# EFFECTS OF *METARHIZIUM ANISOPLIAE* APPLICATION ON THE DIVERSITY OF PLANTS, ANTS, COCKROACHES AND MANTIDS ASSOCIATED WITH *ODONTOTERMES* TERMITE MOUNDS AT MPALA RESEARCH CENTRE - LAIKIPIA DISTRICT (CENTRAL KENYA)

**By**

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**I56/13770/05**

**A THESIS SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE IN AGRICULTURAL ENTOMOLOGY IN THE SCHOOL OF PURE AND APPLIED SCIENCES OF KENYATTA UNIVERSITY.**

**May, 2011**

## DECLARATION

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

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## DEDICATION

I would like to dedicate this accomplishment to the Lord, God Almighty for His sufficient grace through the whole time of this study during which I learnt two very important lessons: Hard work pays and never ever give up - always try, try, try again!

## ACKNOWLEDGEMENTS

I am greatly indebted to my university supervisor, Prof. Elizabeth D. Kokwaro of Department of Zoological Sciences (Kenyatta University), for her excellent supervision and encouragement during data collection and thesis writing.

I would like to express my most profound gratitude to Prof. Alison K. Brody of the University of Vermont, U.S.A., and Dr. Jean Nguya Kalemba Maniania of the Arthropod Pathology Unit, *icipe,* for granting me the opportunity to work within the termite project and for providing funds that supported the completion of this research. I am sincerely grateful for their professional guidance and constructive criticisms during the research period. My sincere appreciation also goes to the Arthropod Pathology Unit staff at *icipe* for their direct and indirect help until the successful completion of my study and for the friendly atmosphere that existed in the laboratory.

My special thanks also go to Dr. Charles Warui, Invertebrate Biology Unit, National Museums of Kenya who facilitated the taxonomic identification process of species of ants. I extend my appreciation to Dr. Margaret Kinnaird, Director, MRC, for the permit to conduct my fieldwork at MRC. I do appreciate the invaluable help I received from the field assistants Messrs Simon Akwam, Gilbert Busienei, Joseph Kabila, Raphael and Fredrick, all of Mpala Research Centre, Kenya.

My most heartfelt thanks go to my husband, Dr. George Ong'amo, Institut de Recherche pour le Dèveloppement (IRD), for the encouragement, support, guidance and advice throughout this work. My deepest appreciation goes to my mother, Mrs. Grace Ondigo Shikuku, for her constant prayers, support and inspiration and my son Master Fidel Jones Ong'amo for the inspiration and challenge. God bless you all.

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## ACRONYMS AND ABBREVIATIONS

| | |
|---|---|
| **ANOVA** | Analysis of variance |
| **EPN** | Entomopathogenic nematodes |
| **GPS** | Global Positioning System |
| **GV** | Granulovirus |
| **ICIPE** | International Centre of Insect Physiology and Ecology |
| **LSM** | Least Square Means |
| **NMK** | National Museums of Kenya |
| **NPV** | Nuclear Polyhedrovirus |
| **POPs** | Persistent Organic Pollutants |
| **SAS** | Statistical Analysis Software |
| **SDA** | Sabouraud Dextrose Agar |
| **SNK** | Student Newman-Keuls test |

## ABSTRACT

Termites are an important component of savanna ecosystems throughout Africa. They feed on dead and living plant cell wall material (wood, leaf litter, roots, dead herbs, grasses, dung and humus). They directly or indirectly modulate the availability of resources such as minerals, fatty acids, vitamins and organic carbon to other species by creating, modifying and maintaining habitats. Their removal from the ecosystem is anticipated to affect the community structure. However, some species can be serious pests of structures, houses, rangelands, tropical forestry and agriculture. In an attempt to manage the damage these species cause, persistent organic pollutants (POPs) including aldrin, dieldrin, endrin, heptachlor and chlordane (cyclodienes) have often been used. In view of the growing concern on the effects of such chemicals on the environment, they have been banned and other alternative strategies have been sought. Alternatives such as chlorpyrifos, isofenphos, and permethrin, are less persistent, but not as effective and need to be frequently applied. It is in this context that biological termiticides with an entomopathogenic fungus *Metarhizium anisopliae* as the active ingredient has gained popularity. *Metarhizium anisopliae* is a cosmopolitan, naturally occurring pathogen, which infects over 200 insect species. Since it infects a variety of insect species, there are concerns that it could potentially cause mortality in populations of non-targets including beneficial insects. This study was undertaken to determine the effects of *M. anisopliae* on the diversity of plants and selected insect species associated with *Odontotermes* termite mounds when the fungus is used for termite control. The selected insect groups were Hymenoptera and Dictyoptera. Effects on plants were also assessed. The research was carried out at the Mpala Research Centre (MRC) in Laikipia District of Kenya. The centre is located in a savannah ecosystem that has been maintained in a relatively undisturbed state, aside from cattle grazing. The diversity of selected insect groups and plants on termite mounds that had been treated with spores of *M. anisopliae* were compared to that of control mounds that had not been treated with the fungus. Invertebrates were sampled using pitfall traps and sweep nets over a period of one and a half years. There was no significant variation in diversities between treated and control mounds ($F = 0.016$, df $= 1$, $p = 0.8989$,). Laboratory assays were conducted to assess the fungus' direct effects on two different species of ants (*Crematogaster mimosae* and *Camponotus* sp.). Results indicated that the fungus has varied pathogenicity towards different species of ants. For *C. mimosae*, the mean mortality for ants exposed to *M. anisopliae* was 28.47 ± 1.08% compared to control mortality of 23.33 ± 1.57% ($F = 7.29$; $p = 0.0072$). However, the result could be attributed to the optimized infection conditions in the laboratory. In *Camponotus* sp., significantly higher mean mortalities were recorded in controls (60) relative to treatments (50) $F = 13.01$; $p = 0.0004$). There was no evidence of variation in vegetation cover across the fungal treatments ($F = 0.003$, df $= 1$, $p = 0.96$). It can be concluded that *M. anisopliae* isolate ICIPE 30 does not have negative effects against non-target organisms associated with *Odontotermes* sp. termites, and can therefore be used for the control of termites.

# CHAPTER ONE: INTRODUCTION

## 1.1 Background

Termites also known as white ants, are soft-bodied social insects in the order Isoptera (Richards and Davies, 1977). Termites are detritivores and contribute significantly to many of the world's ecosystems through their role in nutrient recycling. They also are major ecosystem engineers in the tropics due to their pivotal role as mediators of ecological processes in the soil (Jones and Eggleton, 2000). They have been recognized as "ecosystem engineers" (Dangerfield *et al.*, 1998), a phenomenon whereby organisms create or alter resource flows that affect the composition and spatial arrangement of current and future organismal diversity. They feed on dead plant cell wall material, such as wood, leaf litter, roots, dead herbs, grasses, dung and humus which are largely lignocellulosic matter. In addition, by ingesting and redistributing minerals, fatty acids, vitamins, and 20 amino acids they play an important role in nutrient dynamics (Pearce, 1997). The contents of organic carbon, clay and nutrients, pH and microbial population have been found to be higher in termite mounds in relation to adjacent soils (Bruyn and Conacher, 1990; Black and Okwakol, 1997). The vast network of galleries they build increases soil porosity and water infiltration (Mando and Stroosnijder, 1999). Through their activities they regulate soil processes that in turn help to promote biodiversity by creating suitable conditions for plants and other biota. Termites are one of the most ubiquitous modifiers of habitats and have consequently been described as keystone species (Whitford, 1991; Black and Okwakol, 1997) in various ecosystems.

A keystone species (Paine, 1969) is one that is important in determining the ecological functioning and structure of a community. Its removal causes massive changes in species

composition and other ecosystem attributes (Jones *et al.*, 1994). Although not formally tested, one would assume that if termites were removed from an ecosystem, the biological diversity of the community would be affected.

Termites have thrived on earth for the past 250 million years and have evolved into two basic types, those that live entirely in wood and those that tunnel into the ground (subterranean type). Most termites belong to the latter type. *Odontotermes* sp. are subterranean termites belonging to the super family Termitoidea, family Termitidae and subfamily Macrotermitinae. Their mounds are low-lying, generally 10-20m in diameter, no more than 0.5m high (Darlington and Bagine, 1999). Subterranean termites exert a strong influence over soil structure formation and maintenance (Vivian-Smith, 1997). These effects can flow through to promote biodiversity and maintain the health of tropical savannas.

Termite species however gain pest status as they fulfill their ecological role of recycling plant material. They cause damage to timber, timber products and living plants (Watson and Gay, 1991). They utilize the materials used in building construction, can be devastating to rangelands, tropical agriculture and forestry. For many decades chemical insecticides have formed the backbone of termite management worldwide (Lenz, 2005). These involve soil barrier termiticides, treated-zone termiticides, and baits impregnated with slow acting toxic chemicals. Following the ban of organochlorines in many countries over the years (Rath, 2000), there has been renewed interest in the development

of alternative termite control measures. Among the biological agents reported against termites, entomopathogenic fungi are considered the most suitable (Jones *et al.*, 1996). *Beauveria bassiana* and *Metarhizium anisopliae* have shown great potential for termite control (Dong *et al.*, 2007). *Beauveria bassiana* is reported to be less pathogenic to termites compared to *Metarhizium anisopliae* (Sajap and Jan, 1990; Grace, 1991; Jones *et al.*, 1996). However, *M. anisopliae* has been isolated from a wide variety of insect species (Humber, 1992), is pathogenic to diverse arthropods (Zimmerman, 1993; Genthner *et al.*, 1997) and could potentially cause mortality in populations of non-target species. This possibility is of special concern where protection of non-target species is an important mandate.

The study was designed to investigate whether *M. anisopliae* applied to control *Odontotermes* sp. would have effects on the diversity of non-target insect groups. The groups chosen are associated with termite mounds and include ants, cockroaches and mantids. Species composition, species richness and abundance of these insects was monitored before and after the application of *M. anisopliae* spores. The diversity of plants associated with these mounds was also compared before and after the fungus spore application.

### 1.2 Statement of the problem and justification of the study

Termites play important roles in sustaining savannah ecosystem health by creating heterogeneous habitats, modifying the magnitude and direction of resource flows in both natural and managed ecosystems. However, they gain pest status when they cause

structural damage to houses, rangelands, tropical forestry and tropical agriculture. Several chemical termiticides have been used to manage their populations but there are growing concerns over effects of such chemicals on ecosystem equilibrium and operations, other alternatives have been sought including biological control with entomopathogenic fungi (EPF). *M. anisopliae* has been reported as an effective biological control agent against termites (Sekamatte, 2000; Maniania *et al.*, 2002; Langewald *et al.*, 2003). However, studies have shown that introduced biological control agents affect some non-target species under field conditions (Flexner *et al.*, 1986; Laird *et al.*, 1990; Dobel *et al.*, 2004; Ginsberg *et al.*, 2004) though Hopper (1998) pointed out that few studies demonstrated that such attack had any impact on population density of non-target species. This study, therefore, evaluated the potential effects of *M. anisopliae* as mediated by reduction in termite population on dictyopterans, hymenopteran ants and plants.

### 1.3 Research questions

a) How does application of entomopathogenic fungus *M. anisopliae* in *Odontotermes* sp. mounds affect the diversity (abundance, species composition and species richness) of superorder Dictyoptera and ants (Hymenoptera) that are associated with these termites?

b) Does the application of *M. anisopliae* in *Odontotermes* sp. mounds affect the diversity (species composition) and relative cover of plants found on these mounds?

## 1.4 Null hypotheses

a) The application of *M. anisopliae* in *Odontotermes* sp. mounds does not affect the diversity of insects of the superorder Dictyoptera and ants (Hymenoptera) associated with the mounds.

b) The application of *M. anisopliae* in *Odontotermes* sp. mounds does not affect the diversity and relative cover of plants growing on the mounds.

## 1.5 Objectives of the study

### 1.5.1 General objective

To assess the effect of *M. anisopliae* application for termite control on the diversity of non-target plants and selected insects associated with *Odontotermes* mounds at Mpala Research Centre, Laikipia district, Kenya.

### 1.5.2 Specific objectives

a) To determine the effects of *M. anisopliae* application in mounds on the diversity of members of superorder Dictyoptera and ants (Hymenoptera) that are associated with termites of the genus *Odontotermes*, and how this correlates on-and off-mound.

b) To determine the effects of *M. anisopliae* application on the diversity and relative vegetation cover of plants found on mounds of termites of the genus *Odontotermes*.

## 1.6 Significance of study and output achieved

Understanding the links between species, structure and function of ecological communities has long been a primary goal of ecologists. As human modification of the biosphere intensifies, understanding these linkages has become a fundamental conservation concern. In an attempt to manage termite populations, the use of biological control agents such as *M. anisopliae* may undermine the delicate integrity of interactive relationships within an ecosystem. The effects of application of *M. anisopliae* on target and non-target selected insect groups are hereby documented to give guidelines to users on the extent to which this biocontrol agent can trigger population fluctuations or changes in ecosystem functioning. The manipulative field experiments in this study were especially valuable in furthering our understanding of the influence of *M. anisopliae* on a widespread model ecosystem, the "black cotton savanna" of East Africa and how these effects are mediated by interactions with plants, hymenopteran ants and dictyopterans. The study is documenting how these groups of organisms that are important in overall species interactions and savannah ecosystem functioning are affected and hence the local and regional biodiversity. Before any product is released into the environment to be used for pest control, caution must be exercised. The effects that it may have, not only on the target (the pest) but also on the surrounding biota is critical to the long term management of natural systems, since changes in the distribution and abundance of these species may have far reaching consequences on the ecosystem.

# CHAPTER TWO: LITERATURE REVIEW

## 2.1 Termite morphology

Termites are soft-bodied, small to medium sized insects ranging from 3-20 millimetres in body length. They can be distinguished by the following features: a pale elongate body, two pairs of membranous wings of equal length present in reproductive castes only, mandibulate (biting and chewing) mouth parts and antennae about as long as the head (Meyer, 2005). They are sometimes referred to as white ants, however they are unrelated to the ants (Order: Hymenoptera). They can be differentiated from ants by morphology of the antennae, abdomen and wings. The termite antenna is moniliform or filiform, while ants have geniculate antennae (Fig. 1). The abdomen is broadly joined to the thorax while ants have a slender connection (petiole). If present, the two pairs of termite wings are the same in size and shape while in ants the outer pair of wings are smaller than the inner pair (Richards and Davies, 1977).

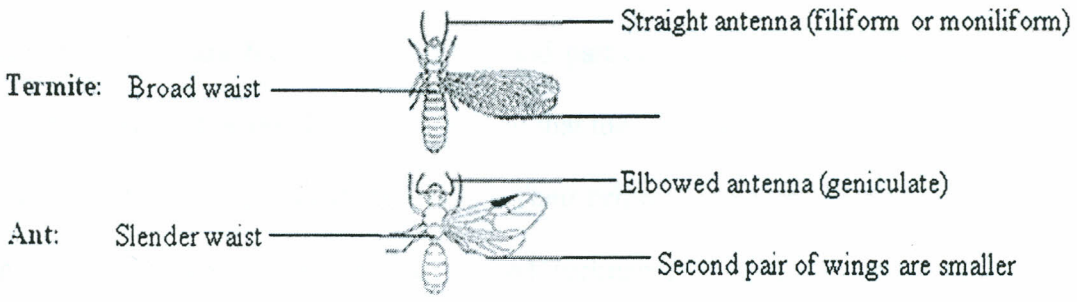



**Figure 1**: Morphological differences between termites and ants

**2.2 Scientific classification of termites** (Richards and Davies, 1977)

| | |
|---|---|
| Kingdom: | Animalia |
| Phylum: | Arthropoda |
| Class: | Insecta |
| Subclass: | Pterygota |
| Infraclass: | Neoptera |
| Order: | Isoptera |
| Suborder: | Apocrita |

**2.3 Termite behaviour and social structure**

Termites are the only hemimetabolous insects that exhibit true social behaviour. They build large communal nests called termitaria that house an entire colony (Meyer, 2005). Each colony contains three forms or castes, which are the workers, soldiers and reproductives. These castes are physically distinct and perform different tasks in the termite colony. Workers are blind, wingless and the most numerous. These sterile individuals forage for food and water, construct and repair shelter tubes, feed and groom other termites, care for eggs and young and participate in colony defence. Soldiers are also wingless and resemble workers except that they have a large, rectangular, yellowish-brown head with large mandibles (jaws). Their primary function is colony defence. Male and female reproductives can be winged (primary) or wingless (neotenic). Winged primary reproductives are called alates or swarmers. However, they shed their wings soon after the mating flight. A pair of primary reproductives head the colony, and these are referred to as the king and queen. Neotenic reproductives often serve as replacements if something happens to the king and queen.

## 2.4 Types of termites

Termites have thrived on earth for the past 250 million years and have evolved into two basic types (Watt *et al.*, 2002); those that live entirely in wood and those that can tunnel into the ground (subterranean type). Among the wood inhabiting termites there are various specialists such as rotten wood termites, damp wood termites and dry wood termites. Most termites are of the subterranean type and they have specialized diets, may eat plant litter, grass, dung and humus instead of wood. All types of termites survive by ingesting cellulose found in wood and wood products.

*Odontotermes* sp. are subterranean termites belonging to the super family Termitoidea, family Termitidae and subfamily Macrotermitinae. It is a large genus comprising three subgenera, *Odontotermes, sensu stricto*, *Hypotermes* and *Euscaiotermes* (Ahmad, 1949). Their mounds are low-lying, generally 10-20m in diameter, no more than 0.5m high (Darlington and Bagine, 1999) (Plate 1). Species belonging to this genus include: *Odontotermes badius* (Haviland), *Odontotermes classicus* (Sjöstedt), *Odontotermes javanicus* (Holmgren), *Odontotermes planiceps* (Sjöstedt), *Odontotermes redemanni* (Wasmann), *Odontotermes vulgaris* (Haviland), *Odontotermes almorensis* (Snyder), *Odontotermes distans* (Holmgren), *Odontotermes formosanus* (Shiraki) and *Odontotermes obesus* (Rambur) among others.

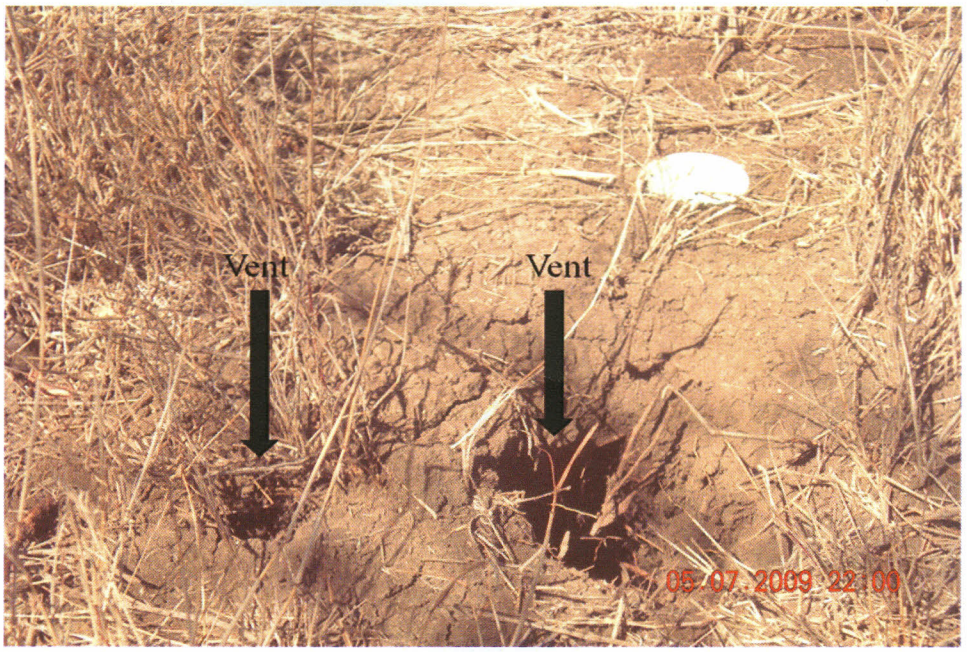



**Plate 1**: *Odontotermes* termite mound (arrows showing various vents)

## 2.5 Termites as "ecosystem engineers"

According to Jones *et al.* (1994), ecosystem engineers are organisms that directly or indirectly modulate the availability of resources to other species, by causing physical state changes in biotic or abiotic materials. In so doing they create, modify and maintain habitats. Termites alter local infiltration rates, create landscape mosaics and their impacts accrue because of the initiation of biophysical processes that often include feedback mechanisms (Dangerfield *et al.*, 1998). These changes to resource flows are likely to persist for long periods and constrain the biological structure of the habitat. By feeding on living and dead plant materials, such as wood, leaf litter, roots, dead herbs, and grasses, dung and humus, they convert lignocellulosic matter into minerals and organic matter that then enriches the soil. In converting this biomass to insect biomass, termite production supports a large proportion of tropical vertebrate biodiversity (Okwakol and Sekamatte, 2007). They also play an important role in nutrient dynamics by ingesting and

redistributing minerals, fatty acids, vitamins and amino acids (Pearce, 1997). They promote soil transformation by disturbance processes so that the contents of organic carbon, clay and nutrients, pH and microbial population increase in termite mounds in relation to adjacent soils (Bruyn and Conacher, 1990; Black and Okwakol, 1997). The accumulated material is later redistributed by erosion causing changes in soil microstructure and fertility (Lee and Wood, 1971; Black and Okwakol, 1997; Dangerfield *et al.*, 1998). The vast network of galleries they build increases soil porosity and water infiltration (Mando and Stroosnijder, 1999) and these may be filled up with top soil after rainfalls, contributing to the process of formation of latosols. It is thus comprehensible that through their activities they play a critical role in the regulation of soil processes that in turn helps to promote biodiversity by creating suitable conditions for the growth of plants and other biota.

**2.6 Termites as keystone species**

The removal of a keystone species from an ecosystem causes massive changes in species composition and other ecosystem attributes (Jones *et al.*, 1994). Termites control the rates and directions of many community and ecosystem processes. They are crucial to communities because they typically provide the major energy flow and the three dimensional structure that supports and shelters other organisms (Duran and Castilla, 1989; Ashton, 1992). Termite-related processes also profoundly influence key determinants of primary production and vegetation dynamics in savannas (Schlesinger, 1997).

## 2.7 Interactions of termites with plants and other insects

Interactions between organisms are a major determinant of the distribution and abundance of species (Jones *et al.*, 1994). Ecology textbooks summarize these important interactions as intra- and interspecific competition for abiotic and biotic resources, predation, parasitism and mutualism (Ricklefs, 1984; Krebs 1985; Begon *et al.*, 1990) Activities that do not involve such direct trophic interactions between species (for example the role organisms play in creation, modification and maintenance of habitats) lack in these texts but they are nevertheless important and common. Termites provide food and shelter to an extraordinary number of associated organisms. They build nests (termitaria) which are among the most impressive examples of animal architecture. These termitaria house several other creatures. They modulate resource flows which in turn help to promote biodiversity by creating suitable conditions for the growth of plants and other biota.

### 2.7.1 Termite - plant interactions

Termite colonies and large earth mounds constructed by termites have been recognized as important determinants of vegetation pattern in tropical savannas and woodlands (Fanshawe, 1968: Malaise, 1978: Pullan, 1979; Arshad, 1982). For instance, studies carried out near Nanyuki, approximately 1950 m on the northwest base of mount Kenya revealed that large termite mounds in *Themeda* grassland bore a characteristically different flora of a ruderal nature, including species of *Solanum, Stachys, Achyropsis and Laggera* (Fries and Fries, 1948). They suggested that mound soils possessed better drainage and nutrient availability. Glover *et al.* (1964), describing vegetation zonation

around low (<0.6m) colony mounds formed by *Odontotermes* sp. at elevations of 1700-1900 m on the Loita Plains of southern Kenya observed that the innermost zone, corresponding to the mound proper, was dominated by *Cynodon dactylon* and a low weedy shrub (*Achyropsis greenwayi*). Surrounding these were zones of short and tall grasses. This zonation was attributed to the dense, clayey nature of the mound soil in contrast to the more friable, loamier soil away from the mound. Termite colony mounds are formed of clay-rich subsoil (Pullan, 1979) and are richer in the clay textural fraction than are adjacent surface soils. Mound soils end up lighter in texture with greater depth, better drainage and higher intrinsic fertility of mound soils (Cox and Gakahu, 1985).

### 2.7.2 Termite - ant interactions

Ants (Hymenoptera: Formicidae) are diverse, abundant and important components of ecosystems not only because they constitute a great part of animal biomass but also because they act as ecosystem engineers. They turn soil, disperse seed, and affect energy flow (Hölldobler and Wilson, 1990). Ants are also important in below ground processes where they alter the physical and chemical properties of soil, affect plants, microorganisms and other soil organisms (Folgarait, 1998). Termites and ants are therefore of extreme importance and their elimination would fundamentally change the character of ecosystems.

The relationship between ants and termites has been considered as either commensal or parasitic, with ants being the only beneficiaries using termite mounds to nest and /or obtain food. Dejean and Bolton (1995); Dejean and Féneron (1999) observed that the

fauna found in the nests of *Procubitermes niapunesis* and *Cubitermes subarquatus*, were mostly ants. Other authors assert that ants are the most significant enemies of termites (Hölldobler and Wilson, 1990; Cornelius *et al.*, 1995). They are effective termite predators, heavily compete for nesting space and are an important disturbance factor in resource exploitation by termites (Goncalves *et al.*, 2005). However, termites may use fire ant mounds as incubators to shorten their reproductive development (Shelton *et al.*, 1999) and thus termites may receive benefits from ants as well. Diehl *et al.* (2005) reported ant and termite species inhabiting mounds found in three wetland sites in Santo Antonio da Patrulha. Higashi and Ito (1989) and Jaffé *et al.* (1995) noted that some ant species co-inhabited termite nests and provided protection against predator attacks thus suggesting a mutualistic kind of interaction. The ant colony can be considered a superorganism and in this way ants offer special advantages for important kinds of basic biological research. Due to the strength of these interactions, they were chosen as indicator organisms for this study.

### 2.7.3 Termite - Dictyoptera interactions

Termites are closely related to cockroaches and mantids (Myers *et al.*, 2006). A study by Inward *et al.* (2007) showed that termites are social cockroaches, no longer meriting being classified as a separate order (Isoptera) from the cockroaches (Blattodea). There are over 4000 species of cockroaches worldwide belonging to six families: Nocticolidae, Polyphagidae, Blattidae, Cryptocercidae, Blattellidae and Blaberidae (Bell *et al.*, 2007).

Cockroaches are an important part of the decomposer component of many ecosystems – they are omnivorous scavengers and sometimes cannibalistic. They take part in organic debris breakdown by feeding on decaying wood and leaves and add nutrients to the soil through their excrement.

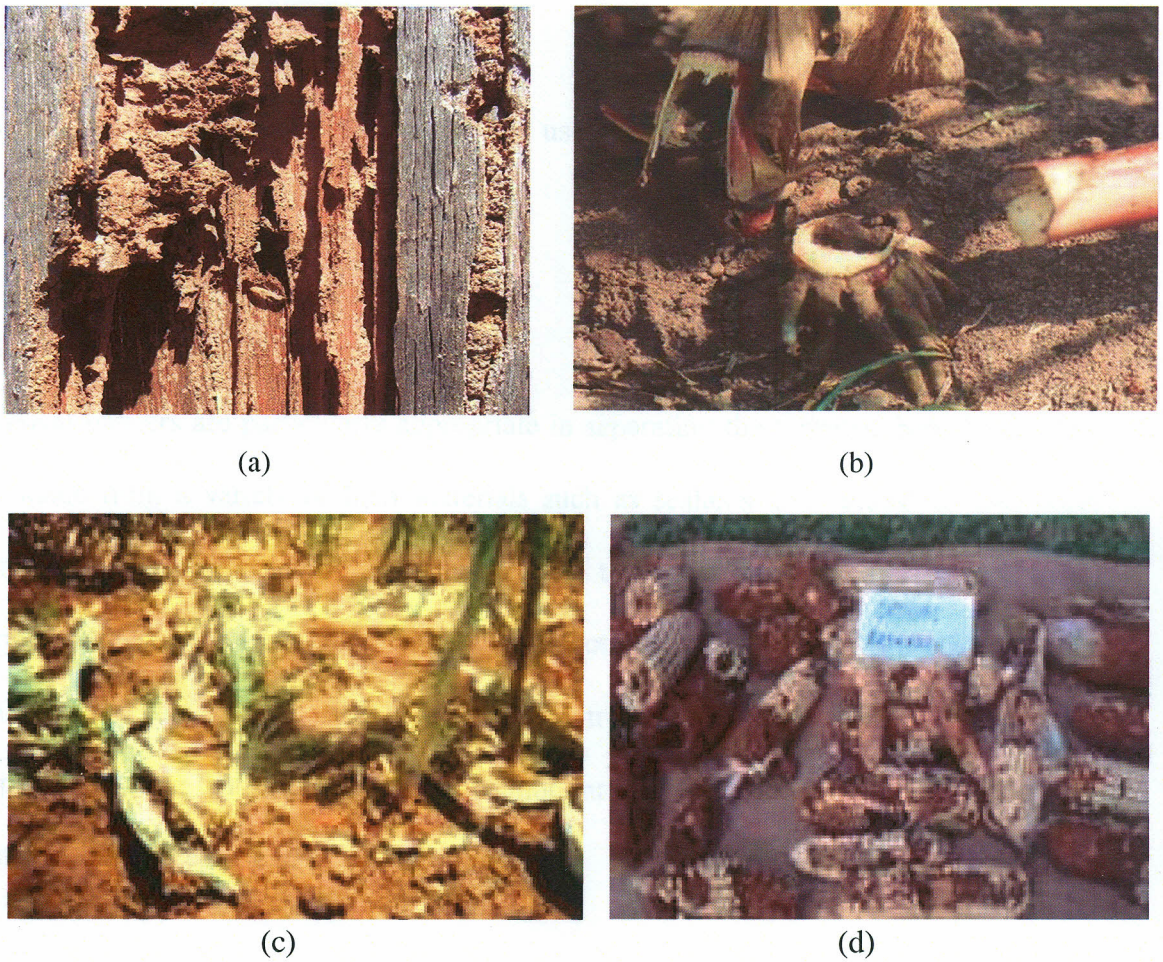
Mantids also belong to the superorder Dictyoptera. The closest relatives of mantises are the orders Isoptera and Blattodea. All mantids are carnivorous and use their front raptorial legs to catch their food. They feed on a wide range of insects and thus convert the protein matter into nutrients later on released to the soil through excrement.

**2.8 Termites as pests**

Termite species gain pest status as they fulfil their ecological role of recycling plant material they encounter and endeavour to utilise the materials used in building (Plate 2), construction or agronomic and forestry commodities. Subterranean termites cause significant damage to structures like houses, bridges, dams and roads. They also attack rangelands, tropical forestry (eucalyptus and acacia) and agricultural crops (maize, sorghum, beans, cowpeas, groundnuts, pigeon peas, cassava, banana, sugarcane, yams and cotton). They can attack plants at any stage of development from the seed to the mature plant (UNEP, 2000).

Damage to seedlings occur as termites directly forage on underground plant material. Seedlings are either cut just below or above the soil surface (Plate 2c). Damage to a maturing plant is largely caused by root system consumption (Plate 2b). This directly

kills the plant and indirectly lowers yield through decreased translocation of water and nutrients. Termite damage to stored products (Plate 2d) generally results in invasion by *Aspergillus* leading to indirect yield losses and contamination with aflatoxins (UNEP, 2000).



(a) (b)

(c) (d)

**Plate 2**: Damage caused by termites (a) galleries on buildings (b) seedling cut just above the soil surface by foraging termites (c) feeding on the root system of maize plants and (d) harvested maize cobs (Photos courtesy of UNEP, 2000).

### 2.8.1 Economic losses

The negative impact of termites is often cited in economic terms as expenditures for damage, repair and preventative treatment costs. Economic losses caused by termites in

crops have been estimated at US$ 15-20 billion worldwide, when combined with damage in forestry, the value may exceed US$ 30 billion per year (Meyer, 2005). Losses range from 7.2-90% in annual crops such as maize (Wood and Pierce, 1991), cassava (Greathead *et al.*, 1984), ground nuts (Johnson *et al.*, 1981) and sorghum (Logan, 1991).

## 2.9 Termite management strategies

Several management strategies have been used and these include physical, cultural, chemical and biological control.

### 2.9.1 Physical control

Physical barriers are particularly appropriate in separating the termites from food. They are made from a variety of inert materials such as sealants and "glues" to join sheet material or woven mesh to bricks and concrete to provide a strong and durable bond. Strip shielding, pie plates, posts on stirrups, electrocution in soil, heating, freezing and plastic films can also be used in physical controls (Lewis and Haverty, 1996). It is important to note that physical barriers cannot entirely exclude the possibility of termite attack as barriers can be breached or bridged.

### 2.9.2 Cultural control

Deep ploughing and or hand tillage may result in the exposure of termites to dessication, thus reducing their number in the field crops. Pre-planting tillage also destroys tunnels built by termites and restricts their foraging activities and associated damage to crops. Removal of the queen and/or destruction of the nest, flooding or burning with straw to

suffocate and kill the colony have frequently been used by farmers as a traditional method for control of mound-building termites. Crop rotation may be useful in reducing the build up of termites since intensive monoculture for long periods makes plants more susceptible to attack. Intercropping is the most effective cultural practice used by small-scale farmers in Sub- Saharan Africa to manage insects that have specific host ranges. However, controversial results have been reported for termites. For example, intercropping maize and beans resulted in significant reduction of ground tunnelling by termites but did not reduce termite damage on the plants. The removal of residues and other debris from the field may reduce potential termite food supplies and hence lead to a reduction in termite numbers and subsequent attack. Mulches may either increase or decrease the incidence of termites depending on whether they have any repellent properties (UNEP, 2000).

### 2.9.3 Chemical control

For many decades, the prevention and treatment of termites relied heavily on the use of organochlorine insecticides such as aldrin, dieldrin, endrin, chlordane, mirex and heptachlor. Though they provided 20-30 years of protection against termites, are chemically stable and extremely effective, these chemicals were largely withdrawn from use over their environmental persistence, metabolic stability, lipid solubility, resultant bioaccumulation (accumulation in the fats of animals and humans) concerns (Australian case study, 1994). They were thus labeled persistent organic pollutants (POPs). Other insecticides currently being marketed for termite control though not as effective include chlorpyrifos, isofenphos, and the pyrethroids (Wood *et al.*, 1987).

The active ingredients in the available termiticides can be broadly classified as repellent and non-repellent. Pyrethroids and synthetic pyrethroids are considered repellent. Termites detect the barrier and avoid treated structures. However, termites may continue to forage until they find a break in the barrier and tunnel into the structure that is otherwise protected. Examples of non-repellent chemicals include organophosphates, imidacloprid, fipronil and chlorphenapyr. Termites can tunnel through this soil and contact the chemicals or ingest them and die (www.allpest.info).

### 2.9.4 Biological control agents

In recent years, the shortcomings associated with conventional chemical control methods have prompted policy makers and scientists to evaluate the potential of biological control of termites. Live biological agents used for insect control include various species of bacteria, viruses, nematodes and fungi (Culliney and Grace, 2000). All classes of biological control must be carefully and fairly evaluated as candidates for termite control. Various biological agents have been explored. Ants are the greatest predators of termites, and may have a considerable local impact on termite populations in some areas of the world (Culliney and Grace, 2000). A few parasitoids of termites are known for instance, *Verticia fasciventris* (Order: Diptera) but their potential for regulating termite populations seems negligible (Tsang, 2006). The protected underground location of the colony is likely to limit the impact predators and parasitoids have on subterranean termites. Entomopathogenic nematodes (EPN) in the families Steinernematidae and Heterorhabditidae have been found to evoke avoidance responses in isolated species. EPNs are virulent enough to produce 100% mortality within an inoculated termite

colony, yet their virulence is limited to the point that single inoculations are unable to produce colony nullification. Nullification of the termite colony is only assured if inoculation is done repeatedly over a minimum period of twelve to twenty four months (Jerry, 2007). Soil moisture and soil type also limit the nematodes ability to move in the soil and locate termites. For various reasons, viruses, bacteria and protozoa have shown little promise for use in biological control of termites (Culliney and Grace, 2000). Bacterial strains in the species *Bacillus thuringiensis* appear unsuitable for use in termite control because they evoke complex and efficient avoidance responses (Jerry, 2007). Baculoviruses in the genera *Nucleopolyhedrovirus* (NPV) and *Granulovirus* (GV) may have promise in the future if virulence concerns can be resolved. Recent studies suggest that natural products, such as ant semiochemicals and fungal metabolites (siderophores), or their synthetic analogues, might eventually find use as termite repellents or insecticides if stable formulations can be developed (Jerry, 2007).

Research suggests that strains of entomopathogenic fungi, *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) Sorokin, are most suitable for termite control (Dong *et al.*, 2007). *M. anisopliae* has been commercialized under the trade name Bioblast® in the USA and Bio Green® in Australia (Milner, 2000). In Africa, one isolate of *M. anisopliae,* isolate ICIPE 30, has been found to be highly pathogenic against many species of termites and is under development as biopesticide at the International Centre of Insect Physiology and Ecology (*icipe*) (Sekamatte, 2000; Maniania *et al.*, 2002; Langewald *et al.*, 2003).

### 2.9.4.1 The fungus, *Metarhizium anisopliae*, mode of action

*Metarhizium anisopliae* is a naturally occurring pathogen, which infests over 200 insect species (Tanada and Kaya, 1993). It is a promising agent for the biological control of termites because the conidia are viable in the soil (Zimmerman, 1982), they naturally adhere to the insect cuticle, and are easily transferred to other termites through ordinary, interactive colony behaviour (Kramm *et al.,* 1982; Hanel and Watson, 1983). Studies done on termites using this fungus have shown that there appears to be very little host specificity among *M. anisopliae* fungal isolates with many isolates being highly virulent to many species of termites (Rath, 2000).

*M. anisopliae* normally inhabits the soil as dormant conidia which infects susceptible host on contact with the cuticle. Conidia then germinate and the hyphae that emerge penetrate the cuticle. The fungus then develops while consuming internal contents of the insect, establishing an infection (mycoses), which kills the termite in 2-10 days depending on conditions such as temperature, dose and humidity. The lethal effect is very likely aided by the production of insecticidal cyclic peptides (destruxins). The cuticle of the cadaver often becomes red, a white mold then grows that soon turns green as spores are produced (Hanel, 1982). Termites being highly social insects, engage in a variety of activities that involve frequent, direct physical contact with other colony members. Through grooming the infective propagules are transferred from one individual to another.

### 2.9.4.2 Effects of *M. anisopliae* on non-target organisms

*M. anisopliae* may have been isolated from a number of hosts and it is the wide host ranges of some species that have caused concern regarding safety to non-target invertebrates. The pathogen can have direct effects on target and potentially non-target arthropods. In addition, there can be indirect effects due to depletion of the target host population which in turn influences non-target arthropods either directly associated with the pest e.g. predators and parasitoids or non-targets indirectly associated but impacted by presence of the pest (Goettel and Hajek, 2001). The best documented cases of fungal biocontrol agents are indirect effects on the predators and parasitoids of the target pest through host depletion (Goettel *et al.*, 1990)

In general, it is not possible to reduce a pest population using fungi without also affecting another component of the ecosystem. Most insects living near treated soil evolve natural defenses against *M. anisopliae* but some reports (Flexner *et al.*, 1986; Laird *et al.*, 1990; Cloyd, 1999; Vestergaard *et al.*, 2003; Ginsberg *et al.*, 2004; Dobel *et al.*, 2004) suggest that the defence mechanisms may not suffice to shield them from the fungus effects which do pose inherent, albeit minimal risks to non-targets.

# CHAPTER THREE: MATERIALS AND METHODS

## 3.1 Study site and sampling design

This study was carried out at Mpala Research Centre, situated in Laikipia District, central Kenya, latitude 0° 15′ N, longitude 36° 50′ E and 1800m above sea level (Figure 2). Average yearly rainfall is 500–550mm. The rainfall is usually low in December to February and has three small peaks in April, August and November. Maximum temperatures range from 25 to 30°C the minimum from 12 to 17°C. July and August are often the coldest months.

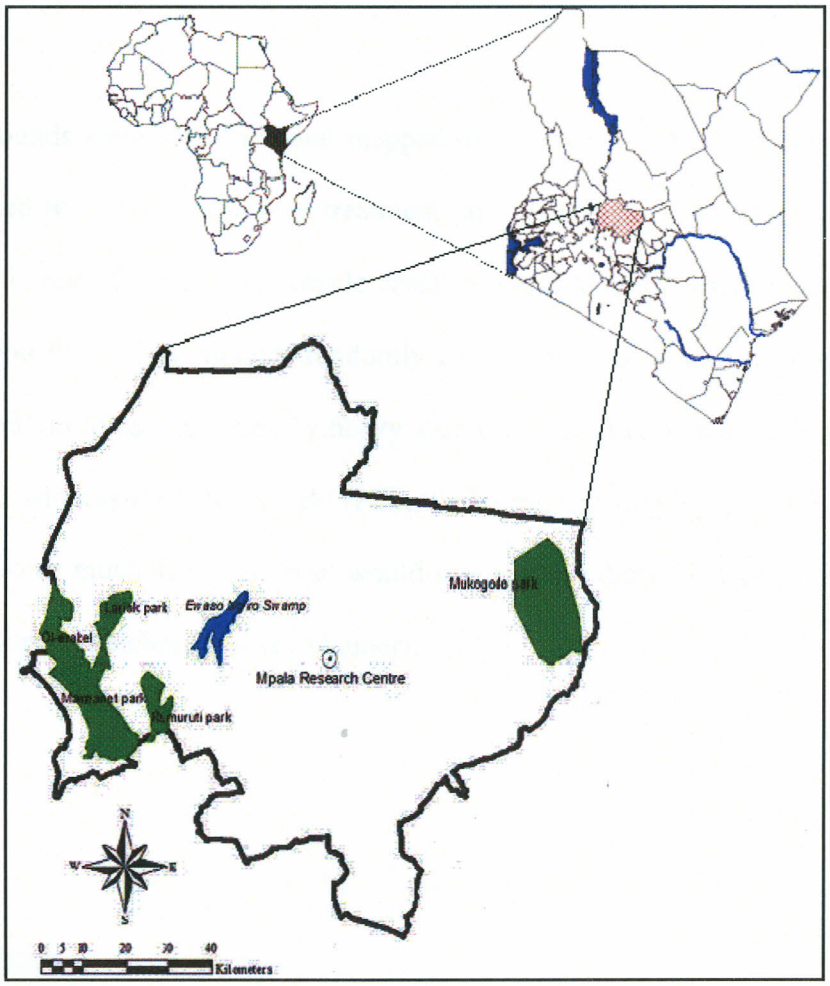



**Figure 2**: A map showing the location of Mpala Research Centre in Laikipia District, Central Kenya.

The research centre is located on a level area of black cotton soil with impeded drainage. *Acacia drepanolobium* forms a virtual monoculture in the overstory accounting for over 97% of canopy cover (Young *et al.*, 1997). The climatic conditions in the area were considered while designing the project. The Random Complete Block Design (RCBD) was chosen whereby the field was divided into a number of units equal to the number of treatments to account for any variation in the field. Further to that, the design included a control group practically identical to the experimental group (and thus affected by the same climatic conditions) except that the experimental group was tested on using variables.

Forty termite mounds were identified and mapped using a GPS. Twenty mounds were randomly assigned to a termite removal treatment, and twenty mounds were randomly assigned to be fenced. Smooth wire (made from galvanized mild steel) fences were constructed around the twenty mounds randomly chosen for this treatment. The fences were 2m high and 8m long, supported by heavy strutted posts at corners and at intervals of 2m to keep the wires spaced and upright (Plate 3). To prevent sagging of the fence, the wire was stretched as much as the material would safely allow during construction using a hand operated fence stretcher (monkey strainer).

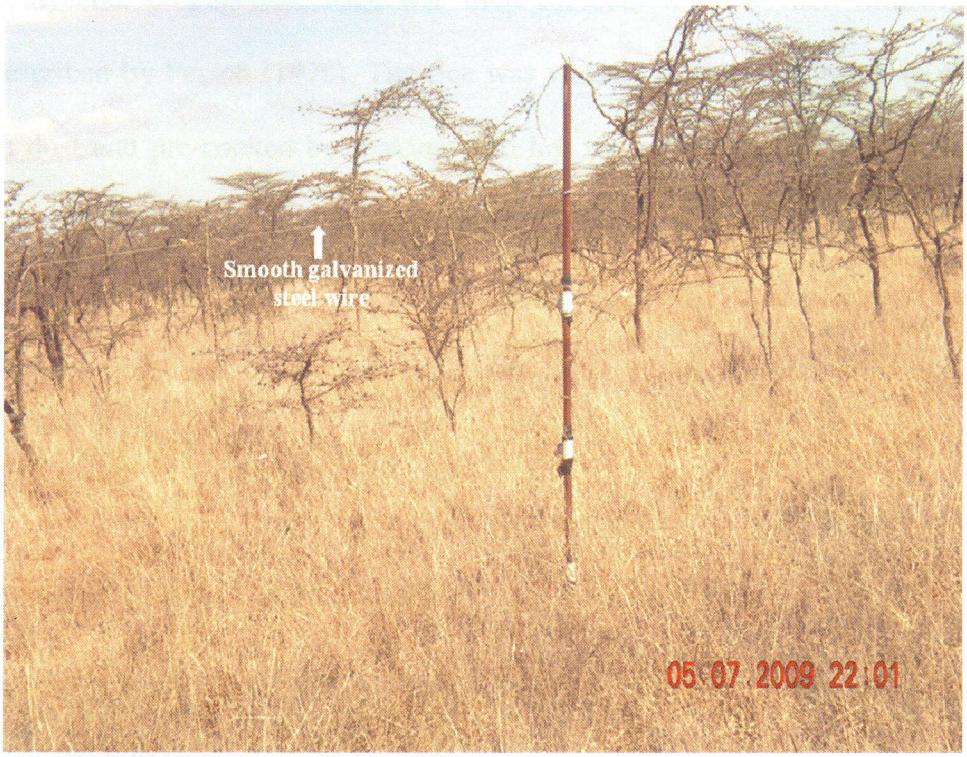



**Plate 3**: Picture showing part of one of the mounds fenced using smooth galvanized wire.

The purpose of the fencing was to keep away the megaherbivores found in the area that were likely to use the mounds as resting areas thus depositing dung that would eventually affect invertebrate diversity on mounds. The design was a full-factorial with two treatments – termite removal (yes/no) and vertebrates excluded (yes/no). The design thus allowed for the separation of the effects of termites from those of vertebrate herbivores on plant and invertebrate patterns of diversity.

### 3.2 Mass production and application of the fungus

*Metarhizium anisopliae* isolate ICIPE 30 was mass-produced in the laboratory by culturing spores onto Sabouraud Dextrose Agar (SDA). The culture was inoculated into a liquid broth prepared from peptone, glucose and yeast extract and left to incubate for 72

hours for mycelial propagation. Rice was used for conidia production following the method described by Ferron (1978). The rice was washed in clean tap water to remove any starch dust and pre-cooked by soaking with hot water. It was then transferred into polyethylene plastic bags and autoclaved at 121°C for an hour, left to cool and later inoculated with the liquid broth. This was then incubated (Plate 4) at room temperature (26 ± 2°C and 60-70% RH) and substrate allowed to dry (Plate 5). Harvesting was done by sifting through a sieve. A glass dessicator containing dried silica gel was used for final drying (Jenkins, 1996). Dry conidia of *M. anisopliae* (Plate 6) were applied into the termite mound vents using a bicycle pump.

**Plate 4**: Rice inoculated with conidia incubating at room temperature in polyethylene plastic bags.

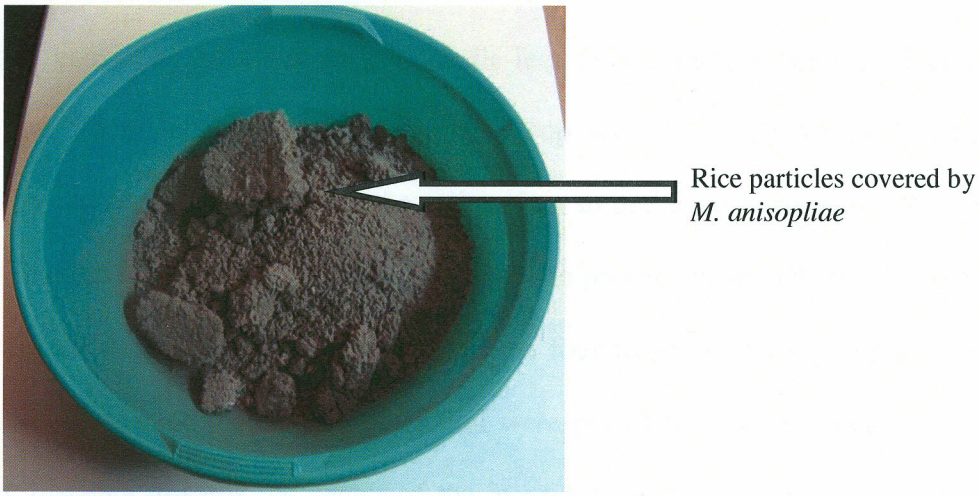



**Plate 5:** Rice substrate drying

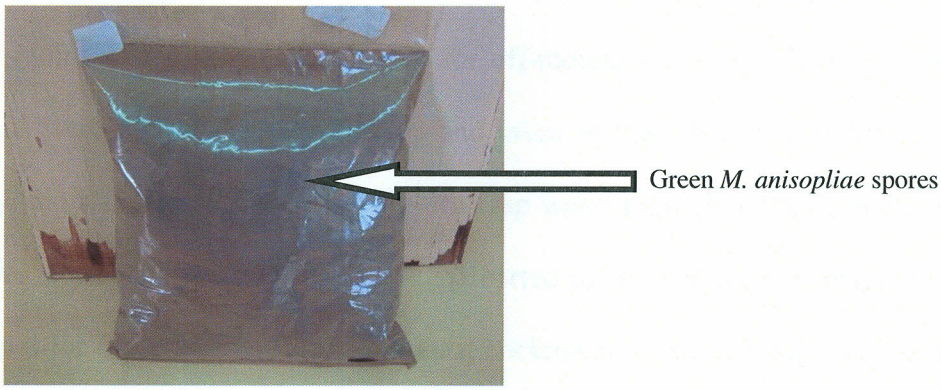



**Plate 6:** End product, dry, dusty conidia of *M. anisopliae*

### 3.3 Sampling procedures

Line transects were established for invertebrate and plant sampling purposes. The transects were marked using short metal posts from the mound centre, every five metres in a straight line, ending off-mound. To tell the difference on- and off mound, the type of vegetation, soil texture and soil depth was used. The insects were sampled in the established transects after every three months over the period of this study (one and a half years). Two sampling methods were used: pitfall trapping and sweep-netting.

### 3.3.1 Pitfall trapping

Pitfall traps consisting of cone-shaped, handle-less plastic cups measuring 5.4cm in diameter and 5.5cm in height were placed into cup size holes dug out using garden trowels. The cup rims were flash with the soil surface so that unsuspecting insects would easily fall into the containers filled with soapy water to prevent escape (Plate 7). The cups were positioned such that four of them lay in the four main compass point directions around the edge of each mound, the other four were placed between each adjacent two cups some distance towards the center of the mound depending on the mound size as depicted in line diagram below (Figure 3). The layout was then repeated at a distance twice the radius of the mound to account for off-mound sampling. Contents from the pitfall traps were collected after 48 hours and taken to the laboratory at MRC. In the laboratory, the samples were rinsed with clean tap water through a plastic sieve (which only allowed the soapy water to filter through), sorted and transferred into nalgene bottles with ethanol for preservation. Recovered ants, cockroaches and mantids were identified up to the order level.

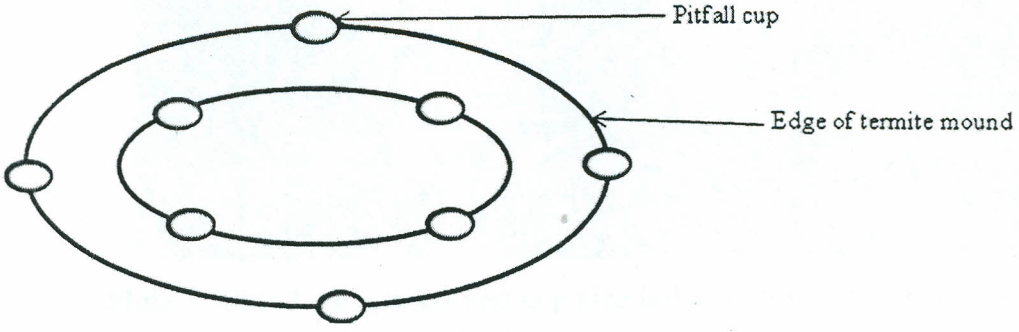



**Figure 3**: Line diagram depicting the pitfall cup layout on a mound

### 3.3.2 Sweep-netting

To sample flying insects, sweeping was done by walking along two parallel lines on either side of the diameter of the mound while sweeping the herbage using sweep nets across the mound. The number of sweeps was determined by the size of the mound. The design was meant to provide maximum surface coverage of the mounds. Contents from the sweeps were then emptied into containers and taken to the laboratory for processing. The same process was duplicated at a distance twice the radius of the mound to account for off-mound sampling.

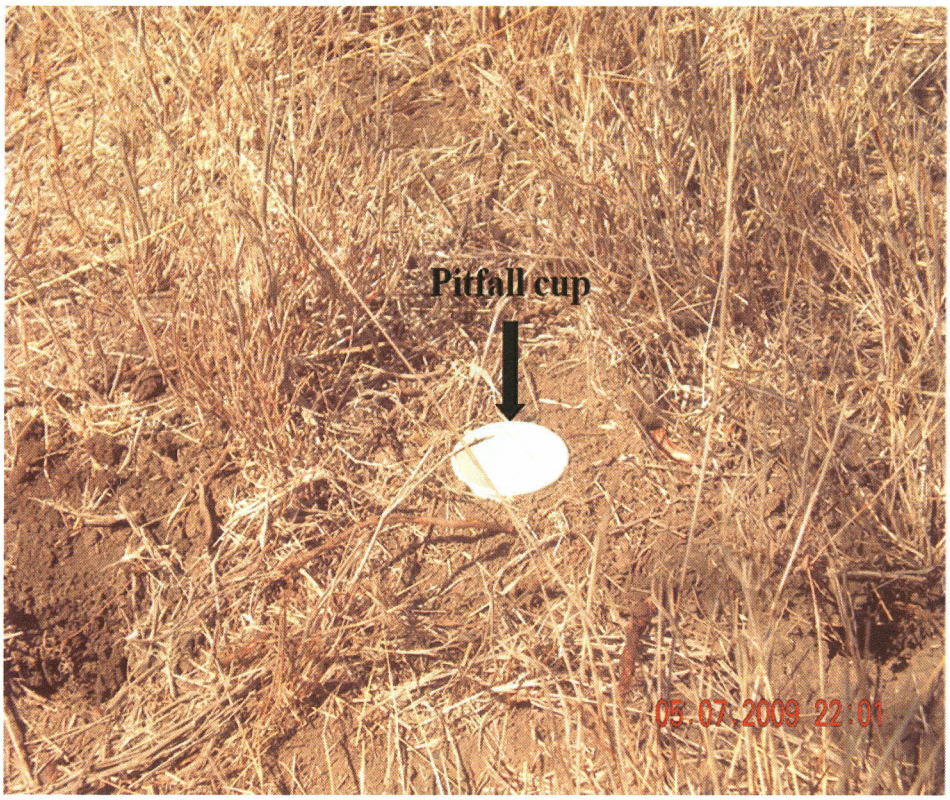



**Plate 7**: Pitfall cup placed into cup size holes with the rim at flush with the soil surface. The cup size holes are dug out using a garden trowel

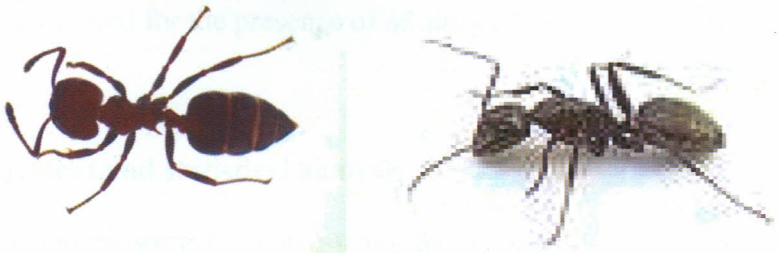
### 3.3.3 Vegetation sampling

Vegetation sampling was done every three months. A point-intercept method, the pin-frame method consisting of 10 pins was placed at the site of each quadrat along a permanent transect and the number of hits recorded by species. The pinframe together with the quadrat were placed starting at the mound centre and then moved outwards towards the edge, records of hits being done every 2m. A 2 x 2m quadrat was chosen due to the small sizes of some of the mounds. The plant species present within the quadrats were also recorded to provide presence/absence vegetation data.

### 3.4 Laboratory assays

Two species of ants, *Crematogaster mimosae* and *Camponotus* sp (Plate 8a and b respectively) were collected from Mpala and brought to *icipe* for laboratory assays in December 2008. Factors considered while choosing the two species assessed were abundance and availability of the ant nests. Three hundred and twenty insects belonging to each species were collected for this assay.

The isolate ICIPE 30 was obtained from the *icipe* Arthropod Germplasm Centre. Spores were harvested from three week old fungal cultures by scraping them off from Sabouraud dextrose agar (SDA) in petri dishes.



(a) (b)

**Plate 8**: Ant species (a) *Crematogaster mimosae* (b) *Camponotus* sp that were used in laboratory assays

The spores were then suspended in 20ml sterile distilled water containing 0.2% Triton X-100 and then vortexed for 5min to produce a homogenous conidial suspension. A viability test was then done by covering a spread of 0.1 ml of the suspension on SDA plates using a sterile microscope cover slip. This was incubated at 26 ± 2°C for 24h after which percentage germination was determined by counting 100 spores for each plate. Concentrations of $3.0 \times 10^5$, $3.0 \times 10^6$ and $3.0 \times 10^7$ conidia $ml^{-1}$ were then formed and sprayed onto filter papers in the Burgerjon spray tower. For the controls, filter papers were sprayed with 0.2% triton water. Test insects were then left to walk on the filter papers. The experiments were replicated four times, each replicate with twenty ants. This experiment aimed at assessing the direct effects of the fungus on the ants. Mortalities were recorded daily for fourteen days. Differences in mortalities in controls were then compared to those in the treatments. Mycosis tests were done on the cadavers in order to authenticate if the deaths resulted from lethal fungal infections (epizootics). This involved surface-sterilization of the cadaver using a solution of 3% sodium hypochloride (jik), then into 70% alcohol for two seconds only. They were then rinsed 2-3 times in

distilled water and placed in petri dishes covered with moistened filter paper. The cadavers were then scored for the presence of *M. anisopliae*.

### 3.5 Data management and statistical analysis

Species diversity indices were generated using the Species Diversity module of Ecosim (Gotelli and Entsminger, 2005). The Shannon index of diversity and the Berger - Parker dominance index were obtained. The diversity indices were analyzed as separate response variables using repeated measures ANOVA with "mound" or "matrix" and time as main effects using JMP® 7.0.2 software. Maximum likelihood parameter estimates and confidence limits were found using least squares. The Least Square Means (LSM) Student's t test was then used to give multiple regression effects for model effects and to compute individual pairwise comparisons of least squares in the model. These analyses aimed at estimating the percentage of total variance in species diversity.

For the laboratory bioassays, the percentage mortality was corrected using Abbot's formula (Abbot, 1925) to cater for natural mortality.

$$\text{Abbott's formula} = \frac{100 \times (Treatment\% - Control\%)}{100\% - Control\%}$$

To compare mortalities in treatments and in controls, ANOVA was used and the means were separated using Student Neuman Keul's test in SAS version 9.1 (2003). Change in plant diversity was assessed by obtaining percentage relative cover from pin-frame data and comparing changes before and after the fungus application. The comparison was done using JMP® 7.0.2 software. The percent cover for each individual species was

calculated by totalling the "hits" for that species and dividing by the total number of hits for total species for the transect and multiplying by 100.

# CHAPTER FOUR: RESULTS

## 4.1 Abundance and diversity of ants (Hymenoptera)

Ants from pitfalls and sweeps were pooled together and a total of 262,030 ants were collected over the study period. Species identified belong to eight genera: *Crematogaster, Camponotus, Dorylus, Leptogenys, Platythyrea, Polyrhachis, Tetramorium,* and *Tetraponera*. Other specimens were identified to the order level. Their diversity and abundance were estimated in the different treatments.

### 4.1.1 Abundance and diversity of ants before mound treatment

A total of 11,363 ants were collected prior to the application of *M. anisopliae* on mounds designated to be treated.

**Table 1**: Ant abundance, species richness and diversity indices obtained in July 2007 preapplication sampling session

| Parameter | Mounds designated to be controls | | Mounds designated to be treated | |
|---|---|---|---|---|
| | On | Off | On | Off |
| Total individuals (*n*) | 3581 | 2146 | 3374 | 2262 |
| Total species (*S*) | 21 | 23 | 22 | 24 |
| Shannon diversity index (*H'*) | 0.9 | 1 | 1 | 0.9 |

| | *F* - ratio | *p* value |
|---|---|---|
| Fungus' treatment | 0.68 | 0.41ns |
| Fence treatment | 0.14 | 0.71ns |
| Mound (on / off) | 0.48 | 0.5ns |

Level of significance set at $p < 0.05$

The diversity indices obtained were high ranging from 0.9 to 1. *Crematogaster mimosae* was the most dominant species by a percentage of 23% of the total collection. There was no significant difference between fenced and unfenced mounds, nor between on and off mound samples before the fungus was applied (Table 1).

### 4.1.2 Abundance and diversity of ants in the control versus the treated mounds

Hymenopteran ants varied in diversity and abundance across the sampling periods. There was evidence of variation in ant diversity across the sampling dates ($F = 10.616$; $p < 0.0001$). However, there was no significant effect of the fungus treatment on ant diversity, ($F = 0.0162$; $p = 0.8989$) and there was no significant difference in diversity on- and off mounds ($F = 2.021$; $p = 0.1558$).

In the control mounds, the highest number of individuals was recorded off mound during the month of October 2007 while the least number of individuals were collected off mound during the months of January, April and July in 2008 (Figure 4). In contrast, the highest Shannon diversity index was obtained in April 2008 (on and off these mounds). The lowest indices were obtained in October 2007 off the mounds where *Dorylus affinis* represented 95.4% of the ants collected off control mounds in October 2007. *Crematogaster mimosae* remained predominant on and off untreated mounds in April, July and October 2008 by percentages ranging from 25 – 35.8%.

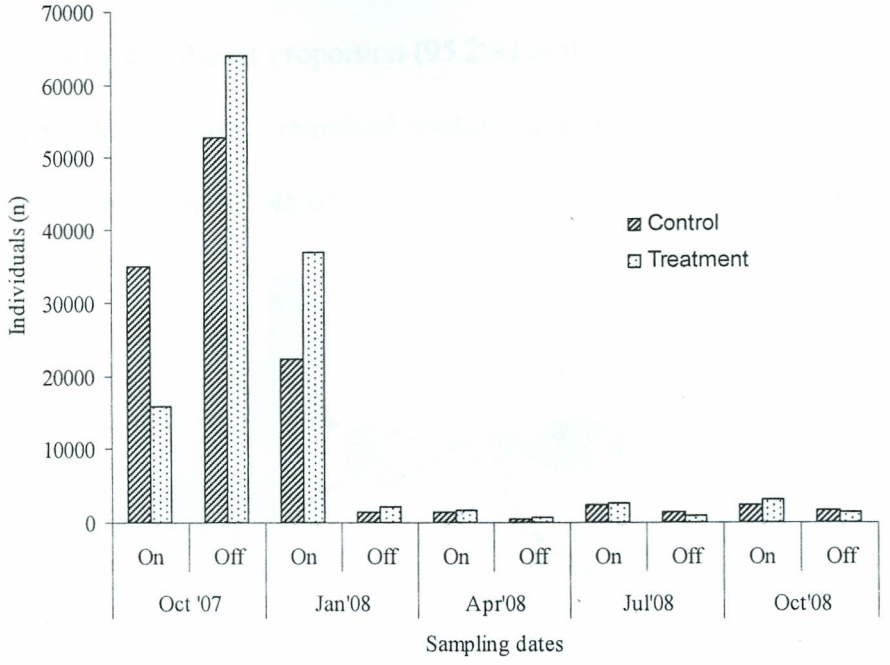



**Figure 4**: Ant abundance obtained from the control and the treated mounds across the sampling sessions.

Ant diversity and abundance in treated mounds also varied across the sampling sessions with the highest total number of individuals (63,936) being recorded off mounds during the month of October 2007, two months after application of *M. anisopliae*. Generally, low number of individuals was recorded in April 2008 though the number gradually increased in the subsequent sampling months of July and October 2008.

Species richness also varied across the sampling periods (Figure 5). Highest number of species was recorded in April 2008 and this corresponded to the highest Shannon index. Diversity (*H'*) was generally low in collections made in the month of October 2007 and January 2008. Inversely, the highest number of total ant collection was made during the two months, (79,705 in October 2007 and 38,965 in January 2008). There was variation

in the composition of ant community across the sampling dates. *Dorylus affinis* (Plate 9) constituted a significant proportion (95.2%) of the total collection in October 2007 while *Crematogaster mimosae* remained predominant in April, July and October 2008 where it constituted between 27- 46.6% of the total collection.

**Plate 9:** *Dorylus affinis*

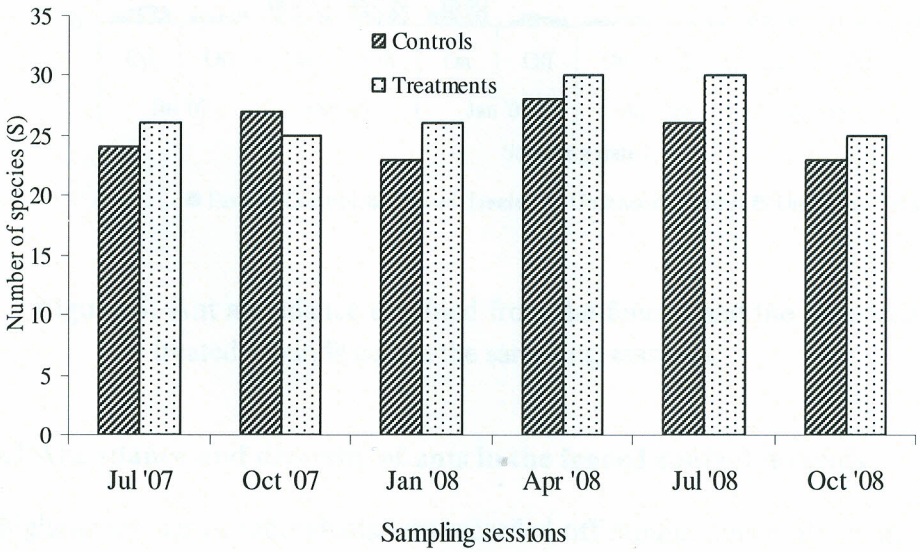



**Figure 5**: Species richness of ants obtained from the control and the treated mounds across the sampling sessions.

### 4.1.3 Abundance and diversity of ants in the fence treatments across the mounds

The diversity and abundance of the ants sampled were compared to determine if the fence treatment resulted in variations. The results obtained indicated that the fence treatment did not have a significant effect ($F = 0.379$; $p = 0.5385$). However, there was variation in the number of individuals collected (Figure 6) and the general species composition.

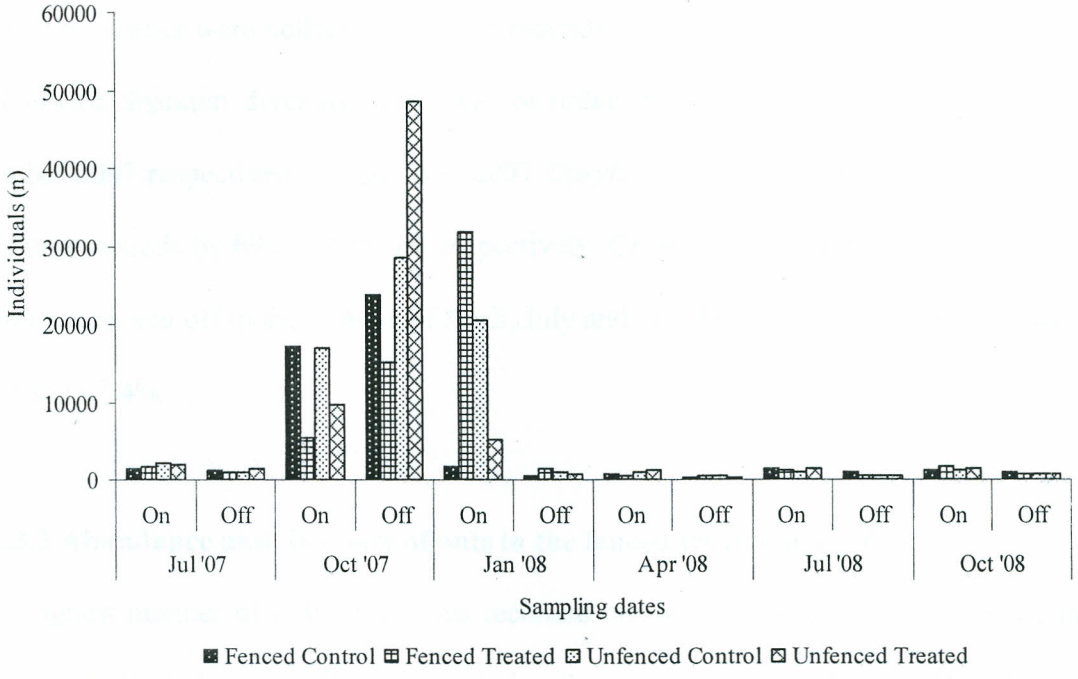



**Figure 6:** Ant abundance obtained from the fenced and the unfenced control and treated mounds across the sampling sessions.

#### 4.1.3.1 Abundance and diversity of ants in the fenced control mounds

The highest number of individuals was recorded off mound during the month of October 2007 with the least being collected off mounds in April 2008 (Fig. 6). In contrast, the highest Shannon diversity index was off the mounds in April 2008 and lowest off the mounds in October 2007. *Dorylus* sp. constituted 58 and 94% of the ant population

sampled both on and off fenced control mounds in October 2007. *Crematogaster mimosae* was the most dominant in the months of July and October 2008, constituting between 37-47% of the total collection.

#### 4.1.3.2 Abundance and diversity of ants in the unfenced control mounds

The highest number of ants was recorded off mound during the month of October 2007. The least number were collected off these mounds in April 2008 (Figure 6). The highest and lowest Shannon diversity index was recorded off the mounds in April 2008 and October 2007 respectively. In October, 2007 *Dorylus* sp. of ants dominated both on and off these mounds by 69.2 and 96.1% respectively. *Crematogaster mimosae* was the most dominant on and off in the months of April, July and October, 2008, constituting between 27.9 and 47.4%.

#### 4.1.3.3 Abundance and diversity of ants in the fenced treated mounds

The highest number of individuals was recorded on mounds in January 2008 while the least was collected on and off mounds during the month of April (Fig. 5). The Shannon diversity index was highest on the mounds in April 2008 while the lowest was obtained on the mounds in January 2008. *Dorylus affinis* predominated the ant collection in October 2007 by 90.3% and January 2008 by 97.2%. *Crematogaster mimosae* remained predominant on and off these mounds in April, July (off) and October, 2008.

#### 4.1.3.4 Abundance and diversity of ants in unfenced treated mounds

Ant species diversity did not vary across the different treatments. However, within the treated mounds, diversity values varied across different sampling dates with low values being recorded in January 2008 (Table 2). The highest number of individuals was recorded off mound during the month of October, 2007 while the least number was collected off mound during the month of April 2008. The Shannon diversity index was highest on the mounds in July 2008. The lowest index was obtained on the mounds in October 2007. Like in the fenced mounds, *Dorylus affinis* constituted 97.4% of the off mound collection in October 2007. *Crematogaster mimosae* remained predominant on and off these mounds in April, July and October, 2008 with proportions ranging from 28.5- 63.1%.

**Table 2**: Least square mean values (LSM), *F* ratio and *p* values obtained after crossing all the parameters with regard to ants diversity (*H'*)

| Sampling date | Fence treatment | Fungus treatment | | On | Off |
|---|---|---|---|---|---|
| July 2007 | Fenced | Treated | | 0.68 | 0.64 |
| | | Control | | 0.71 | 0.71 |
| | Non fenced | Treated | | 0.73 | 0.66 |
| | | Control | | 0.69 | 0.71 |
| | | | ***F*** | **0.526** | |
| | | | ***p*** | **0.787** | |
| October 2007 | Fenced | Treated | | 0.57 | 0.56 |
| | | Control | | 0.50 | 0.54 |
| | Non fenced | Treated | | 0.60 | 0.55 |
| | | Control | | 0.55 | 0.55 |
| | | | ***F*** | **0.16** | |
| | | | ***p*** | **0.99** | |
| January 2008 | Fenced | Treated | | 0.51 | 0.53 |
| | | Control | | 0.53 | 0.51 |
| | Non fenced | Treated | | 0.53 | 0.51 |
| | | Control | | 0.51 | 0.53 |
| | | | ***F*** | **0.095** | |
| | | | ***p*** | **0.758** | |
| April 2008 | Fenced | Treated | | 0.68 | 0.67 |
| | | Control | | 0.67 | 0.68 |
| | Non fenced | Treated | | 0.68 | 0.68 |
| | | Control | | 0.67 | 0.67 |
| | | | ***F*** | **0.077** | |
| | | | ***p*** | **0.782** | |

The *F* and *p* values shown are results of crossing fence, fungus and mound effects at each sampling date ($p = 0.05$).

### 4.2 Effect of *M. anisopliae* on *Crematogaster mimosae* and *Camponotus* sp. in laboratory bioassays.

*Crematogaster mimosae* were susceptible to *M. anisopliae* in the laboratory bioassays. For *C. mimosae*, the mean mortality for the ants exposed to *M. anisopliae* was 28.47 ± 1.08% compared to the control mortality of 23.33 ± 1.57% ($F = 7.29$; $p = 0.0072$). However, the opposite pattern was observed in *Camponotus* sp., significantly higher

mean mortalities were recorded in controls (60) relative to treatments (50) $F = 13.01$; $p = 0.0004$) (Table 3).

**Table 3**: Mean mortalities obtained from laboratory assays on *Crematogaster mimosae* and *Camponotus* sp of ants

| Ant species | Mean mortalities % | | *F* value | *p* value |
|---|---|---|---|---|
| | Treatments | Controls | | |
| *C. mimosae* | 28.47 ± 1.08 | 23.33 ± 1.57 | 7.29 | 0.0072 |
| *Camponotus* sp. | 50 | 60 | 13.01 | 0.0004 |

### 4.3 Abundance and diversity of cockroaches

Cockroaches were identified up to the order level (Order Blattoidea). Generally, the number of individuals recovered was low.

#### 4.3.1 Abundance and diversity of cockroaches before mound treatment

Sampling prior to the application of *M. anisopliae* on mounds designated to be treated was done in July 2007. The highest total number of cockroaches (16) was recorded on the mounds. The diversity indices obtained showed no significant differences in the mounds designated to be controls from those designated to be sprayed with *M. anisopliae* ($F$= 106.52; $p = 0.062$). Similarly, there was no evidence of variation on and off the mounds ($F = 3.349$; $p = 0.318$) (Figure 6).

### 4.3.2 Abundance and diversity of cockroaches in the control versus the treated mounds

From the control mounds, the highest number of cockroaches was recorded on and off mounds in January 2008 (12 each) while the least were on the other hand collected off mound during the month of October 2007 and July 2008. The Shannon diversity index was correspondingly high in January 2008. In October 2007 and July 2008, the figures obtained both on and off mounds were too low (Fig. 7) for the respective diversity indices to be calculated.

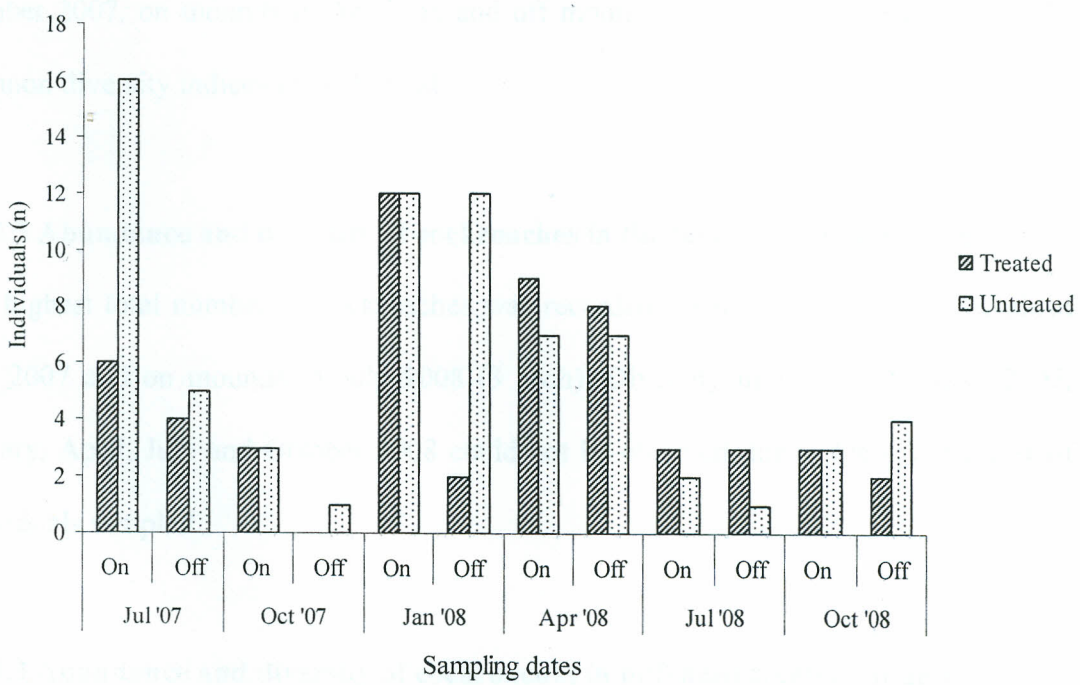



**Figure 7**: Cockroach abundance obtained from the control and the treated mounds across the sampling sessions.

### 4.3.3 Abundance and diversity of cockroaches in the fence treatments across the mounds

The diversity and abundance of the cockroaches sampled were compared to determine if the fence treatment resulted in variations. The results below were obtained.

#### 4.3.3.1 Abundance and diversity of cockroaches in the fenced control mounds

The number of cockroaches varied across the study period with the highest number being recorded on mounds in July 2007 (11). Numbers were too low on and off mounds in October 2007, on mounds in April, on and off mounds in July 2008 (Figure 7) for the Shannon diversity indices to be derived.

#### 4.3.3.2 Abundance and diversity of cockroaches in the fenced treated mounds

The highest total number of cockroaches was recorded off mound during the month of July 2007 and on mounds in July 2008 (3 each). Diversity indices for October 2007, January, April, July and October 2008 could not be obtained due to the low number of individuals sampled.

#### 4.3.3.3 Abundance and diversity of cockroaches in unfenced treated mounds

The highest total number of cockroaches was recorded on mounds in January 2008 (10). No cockroaches were recovered off mounds during the months of October 2007, January 2008 and on mounds in July 2008 (Figure 7).

Overall, the cockroaches were consistently more abundant on the mounds than off the mounds in both treated and untreated mounds. The fungus pre-application sampling session yielded the highest number of cockroaches ($n$ = 31). Species richness was higher in the controls before the fungus was applied. In October, 2007 there was a reduction in the number of cockroaches comparable to what was being observed in the treated mounds too. The number of species was higher in the controls ($n$ = 30) compared to that of the treated mounds ($n$ = 26). The difference was however not significant. The reduction in species richness over the October sampling session was observed in both the controls and the treated mounds.

### 4.4 Abundance and diversity of mantids

The sampled mantids were identified down to the order level (Order Mantodea).

#### 4.4.1 Abundance and diversity of mantids before mound treatment

Sampling prior to the application of *M. anisopliae* on the mounds designated to be treated was done in July 2007 during which the highest total number of mantids was recorded on the mounds (61). There was no significant difference in species diversity among the mounds designated for different treatments ($F$ = 0.00, $p$ = 1.0). There also were no significant variations on and off the mounds either ($F$ = 0.00, $p$ = 1.0).

### 4.4.2 Comparison of abundance and diversity of mantids in the control versus the treated mounds

From the control mounds, the highest number of mantids was recorded on mound during the month of July 2007. The least number of mantids were on the other hand collected off mound in April, July 2008. The highest Shannon diversity index was recorded off the mounds in October 2007 while the lowest was obtained on mounds in April 2008 (3). In January and April, 2008, the figures obtained both on and off mounds were too low for the respective diversity indices to be calculated (Figure 8).

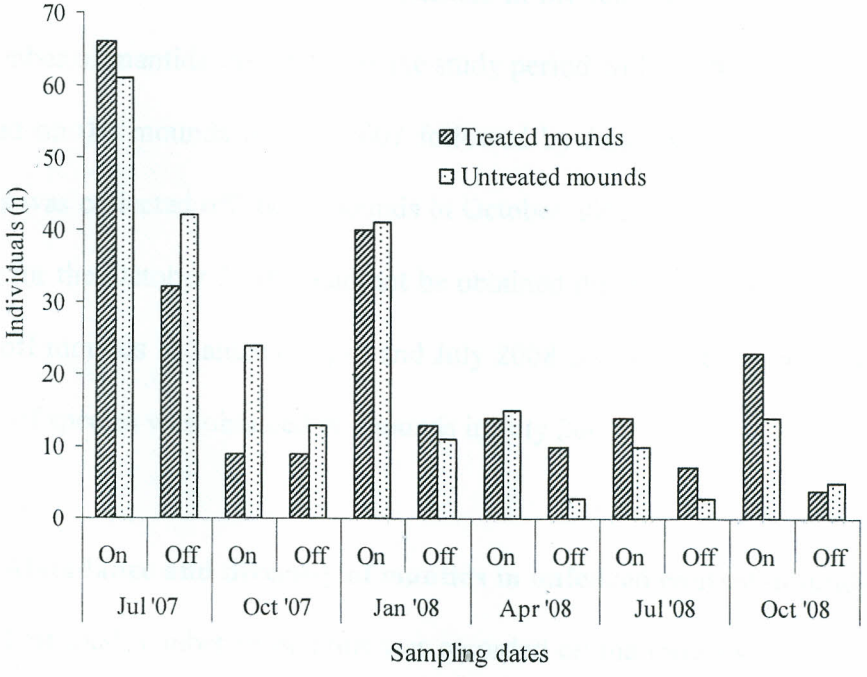



**Figure 8**: Mantid abundance obtained from the control and the treated mounds across the sampling sessions.

In the treated mounds, the highest total number of mantids (40) was recorded on the mounds in January 2008 while the least number were on the other hand collected off

mound in July (7) and October 2008 (4). The Shannon diversity index was highest on the mounds in October 2007 while the lowest indices were obtained in October 2008 on and off the mounds.

### 4.4.3 Comparison of abundance and diversity of mantids in the fence treatments across the mounds

The diversity and abundance of the mantids sampled were compared to determine if the fence treatment had resulted in variations.

#### 4.4.3.1 Abundance and diversity of mantids in the fenced control mounds

The number of mantids varied across the study period with the highest total number being recorded on the mounds in July 2007 followed by January 2008. The least number of mantids was collected off these mounds in October 2008 (Fig. 9). The Shannon diversity indices for the October 2008 could not be obtained due to the low numbers. The $H'$ was higher off mounds in January, April and July 2008 compared to on mounds. The highest number of species was obtained on mounds in July 2007.

#### 4.4.3.2 Abundance and diversity of mantids in unfenced control mounds

The highest total number of mantids was recorded on the mounds in July 2007 followed by January 2008 while the least numbers were collected off these mounds in April and July 2008 (Fig. 9). The Shannon diversity indices for the April and July 2008 could not be obtained due to the low numbers of individuals recovered. The highest number of species was obtained on and off mounds in July 2007

#### 4.4.3.3 Abundance and diversity of mantids in the fenced treated mounds

The highest total number of mantids was recorded on the mounds in the month of January 2008 (22) while the least number of individuals were collected off these mounds in April and July 2008 (1 each) (Fig. 9). The Shannon diversity indices could not be derived in April and July 2008. The highest number of species was obtained on the mounds in July 2007 before the fungus was applied.

#### 4.4.3.4 Abundance and diversity of mantids in the unfenced treated mounds

The highest total number of mantids was recorded on the mounds in January 2008 (18). The least number of individuals were collected off these mounds in October 2008 (2) (Fig. 8). The Shannon diversity indices for October 2008 could not be obtained due to the low numbers. There $H'$ was higher off mounds in January, April and July 2008 compared to on mounds. The highest number of species was obtained on mounds in July 2007.

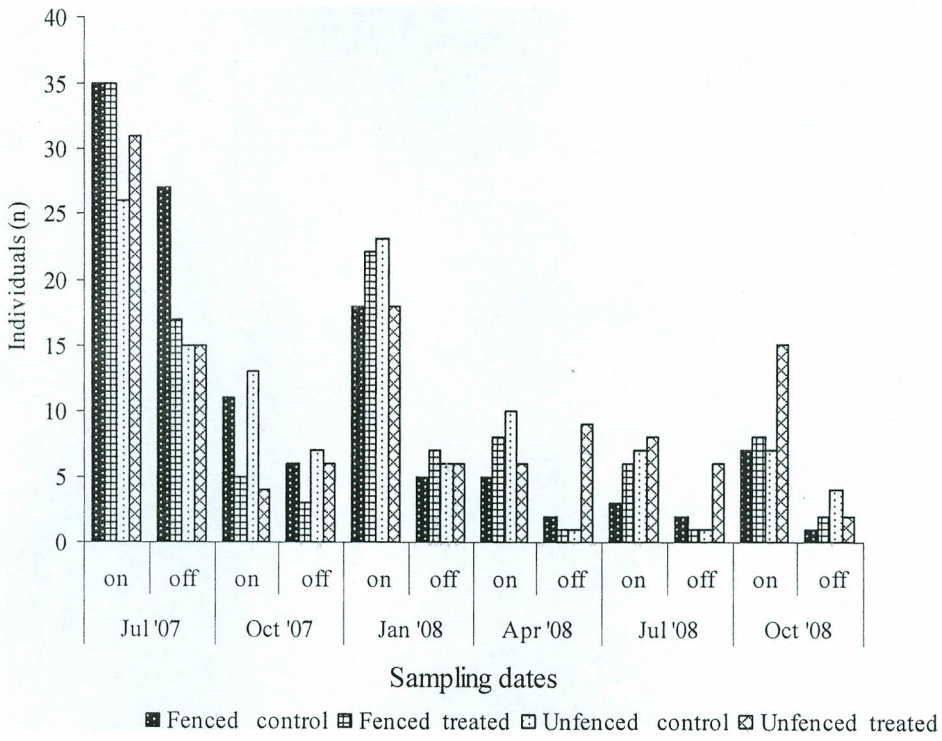



**Figure 9**: Mantid abundance obtained from the fenced and unfenced control and treated mounds across the sampling sessions.

### 4.4.4 Effects of the application of *M. anisopliae* on plant diversity

The effects of application of *M. anisopliae* on plant diversity assessed in terms of relative plant cover across different sampling categories revealed a total of 66 plant species. Plant species varied in distribution across the different categories as reflected in variation in the areas covered by respective species (Appendix 1 a – f). The most dominant species included *Brachiaria lachnatha*, *Pennisetum mezianum, Pennisetum starmineum* and *Themeda triandra* (Plates 10, 11, 12 and 13 respectively). These plant species occurred mainly around *Odontotermes* sp. termite mounds.

**Plate 10**: *Brachiaria lachnatha*

**Plate 11**: *Pennisetum mezianum*

**Plate 12**: *Pennisetum straminium*

**Plate 13**: *Themeda triandra*

Generally, the vegetation cover varied across the sampling dates ($F = 15.48$; $p < 0.0001$) with evidence of variation among plant species ($F = 72.69$; $p = 0.0000$). The highest vegetation cover was recorded in July (LSM = 4.19) followed by October 2007 (LSM = 4.12) and October 2008 (LSM = 3.66) with the lowest cover recorded in July 2008 (LSM = 1.27; Table 4). However, within the individual sampling dates, there was no evidence of variation in vegetation cover around the treated and untreated mounds ($F = 0.003$; $p = 0.96$), suggesting that effects of fungus on insect species did not translate to changes in vegetation cover. Similarly, there was no evidence of variation in vegetation cover with respect to fence treatments ($F = 2.29$; $p = 0.13$).

**Table 4**: Relative vegetation cover across the different sampling dates.

| Sampling session | Back transformed LSM (± SE) |
|---|---|
| July 2007 | $4.19 \pm 0.47^{A}$ |
| October 2007 | $4.12 \pm 0.47^{A}$ |
| January 2008 | $2.44 \pm 0.50^{B}$ |
| April 2008 | $2.24 \pm 0.48^{B}$ |
| July 2008 | $1.27 \pm 0.48^{C}$ |
| October 2008 | $3.66 \pm 0.46^{A}$ |

Levels not having the same letter are significantly different ($p < 0.05$)

# CHAPTER FIVE: DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

## 5.1 Discussion

The main objective of this study was to investigate whether *M. anisopliae* applied to control *Odontotermes* sp. of termites has the potential to cause effects on the diversity of hymenopteran ants, cockroaches, mantids and plants that are associated with these termite mounds. Prior to the application of the fungus *M. anisopliae*, there was no significant difference in ant diversity between the mounds regardless of whether they were designated to be the controls or treatments. Two months after the fungal treatment, there was an increase in ant population suggesting a quick response of ants to ecosystem changes. However, the increase in ant abundance was not accompanied by a similar increase in species richness. These results suggest that ecological changes effected by the application of *M. anisopliae* might have favoured some ant species. For instance, species of ants belonging to the *Dorylus* genus (*D. molestus* and *D. affinis*) ended up dominating the community by high percentages. The *Dorylus* sp. also known as safari ants, driver ants or army ants, are primarily found in Central and East Africa where they are nomadic, forming seasonal temporary ant hills (Kronauer *et al.*, 2007). When food supplies dwindle, they leave the hills and form marching columns of up to 50,000 ants (Schöning *et al.*, 2005, Leroux, 1982). Their presence, though a menace to people can conversely be considered beneficial to certain human communities as they perform a pest prevention service in farming communities. *Dorylus* species are capable of feeding on crop pests. They overwhelm most invertebrates that do not get out of their way and occasionally also small vertebrates (Gotwald, 1995).

Infection by entomopathogenic fungi can result in behavioural changes such as sluggishness before insect death (Rath, 2000; Culliney and Grace, 2000). The epizootic might have rendered termites weak. Infected termites are avoided and sealed off from the rest of the colony (Rath, 2000) thus rendering them as easy targets / prey and could have attracted the aggressive, predatory foraging groups thus the 'raids' or 'invasions'. Application of *M. anisopliae* appears to have directly affected the population of termites while indirectly favouring some ant species which are mainly predatory in behaviour.

The increase in ant population was observed in both the control and the treated mounds. This may be attributed to ecological characteristics of the dominant ant species, *D. molestus* and *D. affinis*. Due to the invasive nature of these species, they appear to have a potential for dispersal and re-distribution between plots (Matteson, 1992). They did not discriminate between fungus - infected and uninfected termites. Vosseler (1905) reported that army ant colonies change their bivouac sites (being temporarily camped in more or less exposed position) whenever the surrounding food supply is exhausted and this could have led to the indiscriminate spread across all the termite mounds.

Generally, the number of ants collected in the April, July and October 2008 sampling sessions compared well to the pre-application session in July 2007 suggesting that populations of termites may have recovered. A number of theories have been put forward to explain this. Several authors have reported that the conidia of virulent strains of *M. anisopliae* are repellent to termites (Rath and Tidbury, 1996; Staples and Milner, 2000; Myles, 2002). The conidia may trigger alarm and aggregation around spore-dusted

individuals who are then groomed, bitten, dismembered, defecated upon or even buried. Such reactions limit the impact of *M. anisopliae*. The other theory would be that the fungus did not reach the colony nursery or most importantly, the queen. This led to partial elimination of the termite colonies thus the population recovery. Recovery is also possible where the section with infected individuals is sealed off and supplementary queens continue with reproduction in another section of the mound.

*Crematogaster mimosae* numbers were higher on the mounds and decreased away from the mounds in both the treated and the untreated mounds. However, their numbers were not consistent in all sampling sessions as these considerably reduced in October 2007 and January 2008, coinciding with the time when *Dorylus* species greatly increased in numbers. *Dorylus* species are known to sweep almost all forms of animal life on their way including insects and sluggish ground dwelling creatures (Hölldobler and Wilson, 1990). This may explain the trend observed in *Crematogaster* population fluctuations.

Although the indirect effect trials in the field showed no evidence of acute toxicity of *M. anisopliae* on non-target organisms, the fungus had lethal effects on *Crematogaster mimosae*, but not *Camponotus* sp., in laboratory bioassays meant to assess the direct effects. In *C. mimosae*, the mean deaths were higher in treated ants compared to controls suggesting some degree of direct effect. In contrast, there was a relatively higher mean mortality among the untreated *Camponotus* species, results that may not be attributed to applied fungus but to stressful experimental conditions. It is possible that a host can be infected in the laboratory due to the optimized conditions but the same species may not

be infected in the field due complex biotic and abiotic interactions that occur in the field (Hajek and Butler, 1999). Consequently, great caution must be exercised when attempting to extrapolate laboratory results in field trials (Goettel and Hajek, 2001).

A dozen or so genera of cockroaches have been found in a state of either known or suspected commensalism, in the nests of ants, wasps or termites (Cornwell, 1968). The majority of cockroach species are solitary, however, a number of them are gregarious or subsocial (Bell *et al.*, 2007). Their persistence on termite mounds appears to be driven by their use of mounds as a refuge. On many occasions the cockroaches were observed lining the termite mound vent walls.

There was a general decrease in their numbers after fungal application. Probably, the predatory and scavenging ants that raided the mound areas to feed on dead and weakened termites also fed on the cockroaches. The fact that the decrease was observed in both treated and untreated mounds further emphasizes the indiscriminate nature of the raids carried out by the *Dorylus* species. The increase in number of cockroaches in January 2008 suggested recovery of cockroach population from the fungus effect and it was not surprising that it coincided with the decrease in *Dorylus* sp. The reduction in the number of *Dorylus* sp. could have eased off the predatory pressure exerted on the cockroaches resulting in population recovery.

In the treated mounds, more cockroaches were sampled on the mounds compared to off the mounds. If the fungus had any repellent effects on the cockroaches, then more would have been found further away from the mounds (off mound).

The order Mantodea (Mantids) is mainly known for low mobility, high mimicry and low population density. Though mantids were generally low in numbers, relatively higher numbers were recorded around the mounds in July 2007 just before the application of the fungus. What could they be benefiting from their heavy presence on mounds? They are carnivorous and feed on many species of insects including soft-shelled turtles, mice, frogs, birds and newts (Prete, 1990). Preliminary studies showed that invertebrates are three times more abundant on mounds compared to off mounds. Thus, mantids are associating with mounds as sources of high prey density.

The number of mantids was lower in October than in July 2007. It is possible that *Dorylus* sp. of scavenging and predatory ants that were abundant in October fed on them and reduced their numbers. Alternatively, the *Dorylus* sp. of ants swept almost all forms of animal life on their way thus drastically reducing the amount of food available to the mantids. A synergism between the two explanations does not seem impossible as both factors could have led to the decrease in mantid numbers. The recovery in numbers over the January 2008 sampling session could have occurred as a result of a reduction in *Dorylus* sp. This could have led to either a reduction in resource exploitation competition between the two predators or a reduction in predatory pressure on mantids by *Dorylus* sp.

Termites mediate several ecological processes in the soil and in their absence, vegetation could be affected. The vegetation characteristics were used to detect changes in vegetation cover of which results revealed a significant difference in the species encountered across the sampling times. Observed variation in species diversity and vegetation cover may be attributed to the seasonality of some plant species. The seasonal variations could have led to a reduction or increase of particular species at different times during the study period.

However, within the individual sampling dates, there was no significant difference in diversity and relative vegetation cover across the treated and control mounds implying that the fungus had no effect on the vegetation found on these mounds despite a general reduction in the relative cover over time. There are two possible explanations:

i) Plants take a relatively longer time to respond to such ecosystem changes. The fungus was applied in the month of August, 2007 and from the previous discussion, there seems to have been a recovery or re-establishment of the colonies within a period of approximately eight months. Recovery from the environmental changes could have begun before the effect established. Mando *et al.* (1999) observed that efficient use of infiltrated water and plant diversity did not vary among treatments until the third year of study. Time taken during this study could have been short to reverse changes termites had effected in the soil.

ii) Authors assert that these modifications have a great impact on the vegetation through spatial and temporal effects, even when the termite colony is dead and the mound material subject to erosion (Glover *et al.*, 1964; Belsky *et al.*, 1983).

The overall picture given from crossing of the test effects indicate that the fungal and fencing treatment did not have any significant effects on the relative vegetation cover across the sampling sessions.

**5.2 Conclusions**

The importance of termites and ants in the ecosystem cannot be overemphasized. By definition, a keystone species is an animal or plant species whose removal or extinction has a wide ranging influence on community composition and this can alter competitive relationships and relative abundances of other species in a community. If these are guidelines to meet to qualify as a keystone species, then from this study, the termites are here confirmed as keystone species.

Human disturbance results in a change in species richness, disruption in patterns of endemism, change in abundance patterns and modification of ecosystem structural properties. Removal of termites from termite mounds altered their competitive relationships with the ants. Pesticide hazard to a non-target species is evaluated by combining data on pesticide toxicity and exposure. The risk is then interpreted in the light of life history, application patterns, habitat and other factors that will determine the severity of the effects and the ability of the population to recover. The field experiment which was designed to address the indirect effects of *M. anisopliae* on the non-targets

showed that the fungus had no significant effect on the diversity of ants, cockroaches, mantids and vegetation. However, laboratory assays designed to assess direct effects of *M. anisopliae* on the selected ant species showed that the fungus had no direct effect on the ants. The higher mortality observed in the treated *C. mimosae* was attributed to optimum conditions supplied in the laboratory that could have favoured fungal infections. There is need to exercise great caution while extrapolating such results to the field.

Indirect estimates based on ant diversity suggest that the termite colonies may have recovered within eight months of fungus application. Based on cockroaches and mantids data, this time may have been even shorter. This shows that the degree of severity of *M. anisopliae* on termite colonies is trivial; a conclusion based on the time the system snapped back to normality. An ecosystem previously dominated by a keystone species, an ecosystem engineer and a detritivore (the termites) was for a few of months swapped with another keystone species, ecosystem engineer and a predator (*Dorylus* sp. of ants) corroborating findings that if a competitive keystone disappears, other plants or animals that play similar roles in the community prosper.

The results of this study indicate that *M. anisopliae* isolate ICIPE 30 can safely be used for the control of *Odontotermes* species as it does not show indirect effects on the selected non-targets that interact with termites under field conditions. The species that was infected in the laboratory may be included in the fungus' host range but extreme caution must be exercised when extrapolating laboratory results to the field. Besides, it is

in the field that the most definitive and useful information is obtained when it comes to pathogen effects on non-targets.

**5.3 Recommendations**

1) Due to advantages associated with microbial fungi, control of *Odontotermes* sp. of termites using ICIPE 30 still be taken up. Apart from ICIPE 30 being safe to non-targets tried in this study, fungal microbial control agents have several advantages to offer compared to chemical insecticides. They can be integrated with other biocontrol agents, can establish in the pest population thereby offering a prolonged period of control and are biodegradable (Goettel and Johnson, 1992).
2) There is need to establish optimum fungus dosage and the frequency of application suitable for complete elimination of the termite colonies. This stems from the observation that termite colonies appear to have recovered eight months after fungus application, probably due to partial elimination of the colonies.
3) Further research be undertaken on the diversity of plants to allow for the experiment to be repeated over a longer period of time. Diversity changes need to be monitored over a longer period than this study allowed.

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# APPENDICES

**Appendix 1 (a)**: Identified plant species and their respective covers (%) with respect to different sampling categories in July 2007 pre-application session.

| Plant species | Fenced | | Non-fenced | |
|---|---|---|---|---|
| | Treated | Control | Treated | Control |
| *Acacia drepanolobium* | 0.07 ± 0.00 | 1.04 ± 1.84 | 0.14 ± 0.00 | 0.64 ± 2.5 |
| *Acacia mellifera* | — | 0.06 ± 0.00 | — | — |
| *Aerva lanata* | — | 0.14 ± 0.00 | 0.32 ± 0.05 | 0.34 ± 0.49 |
| *Aristida* | — | 0.21 ± 0.00 | — | 0.14 ± 0.00 |
| *Armania ulughi* | 0.70 ± 0.00 | 0.69 ± 0.01 | 0.68 ± 0.02 | 0.62 ± 0.01 |
| *Aspilia* | 0.06 ± 0.00 | 0.13 ± 0.01 | 0.14 ± 0.00 | — |
| *Bothriochloa* | 1.90 ± 1.27 | 0.44 ± 3.21 | 1.28 ± 0.74 | 2.00 ± 0.97 |
| *Brachiaria* 3 | — | 0.33 ± 0.00 | 0.26 ± 0.00 | — |
| *Brachiaria eruciformius* | — | — | 0.07 ± 0.00 | — |
| *Brachiaria lachnatha* | 14.0 ± 2.72 | 15.2 ± 2.25 | 16.3 ± 0.13 | 15.5 ± 2.48 |
| *Cadaba farinosa* | 0.42 ± 0.71 | 0.06 ± 0.00 | — | — |
| *Commelina* | 0.14 ± 0.00 | 0.11 ± 0.00 | 0.07 ± 0.00 | 0.28 ± 0.23 |
| *Commicarpus pedunculosus* | — | — | 0.07 ± 0.00 | — |
| *Conyza* | 0.06 ± 0.00 | — | — | — |
| *Cynodon plectostachyus* | — | 0.28 ± 0.00 | 0.28 ± 0.00 | — |
| *Digitaria* 2 | 1.28 ± 0.71 | 3.61 ± 3.76 | 3.07 ± 2.35 | 2.14 ± 3.27 |
| *Dischoriste radicans* | 0.21 ± 0.35 | 0.07 ± 0.00 | — | — |
| *Eragrostis* 2 | — | — | 0.30 ± 0.20 | — |
| *Helichrysum pseudognaphalium* | 2.90 ± 0.94 | 3.73 ± 0.94 | 1.59 ± 0.66 | 3.07 ± 0.97 |
| *Hermania ulugni* | — | 0.06 ± 0.00 | 0.04 ± 0.00 | 0.14 ± 0.00 |
| *Hibiscus flavifolius* | — | 0.07 ± 0.00 | 0.09 ± 0.00 | — |
| *Indigofera brew* | — | — | 0.06 ± 0.00 | — |
| *Indigofera schimperi* | 0.21 ± 0.35 | — | — | 0.14 ± 0.00 |
| *Leucus spp* | — | — | 0.07 ± 0.00 | — |
| *Lintonia nutans* | 3.78 ± 1.28 | 4.72 ± 1.37 | 3.42 ± 0.69 | 7.37 ± 1.14 |
| *Lycium spp* | — | — | — | 0.28 ± 0.00 |
| *Mariscus (Cyperus sp.)* | — | 0.07 ± 0.00 | 0.09 ± 0.00 | 0.21 ± 0.00 |
| *Misopates* | — | 0.07 ± 0.00 | — | — |
| *Monechima* | 0.92 ± 0.83 | 0.48 ± 0.16 | 0.21 ± 0.35 | 0.50 ± 0.17 |
| *Pavonia* | — | 0.06 ± 0.00 | 0.04 ± 0.00 | — |
| *Pennisetum mezianum* | 3.80 ± 0.81 | 5.88 ± 1.68 | 4.15 ± 1.48 | 4.42 ± 1.07 |
| *Pennisetum straminium* | 31.0 ± 5.10 | 34.0 ± 3.10 | 40.1 ± 3.98 | 36.8 ± 4.36 |
| *Phyllanthus maderaspatensis* | 0.20 ± 0.30 | 0.20 ± 0.00 | 0.14 ± 0.00 | 0.14 ± 0.00 |
| *Plectranthus* | — | 0.13 ± 0.00 | — | — |
| *Polygala sp* | 0.21 ± 0.35 | — | — | 0.07 ± 0.00 |
| *Rhinacanthus* | 0.21 ± 0.00 | 0.28 ± 0.00 | 0.09 ± 0.00 | 0.46 ± 0.51 |
| *Rhynchosia usambarensis* | 0.40 ± 0.00 | 0.14 ± 0.00 | — | 0.07 ± 0.00 |
| *Solanum big (incanum)* | 0.07 ± 0.00 | — | 0.14 ± 0.00 | — |
| *Sporobolus* | — | — | 1.23 ± 0.00 | — |
| *Themeda triandra* | 11.0 ± 2.97 | 10.3 ± 1.70 | 9.15 ± 2.53 | 9.84 ± 1.60 |
| Bare | 3.56 ± 0.82 | 3.33 ± 0.75 | 3.26 ± 0.76 | 4.98 ± 2.38 |

**Appendix 1 (b)**: Identified plant species and their respective covers (%) with respect to different sampling categories in October 2007.

| Plant species | Fenced | | Non-fenced | |
|---|---|---|---|---|
| | Treated | Control | Treated | Control |
| *Acacia drepanolobium* | — | 0.85 ± 2.14 | 0.07 ± 0.00 | 0.57 ± 0.00 |
| *Acacia mellifera* | — | 0.26 ± 0.00 | — | — |
| *Aerva lanata* | 0.06 ± 0.00 | 0.21 ± 0.20 | 0.14 ± 0.00 | — |
| *Aristida* | — | 0.21 ± 0.35 | — | 0.21 ± 0.00 |
| *Armania ulughi* | 0.70 ± 0.00 | 0.69 ± 0.01 | 0.69 ± 0.02 | 0.70 ± 0.00 |
| *Aspilia* | 0.20 ± 0.01 | 0.07 ± 0.00 | — | 0.14 ± 0.00 |
| *Bothriochloa* | 2.03 ± 2.78 | 2.68 ± 1.03 | 0.75 ± 0.26 | 1.18 ± 0.65 |
| *Brachiaria eruciformius* | — | 0 ± 0 | 0.21 ± 0.00 | 0.19 ± 0.26 |
| *Brachiaria lachnatha* | 14.2 ± 2.91 | 14.2 ± 2.90 | 15.4 ± 2.37 | 15.8 ± 2.84 |
| *Cadaba farinosa* | 0.64 ± 2.5 | 0.07 ± 0.00 | — | — |
| *Commelina* | — | — | 0.14 ± 0.00 | 0.12 ± 0.00 |
| *Commicarpus pedunculosus* | — | 0.06 ± 0.00 | — | — |
| *Digitaria* 2 | 1.71 ± 2.47 | 4.24 ± 2.90 | 3.22 ± 2.69 | 3.12 ± 3.32 |
| *Dischoriste radicans* | 0.14 ± 0.00 | 0.07 ± 0.00 | 0.14 ± 0.00 | 0.13 ± 0.04 |
| *Eragrostis* 2 | — | — | 0.40 ± 0.24 | 0.06 ± 0.00 |
| *Eurphobia inaequilatera* | 0.07 ± 0.00 | — | — | — |
| *Helichrysum, Pseudognaphalium* | 2.30 ± 0.73 | 3.12 ± 1.01 | 1.76 ± 0.64 | 2.49±0.70 |
| *Hermania ulugni* | 0.14 ± 0.00 | 0.07 ± 0.00 | 0.05 ± 0.00 | — |
| *Hibiscus flavifolius* | — | — | 0.17 ± 0.14 | — |
| *Indigofera schimperi* | 0.07 ± 0.00 | — | 0.07 ± 0.00 | 0.07 ± 0.00 |
| *Ipomoea sinensis* | 0.07 ± 0.00 | — | — | — |
| *Lintonia nutans* | 4.50 ± 1.85 | 3.40 ± 0.82 | 3.86 ± 0.89 | 7.75 ± 1.45 |
| *Lycium* | — | — | — | 0.28 ± 0.00 |
| *Mariscus (Cyperus sp.)* | 0.06 ± 0.00 | 0.14 ± 0.00 | 0.17 ± 0.14 | — |
| *Microchloa kunthii* | — | — | — | 0.14 ± 0.00 |
| *Monechima* | 0.64 ± 0.71 | 0.71 ± 0.85 | — | 0.21 ± 0.35 |
| *Pavonia* | — | 0.13 ± 0.02 | — | — |
| *Pennisetum mezianum* | 3.31 ± 0.64 | 4.01±1.30 | 5.55 ± 2.02 | 4.66 ± 1.01 |
| *Pennisetum straminium* | 32.8 ± 4.50 | 32.1±3.25 | 39.0 ± 4.27 | 33.8 ± 4.02 |
| *Phyllanthus maderaspatensis* | — | — | 0.07 ± 0.00 | 0.07 ± 0.00 |
| *Plectranthus* | — | 0.07 ± 0.00 | — | — |
| *Polygala sp* | — | — | — | 0.07 ± 0.00 |
| *Rhinacanthus* | 0.28 ± 0.00 | 0.14 ± 0.00 | — | 0.25 ± 0.00 |
| *Rhynchosia usambarensis* | 0.60 ± 2.30 | 0.14 ± 0.00 | — | 0.14 ± 0.00 |
| *Solanum big (incanum)* | 0.07 ± 0.00 | 0.13 ± 0.00 | 0.21 ± 0.35 | — |
| *Solanum small* | 0.07 ± 0.00 | — | — | — |
| *Sporobolus* | 1.5 ± 0.00 | — | — | — |
| *Themeda triandra* | 10.4 ± 3.32 | 11.5 ± 1.98 | 11.0 ± 2.35 | 11.1 ± 1.92 |
| *Tribulus terrestris* | — | 0.78 ± 0.00 | — | — |
| *Zyenedon* | 0.14 ± 0.00 | 0.21 ± 0.00 | 0.35 ± 0.00 | — |
| Bare | 4.33 ± 0.90 | 4.31 ± 0.99 | 4.17 ± 0.99 | 6.26 ± 1.96 |

**Appendix 1 (c)**: Identified plant species and their respective covers (%) with respect to different sampling categories in January 2008.

| Plant species | Fenced | | Non-fenced | |
|---|---|---|---|---|
| | Treated | Control | Treated | Control |
| *Acacia drepanolobium* | 0.11 ± 0.00 | 0.70 ± 0.72 | — | 0.05 ± 0.00 |
| *Aerva lanata* | 0.05 ± 0.00 | 0.17 ± 0.00 | 0.17 ± 0.29 | 0.05 ± 0.00 |
| *Aristida* | — | 0.05 ± 0.00 | — | 0.17 ± 0.29 |
| *Aspilia* | — | 0.05 ± 0.00 | — | 0.11 ± 0.00 |
| *Bothriochloa* | 1.17 ± 2.18 | 2.29 ± 0.74 | 0.56 ± 0.11 | 0.70 ± 0.41 |
| *Brachiaria lachnatha* | 11.1 ± 2.36 | 12.2 ± 1.97 | 11.6 ± 2.10 | 12.2 ± 2.33 |
| *Cadaba farinosa* | 0.76 ± 3.23 | 0.05 ± 0.00 | — | — |
| *Commelina* | — | 0.11 ± 0.00 | 0.05 ± 0.00 | — |
| *Conyza* | — | — | — | 0.05 ± 0.00 |
| *Cynodon plectostachyus* | 0.05 ± 0.00 | — | 0.05 ± 0.00 | — |
| *Cyperus spp* | 0.05 ± 0.00 | 0.05 ± 0.00 | 0.15 ± 0.00 | 0.05 ± 0.00 |
| *Digitaria* 2 | 0.94 ± 1.28 | 3.52 ± 3.04 | 2.38 ± 1.54 | 2.11 ± 3.01 |
| *Dyschoriste radicans* | 0.17 ± 0.00 | 0.05 ± 0.00 | — | — |
| *Eragrostis spp* | — | — | 0.22 ± 0.01 | 0.17 ± 0.29 |
| *Helichrysum pseudognaphalium* | 1.76 ± 0.56 | 1.64 ± 0.72 | 0.78 ± 0.46 | 1.05 ± 0.77 |
| *Hibiscus flavifolius* | — | 0.05 ± 0.00 | 0.10 ± 0.00 | — |
| *Indigofera brew* | 0.05 ± 0.00 | — | — | — |
| *Indigofera schimperi* | 0.05 ± 0.00 | — | — | — |
| *Lintonia nutans* | 2.82 ± 1.09 | 2.88 ± 0.54 | 3.46 ± 0.71 | 5.52 ± 0.95 |
| *Lycium spp* | — | — | — | 0.17 ± 0.00 |
| *Monechima* | 0.17 ± 0.29 | 0.23 ± 0.00 | 0.05 ± 0.00 | 0.23 ± 0.19 |
| *Pennisetum mezianum* | 2.00 ± 0.45 | 4.35 ± 1.28 | 3.65 ± 1.32 | 3.82 ± 0.86 |
| *Pennisetum straminium* | 25.0 ± 3.85 | 27.0 ± 3.32 | 30.3 ± 3.74 | 25.4 ± 2.80 |
| *Phyllanthus maderaspatensis* | 0.05 ± 0.00 | — | 0.05 ± 0.00 | — |
| *Plectranthus* | — | 0.23 ± 0.00 | — | — |
| *Rhinacanthus* | 0.05 ± 0.00 | 0.05 ± 0.00 | 0.05 ± 0.00 | 0.11 ± 0.00 |
| *Rhynchosia usambarensis* | — | 0.05 ± 0.00 | — | — |
| *Solanum big (incanum)* | — | — | 0.11 ± 0.00 | — |
| *Sporobolous* 2 | — | — | — | 0.11 ± 0.00 |
| *Sporobolus* | — | — | 0.88 ± 0.00 | — |
| *Themeda triandra* | 8.82 ± 2.06 | 9.41 ± 1.65 | 7.24 ± 2.15 | 7.76 ± 1.40 |
| Bare | 7.76 ± 1.60 | 6.23 ± 0.95 | 6.84 ± 1.15 | 9.64 ± 2.45 |

**Appendix 1 (d)**: Identified plant species and their respective covers (%) with respect to different sampling categories in April 2008.

| Plant species | Fenced | | Non-fenced | |
|---|---|---|---|---|
| | Treated | Control | Treated | Control |
| *Acacia drepanolobium* | 0.12 ± 0.00 | 0.88 ± 0.87 | — | 0.35 ± 1.17 |
| *Acacia mellifera* | — | 0.12 ± 0.00 | — | — |
| *Aerva lanata* | 0.06 ± 0.00 | 0.06 ± 0.00 | 0.18 ± 0.00 | 0.06 ± 0.00 |
| *Aristida* | — | 0.12 ± 0.00 | — | — |
| *Aspilia* | — | — | 0.06 ± 0.00 | — |
| *Bothriochloa* | 1.12 ± 1.37 | 1.76 ± 0.93 | 0.52 ± 0.22 | 0.94 ± 0.86 |
| *Brachiaria* 3 | 0.06 ± 0.00 | — | 0.11 ± 0.03 | 0.17 ± 0.29 |
| *Brachiaria eruciformius* | — | — | 0.05 ± 0.00 | — |
| *Brachiaria lachnatha* | 11.4 ± 2.29 | 11.2 ± 1.90 | 10.1 ± 1.75 | 9.58 ± 1.96 |
| *Cadaba farinosa* | 0.82 ± 3.52 | — | — | — |
| *Commelina* | 0.06 ± 0.00 | — | 0.06 ± 0.00 | — |
| *Commicarpus pedunculosus* | — | 0.06 ± 0.00 | — | — |
| *Conyza* | — | 0.06 ± 0.00 | — | — |
| *Cynodon plectostachyus* | — | 0.12 ± 0.00 | 0.18 ± 0.00 | — |
| *Cyperus spp* | 0.12 ± 0.00 | 0.24 ± 0.19 | 0.38 ± 0.68 | 0.05 ± 0.00 |
| *Digitaria* 2 | 0.53 ± 0.67 | 2.06 ± 1.93 | 1.92 ± 1.90 | 1.82 ± 2.63 |
| *Dyschoriste radicans* | 0.18 ± 0.29 | 0.06 ± 0.00 | — | 0.05 ± 0.00 |
| *Eurphobia inaequilatera* | 0.06 ± 0.00 | — | — | — |
| *Evolvulus alsinoides* | — | — | — | 0.05 ± 0.00 |
| *Helichrysum pseudognaphalium* | 1.82 ± 0.80 | 1.71 ± 0.68 | 0.62 ± 0.30 | 0.70 ± 0.29 |
| *Hermania ulugni* | 0.18 ± 0.00 | — | — | 0.05 ± 0.00 |
| *Hibiscus flavifolius* | — | 0.06 ± 0.00 | 0.21 ± 0.00 | — |
| *Indigofera schimperi* | 0.06 ± 0.00 | 0.06 ± 0.00 | — | 0.05 ± 0.00 |
| *Leucus spp* | — | — | 0.06 ± 0.00 | — |
| *Lintonia nutans* | 2.65 ± 1.40 | 2.94 ± 0.97 | 2.56 ± 0.84 | 4.29 ± 0.75 |
| *Lippia javanica* | 0.12 ± 0.00 | — | — | — |
| *Lycium spp* | — | 0.06 ± 0.00 | — | — |
| *Microchloa kunthii* | 0.06 ± 0.00 | — | — | 0.11 ± 0.00 |
| *Monechima* | 0.06 ± 0.00 | 0.12 ± 0.00 | 0.06 ± 0.00 | 0.05 ± 0.00 |
| *Monsonia angustifolia* | — | — | 0.06 ± 0.00 | 0.05 ± 0.00 |
| *Pennisetum mezianum* | 2.24 ± 0.49 | 3.41 ± 1.42 | 3.01 ± 1.16 | 3.17 ± 0.00 |
| *Pennisetum straminium* | 23.6 ± 3.65 | 26.7 ± 3.30 | 29.6 ± 3.12 | 23.7 ± 3.17 |
| *Phyllanthus maderaspatensis* | 0.06 ± 0.00 | 0.24 ± 0.00 | 0.05 ± 0.00 | — |
| *Polygala sphenoptera* | 0.06 ± 0.00 | — | — | — |
| *Portulaca oleracea* | — | — | — | 0.05 ± 0.00 |
| *Rhinacanthus* | 0.06 ± 0.00 | 0.06 ± 0.00 | — | 0.05 ± 0.00 |
| *Rhynchosia usambarensis* | 0.76 ± 0.00 | 0.24 ± 0.58 | — | 0.11 ± 0.00 |
| *Solanum big (incanum)* | — | — | 0.12 ± 0.00 | — |
| *Solanum small* | — | — | — | 0.11 ± 0.00 |
| *Sporobolous* 2 | — | — | — | 0.05 ± 0.00 |
| *Themeda triandra* | 7.24 ± 2.24 | 7.88 ± 1.53 | 6.39 ± 1.30 | 6.05 ± 1.05 |
| Bare | 8.71 ± 1.32 | 8.18 ± 0.82 | 11.5 ± 0.91 | 14.2 ± 2.48 |

**Appendix 1 (e)**: Identified plant species and their respective covers (%) with respect to different sampling categories in July 2008.

| Plant species | Fenced | | Non-fenced | |
|---|---|---|---|---|
| | Treated | Control | Treated | Control |
| *Acacia drepanolobium* | 0.20 ± 0.16 | 0.7 ± 0.52 | 0.15 ± 0.25 | 0.5 ± 2.00 |
| *Acacia mellifera* | — | 0.1 ± 0.00 | — | — |
| *Aerva lanata* | 0.10 ± 0.00 | 0.4 ± 0.60 | 0.2 ± 0.16 | 0.05 ± 0.00 |
| *Aspilia* | — | 0.05 ± 0.00 | 0.05 ± 0.00 | — |
| *Bothriochloa* | 0.85 ± 1.64 | 1.35 ± 0.46 | 0.25 ± 0.16 | 0.55 ± 0.44 |
| *Brachiaria* 3 | 0.4 ± 0.00 | — | 0.25 ± 0.75 | 0.55 ± 0.60 |
| *Brachiaria eruciformis* | — | 0.10 ± 0.00 | 0.10 ± 0.00 | 0.05 ± 0.00 |
| *Brachiaria lachnatha* | 8.80 ± 1.99 | 8.90 ± 1.56 | 7.65 ± 1.43 | 7.40 ± 1.41 |
| *C* | — | — | 0.05 ± 0.00 | — |
| *Cadaba farinosa* | 0.65 ± 1.25 | — | — | — |
| *Commelina* | 0.05 ± 0.00 | 0.05 ± 0.00 | — | — |
| *Commicarpus pedunculosus* | — | 0.05 ± 0.00 | — | — |
| *Cynodon plectostachyus* | — | 0.10 ± 0.00 | — | — |
| *Cyperus spp* | 0.10 ± 0.00 | 0.05 ± 0.00 | 0.15 ± 0.00 | — |
| *Digitaria* 2 | 0.45 ± 0.75 | 1.40 ± 0.98 | 1.70 ± 1.06 | 1.20 ± 1.04 |
| *Dinebra retroflexa* | — | — | — | 0.05 ± 0.00 |
| *Dyschoriste radicans* | 0.10 ± 0.00 | 0.05 ± 0.00 | 0.05 ± 0.00 | 0.05 ± 0.00 |
| *Eurphobia inaequilatera* | 0.05 ± 0.00 | 0.05 ± 0.00 | — | 0.05 ± 0.00 |
| *Helichrysum pseudognaphalium* | 1.55 ± 0.55 | 1.45 ± 0.56 | 0.80 ± 0.26 | 1.50 ± 0.70 |
| *Hermania ulughi* | 0.05 ± 0.00 | — | 0.10 ± 0.00 | — |
| *Hibiscus flavifolius* | 0.05 ± 0.00 | — | 0.15 ± 0.00 | — |
| *Indigofera schimperi* | 0.10 ± 0.00 | 0.10 ± 0.00 | 0.05 ± 0.00 | 0.10 ± 0.00 |
| *Leucus spp* | 0.05 ± 0.00 | — | — | — |
| *Lintonia nutans* | 2.20 ± 1.03 | 2.05 ± 0.73 | 2.05 ± 0.65 | 3.30 ± 0.53 |
| *Melhania* | — | 0.05 ± 0.00 | — | — |
| *Microchloa kunthii* | — | — | — | 0.10 ± 0.00 |
| *Misopates* | — | — | 0.05 ± 0.00 | — |
| *Monechima* | 0.20 ± 0.5 | 0.25 ± 0.12 | 0.15 ± 0.00 | 0.10 ± 0.00 |
| *Pavonia* | — | 0.05 ± 0.00 | — | — |
| *Pennisetum mezianum* | 1.65 ± 0.41 | 2.85 ± 0.81 | 2.65 ± 0.96 | 2.45 ± 0.61 |
| *Pennisetum straminium* | 20.5 ± 3.30 | 22.1 ± 2.74 | 22.8 ± 2.74 | 20.9 ± 2.52 |
| *Phyllanthus maderaspatensis* | 0.05 ± 0.00 | 0.10 ± 0.00 | 0.10 ± 0.00 | 0.00 ± 0.00 |
| *Rhinacanthus* | 0.05 ± 0.00 | 0.20 ± 0.00 | — | — |
| *Rhynchosia usambarensis* | 0.25 ± 0.00 | 0.10 ± 0.00 | 0.05 ± 0.00 | 0.00 ± 0.00 |
| *Solanum small* | 0.05 ± 0.00 | — | — | — |
| *Themeda triandra* | 5.80 ± 1.79 | 7.15 ± 1.19 | 6.00 ± 1.35 | 5.70 ± 1.39 |
| Bare | 8.85 ± 1.26 | 9.5 ± 0.93 | 12.6 ± 1.02 | 17.9 ± 3.82 |

**Appendix 1 (f)**: Identified plant species and their respective covers (%) with respect to different sampling categories in October, 2008.

| Plant species | Fenced | | Non-fenced | |
|---|---|---|---|---|
| | Treated | Control | Treated | Control |
| *Acacia brevispica* | – | – | 1.07 ± 0.00 | – |
| *Acacia drepanolobium* | 0.14 ± 0.00 | 0.76 ± 0.63 | 0.14 ± 0.00 | 0.50 ± 1.78 |
| *Acacia mellifera* | – | 0.13 ± 0.00 | – | – |
| *Aerva lanata* | 0.21 ± 0.00 | 0.62 ± 0.28 | 0.07 ± 0.00 | 0.07 ± 0.00 |
| *Aspilia* | 0.07 ± 0.00 | – | – | – |
| *Bothriochloa* | 1.43 ± 1.60 | 3.23 ± 1.23 | 1.97 ± 0.82 | 1.62 ± 1.33 |
| *Brachiaria* 3 | 0.28 ± 0.71 | – | 0.21 ± 0.32 | 0.42 ± 0.42 |
| *Brachiaria eruciformius* | 2.50 ± 6.56 | 0.40 ± 0.38 | 0.40 ± 0.74 | 0.06 ± 0.00 |
| *Brachiaria lachnatha* | 13.0 ± 3.04 | 13.9 ± 2.22 | 10.3 ± 1.87 | 11.9 ± 2.55 |
| *C* | 0.06 ± 0.00 | 0.13 ± 0.00 | – | 0.07 ± 0.00 |
| *Cadaba spp* | 1.28 ± 3.57 | – | – | – |
| *Commelina* | – | 0.26 ± 0.08 | 0.07 ± 0.00 | 0.21 ± 0.01 |
| *Commicarpus pedunculosus* | – | 0.06 ± 0.00 | 0.07 ± 0.00 | – |
| *Cynodon plectostachyus* | – | – | 0.07 ± 0.00 | – |
| *Cyperus spp* | 0.13 ± 0.02 | 0.14 ± 0.00 | 0.22 ± 0.45 | 0.35 ± 0.24 |
| *Digitaria* 2 | 0.78 ± 0.35 | 3.06 ± 2.12 | 3.64 ± 2.51 | 3.07 ± 2.87 |
| *Dyschoriste radicans* | 0.07 ± 0.00 | 0.21 ± 0.35 | 0.14 ± 0.00 | 0.22 ± 0.43 |
| *Eragrostis* 2 | – | – | 0.07 ± 0.00 | – |
| *Eragrostis spp* | – | – | – | 0.07 ± 0.00 |
| *Eurphobia inaequilatera* | 0.07 ± 0.00 | – | 0.07 ± 0.00 | 0.14 ± 0.00 |
| *Harpachne schmperi* | – | – | 0.78 ± 0.00 | – |
| *Helichrysum pseudognaphalium* | 4.29 ± 1.03 | 2.78 ± 1.25 | 1.99 ± 0.77 | 2.63 ± 2.42 |
| *Hermania ulugni* | 0.28 ± 0.00 | – | – | – |
| *Hibiscus flavifolius* | – | – | 0.34 ± 0.47 | – |
| *Indigofera schimperi* | 0.07 ± 0.00 | 0.07 ± 0.00 | 0.07 ± 0.00 | 0.07 ± 0.00 |
| *Lily* | – | – | 0.07 ± 0.00 | – |
| *Lintonia nutans* | 1.89 ± 1.53 | 1.83 ± 0.79 | 5.36 ± 3.92 | 3.37 ± 0.35 |
| *Lippia javanica* | 0.14 ± 0.00 | 0.18 ± 0.00 | 0.14 ± 0.00 | – |
| *Melhania* | – | 0.06 ± 0.00 | 0.07 ± 0.00 | – |
| *Misopates* | – | – | 0.07 ± 0.00 | – |
| *Monechima* | 0.36 ± 0.25 | 0.27 ± 0.25 | 0.13 ± 0.02 | 0.07 ± 0.00 |
| *Pavonia* | – | 0.06 ± 0.00 | – | – |
| *Pennisetum mezianum* | 2.30 ± 0.45 | 2.82 ± 1.22 | 2.33 ± 0.94 | 2.70 ± 0.53 |
| *Pennisetum straminium* | 30.6 ± 4.24 | 29.1 ± 2.36 | 30.1 ± 4.58 | 29.2 ± 4.57 |
| *Phyllanthus maderaspatensis* | – | – | – | 0.21 ± 0.00 |
| *Polygala sphenoptera* | 0.21 ± 0.35 | – | – | – |
| *Rhinacanthus* | 0.14 ± 0.00 | 0.35 ± 0.00 | – | 0.13 ± 0.00 |
| *Rhynchosia usambarensis* | 1.06 ± 0.00 | – | – | – |
| *Rhynchosia usambarensis* | – | 0.45 ± 1.58 | 0.07 ± 0.00 | – |
| *Solanum big (incanum)* | – | 0.20 ± 0.00 | 0.32 ± 0.19 | – |
| *Solanum small* | 0.14 ± 0.02 | – | – | 0.14 ± 0.00 |
| *Sporobolous* 2 | – | – | 0.14 ± 0.00 | 0.06 ± 0.00 |
| *Sporobolus (Panicum atroso..)* | – | – | – | 0.13 ± 0.00 |
| *Themeda triandra* | 9.28 ± 2.29 | 10.0 ± 1.63 | 9.77 ± 2.58 | 8.57 ± 1.54 |
| *Tragus bertoronianus* | – | – | 0.07 ± 0.00 | – |
| Bare | 9.42 ± 1.92 | 10.2 ± 1.31 | 11.9 ± 1.71 | 17.1 ± 4.46 |