also, reported the efficacy and compatibility of *M. anisopliae* against *Megalurothrips sjostedti* in cowpea–maize intercrop. Study therefore will exploit effect of intercropping maize with legume crop (soyabean and common beans) on the performance of fungus against termites in Siaya County. The two legumes are house hold food crops grown by almost all farmers in the county as intercrop with maize in both long and short rain seasons (MOA, 2010). Also, the two legumes have sheltering effect during their vegetative stage and therefore the sheltering effect will be exploited to protect the fungus from harsh environmental conditions in the field.

Persistence of entomopathogenic fungi has also been shown to be dependent on weather conditions, particularly temperature and moisture (Inglis *et al.*, 2001; Coombes, 2013), although Ibrahim *et al.* (1999) reported that solar radiation also affected persistence of *M. anisopliae* by inactivating the inoculum in the environment where conidia are exposed to radiation. However, fungal granules applied in the soil are usually protected from these harmful radiations (Scheepmaker and Butt, 2010). Optimal growth temperature for *M. anisopliae* is between 20^{0}C and 30^{0}C (Ferron 1981; Goettel *et al*. 2000). Excessive high temperatures reduce fungal spore viability (Goettel *et al*. 2000; Meyling and Eilenberg, 2007) while lower temperatures than the optimal distinctly retards the development of mycosis thus affecting host mortality.

While low relative humidity (below 45%RH) lowers fungal sporulation in the cadavers and initiates production of conidia with low viability in the field (Arthurs and Thomas, 2001). Combination of these two factors therefore adversely affects the efficiency and persistence or production of additional spore (sporulation) in the soil (Goettel *et al*. 2000; Meyling and Eilenberg, 2007; Scheepmaker and Butt, 2010). Therefore manipulating microhabitats will play an important role in enhancing the efficacy of *M. anisopliae* conidia and persistence.

The study therefore seeks to explore the effect of integrating *M. anisopliae* granule application with food legume (soyabeans + maize or common beans + maize) intercrop on performance of fungus in the management of termite in maize crop in Siaya County. The dense canopy cover of food legumes will aid to moderate microhabitat environment (low relative humidity and high temperature) at the base of maize plant which is the point of the fungus placement hence its action area, thus increasing the inoculum pathogenicity and persistence in the field. Also the use of maize–legume intercrop has an additional advantage of buffering the fungus against ultra violet radiation which adversely affects its survival (Langewald *et al*., 2003).

CHAPTER THREE

Evaluation of Application Rates and Influence of Different Maize-based Cropping System on the Efficacy of *Metarhizium anisopliae* for Integrated Termite Management.

3.0. Abstract.

Studies have demonstrated the ability of *Metarhizium anisopliae* isolate ICIPE 30 to control termites, yet little information is known on the application rate of the fungus. Application rate is necessary for development of an effective and sustainable termite management using *M. anisopliae* fungus as bio pesticide in the field. Studies also, have shown that pathogenicity of *M. anisopliae* is influenced by temperature and relative humidity. Different cropping systems have influence on micro environmental condition within the field. The study evaluated different application rates of *M. anisopliae* and the influence of different maize-based cropping system on the efficacy of the fungus for control of termite in maize fields at Siaya ATC and farmer's field in Ligega, Siaya County for two seasons. Three application rates of the fungus 40.0 kg/ha, 60.0 kg/ha 80.0 kg/ha plus untreated (control) were evaluated in the field under 3 maize-based cropping systems; maize monocrop, maize + soybean intercrop and maize +common bean intercrop. Treatments were replicated three times in a RCBD in both sites. The following data was recorded after every two weeks; head count of lodged maize due to termite attack, temperature and relative humidity at the base of maize plant were recorded using hygrothermometer and yields per plot taken at harvest. Collected data was subjected to ANOVA analysis and means were seperated by Tukeys' ($P<0.05$) using Genstat software. Application of *M. anisopliae* significantly reduced maize lodging and increased maize yield. An intercropping system of maize with either soybean or common bean intercrops significantly increased relative humidity during vegetative stage of legumes while temperature remained within optimal range (25-30^0 C) in all cropping systems. Intercropped plots of maize + soybean with application of 40.0 kg/ha of *M. anisopliae* recorded further reduction in maize lodging and increased maize yield to a level comparable with application of 80.0 kg/ha of *M. anisopliae* in maize monocrop of 7.3 t/ha and 7.6 t/ha at Siaya ATC and Ligega respectively. This study demonstrates that application of *M. anisopliae* protects maize against termite attack and the use of legume intercrops enhances the efficacy of the fungus in the field. The results could aid farmers in decision making on available options for controlling termite in maize field using *M. anisopliae.*

3.1. Introduction

Use of *M. anisopliae* as a bio pesticide against termites has been documented extensively in laboratory research. For instance, Fernandes and Alves (1991) achieved 100 % mortality of *Cornitermes cumulans* Kollar colonies within 10 days of application of 5g of dust of *M. anisopliae* in the laboratory. Several other success results have been reported in the laboratory; *Reticulitermes* spp. (Ramakrishnan *et al*., 1999), *Coptotermes* spp. (Ahmed *et al.*, 2009, Hussain *et al*., 2010; Hussain and Tian, 2013), *Nasutitermes* spp. (Milner, 2003) and *Heterotermes tenuis* Hagen (Moino *et al*., 2002). However, limited literature is available on application of the fungus in agro-ecosystems, yet the fungus provides an opportunity for sustainable management of termites in agro-ecosystems; as its production is cheap and facile, does not require high input technology and can be formulated and applied in a variety of ways. The low fungus toxicity to humans compared with chemical pesticides is an advantage too (Rath, 2000; Hussain *et al.,* 2011).

Formulations and application rate of *M. anisopliae* against termites in an agro-ecosystem is critical for both smaller-holder farmer settings and large scale-mechanized farming to enable correct placement of active ingredient (viable conidia) for effective protection of crops in the fields and for economic use of the bio pesticide (Maniania *et al.,* 2002). Hussain *et al*. (2011) using liquid formulation of *M. anisopliae* applied on sugarcane setts at planting reported least lodging of sugarcane plantation comparable to fields applied with thiamethoxam while Maniania *et al.* (2002) using *M. anisopliae* formulated as granules applied at planting reported significant decline in maize destruction by termites. Although liquid formulation has been reported to reduce termite attack on crops in the field, the formulation is less effective compared granule formulation because fungus acts by contact rather than systemic and secondly liquid formulation break down slightly more quicker compared to granule formulation (Rath, 2000).

There is limited information available application rates of the *M. anisopliae* to control termites in maize fields. Only Maniania *et al.* (2002) and Sekamatte (2001) in their studies reported use approximately 30.0- 40.0 kg of *M. anisopliae* (Isolate ICIPE 30) granules/ha at planting to control of termites in maize in Kenya and Uganda respectively and they reported decreased maize lodging and increased grain yield comparable to lindane treatments. While Krueger *et al.* (1992) and Booth *et al.* (2000) talked of the use of 50.0 kg and 60.0 kg granule per hectare of *M. anisopliae* to control of *Odontotermes spp* and *Coptotermes heimi* respectively in forestry and pasture fields. However, all these authors recommended for further studies to establish optimum dosage for field application in different agro-ecosystem.

The efficacy of *M. anisopliae* in an agro ecosystem is affected by environmental temperature and relative humidity (micro climate) because the two factors influence the fungus virulence, sporulation, persistence and conidiogenesis on insect pest body surfaces (Rath, 2000; Kotwal *et al.*, 2012). For instance high temperatures reduce fungal spore viability (Goettel *et al*. 2000; Meyling and Eilenberg, 2007) while lower temperatures than the optimal distinctly retards the development of mycosis thus affecting host mortality on the other hand low relative humidity (below 45%RH) lowers fungal sporulation in the cadavers and initiates production of conidia with low viability in the field (Arthurs and Thomas, 2001).

Cultural practice of intercropping maize with legume crops (maize + soybean or maize + common bean) influences temperature and relative humidity range within the crop cover canopy and the immediate environment at base of the crop (Studdert and Kaya, 1990; Ekesa *et al,* 1999). Although, literature review indicates no information on the effect of intercropping on the performance of entomopathogenic fungi against termite, cases of enhanced activity of pathogens like *Bacillus thuringiensis* (Berliner) maize with common bean have been reported (Baliddawa, 1985). Ekesi *et al.* (1998) also, reported improved efficacy and compatibility of *M.anisopliae* against *Megalurothrips sjostedti* in cowpea–maize intercrop. Temperature (20-30^{0}C) and high relative humidity (above75%) increases *M. anisopliae* sporulation and conidiogenesis on termite exoskeleton (Ferron, 1977; Inglis *et al.,* 2001). Therefore integration of these legumes as intercrops in maize fields applied with *M. anisopliae* fungus is expected to regulate these environmental factors especially temperature and relative humidity which influence the efficacy of the fungus in the fields. This study therefore seeks to establish an effective application rate of *M. anisopliae* granules that will control of termite in maize field at Siaya ATC and farmer's field in Ligega location, Siaya County. Further explore the effect of integrating *M. anisopliae* granules with food legume intercrops in maize as means of modifying environmental at the base of maize plant, to influence performance of *M. anisopliae* against termite in maize field.

3.2. Materials and Methods

3.2.1. Study Sites

The study was done in two sites at Siaya ATC (0^{o}03'40.86"N 34^{o}17'13.67"E) and at farmer's field in Ligega location (0^{o}12'57.77"N 34^{o}15'54.30"E) in Siaya County. County is located in the midlands of Western Kenya, with an area which covers approximately 2, 530.5 km^2, and is characterized by high population densities (KNBS 2009, CRA, 2012). The county lies between latitude 0° 26’ to 0° 18’ north, longitude 33° 58’ east and 34° 33’ west and an altitude of 1100 to 1400 meters above sea level. Mean annual temperature of 22.5^{0}C, mean annual relative humidity of 56% and mean annual precipitation of 1800-2000mm (Jaetzold and Schmidt, 2008). Termite existence in the county has been reported to date back to precolonial era, with local population in the county understanding economic importance (food and production of molding clay). However, in the recent past termites have been reported causes huge on crops losses in the county (Gitonga, 1996; MOA, 2007).

3.2.2. Mass Production of fungus

M. anisopliae isolate ICIPE 30 was formulated as granules at International Centre for Insect Physiology and Ecology (icipe) Nairobi, Kenya (Appendix I).

3.3. Determination of Application Rates and Effect of Intercropping on Efficacy of *M. anisopliae* to Manage Termite in Maize Fields.

The following application rates; 40.0 kg/ha, 60.0 kg/ha, 80.0 kg/ha and 0.0 kg/ha of *M. anisopliae* formulated as granules were selected and evaluated in the two study sites in Siaya county based on estimates recorded in the literature (Krueger *et al.*, 1992; Booth *et al.*, 2000; Maniania *et al.*, 2002). Cropping system of maize + soyabean, maize + common bean and maize monocrop were selected based on common maize growing practice in the county (MOA, 2010) and ability to modify micro environmental conditions within crop field set up (Studdert and Kaya, 1990; Ekesa *et al*, 1999).

3.3.1. Experimental Design and Field Layout

The two field sites, Siaya ATC and farmer's field in Ligega location, Siaya County were prepared before the onset of long rains in March 2012 and short rains in September 2012. Thirty six plots each measuring 3 by 3 meters were prepared on each site (Appendix II). Twelve treatments (Table 2) were assigned and replicated three times in a randomized complete block design (RCBD). Maize hills were dug at a spacing of 75cm by 25cm giving a plant population of 32 maize plants per plot. The following model for maize cropping systems and fungal application rates was applied.

i. $\gamma = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \Sigma_{ijk}$

(μ Treatment mean, α_i cropping systems, β_j rate of application, $\alpha\beta_{ij}$ interaction between treatment rates and cropping systems, Σ_{ijk} the error term due to treatments).

Table 2: Table of treatments applied in the field at planting.

Plot	Treatments
1	0.0 kg/ha of *M. anisopliae* granule; Maize monocrop
2	40.0 kg/ha of *M. anisopliae* granule; Maize monocrop
3	60.0 kg/ha of *M. anisopliae* granule; Maize monocrop
4	80.0 kg/ha of *M. anisopliae* granule; Maize monocrop
5	0.0 kg/ha of *M. anisopliae* granule; Maize+ bean intercrop
6	40.0 kg/ha of *M. anisopliae* granule; Maize+ bean intercrop
7	60.0 kg/ha of *M. anisopliae* granule; Maize+ bean intercrop
8	80.0 kg/ha of *M. anisopliae* granule; Maize+ bean intercrop
9	0.0 kg/ha of *M. anisopliae* granule; Maize+ soybean intercrop
10	40.0 kg/ha of *M. anisopliae* granule; Maize+soybean intercrop
11	60.0 kg/ha of *M. anisopliae* granule; Maize+soybean intercrop
12	80.0 kg/ha of *M. anisopliae* granule; Maize+soybean intercrop

3.3.2. Applications Rates and Different Maize Cropping Systems

The following application rates of *M. anisopliae* formulated as granules; 80.0 kg/ha, 60.0 kg/ha, 40.0 kg/ha and 0.0 kg/ha were applied in maize (Hybrid 513) + Soyabeans (*Glycine max* KARI Kakamega var.) intercrop; maize (Hybrid 513) + common bean (*Phaseolus vulgaris* Mwitemania var.) intercrop and maize (Hybrid 513) monocrop at planting (Table 2). These application rates were equivalent to approximately 3.0 g, 2.0 g, 1.0 g and 0.0 g per maize hill. The granules were applied in every maize hill and thoroughly mixed with soil before two maize seeds were sown per hill. Soybeans or common bean intercrops were planted in the intra rows of the maize hills in the intercrop treatments (Plate 3). D.A.P fertilizer at 50.0 kg per hectare was applied at planting and C.A.N applied as a top dress fertilizer to maize at knee high stage. Thinning was done seven days after emergence to leave one maize plant per hill.

Plate 5: A plot with common bean planted intra row of maize seedlings at Siaya ATC farm (Source: Author, 2012).

3.3.2 Data collected

The efficacy of *M. anisopliae* application rates was measured by head count of the lodged maize plant per plot after every fourteen days from germination to harvest. Termite of attack was ascertained by lifting the plant and examining the root and stem for presence termite galleries at the base of lodged maize stem; typical syptoms of *Macrotermes* and *Microtermes* damage on maize crops in the fields (Wood *et al.,* 1980; Umeh and Ivbijaro, 1997; http://www.chem.unep.ch/pops/termites/termite_toc.htm9.7.2011) (Plate 4). Any lodged maize plants that were counted, were marked using stickers to avoid repetition in the succeeding data collection schedules. At harvest, grain yield was recorded and yield per plot was extrapolated to kg/ha.

Plate 6: Damage to plants caused by subterranean termites

Source: http://www.chem.unep.ch/pops/termites/termite_toc.htm 9.7.2011.

To assess the effect of the intercropping on efficacy of *M. anisopliae* granules against termites in maize fields, data on following were collected; temperature and relative humidity using hygrothermometer after every fourteen days from planting to harvest time at the base of maize (Plate 5). At top of soil surface.

Plate 7: Hygrometer sensor at the base of maize crop in a maize monocrop plot (Source: Author, 2012).

3.3. Statistical Analysis.

Percentage of lodged plant and maize yield data were subjected to analysis of variance (ANOVA) for a randomized complete block design and means were compared by using the Tukeys' ($P \leq 0.05$) test Genstat (Genstat, 2010) using ANOVA procedure to test for performance between different fungus application rates and maize-based cropping system, therefore select best combination of application rate and maize-based cropping system. Regression analysis was done to establish relationship between maize lodging and different maize cropping system applied in the study.

3.4. Results.

3.4.1. Application rates of *M. anisopliae* granules and maize –based cropping systems.

Maize lodging was highest in untreated maize monocrop 97.3% per hectare while application of 40.0 kg/ha *M. anisopliae* granules had up to 81.3% of lodged plants per hectare during the long rain season of 2012 at Siaya ATC farm. Maize lodging was less 31.3% in plots applied with 60.0 kg/ha of *M. anisopliae* granules in both site during long and short rain seasons while in plots applied with 80.0 kg/ha *M. anisopliae* granules the least number lodged maize plant was recorded both at Siaya ATC farm and farmer’s field in Ligega location during the long rains and short rains of 2012 (Plate 6and Table 3).

(0.0 kg/ha)	40.0 kg/ha	60.0 kg/ha	80.0 kg/ha
			

Plate 8: Effect of different applications rate of M. anisopliae granules against termite attack on maize at Siaya ATC farm at harvest stage, during the long rains of 2012 (Source: Author, 2012).

Application of *M. anisopliae* granules in maize monocrop at planting significantly decreased maize lodging when compared with the untreated control in both seasons at Siaya ATC and Ligega. Application of 80.0 kg/ha of *M. anisopliae* granules had the least of lodged maize in both Siaya ATC and Ligega during the short and long rain seasons of 2012 ($P \leq 0.05$) (Fig 3 and Table 3), maize lodging at Siaya ATC and Ligega in plots with 80 kg/ha and 60 kg/ha of *M. anisopliae* applications did not differed significantly in both seasons ($P \leq 0.05$). And when the two sites were compared at different treatments application rates (control, 40.0 kg/ha, 60.0 kg/ha and 80.0 kg/ha) Ligega had less maize lodging than Siaya ATC (Table 3).

In all maize cropping system (monocrop, maize +common intercrops and maize +soyabean) maize lodging was high in untreated plots compared to treated plots in both Siaya ATC farm and farmer's field in Ligega location during long and short rains of 2012 ($P \leq 0.05$) (Table 3). When food legume intercrops were introduced, maize lodging further declined in all treatments with *M. anisopliae* granule application although not significantly (Table 3 and appendix 5). Applications of 60.0 kg/ha and 80.0 kg/ha of *M. anisopliae* granule in maize + soyabean intercrops significantly reduced number of lodged maize at Siaya ATC in both short rains and long rains of 2012 (Table 3).

Application of *M. anisopliae* granules significantly increased maize grain yields compared with the untreated treatments at both Siaya ATC farm and farmer's field in Ligega during the short and long rains of 2012. Maize grain yield was not significantly different in plots treated with 60.0 kg/ha and 80.0 kg/ha of *M. anisopliae* granule at farmer's field in Ligega during long rains and in Siaya during short rains of

2012. Maize grain yields were highest in plots treated with 80.0 kg/ha of *M. anisopliae* granule in both sites during short and long rains of 2012 (Table 3).

Maize grain yield increased significantly with application of 80.0 kg/ha of *M. anisopliae* granule in a maize monocrop system from 1.3 to 7.3 t/ha at Ligega and from 1.0 to 7.7 t/ha at Siaya ATC farm (Table 3). Integrating of *M. anisopliae* granule with intercrop of maize of either soybean or common bean significantly increased maize grain yield. For instance application of 40.0 kg/ha of granules in an intercrop of maize + soyabean increased maize grain yield to a level comparable with application of 80.0 kg/ha of *M. anisopliae* in maize monocrop of 7.3 t/ha and 7.6 t/ha at Siaya ATC and Ligega respectively (Table 3). Applications of 60.0 kg/ha and 80.0 kg/ha of *M. anisopliae* granule in maize + common bean intercrops and maize +soybean intercrops recorded high maize yields at Siaya ATC farm and farmer's field in Ligega location both short and long rains of 2012 (Table 3). Although soybean intercrops recorded the highest maize grain yields with the same rates of fungal application. When application of 40.0 kg/ha of *M. anisopliae* granules was integrated with maize + soyabeans intercrops; maize yields increased significantly from 2.2 to 7.5 t/ha at Ligega and 1.8 to 8.8 t/ha at Siaya ATC farm while application of 60 kg/ha of *M. anisopliae* granules integrated with maize + soyabeans intercrop; maize yields increased from 3.5 to 12.8 t/ha at Ligega and 3.1 to 10.2 t/ha at Siaya ATC farm and finally when 80.0 kg/ha of *M. anisopliae* granules was applied in maize + soyabean intercrops maize yield increased from 7.3 to 13.5 t/ha and 7.6 to 11.7 t/ha at Ligega and ATC Siaya respectively. The increment in maize grain yields were also recorded when different rates of *M. anisopliae* applications were integrated in maize + common beans intercrops; @40.0 kg/ha of *M. anisopliae* granules the increment was from 2.2

to 6.7 t/ha at Ligega and 1.8 to 5.5 t/ha at Siaya ATC, @60.0 kg/ha of *M. anisopliae* granules the increment was from 3.5 to 9.7t/ha at Ligega and 3.1 to 8.1 at Siaya ATC while @80.0 kg/ha of *M. anisopliae* granules the increment went from 7.3 to 10.8t/ha at Ligega and from 7.6 to 8.8 t/ha at Siaya ATC. The maize grain yields of the untreated plots in both monocrops and in the two legume intercrops were significantly reduced and ranged between 1.0 to 2.2t/ha ($P \leq 0.05$) both Siaya and Ligega (Table 3).

To assess the effect of integrating cropping system to the performance of *M. anisopliae* relative humidity and temperature were measured (Table 4 &5). Temperatures in all maize monocrops, maize +common beans intercrops and maize +soybeans intercrops did not vary significantly throughout the maize growth, ranging between 26.42^0C to 30.92^0C (Table 4a and 4b).

Table 3: Mean number of lodged maize plant per plot and maize grain yield in tonnes per hectare under different applications rates of M. anisopliae granule and in integrated systems of M. anisopliae granule with different maize-based cropping systems during the long rains and short rains seasons of 2012 at Siaya ATC and Ligega sites

	Long Rains 2012 Lodged Maize per Plot		Short Rains 2012 Lodged Maize per Plot		Grain yield in t/ha Long and Short Rains 2012	
TREATMENTS	Ligega	Siaya	Ligega	Siaya	Ligega	Siaya
Maize monocrop 0 kg/ha	31.3a	31.3a	29.3a	31.5a	1.3f	1.0e
Maize monocrop 40 kg/ha	193b	22.7c	19.3b	23.0c	2.2f	1.8e
Maize monocrop 60 kg/ha	15.3bcd	15.3ef	14.3bc	16.7de	3.5ef	3.2de
Maize monocrop 80 kg/ha	11.0def	14.7ef	11.3cd	15.7ef	7.3cd	7.7bc
Maize + beans 0 kg/ha	27.0a	293ab	27.0a	29.7ab	1.3f	1.3e
Maize + beans 40 kg/ha	18.3bc	19.0d	18.0b	20.0cd	6.7de	5.5cd
Maize + beans 60 kg/ha	15.0bcd	13.7efg	14.3bc	14.0efg	9.7bcd	8.2b
Maize + beans 80 kg/ha	13.7cde	12.0fgh	12.7cd	12.3fgh	10.8abc	8.8b
Maize + soya 0 kg/ha	27.0a	27.3b	27.7a	27.7b	2.2f	2.2e
Maize + soya 40 kg/ha	15.7bcd	17.0de	15.3bc	17.3de	7.5cd	8.8b
Maize + soya 60 kg/ha	8.3ef	11.0gh	8.7d	10.3gh	12.8ab	10.2b
Maize + soya 80 kg/ha	7.7f	9.3h	8.0d	8.7h	13.5a	11.7a
Total mean	17.5	18.9	17.2	18.6	13.1	11.7
Grand mean	17.5	18.9	17.2	18.6	6.6	5.9
CV	10.7	7.4	10.0	6.6	27.4	22.4
S.E	1.46	1.10	1.34	1.22	1.44	1.05
S.E.D	1.52	1.15	1.40	0.99	1.47	1.07

Note: Means in the same column followed by the same letter are not significantly different at P≤0.05 according toTukey's range test. CV – Coefficient of Variation %, SE – Standard Error, SED – Standard Error of difference between means

Maize intercrop with either common beans or soybeans significantly increased relative humidity during vegetative to maturity stages of the two food legume intercrops that is from week four to week twelve compared to maize monocrop in both seasons at Siaya ATC and Ligega (Table 5). Of the two maize-legume intercrop; maize +soybeans recorded the highest relative humidity in both seasons at Siaya ATC and Ligega. However, relative humidity was low during early stage (week 2) and late stage (week 16) of maize growth at Siaya ATC in all plots with maize monocrop, maize +common bean intercrops and maize +soybean intercrops (Table 5). To infer the effect of environmental factor on performance of *M. anisopliae* granule number of lodged maize plant and maize grain yield were recorded. Termites attacked maize crop at all stages of growth although severe attack occured in the early and in the late stage of maize growth, with highest lodging recorded at week 16, 14 (late stages) and 2 (early stage) in that order in both seasons at Siaya ATC and Ligega (Table 6&7). Generally maize monocrop recorded highest level of lodged maize throughout maize growth period. Lodged maize was low in maize intercrops during the vegetative to maturity stage of two food legume intercrops (week 4 to week 12), although maize + soybean intercrops recorded the least number of lodged maize during this period (Table 6&7). Maize +common bean intercrop recorded the least maize lodged in treatments applied with 60.0 kg/ha and 80.0 kg/ha of *M. anisopliae* granules at week 6 and week 8 (period dense canopy of common bean) while maize +soybean intercrops with same quantities of *M. anisopliae* granules had least number of lodged maize at week 10 to week 12 (period dense canopy of common bean) in both Siaya ATC and Ligega during long rains of 2012. Also, maize + soybeans intercrop with application of 80.0 kg/ha of *M. anisopliae* granules had the longest period (week 6 to week 12) with least number of lodged maize (Table 6&7).

Table 4: Temperatures in different maize cropping system at different growth stages at Siaya ATC farm during long rains of 2012

	Temperature at different growth stages of maize in the field							
Treatment	Wk2	Wk4	Wk6	Wk8	Wk10	Wk12	Wk14	Wk16
Maize monocrop 0 kg/ha	30.08a	28.38bcd	28.00ab	26.58b	28.33abc	30.00abc	27.75ab	30.38a
Maize monocrop 40 kg/ha	30.42a	28.42bcd	27.96ab	26.71b	27.75c	30.33ab	27.5abc	30.67a
Maize monocrop 60 kg/ha	34.33a	29.00bc	26.42b	26.79b	28.33abc	30.67a	27.00cd	30.67a
Maize monocrop 80 kg/ha	30.25a	27.75d	28.67ab	26.67b	28.08bc	29.67bcd	28.08a	30.92a
Maize + beans 0 kg/ha	30.00a	28.08cd	28.00ab	26.92b	28.5abc	30.33ab	27.17bcd	30.83a
Maize + beans 40 kg/ha	30.00a	28.5bcd	28.25ab	26.83b	28.67ab	30.08abc	27.25bcd	30.67a
Maize + beans 60 kg/ha	30.17a	28.42bcd	28.08ab	26.83b	28.33abc	29.75bcd	26.58d	30.50a
Maize + beans 80 kg/ha	30.00a	28.75bcd	27.83ab	26.58b	28.50abc	29.00d	26.83cd	30.42a
Maize + soya 0 kg/ha	30.08a	28.92bc	28.25ab	26.5b	28.67ab	30.25ab	27.25bcd	30.58a
Maize + soya 40 kg/ha	30.08a	28.42bcd	27.00b	26.33b	29.08a	30.33ab	27.25bcd	30.96a
Maize + soya 60 kg/ha	34.25a	29.33b	27.42ab	26.67b	28.92ab	30.42ab	27.25bcd	30.33a
Maize + soya 80 kg/ha	30.17a	29.50b	28.25ab	26.58a	28.17bc	29.42cd	27.42abc	30.58a
Grand Mean	30.82	28.71	28.09	27.28	26.75	28.44	30.02	30.63
CV	19.20	3.90	14.90	2.80	2.40	3.20	3.20	2.20
SE	5.64	1.07	4.00	0.77	0.61	0.86	0.78	0.69
SED	2.41	0.46	1.71	0.32	0.26	0.36	0.34	0.29

Note: Means in the same column followed by the same letter are not significantly different at P≤0.05 according to Tukey'srange test. CV – Coefficient of Variation %, SE – Standard Error, SED – Standard Error of difference between means.

Table 5: Temperatures in different maize cropping system at different growth stages at Siaya ATC farm during short rains of 2012

	Mean temperature at different growth stages of maize in the field							
Treatment	Week2	Week4	Week6	Week8	Week10	Week 12	Week14	Week 16
Maize monocrop 0kg	32.2a	29.0bc	28.7ab	26.8ab	31.1a	30.3ab	30.1bc	30.7a
Maize monocrop 40kg	29.4a	28.4bcd	27.9ab	26.5cd	30.3a	30.7a	29.6c	30.9a
Maize monocrop 60kg	31.3a	28.4bcd	26.4b	26.0cd	32.3a	30.3ab	30.3abc	30.7a
Maize monocrop 80kg	31.3a	27.8d	28.0ab	28.1a	31.3a	29.4bc	30.3abc	30.7a
Maize + beans 0kg	30.0a	30.5a	31.3a	26.8bcd	30.0a	30.3ab	30.5abc	30.4a
Maize + beans 40kg	31.0a	28.5bcd	28.3ab	26.3bcd	31.0a	30.1abc	30.3abc	30.7a
Maize + beans 60kg	30.0a	28.4bcd	28.0ab	26.3bcd	30.2a	29.8bcd	30.7ab	30.6a
Maize + beans 80kg	32.2a	28.8bcd	27.8ab	26.2bcd	30.0a	29.0d	30.5abc	30.8a
Maize + soya 0kg	29.2a	28.9bc	28.3ab	26.3bcd	30.1a	30.3ab	30.7ab	30.6a
Maize + soya 40kg	30.2a	28.4bcd	27.0b	26.6bcd	31.1a	30.4ab	30.1a	30.6a
Maize + soya 60kg	30.3a	28.1cd	27.4ab	26.3bcd	31.3a	30.4ab	30.2bc	30.4a
Maize + soya 80kg	31.1a	28.1cd	28.1ab	27.4ab	30.2a	29.7bcd	30.9ab	30.9a
Grand Mean	30.7	28.6	28.1	26.8	30.7	30.0	30.6	30.6
CV	19.1	3.7	14.9	20.8	19.5	3.2	2.3	2.2
SE	5.54	1.07	4.00	0.76	5.54	0.78	0.86	0.68
SED	2.312	0.46	1.71	0.32	2.40	0.33	0.36	0.28

Note: Means in the same column followed by the same letter are not significantly different at P≤0.05 according toTukey's range test. CV – Coefficient of Variation %, SE – Standard Error, SED – Standard Error of difference between means.

Table 6: Relative humidity in different maize cropping system at different growth stages at Siaya ATC farm during long rains of 2012

	Mean relative humidity at different growth stages of maize in the field							
Treatment	Week2	Week4	Week6	Week8	Week10	Week12	Week14	Week16
Maize monocrop 0 kg/ha	42.0bcde	42.2d	51.0d	53.5b	60.8d	55.8e	65.5c	42.0d
Maize monocrop 40 kg/ha	41.4de	41.2d	51.3d	53.6b	61.0d	55.9e	49.8d	45.1bcd
Maize monocrop 60 kg/ha	42.0cde	41.3d	51.9d	53.7b	60.8d	56.2e	50.0d	41.3d
Maize monocrop 80 kg/ha	44.6bcde	41.0d	51.2d	53.4b	60.4d	56.2e	77.6a	42.9cd
Maize + beans 0 kg/ha	42.0cde	55.8 c	71.0c	83.3a	86.0bc	86.2bc	50.3d	41.9d
Maize + beans 40 kg/ha	44.8bcd	66.2ab	72.3abc	83.5a	86.0bc	85.8bcd	68.8b	50.9ab
Maize + beans 60 kg/ha	45.7bc	66.5ab	72.9ab	83.3a	85.9bc	87.4ab	68.5b	48.4bc
Maize + beans 80 kg/ha	45.8b	65.6ab	71.6bc	83.3a	85.4c	84.6cd	75.7a	50.5ab
Maize + soya 0 kg/ha	40.8e	67.3a	71.8abc	83.3a	90.6a	83.7d	50.1d	54.5a
Maize + soya 40 kg/ha	41.9de	64.0ab	72.6abc	82.4a	91.3a	88.6a	66.8bc	50.4ab
Maize + soya 60 kg/ha	41.5de	62.7b	73.4a	83.4a	91.3a	89.5a	75.6a	54.8a
Maize + soya 80 kg/ha	49.8a	64.1ab	72.3abc	83.3a	88.1b	89.1a	75.5a	50.2ab
Grand Mean	43.5	56.5	65.3	73.3	78.9	76.6	64.0	47.7
CV	9.0	7.3	2.8	2.5	2.9	3.1	3.6	13.0
SE	3.75	3.92	1.76	1.75	2.19	2.30	2.22	6.23
SED	1.61	1.67	0.75	0.75	0.94	0.98	0.95	2.54

Note: Means in the same column followed by the same letter are not significantly different at P≤0.05 according to range test.

CV – Coefficient of Variation %, SE – Standard Error, SED – Standard Error of difference between mean

Table 7: Relative humidity in different maize cropping system at different growth stages at Siaya ATC farm during short rains of 2012

	Mean relative humidity at different growth stages of maize in the field							
Treatment	week 2	week 4	week 6	week 8	week 10	week 12	week 14	week 16
Maize monocrop 0kg	44.1bcd	50.9 c	51.2d	53.6b	60.2d	53.8e	49.9d	42.0d
Maize monocrop 40kg	42.3cde	62.7b	51.5d	53.7b	59.0d	53.9e	49.9d	45.1bcd
Maize monocrop 60kg	42.0cde	43.3d	51.9d	53.7b	61.8d	55.4e	49.2d	41.3d
Maize monocrop 80kg	44.6bcde	64.0ab	51.9d	53.4b	61.0d	56.2e	51.3d	42.9cd
Maize + beans 0kg	42.0cde	42.2d	71.1c	83.2a	86.4bc	84.3bc	64.6c	41.9d
Maize + beans 40kg	44.8bcd	66.1ab	72.3abc	83.3a	86.0bc	83.8bcd	66.8bc	50.9ab
Maize + beans 60kg	45.7bc	66.5ab	72.9ab	83.3a	85.9bc	85.4ab	68.8b	48.4bc
Maize + beans 80kg	45.8b	65.6ab	71.6bc	83.3a	85.4bc	85.7cd	68.5b	50.54ab
Maize + soya 0kg	40.9e	65.3ab	71.8abc	83.2a	88.1b	80.6d	75.6a	54.5a
Maize + soya 40kg	42.9cde	43.2d	72.6aba	83.4a	91.3a	88.9a	75.7a	50.4ab
Maize + soya 60kg	42.5cde	45.1d	72.3abc	83.3a	91.3a	89.7a	75.5a	54.8a
Maize + soya 80kg	44.8a	64.1ab	73.4a	83.3a	90.6a	89.9a	75.6a	50.3ab
Grandmean	43.5	56.5	65.4	73.4	78.9	75.6	64.5	47.8
CV	9.01	7.27	2.90	2.47	3.00	3.73	3.63	13.17
SE	3.75	3.92	1.76	1.75	2.19	2.30	2.23	6.24
SED	1.64	1.69	0.76	0.749	0.95	0.98	0.94	2.55

Note: Means in the same column followed by the same letter are not significantly different at P≤0.05 according to Tukey'srange test. CV – Coefficient of Variation %, SE – Standard Error, SED – Standard Error of difference between means

At week 6 with 40 kg/ha	At week10 with 40 kg/ha	At week 10 with 40 kg/ha
 Dense common bean canopy	 Dense soybeans canopy	 Maize lodging in monocrop

Plate 9: Treatment of 40.0 kg/ha of M. anisopliae under different cropping system at Siaya ATC during the long rains season of 2012

(Source:Author,2012)

Table 8: Maize lodging over time in different maize cropping system and under varying application rates of M. anisopliae granules during the long rains of 2012 at farmer's field in Ligega location.

Number of Lodged Maize Plant in % per Hectare Over Time During long rains of 2012								
TREATMENTS	WEEK 2	WEEK 4	WEEK 6	WEEK 8	WEEK 10	WEEK 12	WEEK 14	WEEK 16
Maize monocrop 0 kg/ha	4.2a	3.5a	3.2a	3.5a	3.7a	4.0a	4.2a	5.5a
Maize monocrop 40 kg/ha	2.5cd	2.5abc	1.7bc	1.8cd	2.0cde	2.5bc	3.0bc	4.7ab
Maize monocrop 60 kg/ha	2.0d	1.8bcd	1.3c	1.2cde	1.7de	2.0cd	2.0cde	4.2bc
Maize monocrop 80 kg/ha	2.2d	1.5cd	1.2c	1.0cde	1.2ef	1.7cde	1.8de	3.3cde
Maize + beans 0 kg/ha	3.5abc	3.0ab	2.5ab	3.0ab	3.2ab	3.7ab	4.0ab	5.0ab
Maize + beans 40 kg/ha	2.3cd	2.3abcd	1.7bc	1.3cde	2.3bcd	2.5bc	2.7cd	3.8bcd
Maize + beans 60 kg/ha	1.7d	1.7cd	0.7c	0.3e	1.7de	2.2c	2.0cde	2.8de
Maize + beans 80 kg/ha	1.8d	1.2d	0.7c	0.3e	1.3def	1.3cde	2.2cde	2.8de
Maize + soya 0 kg/ha	3.8ab	3.0ab	3.0a	3.0ab	3.0abc	3.7ab	3.8ab	4.5abc
Maize + soya 40 kg/ha	2.7bcd	1.8bcd	1.7bc	2.0bc	1.7de	2.0cd	2.2cde	3.3cde
Maize + soya 60 kg/ha	2.0d	1.3cd	1.2c	1.2cde	0.5fg	0.8de	1.5e	2.2e
Maize + soya 80 kg/ha	1.83d	1.2d	0.8c	0.8de	0.0g	0.7e	1.3e	2.2e
Total mean	5.1	4.1	3.3	3.3	3.6	4.5	5.1	7.4
Grand mean	2.5	2.1	1.6	1.6	1.9	2.3	2.6	3.7
CV	25.60	31.80	34.30	31.10	29.00	26.40	22.90	18.10
S.E	0.65	0.53	0.45	0.40	0.54	0.48	0.47	0.54
S.E.D	0.53	0.54	0.45	0.41	0.44	0.49	0.48	0.55

Note: Means in the same column followed by the same letter are not significantly different at P≤0.05 according to Tukey's range test. CV – Coefficient of Variation %, SE – Standard Error, SED – Standard Error of difference between means

Table 9: Maize lodging over time in different maize cropping system and under different application rates of M. anisopliae granules during the short rains of 2012 at a farmer's field in Ligega location

	Number of Lodged Maize Plant in % per Hectare Over Time During short rains of 2012							
TREATMENTS	WEEK 2	WEEK 4	WEEK 6	WEEK 8	WEEK 10	WEEK 12	WEEK 14	WEEK 16
Maize monocrop 0 kg/ha	4.2a	3.8a	3.2a	3.5a	3.6a	4.0a	4.2a	5.500a
Maize monocrop 40 kg/ha	3.5cd	2.5abc	1.7bc	1.8cd	2.1cde	2.5bc	3.0bc	4.670ab
Maize monocrop 60 kg/ha	2.0d	1.8bcd	1.3c	1.2cde	1.7de	2.0cd	2.0cde	4.165bc
Maize monocrop 80 kg/ha	2.2d	1.5cd	1.2c	1.0cde	1.2ef	1.7cde	1.8de	3.330cde
Maize + beans 0 kg/ha	3.5abc	3.0ab	2.5ab	3.0ab	3.2ab	3.7ab	4.0ab	5.000ab
Maize + beans 40 kg/ha	2.3cd	2.3abcd	1.7bc	1.4cde	2.3bcd	2.5bc	2.7cd	3.835bcd
Maize + beans 60 kg/ha	1.7d	1.7cd	0.7c	0.5e	1.7de	2.2c	2.0cde	2.835de
Maize + beans 80 kg/ha	1.8d	1.2d	0.7c	0.5e	1.3def	1.3cde	2.2cde	2.835de
Maize + soya 0 kg/ha	3.8ab	3.0ab	3.0a	3.0ab	3.0abc	3.7ab	3.8ab	4.500abc
Maize + soya 40 kg/ha	2.7bcd	1.8bcd	1.7bc	2.0bc	1.6de	2.0cd	2.2cde	3.330cde
Maize + soya 60 kg/ha	1.0d	1.3cd	1.2c	1.2cde	0.5fg	0.8de	1.5e	2.165e
Maize + soya 80 kg/ha	1.8d	1.2d	0.8c	0.8de	0.10g	0.6e	1.3e	2.165e
Total mean	5.1	4.1	3.3	3.3	3.7	4.5	5.1	7.4
Grand mean	2.5	2.1	1.6	1.6	1.9	2.3	2.6	3.7
CV	25.63	31.80	34.30	31.10	39.00	36.40	22.90	28.10
S.E	0.66	0.54	0.45	0.40	0.54	0.45	0.47	0.54
S.E.D	0.53	0.56	0.45	0.41	0.44	0.45	0.48	0.55

Note: Means in the same column followed by the same letter are not significantly different at P≤0.05 according to Tukey's range test. CV – Coefficient of Variation %, SE – Standard Error, SED – Standard Error of difference between means

Table 10; Maize lodging over time in different maize cropping system and under different application rates of M. anisopliae granules during long rains of 2012 at Siaya ATC farm.

Number of Lodged Maize Plant in % per Hectare Over Time During long rains of 2012								
TREATMENTS	WEEK 2	WEEK 4	WEEK 6	WEEK 8	WEEK 10	WEEK 12	WEEK 14	WEEK 16
Maize monocrop 0 kg/ha	3.5ab	3.3a	3.0a	3.5a	3.7a	3.8a	4.0a	5.5a
Maize monocrop 40 kg/ha	2.3bc	2.3abc	1.8bc	1.8b	2.0cde	2.5cd	3.0ab	4.5abc
Maize monocrop 60 kg/ha	2.0c	1.8c	1.3c	1.0bcd	1.3def	1.5def	2.0bc	3.8bcd
Maize monocrop 80 kg/ha	2.0c	1.3c	0.8c	1.0bcd	1.7ef	1.5def	1.8c	3.3cde
Maize + beans 0 kg/ha	3.5ab	3.0abc	2.5ab	3.0a	3.7ab	3.7ab	3.8a	5.0ab
Maize + beans 40 kg/ha	2.3bc	2.3abc	1.7bc	1.3bcd	2.3bcd	2.7bc	2.5bc	3.8bcd
Maize + beans 60 kg/ha	1.7c	1.7bc	0.8c	0.5d	1.7de	2.7cde	2.0bc	2.8de
Maize + beans 80 kg/ha	1.8c	1.7c	0.8c	0.5d	1.0ef	1.7cdef	2.2bc	2.8de
Maize + soya 0 kg/ha	3.8c	3.3a	3.0a	3.0a	3.0abc	3.3a	3.7a	4.3abc
Maize + soya 40 kg/ha	2.7abc	2.0abc	1.5bc	1.7bc	1.7de	1.3efg	2.2bc	3.3cde
Maize + soya 60 kg/ha	2.0c	1.7bc	1.0c	1.0bcd	0.5f	0.7fg	1.5c	2.2e
Maize + soya 80 kg/ha	1.8c	1.3c	0.8c	0.8cd	0.3f	0.3g	1.5c	2.0e
Total mean	4.9	4.2	3.2	3.2	3.6	4.3	5.0	7.3
Grand mean	2.5	2.1	1.6	1.6	1.8	2.1	2.5	3.7
CV %	24.20	33.40	35.70	31.50	28.80	25.50	21.10	19.00
SE	0.59	0.56	0.46	0.40	0.52	0.44	0.42	0.55
SED	0.34	0.58	0.47	0.41	0.30	0.45	0.31	0.39

Note: Means in the same column followed by the same letter are not significantly different at P≤0.05 according to Tukey's range test. CV – Coefficient of Variation %, SE – Standard Error, SED – Standard Error of difference between means.

Table 11: Maize lodging over time in different maize cropping system and under different application rates of M. anisopliae granules during short rains of 2012 at Siaya ATC farm.

Number of Lodged Maize Plant in % per Hectare Over Time During short rains of 2012								
TREATMENTS	WEEK 2	WEEK 4	WEEK 6	WEEK 8	WEEK 10	WEEK 12	WEEK 14	WEEK 16
Maize monocrop 0 kg/ha	3.3ab	3.3a	3.3a	3.5a	3.7a	3.8a	4.0a	5.5a
Maize monocrop 40 kg/ha	2.3bc	3.3a	1.8bc	1.8b	2.0cde	2.5cd	3.0ab	4.5abc
Maize monocrop 60 kg/ha	2.0c	1.8bc	1.0c	1.0bcd	1.3def	1.5def	2.0bc	3.8bcd
Maize monocrop 80 kg/ha	2.0c	1.3c	0.8c	1.0bcd	1.2ef	1.5def	1.8c	3.3cde
Maize + beans 0 kg/ha	3.5ab	3.0abc	2.5ab	3.0a	3.2ab	3.7ab	3.8a	5.0ab
Maize + beans 40 kg/ha	2.3bc	2.3abc	1.7bc	1.3bcd	2.3bcd	2.7bc	2.5bc	3.8bcd
Maize + beans 60 kg/ha	1.8c	1.7bc	0.8c	0.5d	1.7de	2.2cde	2.0bc	2.8de
Maize + beans 80 kg/ha	1.8c	1.2c	0.8c	0.5d	1.0ef	1.7cdef	2.2bc	2.8de
Maize + soya 0 kg/ha	3.8c	2.3abc	3.0a	3.0a	3.0abc	3.8a	3.7a	4.3abc
Maize + soya 40 kg/ha	2.7abc	2.0abc	1.5bc	1.7bc	1.7de	1.3efg	2.2bc	3.3cde
Maize + soya 60 kg/ha	2.0c	1.6bc	1.0c	1.0bcd	0.5f	0.7fg	1.5c	2.2e
maize + soya 80 kg/ha	1.8c	1.3c	0.8c	0.8cd	0.3f	0.3g	1.5c	2.0e
Total mean	4.9	4.2	3.2	3.2	3.6	4.3	5.1	7.3
Grand mean	2.5	2.1	1.7	1.8	1.80	2.2	2.5	3.7
CV %	24.2	33.4	35.7	31.5	28.8	35.5	31.1	39.0
SE	0.585	0.563	0.456	0.402	0.524	0.435	0.425	0.552
SED	0.343	0.575	0.466	0.411	0.303	0.445	0.305	0.395

Note: Means in the same column followed by the same letter are not significantly different at P≤0.05 according to Tukey's range test. CV – Coefficient of Variation %, SE – Standard Error, SED – Standard Error of difference between means

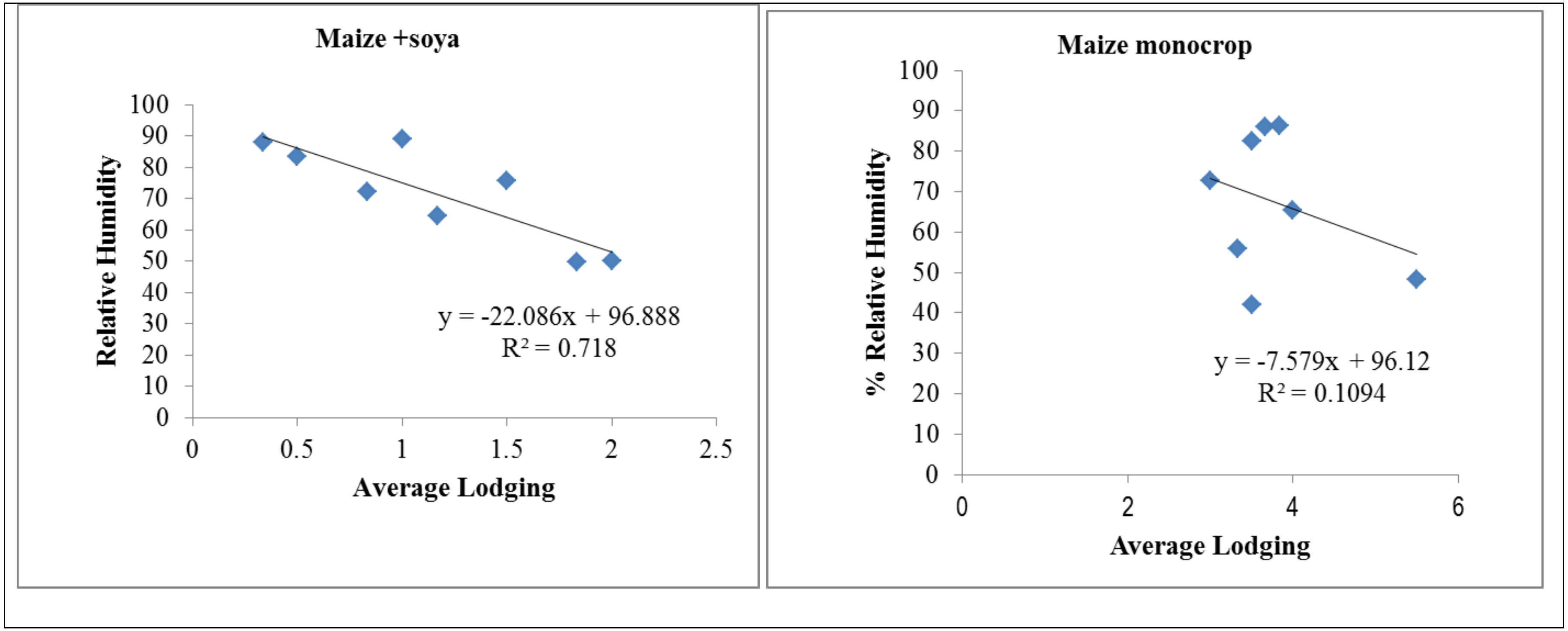


Figure 1: Relationship between maize lodging and Rh in different maize cropping systems (maize +soyabean intercrop and maize monocrop)

Regression analysis showed that maize lodging decreased with increase in relative humidity both in Siaya and Ligega (Fig 5) irrespective of the prevailing weather condition in terms of daily rainfall amount (appendix 10). Regression for relative humidity with introduction of soybeans intercrop on maize lodging showed a high positive significant trend ($P \leq 0.05$) with a coefficient of determination of R^2=0.72. While regression for relative humidity under maize monocrop on maize lodging showed a low positive not significant trend ($P \leq 0.05$) with coefficient of determination of R^2= 0.10. This implying that relative humidity in maize monocrop only reduced maize lodging by 10% unlike in the maize+ soyabean intercrops where relative humidity reduced maize lodging by huge range of 72% (Fig 5).

3.5. Discussion

Application of granules of *M. anisopliae* at planting significantly decreased maize lodging and increased maize grain yield when compared with the untreated plots at both Siaya ATC farm and farmer's field in Ligega location. *M. anisopliae* act as a twofold bio control agent, contact and repellence (Rath, 2000; Ahmed *et al.,* 2009; Mburu *et al.,* 2009; Mburu *et al.,* 2010). By contact *M. anisopliae* inoculum directly attaches on termite exoskeleton and initiate pathogenesis resulting in termite mortality (Milner, 2003; Ahmed *et al.,* 2009; Sileshi *et al.,* 2013) while by repellence; Rosengaus *et al*. (1999) and Staples and Milner (2000) reported that termite detect presence of *M. anisopliae* spore and show an alarm response by striking vibratory display which appears to convey information about the presence of a pathogen to nearby unexposed nest mates through substrate vibration and the nest mates not directly in contact with spores that perceive the vibrational signal increase significantly their distance from the spore-exposed termites and Rosengaus *et al*. (1999) termed this response as "Pathogen Alarm Response" while Mburu *et al*. (2009) showed that the *Macrotermes michaelseni* detects at a distance the presence of *M. anisopliae* through olfaction and thus avoids direct physical contact with the fungus.

The inherent potential of termite to detect specific sickening signatures from potentially infective fungi and avoid it is therefore exploited as means to repel termite from crop. We can therefore, deduce that application of *M. anisopliae* spore formulation forms a 'barrier' similar to currently used chemical termite barrier to protect utility or crops from termite invasion and this agrees well with Milner *et al.* (1998) who reported that *M. anisopliae* protects a crop by avoidance as a result of repellent action of the spores. These results agree well with previous studies by Maniania *et al.* (2002) who showed that application of *M. anisopliae* isolate ICIPE 30 at maize planting decreased maize lodging and increased maize yield, also trials carried out in Uganda with the same isolate (ICIPE 30) against termites demonstrated a 70 % increase in maize yield; this was comparable to results obtained with the use of the chemical insecticide lindane (Sekamatte, 2001) while Hussain *et al*. (2011) reported that application of *M. anisopliae* on sugarcane setts at planting protected the canes against termite infection/bud damage significantly compared to the untreated control.

Maize lodging at both sites (Siaya ATC and Ligega) declined with increase in amount of *M. anisopliae* granule applied (0.0 kg/ha, 40.0 kg/ha, 60.0 kg/ha and 80.0 kg/ha). The additional quantities of *M. anisopliae* granules significantly increased maize yield as a result of decreased maize lodging. We can therefore infer that *M. anisopliae* repellence and/or termite mortality increased with increasing treatment application rates. This agrees well with the findings of Ahmed *et al.* (2008), Mburu *et al*. (2009) and Sileshi *et al.* (2013), these authors showed that the repellence or infection of *M. anisopliae* like most of EP fungi is density dependent. In this study application of 80 kg/ha of *M.anisopliae* had the least number of lodged maize and hence the highest maize yield, this resulted from protection of maize plant from termite attack causing less or no maize lodging at all. Application of 80 kg/ha of *M. anisopliae* is still within economical and practical range as recommended by Jaronskel *et al.* (2007) who concluded that critical concentration of 40g granules/cc of soil could still be achieved when between 70.0 and 112.0 kg/ha of *M. anisopliae* granules were applied using in-furrow application to control larvae of sugarbeets maggot in citrus. Anything less than this critical concentration, sharply decreased the infection rates observed in sugarbeet root maggot larvae.

Farmers in Siaya County intercrop maize with soyabean or with common bean to increase total farm output and protect farmers against total crop failure in case of pest outbreak. However, studies have shown that the two intercrops can also play an important role in modification of micro climatic factors (temperature and relative humidity) in an agro-ecosystem. In the study, intercrops of maize with soyabean or common bean had no significant effect on environmental temperature measured at the base of maize plant. Field temperature remained within the optimal range (25^0C-30^0C) for *M. anisopliae* infection in termite throughout the maize growth period. This agrees well with the findings of Ekesi *et al*. (1999) who reported that intercropping maize with cowpea did not significantly increase or decrease environmental temperature.

These unchanged temperature range within the three cropping system could be attributed to very constant but slight thermal variations in the fields. Field temperature is not vegetation type cover dependent but rather depends on the present prevailing weather conditions (Ekesi *et al*., 1999). On the other hand introduction of the two food legume intercrops increased significantly relative humidity at base of maize plants to optimal level (>75%RH) for *M. anisopliae* conidia germination and sporulation on termite cadavers (Milner *et al.*, 1997; Inglis *et al.*, 2001; Authurs and Thomas, 2001). These results concur well with results of Sprenkle (1979) and Studdert and Kaya (1990), who reported that closely sown cotton and soybean crop respectively, increased relative humidity at the base of the plant during the vegetative stage. Sprenkle (1979) suggested that increase of relative humidity is a result of dense canopy cover which limits moisture movement within the crop cover thus increasing moisture level at base of the plant. High moisture plays a key role as it directly affects pathogenicity of *M. anisopliae* conidia in agro-ecosystem, high moisture level within intercrop treatments made *M. anisopliae* propagules in the rhizosphere (upper soil level) to be very contagious against termites thus reducing termite numbers in the field hence decline maize lodging resulting to increase in maize grain yields.

Also, Ekesi *et al.* (1999) reported that application of *M. anisopliae* conidia to control *Megalurothrips sjostedti* (legume flower thrips) in cowpea increased yields in intercropped cowpea with maize to levels comparable to cowpea monocrop treated with synthetic insecticide, karate (Lambda-cyhalothrin). The increase in yields in intercropped treatments was attributed to high relative humidity and increased light interception within the crop canopy (Kyamanywa and Ampofo, 1988; Terao *et al.,* 1997). A high relative humidity is an essential factor for fungal infection in insects

(Benz, 1987). It is suggested that high relative humidity within the intercrop favoured the fungal infection process against legume flower thrips; while increased light interception within the intercrop ensured degradation of *M. anisopliae* by UV light is reduced (Ekesi *et al.,* 1999). Therefore previous authors suggested that the combination of the two factors (increased relative humidity and shielding of light) accelerated pathogenesis process hence decline of pest population which resulted increased crop yields. This could also explain decline in maize lodging in plots where *M. anisopliae* granules was integrated with intercrop treatments during vegetative to maturity stage (week 4 to week 12) in the study hence high yields of maize grains at harvest because fewer number of maize plants were destroyed by termites.

Temperature and relative humidity are very critical environmental factors which influence the pathogenicity and repellence of *M. anisopliae* conidia like any other entomopathogenic fungi (Rath, 2000; Inglis *et al.,* 2001; Authurs and Thomas, 2001). These two environmental factors influence epizootic quality of *M. anisopliae*, conidia germination on termite integument and sporulation on termite cadavers (Inglis *et al.,* 2001). As a result of this ideal condition, there might have been an additional of production inoculum within the intercrop plots to induce more fungal epizootiology in termite. This therefore explains the low maize lodging in the intercrop treatments during the vegetative stage (dense canopy stage) of the two food legumes used as intercrop with maize. Whereas, the maize monocrop had low relative humidity although thermal range was within the optimum of 25-30^{0} C. This could have caused reduced conidia germination and sporulation on termite cadavers resulting in fewer conidia. Arthurs and Thomas (2001) reported that conidia sporulated on cadavers under harsh condition (low relative humidity) would almost certainly have low infectivity under field conditions because of incomplete development. While Hallsworth and Magan (1999) in their study reported that spore germination, germ tube extension and infection for most entomopathogenic fungi require high relative humidity at the insect surface. This implies conidia within the maize monocrop were exposed to very harsh environment throughout maize period thus reducing their efficacy against termite hence high maize lodging in maize monocrop plots.

There was notably high percentage of maize lodging in the early and in late stage of maize growth both in maize monocrops and maize intercrops treatments at both Siaya ATC and Ligega during the long and short rain season of 2012. This agrees well with the findings of Maniania *et al.*(2002) who reported high maize lodging early and late stage of maize growth in plots applied with *M. anisopliae* granules at planting. The early increased maize lodging could be attributed to termite populations within the plots which are still high at this stage because *M. anisopliae* like most biological control agent does not cause death of the target host organism (pest) immediately rather mortality results from progressive process of infection, colonization and/or consumption host organ(s) leading to their depletion hence host mortality (Wright *et al.,* 2005; Yanagawa *et al.,* 2008; Ahmed *et al.*, 2009; Balachander *et al.,* 2009). Termite mortality caused *M. anisopliae* inoculums is a slow and long process, and during this mortality process termites were still attacking and causing maize damage unlike the conventional chemical (insecticides) application which cause abrupt death of the target pest thus crop damage stops a few minutes or hours after insecticide application. Infection of termites by *M. anisopliae* under optimum temperature and relative humidity causes mortality after 3 to 7days (Wright *et al.*, 2005; Ahmed *et al.*, 2009; Balachander *et al.*, 2009; Sileshi *et al.*, 2013). Alternatively high maize lodging in treated plots at early stage could be as associated with low relative humidity (harsh environmental conditions) (table 7) as plots at this stage are still exposed with no vegetation to shield *M. anisopliae* conidia from the low relative humidity and this could have affected the efficacy *M. anisopliae* inoculum. However, after the second week, maize lodging declined in the maize intercrops and this could be that once infected, the cadavers decay relatively quickly after sporulation because of increase in relative humidity which associated with the intercrop treatments because they increased the pathogenesis process. Secondly sporulation resulted in additional *M. anisopliae* inoculums, these additional inoculums were then redeployed throughout the maize hill via exposed termite to unexposed individual resulting in increased infection and/or repellency of the termite hence protecting maize crop from termite damage.

The increased maize lodging in the late stage of maize growth could be associated with persistence of *M. anisopliae* spores in the soil. There was very low persistence of fungus spores in all the treated plots (unpublished personal observation). Decline in spore density negatively affects the efficacy of *M. anisopliae* conidia in the control of termite against maize attack because like mentioned early the effective performance of *M. anisopliae* against termite is conidia density dependent (Ahmed *et al.*, 2009; Balachander *et al.*, 2009 Hussain and Tian, 2013). This compare well with the study of Vanninen *et al.* (2000) and Bruck (2005) who reported that external applied entomopathogenic fungi in an agro ecosystem drop in persistence over time and Vanninen *et al.* (2000) attributed the decline to the following processes biodegradation (considered the most important factor), physical weathering and percolation into deeper soil layers. Although, percolation into deeper soil layers is thought to be the least important factor because most conidia are retained in the top 5 cm regardless of soil texture (Ekesi *et al.* 2007). Alternatively this increase in maize lodging could be attributed to harsh environmental factors which have negative effects on performance of *M. anisopliae* propagules in the soil. As the maize grows the micro climate effects resulting from different cropping system change with time as they (the two food legumes and maize) mature and the vegetative part is detached hence the sheltering effect is no longer felt thus exposing the *M. anisopliae* inoculum to harsh environment (low relative humidity and high temperature) which might have hindered their sporulation and infectiousness. For instance high temperature reduces spore viability (Rath, 2000; Inglis *et al.,* 2001) and this could explain why high lodging maize percentages at this late stage of crop growth. In this study both during long and short rains, the late maize growth stage were July –August 2012 and December 2012 –February 2013 respectively (appendix 9). These months were dry and windy in both Siaya and Ligega. Therefore the conidia were exposed to this harsh environment (low relative humidity) thus reducing their pathogenicity and/or repellency greatly. This concurs well with the finding of Rath (2000) and Authurs and Thomas (2001) who reported that low relative humidity reduces infectivity of *M. anisopliae* inoculum.

When the two sites were compared, maize lodging in Siaya ATC was relatively higher compared to maize lodging in Ligega. This could be attributed to the different termite species in the two sites. Siaya ATC site was predominantly infested by the smaller and numerous *Microtermes specie*s which begin their attack at the seedling stage and continues to plant maturity while Ligega had the large headed *Macrotermes* species which start their attack at later stage of maize growth and are usually less in number compared to *Microterme* species. It had been reported that *Microtermes* species cause great damage in maize compared to *Macrotermes* species (Maniania *et al*., 2002)

3.6. Conclusions and Recommendations

3.6.1. Conclusions

- Application of *M. anisopliae* reduced maize lodging and increased maize yields both in Siaya and Ligegs. The study identified two application rates (60.0 kg/ha and 80.0 kg/ha) of *M. anisopliae* as effective in reducing maize lodging due to termite attack from planting to harvest.
- Introduction of the two food legumes as intercrop with maize to the different treatment application rates of *M. anisopliae* further reduced cumulative maize lodging resulting in increased maize yields.
- Application of 40.0 kg/ha of *M. anisopliae* in the intercrop of soyabean with maize increased maize yields to a level comparable with application 80.0 kg/ha of *M. anisopliae* in maize monocrop. Therefore the use of soyabean intercrops with maize offer protection to maize against termite attack in an agro-ecosystem at a lower application rate of *M. anisopliae* granules.

3.6.2. Recommendations

- Application of *M. anisopliae* granules at planting even as low 40 kg/hacan protect maize against termite attack, however optimum protection is achieved when 60 kg/ha or 80 kg/ha rate is applied in the maize monocrop.
- The study therefore recommends the use of 40.0 kg/ha integrated with maize +soybean to manage termite in Siaya ATC farm and Ligega location.

CHAPTER FOUR

Assessment of Persistence of *Metarhizium anisopliae* inoculum in the Soils.

4.0. Abstract.

Soil by large is the natural niche where propagules of entomopathogenic fungi persist. However, this edaphic environment encompasses a complex set of abiotic and biotic factors which affect persistence of *M. anisopliae* inoculum. Studies have shown that dense crop canopy cover enhances persistence of entomopathogenic fungi in the soil. However, literature has only been documented for the fungus occurring in their natural habitat while little is known about persistence of fungus applied in foreign location. The study assessed the persistence of entomopathogenic fungi; *M. anisopliae* isolate ICIPE 30 in the soil under three different maize-based cropping systems; maize monocrop, maize–soybean intercrop and maize–common bean intercrop at Siaya ATC and farmer's field at Ligega. The following applications rates of *M. anisopliae* granules 80.0 kg/ha, 60.0 kg/ha, 40.0 kg/ha and 0.0 kg/ha were applied at planting in all 3 maize based cropping systems. Treatments were replicated three times in a RCBD. Four soil samples were picked randomly per plot at planting and at harvest of maize. Number of viable conidia was assessed by plating out a suspension of the sampled soil onto a selective medium and the number of colony forming units per gram of fresh soil recorded after seven days. Fungus persistence was determined by analysis of variance (ANOVA) for a randomized complete block design and means of $CFU.g^1$ of soil separated by Tukeys' ($P \leq 0.05$) test using Genstat software. Soils from both sites had no naturally occurring *M.anisopliae* inoculum at planting and very small proportion of conidia survived from planting to harvest, although maize + soybean and maize + common bean intercrops had relatively high conidia density compared conidia density in maize monocrop at harvest. However, there is need for further study to establish if soil organism in Siaya county affected *M.anisopliae* conidia persistence in the before intercrops are adopted for future use.

4.1. Introduction

Entomopathogenic fungi; *M. anisopliae* is soil borne and has been used successful to control a number insect pests both above and below the ground on global scale (Shah and Pell, 2003; de Faria and Wraight, 2007; Pilz *et al.,* 2008; Pilz *et al.,* 2010). Studies have shown that external applied entomopathogenic fungi in an agro ecosystem drop in persistence over time (Milner *et al.,* 2003; Bruck 2005; Ekesi *et al.,* 2011; Coombes *et al.,* 2013). Scheepmaker and Butt (2010) attributed this decline to numerous factors which the authors grouped into five distinct categories: intrinsic, edaphic (physical soil properties), agricultural practices and climatic. While Vanninen *et al.* (2000) mentioned that percolation of the fungal conidia into deeper soil layers also a contributed to their decline. However, percolation into deeper soil layers is thought to be the least important factor because most conidia are retained in the top 5 cm regardless of soil texture (Ekesi *et al.* 2007).

Agricultural practice and weather condation are of particular in determination of persistence of *M. anisopliae* inoculum in the soil (Scheepmaker and Butt, 2010; Coombes, 2013). Although fungal granules applied in the soil are usually protected from these harmful radiations (Scheepmaker and Butt, 2010), agricultural practices like field ploughing or weeding usually exposes fungal propagules to harsh environmental conditions. Optimal growth temperature for *M. anisopliae* is described between 20^0C and 30^0C (Ferron 1981; Goettel *et al*. 2000). Very high temperatures reduce fungal spore viability (Goettel *et al*. 2000; Meyling and Eilenberg, 2007) while low relative humidity (below 45%RH) lowers fungal sporulation in the cadavers also, causes production of conidia low with viability in the field (Authus and Thomas, 2001). Combination of these two factors therefore adversely affects the persistence or production of additional spore (sporulation) in the soil (Goettel *et al*. 2000; Meyling and Eilenberg, 2007; Scheepmaker and Butt, 2010).

Recently, the discovery of microslerotia (compact hyphal aggregates which are often melanised and highly resistant to desiccation), have shown ability to increase persistence of externally applied *M. anisopliae* conidia (Jackson and Jaronski 2009). However, the preparation of these hyphal aggregates is cost inhibiting (Jackson and Jaronski 2009). Cultural practices of intercropping or closing sowing have shown the effect of modifying environmental conditions at areas below the canopy. Use of food legume intercropped with maize in an agro-ecosystem, to regulate temperature and relative humidity to influence persistence of externally applied *M. anisopliae* conidia in the soil are yet to be explored, although Quesata-Moraga *et al*. (2007) reported increased density of *M. anisopliae* conidia in soil from fruit orchard with dense leave canopy cover compare to density of *B. bassiana* conidia. The present study therefore, established the persistence of *M. anisopliae* conidia in maize field under; maize intercrop with food legume and maize monocrop.

4.2. Materials and Methods

4.2.1. Study Site

The study was done in two sites (Siaya ATC and farmer's field in Ligega) in Siaya County for two seasons as in the preceded section. The fertility of the soils in county range from moderate to low, levels of nitrogen and phosphorus are particularly low (Ogaro *et al*., 1997). Vertisols and ferralsols are the most common soils in the county (Okalebo *et al.,* 2007; Mango, 1999). Most soils are underlain by plinthite (Murram) at a shallow depth, resulting in low moisture retention (Mango 1996; Mango, 1999**)**.

4.2.2. Soil Sampling.

Using protocol in section 3.3.1 set up; soil samples were picked at planting before application of *M. anisopliae* granules in maize hills, after application of *M. anisopliae* granules in maize hills and at harvest. To avoid effect of run off four soil samples were picked randomly from four maize hills inside line 2 and 3 of maize per plot using core borer at both sites. To limit fungus exposure to destructive weather conditions soil samples were kept in black polythene bags under cool condition and immediately transported laboratory for analysis.

4.2.3. *Metarhizium anisopliae* Conidia Count.

Soil samples of 25 cm depth and 2 cm diameter were randomly taken from four maize hills in the treated and untreated plots with a core borer. A sample consisted of three insertions; the uppermost three cm of the soil core were discharged and the remaining soil was placed in a plastic bag and stored at 4^oC ($\pm 2^oC$) till analysis, but no longer than 3 weeks. Stones and organic material such as plants and roots were removed and the soil was crushed to small fractions. 1.0 g of soil was weighed, suspended in 1 ml 2% saline solution and vortexed. The suspensions were diluted via serial dilution and 100 μl was spread on Sabouraud dextrose agar (SDA) plates supplemented with 1 ml dodine, 50 $mg.L^{-1}$ rifampicin and 50 $mg.L^{-1}$ chloramphenicol to prevent the growth of bacteria and saprophytic fungi which may have being present in the soil samples given the length of time in the field. Three plates with selective medium were used per sample (Veen's medium: 35.0 g mycological agar, 1.0 g chloramphenicol, 0.5 g cyclohexamide in 1 L of water). The plates were incubated in darkness at 22^0C ($\pm 2^0C$) and 55% RH. After 7 days, the number of noticeable colonies was counted. For ease of counting, plates were divided into twenty grids and the number of colonies within each grid was counted. The total number of colonies was therefore the sum of the grid counts and multiplying the number of colonies from every sample with the dilution factor (10^3). Colonies which were evidently not the species of interest were not included in the CFU count. Due to the selectivity of the media however, this was minimal.

4.3. Statistical Data Analysis

To determine persistence of fungal spore in the soil Colony Forming Units (CFU)/g fresh soil were counted. The data were subjected to analysis of variance (ANOVA) for a randomized complete block design and means of treatments at planting and at harvest were compared by Tukeys' ($p<0.05$) test using Genstat software (Genstat, 2010).

4.4. Results.

Conidia density at planting was significantly different in all treatments ($p<0.05$) (table 8). There was significant decline of *M. anisopliae* conidia density from planting to harvest time (after 4-5 months) in all the treatments (Table 8). Treatments with 40.0 kg/ha and 60.0 kg/ha in maize monocrop had the greatest decline of conidia density ($p<0.05$) to a level comparable to untreated plots (0.0 kg/ha) at harvest. Application of 40.0 kg/ha, 60.0 kg/ha and 80.0 kg/ha of granules of *M. anisopliae* in the maize + soyabean intercrop had significantly high conidia density (5.4×10^2 ±13.6CFU/g of soil) at harvest compared to the maize monocrop with same amount of granules.

Table 12: Mean of M.anispoliae colony forming units per gram of soil (CFU.g-1) present at planting and harvest at Ligega in long rains of 2012.

Mean of *M.anispoliae* present at planting and after 4-5 months (harvest) at Ligega in long rains of 2012				
Treatment	Planting Before fungus application) (CFU/g)	Planting After fungus application) (CFU/g)	Harvest (After 4-5months) (CFU/g)	Losses (CFU/g) after 4-5months
Maize monocrop 0kg	0	0d	0a	0
Maize monocrop 40kg	0	4626c	72.8a	$4.6x10^3$
Maize monocrop 60kg	0	8895b	80.2a	$8.8x10^3$
Maize monocrop 80kg	0	19768a	722.1c	$1.9x10^4$
Maize + beans 0kg	0	0d	0a	0
Maize + beans 40kg	0	4717c	80.0a	$4.6x10^3$
Maize + beans 60kg	0	9142b	548.1b	$8.6x10^3$
Maize + beans 80kg	0	19848a	759.5c	$1.9x10^4$
Maize + soya 0kg	0	0d	0a	0
Maize + soya 40kg	0	4584c	541.7b	$4.0x10^3$
Maize + soya 60kg	0	9126b	566.7b	$8.6x10^3$
Maize + soya 80kg	0	19817a	761.7c	$1.9x10^4$
Grand Mean	0	8377.0	345.9	$8.0x10^3$
CV%	-	27.0	35.4	
SE	-	2153.0	116.8	
SED	-	921.7	49.9	

Note: Means in the same column followed by the same letter are not significantly different at P≤0.05 according to Tukey's range test. CV – Coefficient of Variation %, SE – Standard Error, SED – Standard Error of difference between means

While application of 40 kg/ha of *M. anisopliae* granules in maize + soybean intercrop had significantly high spore density (5.41×10^2CFU/g of soil) comparable to application of 80 kg/hagranules in maize monocrop after 4-5months (at harvest). And finally application of 80 kg/ha of *M. anisopliae* granules in maize-legume intercrops had the highest amount of conidia density ($7.2 \times 10^2 \pm 41.7$CFU/g of soil) of all treatments at harvest (Table 9).

4.5. Discussion

Initial samples soil collected from both sites before application of *M. anisopliae* granules at planting showed that the fungus was not present. Although *M. anisopliae* is natural occurring soil microorganism, studies have shown that the fungus is not universally present in all regions or sites (Scheepmaker and Butt, 2010). Therefore, there is likilyhood that *M. anisopliae* did not inhabit in soils from both Siaya ATC and a farmer's field in Ligega. Application of *M. anisopliae* granules at planting increased spore density in plots applied, however there were significant drop of spore density at harvest (after 4-5months). This agrees well with previous studies by Bruck (2005), Meyling and Eilenberg (2007), Bruck and Donahue (2007), Scheepmaker and Butt (2010) and Coombes *et al*. (2013) who reported that *M. anisopliae* applied in soil under field condition decreases significantly after 4-6 months although still continues to initiates infection. The decline of EPF in the soil has been attributed to biodegradation, physical weathering and percolation into deeper soil layers (Vänninen *et al.,* 2000). Percolation results from rainfall which dislodges and disperses conidia on soil surface into deeper soil layers (Inyang *et al.,* 2000; Vänninen *et al.,* 2000; Vestergaard *et al.*, 2002). However, percolation into deeper soil layers is thought to be the least important factor because most conidia are retained in the top 5 cm regardless of soil texture (Ekesi *et al.* 2007).

Biodegradation and physical weathering are subject to environmental factors like solar radiation, temperature and relative humidity (Ekesi *et al.*, 2005). Although soil applied *M. anisopliae* granules were protected from solar radiation, but cultural practices such weeding, in this case 1st maize weeding could have exposed limited amount of applied *M. anisopliae* propagules to injurious solar radiation propagules causing their inactivation, thus affecting the fungus viability (Scheepmaker and Butt, 2010). The trend reported in this study (maize monocrop had largest decrease of conidia density followed by maize intercrop treatments) is a common trend reported in other studies investigating the persistence of entomopathogenic fungi as biological control agents in a mixed cropping system (Ekesi *et al.*, 1999). A suggestion that the use of legume intercrops improved the persistence of the *M. anisopliae* propagules in the soil. This could be attributed to the leafy canopy shielding effect of the two food legume intercrop. This shielding effect protects the immediate rhizosphere (upper soil layer) which is the habitat of *M. anisopliae* conidia from high temperatures and low relative humidity. Adverse temperature and low relative humidity reduces entomopathogenic fungal sporulation and viability thus affecting its persistence in the soil (Goettel *et al*. 2000; Arthurs and Thomas, 2001; Scheepmaker and Butt, 2010). The effectiveness of entomopathogenic fungi as pest control agent partly depends on the persistence of the applied inoculum in the field (Ekesi *et al.*, 2005; Quesada-Moraga *et al.,* 2007). Contact between the target insect and fungal conidia is essential if infection is to ensue (Shah and Pell, 2003), therefore a decline in the number of infective units (CFU) causes likelihood of interaction between the termite and fungus to diminish thus increase in maize damage by termite, this is agree well with our results on maize lodging where percentage of lodged maize increased by a double percentage in the last one month of maize growth (unpublished MSc. thesis research). These results also confirms with work of Wright *et al*. (2005) and Ahmed *et al.* (2007) who reported that the susceptibility of the termite to fungal infection was often dose dependent while Rosengaus *et al*. (1999) and Ahmet *et al*. (2008) observed that mortality of termite both in the soil and filter paper substrate was infective unit dependant; high dosage of *M. anisoplie* was more lethal to the termite population than low dosage.

It is imperative to note that persistence of *M. anisopliae* isolates is also affected by area where the isolate were originally isolated from, isolate collected from the local soils possess some level of adaptive tolerance to the environment factors (Goble *et al.,* 2011; Coombes *et al.,* 2013). Soil organisms, e.g. earthworms, other fungi, nematodes, protozoa, collembolans and bacteria, tend to reduce fungal persistence through; mycophagy, nutrients and niche competition and via the secretion of antifungal compounds (Scheepmaker and Butt, 2010). For example, Lingg and Donaldson (1981) repeatedly isolated the fungus, *Penicillium urticae* (Bainier) from non-sterile soil. Subsequent analysis found this fungus to be capable of producing a water soluble compound, which inhibits the growth of entomopathogenic fungi. Similarly, Coombes *et al.* (2013) reported high percentage of EPF in sterile soils than in non-sterile soils in citrus orchard after six months. *Metarhizium* isolate ICIPE 30 used in this study was collected from the Democratic Republic of Congo, therefore the isolate could perhaps been affected by soil inhibiting organisms which could have been present in the soils of our trial sites.

4.6. Conclusions and Recommendations

4.6.1. Conclusions

- The study indicates that *M.anisopliae* granules applied in the soil at planting decline over time.
- Inclusion of intercrop (Maize+common bean or maize+ soyabeans) showed increased persistence of *M. anisopliae* inoculum in the soil

4.6.2. Recommendations

- Results in this study showed some promising effect on the use of maize +soyabean intercrops to improve persistence of *M. anisoplie* inoculum in the soil. Therefore integration of maize +soyabean intercrop with *M.anisoplie* application has potential to increase fungus persistence in an agro-ecosystem.
- However, there is need to establish if *Metarhizium* isolate ICIPE 30 is best suited for applications in Siaya county soils as the fungus was not originally isolated from the county soils.

CHAPTER FIVE

GENERAL CONCLUSION AND RECOMMENDATION

5.0. Conclusion

Application of *M. anisopliae* reduced maize lodging and increased maize yields both in Siaya and Ligega. Application of 60 kg/ha and 80 kg/ha of *M. anisopliae* granules offered best maize protection against termite attack from planting to harvest. Introduction of the two food legumes (soyabean intercrop or common bean intercrop) to the different treatment application rates of *M. anisopliae* further reduced maize lodging resulting increased maize yields, for instance application of 40 kg/ha of *M. anisopliae* in the intercrop of maize with soyabean increasing maize yields to a level comparable with application 80 kg/ha of *M. anisopliae* in maize monocrop. The study also, showed that *M.anisopliae* granules applied in the soil decline over time. Inclusion of intercrop (Maize+common bean or maize+ soyabeans) has the potential of increase persistence of *M. anisopliae* as bio-pesticides against termites in an agro-ecosystem.

5.1. Recommendations

The study therefore recommends the use of 40.0 kg/ha integrated with maize +soyabean to manage termite in Siaya ATC and Ligega location in Siaya County. On persistence, results in this study showed some promising effect on the use of maize +soybean intercrops to increase improve of *M. anisoplie* conidia in the soil. However, there is need to find out if *Metarhizium* isolate ICIPE 30 applied in the two trial site was affected by natural soil microorganisms found in Siaya county soils.

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APPENDICES

Appendix I: Mass production of fungus

Production of *Metarhizium anisopliae* conidia on rice, *M. anisopliae* isolate ICIPE 30 was obtained from the ICIPE, Nairobi. Isolate ICIPE 30 was cultured on Potato Dextrose Agar (PDA) One milliliter of a suspension of *M. anisopliae* isolate ICIPE 30 at a concentration of 1×10^6 conidia/ml was poured into a 250 ml flask containing 100 ml of the growth medium (yeast extract, glucose and peptone). Flasks were then put on a rotary shaker at 150 rpm for four days at 27 ± 2°C in complete darkness. Content of flasks were then inoculated at a rate of 150 ml per kg of autoclaved parboiled rice in a Milner plastic bag with the help of a syringe. The bags of inoculated rice were then incubated for 10-14 days at room conditions (24 ± 2°C, 75-85% RH). The substrate were then transferred into a clean basin and conidia allowed to dry for seven days at 30°C (use of a de-humidifier machine fixed in the production room), before conidia were harvested from the rice by sieving through a 300 μm mesh. Plastic sheeting was taped around both the top and bottom edges of the sieve and sealed at the top. A collecting vessel, such as a bucket was fitted to the plastic sheeting at the bottom of the sieve to create a funnel into the collecting vessel. The sieve was shaken until all the loose conidial powder had been removed from the rice and had been collected in the vessel below. The conidial powders were then further sieved using a 10^6 μm sieve to separate the larger rice dust particles from the conidial powder. Conidia were dried further in a desiccator using anhydrous silica gel for two days until the conidia attained relative humidity of 5-13%. Dry conidia were then stored at 4°C until used in field experiments (3day-old conidia were used). A viability test of conidia germination was done on SDA to establish conidia germination percentage within the first 24hours of contact with target pest The concentration of spores per gram (spores/g) of rice granules was assessed prior to incorporation using protocol developed by Goettel and Inglis (1997) to have one gram of rice granule contained approximately 10^8 conidia (recommended concentration)

Viability of the conidia were tested by scrubbing off 2-3 weeks old fungal culture and suspending the inocula in 15ml of sterile 0.0l% triton X-100 (v/v) in universal bottle containing glass beads that measured 3mm diameter to obtain a stock solution. The suspension was vortexed for 5 minutes to obtain homogeneous suspension of conidia. A final concentration of $3x10^6$spores ml^{-1} was prepared by diluting from the stock and quantifying with Bright Line® improved Nuebauer Hemacytomter (Buffalo, New York USA). Volume of 0.1 ml conodial suspension was liquated and spread plated onto clean SDA plates and a thin a layer of lacto-phenol cotton blue added to terminate other fungal growth. A total of 5 plates were cultured for the test, two replicate were made for each of the five plates. The plates were incubated at 26 ± 2^0C in laboratory line imperial incubator (Melrose ILL) for 16-18hours. Six sterile slide cover slips were placed on each plate and 6 viability observations recorded from each plate. Viability percentage was determined by counting a total of 100 conidia (germinated and non-germinated) from each plate.

Appendix II: Block and Plots layout in the field

A	1Maize 2B+M 3MAIZE	2S+M 4B+M 1S+M	3S+M 1B+M 4S+M	4MAIZE 3B+M 2MAIZE
1m				
B	3MAIZE 2S+M 4B+M	1B+M 1S+M 3S+M	4S+M 3S+M 1MAIZE	2MAIZE 4MAIZE 2B+M
1				
C	1S+M 2MAIZE 3MAIZE	2B+M 4S+M 1S+M	4B+M 1MAIZE 2S+M	3B+M 3MAIZE 4MAIZE

Key: (B+M) –Maize + common bean intercrop, (S+M) –Maize + soybean intercrop, (MAIZE) –Maize monocrop stand. A, B and C –blocks.

1, 2, 3 and 4 –treatment plots replicated three times.

1=0Kilogram per hectare/control, 2=40Kilograms per hectare, 3=60Kilograms per hectare

4=80Kilograms per hectare

The distance between blocks is 1m wide and path of 0.5m is left between plots.

Appendix III: Means squares for micro climate factor Temperature measured over time (after every 14days) at both Siaya and Ligega for season 2 2012

Source of variation	df	WK16	WK14	WK12	WK10	WK8	WK6	WK4	WK2
Reps	2	1.3854	1.5417	35.7222	6.5417	4.8472	43.95	3.795	18.51
Trts	11	0.9924*	5.5189***	3.1919***	2.2803***	3.7374	32.53*	11.89***	63.48*
Site	1	0.0139	0.0139	0.0139	0.0139	0	0	0.031	0
Trts.Site	11	0.0063	0.0139	0.0139	0.0063	0	0	0.031	0
Error	262	0.4722	0.6714	0.8183	0.4113	0.5991	17.55	1.264	34.91
Grand Mean		30.625	30.021	28.444	26.75	27.278	28.09	28.705	30.82
CV		2.2	2.7	3.2	2.4	2.8	14.9	3.9	19.2

NB* ** *** indicate significance at $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$ level respectively

Appendix IV: Means squares for micro climate factor Relative Humidity measured over time (after every 14days) at Ligega for short season 2012

Source of variation	df	WK16	WK14	WK12	WK10	WK8	WK6	WK4	WK2
Reps	2	721.95	294.024	155.181	19.733	4.524	7.722	548.45	74.42
Trts	11	582.8***	3084.499***	5607.082***	4452.92***	5100.946***	2547.9***	3189.81***	169.14***
Site	1	8855.59***	0	0	0.17	0.014	0.014	0.06	0
Trts.Site	11	529.84***	0.03	0.008	0.117	0.021	0.006	0.03	33.59
Error	262	38.84	5.436	5.801	5.289	3.37	3.407	16.88	15.47
Grand mean		47.76	64.51	76.58	78.98	73.34	65.26	56.52	43.53
CV		13	3.6	3.1	2.9	2.5	2.3	7.3	9

NB* ** *** indicate significance at P≤0.05, P≤0.01 and P≤0.001 level respectively.

Appendix V: Means squares for maize lodging as a result of termite attack recorded after every 14days at Siaya ATC during for long rains of 2012

Source of Variation	Df	Wk2	Wk4	Wk6	Wk8	Wk10	Wk12	Wk14	Wk16
Blocks	2	1.5	1.0139	0.2917	0.0972	0.6806	1.1667	0.1806	1.125
Treatments	11	3.3674***	3.5101***	3.7273***	6.0745***	6.6806***	6.6705***	4.8018***	7.0644***
Site	1	4.3472***	3.5**	2.5**	2.3472**	2.3472*	2.125*	4.3472**	4.0139**
Trt * Site	11	0.2866	0.0758	0.1667	0.0442	0.0745	0.1553	0.3169	1.1957*
Error	46	0.3551	0.5211	0.3062	0.2566	0.2748	0.3116	0.282	0.4728
CV		24.2	35.6	33.2	30.1	28.8	25.3	21.1	19
Grand mean		2.458	2.028	1.667	1.681	1.819	2.208	2.514	3.625

NB* ** *** indicate significance at P≤0.05, P≤0.01 and P≤0.001 level respectively.

Appendix VI:Anova Table for Yields at Siaya

Siaya	Anova for Yields				
	Source of variation	d.f.	m.s.	v.r.	F pr.
	Rep	2	3.014	1.75	
	Treatments	11	87.207	50.58	<.001
	Seasons	1	40.5	23.49	<.001
	Treat.Seasons	11	3.591	2.08	0.041
	Error	46	1.724		
	Total	71			

Appendix VII: Anova Table for Yields at Ligega

Ligega	Anova for Yields				
	Source of variation	d.f.	m.s.	v.r.	F pr.
	Rep	2	6.764	2.09	
	Treatments	11	119.741	36.93	<.001
	Seasons	1	39.014	12.03	0.001
	Treat.Seasons	11	3.893	1.2	0.314
	Error	46	3.242		
	Total	71			

Appendix VIII: Anova Table for Maize lodging at Ligega during long rains

Ligega	Anova Total lodging				
long rains	Source of variation	d.f.	m.s.	v.r.	F pr.
	Rep	2	2.528	0.73	
	Treatments	11	171.24	49.39	<.001
	Error	22	3.467		
	Total	35			

Appendix IX: Anova Table for Maize lodging at Ligega during short rains

Ligega	Anova Total lodging				
short rains	Source of variation	d.f.	m.s.	v.r.	F pr.
	Rep	2	1.75	0.6	
	Treatments	11	161	54.91	<.001
	Error	22	2.932		
	Total	35			

Appendix X: Anova Table for Maize lodging at Siaya during long rains 2012

Siaya	Anova Total lodging				
long rains	Source of variation	d.f.	m.s.	v.r.	F pr.
	Rep	2	5.861	2.96	
	Treatments	11	176.051	88.81	<.001
	Error	22	1.982		
	Total	35			

Appendix XI: . Anova Table for Maize lodging at Siaya during short rains 2012

Siaya	Anova Total lodging				
short rains	Source of variation	d.f.	m.s.	v.r.	F pr.
	Rep	2	1.194	0.8	
	Treatments	11	166.505	111.19	<.001
	Error	22	1.497		
	Total	35			

Appendix XII: Selection Media (Veen's media)

1% of glucose →5.0g

1% of peptone →5.0g

1.5% of oxgall →7.5g

3.5% of agar →17.5g

10µg/ml of dodine to supress saprophytes

250µg/ml of cycloheximade is an antibacterial agent supress gram +ve and gram –ve bacteria

500µg/ml of CAF

Glucose, peptone, oxgall and agar were placed in an Erlenmeyer flask (1000 ml) with 500ml distilled water. This was heated via water bath for 30minutes before being transfer to an autoclave for 45 minutes. Dodine, cycloheximade and CAF were added to the media under sterile condition before the media was spread on the plates.

Appendix XIII: Weather conditions at study sites

Siaya ATC Rainfall																
Months of 2012&13	Jan-12	Feb-12	Mar-12	Apr-12	May-12	Jun-12	Jul-12	Aug-12	Sep-12	Oct-12	Nov-12	Dec-12	Jan-13	Feb-13	Mar-13	Apr-13	Total 12-13
Rainy Days	4	5	14	**17**	**19**	**19**	**13**	**5**	9	**8**	**15**	**13**	**9**	**1**	3	12	166
Amount (mm)	49.8	50.4	309.1	**226.5**	**171.3**	**259**	**26.2**	**24.1**	182.5	**147.8**	**149.9**	**157.3**	**111**	**1**	18.7	44.4	1929

Ligega Rainfall																
Months of 2012&13	Jan-12	Feb-12	Mar-12	Apr-12	May-12	Jun-12	Jul-12	Aug-12	Sep-12	Oct-12	Nov-12	Dec-12	Jan-13	Feb-13	Mar-13	Apr-13	Total 12-13
Rainy Days	6	6	21	**14**	**13**	**10**	**4**	**11**	11	**13**	**12**	**5**	**6**	**1**	3	17	153
Amount (mm)	64.4	64	166.2	**99.6**	**151.1**	**40**	**17.8**	**75.9**	64.1	**116.1**	**172.2**	**18.2**	**96.9**	**10.4**	15.3	206	1378.2

Cumulative rainfall data for Siaya ATC and Ligega during the year 2012 and part of year 2013. Source: Siaya ATC and Ligega Health Centre **Comment:** Good amount of rain fall was realized during long rain season compared with short rain season.

Key: Bolt number show both long and short rain seasons of 2012**.**