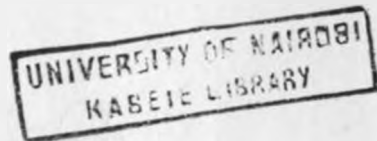


**EFFICACY OF *Metarhizium anisopliae* ICIPE 78 AND ACARICIDES IN THE
MANAGEMENT OF THE TWO-SPOTTED SPIDER MITE, *Tetranychus urticae*
(Acari: Tetranychidae), ON FRENCH BEANS, (*Phaseolus vulgaris* L.)** //



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**Thesis submitted to the University of Nairobi, Plant Science and Crop Protection
Department, in partial fulfillment for the requirements for the award of the Masters
of Science Degree in Crop Protection**

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DECLARATION

I Otilie Taiilombwele Shivolo hereby declare that this thesis is my original work and has not been presented for a degree in any other University.

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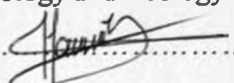
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DEDICATION

This work is dedicated to my beloved husband **Severus**, my daughter **Otilie Michelle Dhiginina** and my son **Joël Ndangi**, who were supportive to me in one way or another throughout the time of study. Their patience, understanding and continued love are highly valued.

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LIST OF ABBREVIATIONS AND ACRONYMS

ANOVA	Analysis of Variance
Df	Degree of freedom
EUROGAP	European Good Agricultural Practices
HCDA	Horticultural Crops Development Authority
ICIPE	International Centre of Insect Physiology and Ecology
IPM	Integrated Pest Management
L: D	Light-Darkness photoperiod proportion
RH	Relative Humidity
SAS	Statistical Analysis System Software
SDA	Sabouraud Dextrose Agar
SE	Standard Error
SNK	Student-Newman-Keuls means separation test
DAT	Days after treatment

ABSTRACT

French bean, (*Phaseolus vulgaris*) L. is key commodity for export in Kenya. The value of export increased from 4,466.7 Kenya Shillings in 2002 to 4,466.7 Kenya Shillings in 2006. Production of French beans is constrained by many factors including pests, lack of capital and credit facilities, fluctuation in demand, and difficult accessibility to the market, and improper grading. The major arthropod insect pests of French beans include bean stem maggots, flower thrips, pollen beetles, foliage beetles, and Tetranychid mites, *Tetranychus urticae* Koch, in particular. Emphasis has been put in breeding lines for resistance and developing IPM for the major insect pests of French beans, but little has been done in developing cost-effective, environmentally safe management strategy for the two-spotted spider mite, *Tetranychus urticae*. The fungal pathogen *Metarhizium anisopliae* (Metschnikoff) Sorokin has been considered a potential candidate for the control of Tetranychid mites including the two-spotted spider mite. To be incorporated in the French bean production system, *Metarhizium anisopliae* has to be compatible with the pesticides used for the control of other pests and diseases.

The *in vitro* compatibility of the entomopathogenic fungus *Metarhizium anisopliae* with 4 acaricide formulations was evaluated in order to incorporate both in the control of two-spotted spider mite. The active ingredients Abamectin, Propargite, Fenpyroximate and Sulphur were tested in three different concentrations: manufacturer's recommended field rate, ten times less recommended rate and hundred times less recommended field rate by incorporating them into SDA media. The effect of acaricides on conidia germination and vegetative growth was compared.

The formulations tested affected conidial germination and vegetative growth of the fungus in different levels. A stimulatory effect was observed with Abamectin on conidial germination. The results indicated that propargite, fenpyroximate and sulphur are not compatible with *Metarhizium anisopliae* because they caused strong inhibition in its development. The compatible formulation with *Metarhizium anisopliae* was Abamectin.

A laboratory study was conducted to evaluate the effects of low rate of Abamectin on the pathogenicity of *Metarhizium anisopliae* against *T. urticae* by transferring adult female mites to leaf discs treated with fungal isolate or acaricide or in combination. Synergism between the fungus and the acaricide was not observed and their combination was as effective as the fungus alone on the mortality of mites. Conclusively, combined application of the fungal formulation with abamectin at the low rate is not economical thus not a potential alternative for sustainable control of the mites.

The efficacy of *Metarhizium anisopliae* and low application rate of Abamectin on *T. urticae* was studied under field and semi-field conditions. Mite density declines and relative efficacy of treatments were determined by sampling all plots at 7 days interval. *Metarhizium anisopliae* reduced the population density of mites as compared to Abamectin and the controls. Fungus-treated plots gave better yields, number of pods/plant and were slightly damaged as compared to Abamectin plots and untreated controls. This study indicates that *Metarhizium anisopliae* hold the potential for the practical management of *T. urticae*.

KEY WORDS: *Tetranychus urticae*, *Phaseolus vulgaris*, *Metarhizium anisopliae*, acaricides, compatibility, pathogenicity

CHAPTER 1

1 INTRODUCTION

1.1 Background Information

French bean (*Phaseolus vulgaris* L.) is a member of Fabaceae family, which is believed to have originated from Mexico. Early domestication of the species occurred in Peru and other Central American countries (Kaplan, 1965). It was introduced to Europe in the 16th century and in tropical Africa shortly afterwards. It is currently widely distributed throughout the world (Mureithi and Onyango, 2005). Young pods and mature seeds are used as cooked vegetables and are rich in vitamins and minerals (Mureithi and Onyango, 2005). They are the most important source of human dietary protein (after maize) and the most important source of calories (after maize and cassava) in Africa (Ampofo and Abate, 1996). French bean forms an important food and cash crop in Africa, particularly in the Eastern, Southern, and Great Lake regions of the continent (Ampofo and Abate, 1996). The crop is grown as a cash crop by both large scale and smallholder farmers.

French bean is an important export vegetable crop in Kenya, Tanzania, Uganda, Zambia, Zimbabwe and North Africa. More than 90 % of the crop produced in Eastern Africa is exported to regional and international markets (CIAT, 2006). French bean is gaining popularity in other countries such as Cameroon, Ethiopia, Rwanda and Sudan. In Kenya, most of the crop is grown by smallholders and virtually all is exported to Europe. Estimates indicate that up to 50,000 smallholder families are involved in French bean production in Kenya (<http://www.infonet. Biovision .org. beans>).

There is a large demand for beans in both fresh and processed form from Western European countries. The European Union (EU) countries, namely The Netherlands, Holland, United Kingdom, Germany and France are the principal importers of Kenya's fresh produce. Other markets are United Arab Emirates and South Africa.

The optimum altitude for French beans is not more than 1800 metres above sea level. They are grown where temperatures are ranging between 12 and 34°C. The optimal temperature for French beans is 20°C. Moderate rainfall of 900 to 1,200 mm per annum is adequate. French beans grow best on friable, medium loam soils that are well drained and have a lot of organic matter. The optimum soil pH is 6.5 to 7.5, but beans can tolerate a low pH of 4.5 to 5.5. In Kenya, French beans are grown in most parts of the country except the arid areas. Most common varieties are Samantha, Julia, Amy, Pualista, Vernando, Tokai, Bakara, Rexas, Cupvert, Sasa, Teresa, and Pekara. Yields of French beans increased from 4.4 tones/ha in 2002 to 7.1 tonnes/ha in 2006. The value of export increased from 4,466.7 million Kenya Shillings in 2002 to 4,466.7 Kenya million Shillings in 2006 (MOA, 2007).

However, production of French beans is constrained by biotic factors including insect pests and diseases such as fusarium root rot, anthracnose, powdery mildew and rust. Among the French beans pests are bean stem maggots, flower thrips, pod borers, pollen beetles, foliage beetles, aphids and Tetranychid mites (Nderitu *et al.*, 1996).

1.2 Problem statement

In Kenya, French bean is a key commodity for export. Two-spotted spider mite, *Tetranychus urticae* is considered as one of the most important pests of French beans. *T. urticae* is highly polyphagous and has a worldwide distribution and it is probably the most important pest species in the family Tetranychidae (Bolland *et al.*, 1998; Migeon and Dorkeld, 2006). It is a serious pest in greenhouse and field crops. Under hot and dry environmental conditions, *T. urticae* can cause significant damage to vegetable, field and orchard crops (Pedigo, 2002). Its phytophagous nature, high reproductive potential and short life cycle facilitate rapid resistance development to many acaricides often after a few applications (Sato *et al.*, 2005).

The main management strategy for the control of mites employs frequent application of chemical acaricides, which are associated with problems such as acaricide resistance and environmental contamination. With the stringent export requirements for Maximum Residue Levels (MRLs) imposed by EUROGAP (European Good Agricultural Practices) mainly in EU markets; farmers are seeking cost-effective, environmentally safe alternatives to chemical pesticides for insect control. A solution to this problem lies in the utilization of integrated control or integrated pest management (IPM).

1.3 Justification

As pest and disease control moves towards more environmentally friendly strategies, there is an increasing attention to the use of biological control agents, including

entomopathogenic fungi, as a logical alternative method to chemical control. However, although effective for the management of many arthropod pests, the use of entomopathogenic fungi will not surpass the need for chemical pesticides in all commercial production systems but can under some instances be applied with sub-lethal levels of chemical pesticides in integrated pest management (IPM) to reduce the amount of insecticides and minimize negative impacts on the environment (Dimbi, 2003; Oliveira *et al.*, 2003). The present study sought to develop a sustainable strategy that can provide effective control of two-spotted spider mites of French beans under field and screen house conditions.

1.4 Objectives

1.4.1 Overall objective

To develop a sustainable strategy for the management of the two-spotted spider mites, *Tetranychus urticae* on French beans

1.4.2 Specific objectives

1. To evaluate the effects of propargite (Omite), abamectin (Dynamec), fenpyroximate (Ortus) and Sulphur (Thiovit) on conidial germination and growth of the entomopathogenic fungus *Metarhizium anisopliae* isolate ICIPE 78 under laboratory conditions

2. To evaluate the effects of abamectin (Dynamec) on the pathogenicity of the entomopathogenic fungus, *Metarhizium anisopliae* ICIPE 78 on *Tetranychus urticae*
3. To determine the efficacy of a reduced rate of abamectin and the entomopathogenic fungus *Metarhizium anisopliae* on *T. urticae* in the field and screen house.

1.4.3 Hypotheses

1. Acaricides commonly used for the control of *Tetranychus urticae* affect conidial germination and vegetative growth of entomopathogenic fungus *Metarhizium anisopliae* ICIPE 78
2. Commonly used acaricides affect infectivity of the entomopathogenic fungus *Metarhizium anisopliae* ICIPE 78 on *T. urticae*
3. A combination of commonly used acaricides and entomopathogenic fungus, *Metarhizium anisopliae* ICIPE 78 is more efficacious in the control of *T. urticae* than the use of acaricides or fungus alone.

CHAPTER 2

2 LITERATURE REVIEW

2.1 Taxonomy of *Tetranychus urticae*

The two-spotted spider mite, *Tetranychus urticae* Koch, has been controversial in its taxonomic placement and about 60 synonyms which have been included under this species have compounded the controversy (Fasulo & Denmark, 2004). Two-spotted spider mite belongs to the class Arachnida, the order Acari, sub order Prostigmata and family Tetranychidae. According to (Zhang, 2003), *Tetranychus urticae* Koch is also known informally by many other names such as the glasshouse spider mite, or the yellow spider mite.

2.2 Origin, geographical distribution and host plants

The two-spotted spider mite was originally described from the European specimens (Fasulo & Denmark, 2004). It is the most widely known member of the family Tetranychidae or spider mites. The two-spotted spider mite, *T. urticae* is a cosmopolitan species with an extremely wide host range and common in greenhouses throughout the world. It is the most polyphagous species of spider mites and has been reported on over 200 host plant species of some economic value (Smith Meyer, 1996; Bolland *et al.*, 1998; Zhang, 2003; Ramasubramanian *et al.*, 2005). The pest is especially destructive on beans, tomatoes, cotton, strawberry, cucurbits and apples.

2.3 Biology of *Tetranychus urticae*

The two-spotted spider mites are oval shaped and may be brown or orange- red or green or greenish-yellow in color with a distinctive pair of dark red spots on the back. These spots, from which the species gets its name, are actually internal food materials seen through the intergument (Pedigo, 2002). The color of mites may vary depending on the host plant and other environmental factors (Zhang, 2003). The female is about 0.4 mm in length with an elliptical body that bears 12 pairs of dorsal setae, while the male is elliptical with the caudal end tapering and smaller than the female (Fasulo & Denmark, 2004).

The female *T. urticae* mites oviposit, and lay eggs in clusters on the under surface of leaves, which hatch in 2-4 days. The life cycle is composed of egg, larva, two nymphal stages (protonymph and deutonymph) and adult. Eggs are spherical in shape and translucent to pale in color, but they become more yellowish and the red eyespots inside the eggshell can be seen as they develop into larva. At larval eclosion, the larva has six legs and develops into protonymph, and subsequently into deutonymph. Both protonymph and deutonymph have 8 legs and appear somewhat similar to the adult. The larvae are pale to yellowish at hatching and later become yellowish green after feeding. Nymphs are yellowish-green with dark spots.

General life cycle of mites is between 10-14 days, but at optimal conditions of 30-32⁰C, the life cycle takes less than one week. Temperature range for development can be between 12 - 40 °C (Smith Meyer, 1996; Zhang 2003).

Adult females may live up to four weeks. There are numerous generations per year and males develop faster than females. Two-spotted spider mite overwinters as adult females resting in protected places in sub-tropical countries.

2.4 Economic importance of *Tetranychus urticae*

The two-spotted spider mite is considered as one of the most economically important spider mite, pest species responsible for significant yield losses in many horticultural, ornamental and agronomic crops around the globe (Ramasubramanian *et al.*, 2005). The mite has been reported to infest over 200 species of plants (Fasulo & Denmark, 2004). The two-spotted spider mite is also a serious pest in greenhouses as well as a pest of trees. All mites have needle-like piercing-sucking mouthparts (Fasulo & Denmark, 2004). The two-spotted spider mites damage plants by feeding on cell chloroplasts on the under surface of the leaf. Destruction or disappearance of chloroplasts leads to basic physiological changes such as stomatal closure and in such a case, uptake of CO₂ decreases, resulting in a marked reduction in transpiration, photosynthesis and increased water loss (Pedigo, 2002). The mesophyll tissue collapses and a small chlorotic spot forms at each feeding site. Although the individual lesions are very small, commensurate with the small size of the mites, the frequently-observed attack of hundreds or thousands of spider mites can cause thousands of lesions. It is estimated that 18 to 22 cells are destroyed per minute (Fasulo & Denmark, 2004). The upper surface of the leaf develops characteristic whitish or yellowish specks, which may join and become necrotic as mites feeding continue. Under heavy infestations, leaves become covered with silk webbing produced by the mites as they move around. In addition, the leaves turn bronze and then

drop from the plants resulting in reduced nutrient transportation, photosynthetic activity and transpiration (Smith Meyer, 1996). Fruits produced by the infested crop are usually reduced in both size and quality. *Tetranychus urticae* causes reduction in plant height, flowering, pod number and length, number of seeds/pod and mean seed weight on beans (Smith Meyer, 1996). Females disperse from a plant of declining food quality on threads of webbing and drift or are blown on to other healthy plants. The secondary economic importance of mites feeding is plant viruses transmission (El Kady, *et al.*, 2007).

2.5 Control methods of *Tetranychus urticae*

2.5.1 Cultural control methods

Cultural control measures include close planting distance, crop rotation, proper field sanitation, burning of infested crops, destroying weeds and volunteer plants. Forceful use of water from a hose pipe (syringing) (Pedigo, 2002) can perform the same task. Water washes off mites from the leaves and also enhances the crop's ability to tolerate spider mite feeding. A regular syringing can keep spider mites under control on most ornamental plants in the landscape. This technique also helps conserve natural predators (Shetlar, 2000). Maintaining proper soil moisture helps prevent high mite populations from developing and dispersing to other plants. High nitrogen levels are often associated with severe mite infestations (Mahr *et al.* 2001). Higher rates of nitrogen resulted in a shorter developmental time and higher fecundity for *T. urticae*. High humidity produced by misting suppresses spider mite populations but favors predatory species such as *Phytoseiulus persimilis* and *Feltiella acarisuga*. The applicability of cultural methods in mite management is limited because it is often preventive in nature rather than control of

an existing mite infestation, thus requires detailed forward planning. Cultural control methods are relatively pest specific and require a thorough knowledge of the life history and habits of the target pest (Fenemore and Prakash, 2006).

2.5.2 Chemical control methods

Chemical control of spider mites generally involves application of pesticides that are developed specifically to target spider mites. Resistance to pesticides is common with *T. urticae*, particularly in greenhouses. The advent of broad-spectrum insecticides helped to elevate the status of this species by killing naturally occurring predators, which play a large role in keeping populations under control in certain areas (Pedigo, 2002). Most miticides are not effective on eggs (Fasulo & Denmark, 2004).

Most spider mites can be controlled with insecticidal oils and soaps. Both horticultural and dormant oils can be used when not phytotoxic. Higher rates of horticultural oil (3 to 4 percent) or dormant oil are useful for killing mite eggs and dormant adults in the fall and spring, while insecticidal soaps are useful in the warm season (Shetlar, 2000). The control of *T. urticae* using 1% mineral oil treatment has been shown to be significantly better than other conventional pesticide treatments. Mineral oils have ovicide activities. At the same time, the comparison of pesticide and oil application costs indicated that the oil based pest and disease management programme would be cheaper than the pesticide programme (Machini, 2005). Chemical control method enables achievement of high levels of control of most pests and production of high quality produce, but involves high ecological and production costs and re-occurrence of pests as control is not permanent.

2.5.3 Biological control methods

2.5.3.1 Use of predators

Mites of the family Phytoseiidae are the most important predators of spider mites and the most commonly used natural enemies for controlling spider mites populations in greenhouses (Mahr *et al.*, 2001; Fasulo & Denmark, 2004). Important genera include the predatory mites, *Phytoseiulus* spp., *Amblyseius* spp. or *Metaseiulus* spp. Others include insects such as ladybird beetles, *Stethorus*; the minute pirate bugs, *Orius*; the thrips, *Leptothrips*; and the lacewing larvae, *Chrysopa*, predatory midges *Feltiella acarisuga*, which are commercially available in many countries and Predatory Hemiptera (*Macrolophus caliginosus*) (Fasulo & Denmark, 2004; Zhang, 2003).

Zhang (2003) further reported that lady bird beetles of the genus *Stethorus* are specialist predators of spider mites and are useful for the control of *T. urticae* in greenhouses. *Stethorus punctillum* is a voracious predator and it can consume over 1000 spider mite eggs over a developmental span of two to three weeks in greenhouses. It is able to find small colonies of spider mites, has very good dispersal ability, and is commercially available. A predatory ant, *Tapinoma melanocephalum* is known to attack *T. urticae* on *Salvia splendens* in central Florida greenhouses and has been shown to be a significant predator of *T. urticae*. The main limitations of predators are insufficient level of control, unavailability and high research costs (Fenemore & Prakash, 2006).

2.5.3.2 Use of pathogens

There is a clear and demonstrated potential for using fungi successfully in insect pest management. Approximately, 750 species of fungi in 56 genera are known to be pathogens or parasites of arthropods (Chandler *et al.*, 2000). Most species are in the Entomophthorales (Zygomycota), Ascomycota, and the Mitosporic fungi. Members of the mitosporic entomopathogens are the most widely used for biological pest control, and important genera include *Beauveria*, *Metarhizium*, *Paecilomyces*, and *Verticillium*. These fungi have global distributions and can be mass-produced readily and over 15 mycopesticides, formulated from these genera, are available commercially for the management of a range of pests in the Homoptera, Coleoptera, Lepidoptera, Diptera, and Orthoptera (Chandler *et al.*, 2000). Entomopathogenic hyphomycetes such as *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) Sorokin are of high potential for control of sucking insect pests (Shi *et al.*, 2007).

Most microbial control agents of *T. urticae* are the entomopathogenic fungi, which invade their hosts by growing through the cuticle (Chandler *et al.*, 2004). Entomopathogenic fungi usually infect their hosts through specialized spores, which they attach to, germinate on, and penetrate the integument. The penetrating fungus multiplies within the haemocoel and soft tissues of the host, and death occurs usually within three to ten days after infection through water loss, nutrient deprivation, gross mechanical damage and the action of toxins (Chandler *et al.*, 2000). There are also those that infect their hosts through digestive tracts (Butt & Goettel, 2000). Several factors control the pathogenicity of the entomopathogenic fungi, and these include pathogenic factors:

virulence, inoculum dose, nutritional antecedents and requirements; host factors: populations, developmental stages and molting influence and the environment factors: temperature, moisture, relative humidity, desiccation, light, soil, host plants (Goettel & Inglis, 1997). Fungal efficacy has been shown in a diverse range of environments, including ones that may be thought of as being hostile to fungal infection processes due to high temperature extremes and conditions of low humidity (Brownbridge *et al.*, 1994). Attempts to manipulate entomophthoran fungi have limited success because of problems with mass production, the fragility of the conidia and the need for suitable field conditions (Milner, 2007).

2.5.3.2.1 Use of *Metarhizium anisopliae*

Metarhizium anisopliae (Metschnikoff) Sorokin is a member of the Mitosporic entomopathogens (Chandler *et al.*, 2000). The fungus occurs naturally in the soil and has potential as a management agent for subterranean insect pests and mites (Langewald, *et al.* 2003). The successful mass culture of *M. anisopliae* and development of methods for mass-production of infective spores has led to the commercial development of this fungus as a microbial insecticide (Cloyd, 2008). *Metarhizium anisopliae* has been reported to cause reduction in feeding, fecundity and increase mortality in a range of insects. For example, it causes reductions in blood feeding and egg fecundity of *Anopheles gambiae* (malaria mosquito) and increased mortality in larvae of stone fruit pest *Capnodis tenebrionis* (Scholte *et al.*, 2005; Marannino *et al.*, 2006). The potential of the entomopathogenic fungus, *M. anisopliae* for control of the legume flower thrips, *Megalurothrips sjostedti* (Trybom) on cowpea was demonstrated by Ekesi *et al.*, (1998).

Metarhizium anisopliae isolate 442.99, caused high mortality against two-spotted spider mite, in a study conducted by Chandler *et al.*, (2004). The susceptibility of *Tetranychus urticae* (Acari: Tetranychidae) developmental stages to *Beauveria bassiana* and *Metarhizium anisopliae* infection under laboratory conditions was studied by Maniania *et al.* (2008). The study showed that egg hatchability was dose-dependent with the lowest hatchability occurring at the highest concentration of 1×10^7 conidia ml⁻¹ at 7 days post treatment. Mortality in all the motile stages was also dose-dependent with the highest concentration of 1×10^7 conidia ml⁻¹ at 10 days post treatment. The use of the entomopathogenic fungus, *M. anisopliae* against adults of three stored-grain beetle species, *Rhyzopertha dominica* (F.) (Coleoptera: Bostrychidae), *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae) and *Tribolium confusum* Jaquelin du Val (Coleoptera: Tenebrionidae) was evaluated in laboratory bioassays. The mortality of adults after 14 days of exposure to the treated substrate was 100% and 96% at the dosages in combination, for the suspension and the powder, respectively (Kavallieratos *et al.*, 2006). *M. anisopliae* and *B. bassiana* have been studied as potential microbial control agents of cattle ticks, which are a major constraint to livestock production in many areas of the world. These fungi subsequently caused up to 100% mortality of free-living ticks in the field (Chandler *et al.*, 2000). *M. anisopliae* is an entomopathogenic fungus widely distributed in Africa and the successful development of methods for mass-producing its infective spores has led to the commercial development of this fungus as a microbial pesticide (Tounou *et al.*, 2007). The biological potential of *M. anisopliae* makes this product an ideal candidate for augmentative biological control of insects and mites.

CHAPTER 3

3. THE EFFECT OF ACARICIDES (PROPARGITE, ABAMECTIN, FENPYROXIMATE AND SULPHUR) ON CONIDIAL GERMINATION AND RADIAL GROWTH OF *Metarhizium anisopliae* IN THE LABORATORY

3.1 Introduction

Spider mites have gained popularity in the world due to their economic importance, wide host range and continuous use of pesticides as a sole method of control, resulting in the development of resistance to most pesticides. Chemical control has potentially high ecological costs, including damage to non-target organisms (Benjamin *et al.*, 2002). Thus, there is a need to find alternative control measures to suppress mite populations. The use of natural enemies, such as entomopathogenic fungi, is generally perceived to be ecologically preferable to chemical pest control (Benjamin *et al.*, 2002). Among entomopathogenic fungi, *Metarhizium anisopliae* is considered an important microbial agent and has been studied in several insect species (Mochi *et al.* 2006). This fungus is usually applied as conidial spray. It has been tested in the laboratory and field against numerous insect pests such as fruit flies, malaria mosquitoes, ticks and tetranychid mites (Chandler *et al.*, 2000; Benjamin *et al.*, 2002; Scholte *et al.*, 2005; Mochi *et al.*, 2006; Shi *et al.*, 2007; and Maniania *et al.*, 2008).

Several studies have demonstrated the importance of pesticides influence on conidial germination, because these fungal structures are responsible for the occurrence of the first disease foci in the field (Oliveira and Neves, 2004). Although entomopathogenic fungi are effective in the management of many arthropod pests, in many instances, they may be

applied in conjunction with pesticides in the integrated pest management programmes (Maniania *et al.*, 2008).

Combined application of mycoinsecticides and synthetic chemical pesticides is an attractive approach, because the fungus and chemical insecticide may act synergistically thus allowing the use of lower concentrations and decreasing the likelihood of resistance to either agent (Irigaray *et al.* 2002). However, the use of several pesticides in agriculture can affect the efficacy of entomopathogens. Chemical products can inhibit vegetative growth, conidiogenesis, sporulation, and cause genetic mutations that can result in low conidial viability and pathogen virulence to certain species (Mochi *et al.* 2006). The effect of pesticides on entomopathogens can vary according to the nature and concentration of the chemical product, and to the pathogen species and lineage. However, few studies on the effect of pesticides used in the control of spider mites have been tested on the entomopathogenic fungi, *M. anisopliae*. The objective of the present study was to investigate possible toxic effects of active ingredients of some acaricides on conidial germination and radial growth of *M. anisopliae*.

3.2 Materials and methods

3.2.1 Media preparation and fungal culture

Sabouraud Dextrose Agar (SDA) medium was prepared and each 500ml portion was dispensed into 1000ml Elenmeyerhoff flasks. The medium was autoclaved at 121°C for 20 minutes and allowed to cool up to 50°C. The acaricides were filter sterilized using 0.2µm syringe and added into each bottle to make the three concentrations (i.e.

recommended field concentrations, ten times less and one hundred times less recommended field concentrations). The suspensions were homogenized by suspending fungal spores in 10ml of Triton water in a universal bottle containing glass beads and 20ml was dispensed into 90 mm Petri dishes. Control dishes consisted of SDA without acaricides.

The isolate of *M. anisopliae* ICIPE 78 was obtained from Microbial Culture Collection of ICIPE and was cultured on Petri dishes containing Sabouraud Dextrose Agar (SDA) at 27°C for 15 days (Plate 1). The conidial suspension was prepared from 2-3 weeks old culture. The spores were removed from the surface of these cultures by scrapping off using a sterile brush and then transferred aseptically to universal bottles containing 10ml of distilled water and 0.02% Triton X-100 and glass beads. The stock conidia suspension was then vortexed to break spore chain to get single spores. Dilution was done by adding 0.1ml of conidia solution into 9ml of distilled water. After vigorous shaking in vortex mixer, the suspension was standardized at a concentration of 1.8×10^8 conidia ml^{-1} by counting the spores in a Neubauer haemocytometer chamber. The final working concentration was obtained by using the following formula: $N/V \times D$ where N = number of conidia, V = volume of the chamber (constant of 2.5×10^5), D = dilution factor.



Plate 1: *Metarhizium anisopliae* pure culture

3.2.2 Acaricides

The acaricides used in the experiments were propargite (Omite), abamectin (Dynamec), fenpyroximate (Ortus) and 80% Sulphur (Thiovit) and they were chosen because they are frequently used in controlling spider mites in French beans production . Twelve different concentrations of these acaricides were prepared by mixing with 100 ml of SDA media. Acaricides were used at the following rates: (i) manufacturer's recommended field rate (MFR), (ii) ten times less recommended rate and (iii) one hundred times less recommended field rate (Table 2). The acaricides trade names, active ingredients, formulations, chemical group and concentrations are indicated in Table 1.

Table 1: Acaricides used in the compatibility studies with *Metarhizium anisopliae* ICIPE78

Trade Name	Active Ingredient	Formulation	Chemical Group	Field rate in 1L of water
Dynamec	Abamectin 18g/L	EC	Avermectin	1.875ml
Omite	57% Propargite	EC	Alkyl sulfate	1.25ml
Ortus	Fenpyroximate 50g/L	SC	Pyrazole	0.04375ml
Thiovit	80% Sulphur	G	Dithiocarbamate	3.5g

Ai – Active ingredient, EC- Emulsifiable Concentration, SC- Soluble Concentrate, G- Granules

Table 2: Different concentrations of acaricides used in the compatibility studies with *Metarhizium anisopliae* ICIPE78

Trade Name	Manufacturer's field rate (MFR) in 100ml of SDA media	Ten times less MFR in 100ml of SDA media	Hundred times less MFR in 100ml of SDA media
Dynamec	188 μ l	18.8 μ l	1.88 μ l
Omite	125 μ l	12.5 μ l	1.25 μ l
Ortus	0.04 μ l	0.004 μ l	0.0004 μ l
Thiovit	0.35g	0.035g	0.0035g

3.2.3 Conidial germination

The effects of acaricides on conidial germination was assessed by spread-plating 0.1 ml of conidial suspension of 3×10^6 conidia ml^{-1} onto SDA plates containing the acaricides and acaricide-free SDA plates as control. 1 ml of lactophenol cotton blue was added onto each plate to halt conidia germination, 20 hours after inoculation. Three replicated sterile microscope cover slips were placed on each plate. The inoculated plates were sealed with parafilm and incubated at room temperature ($26 \pm 2^\circ\text{C}$). Germination of conidia was observed using a compound microscope at x200 magnification and the number of germinated and un-germinated conidia was determined by counting every 100 conidia from each cover slip using a tally counter. Total conidial germination included germinating primary conidia. Germination percentage was determined by dividing the number of germinated conidia with the total number of conidia counted in a specified field and multiplying by 100. The assays consisted of five treatments (four acaricides and the control) replicated five times.

3.2.4 Fungal radial growth

A conidial suspension of 1×10^7 conidia ml^{-1} was spread plated on SDA medium. The plates were sealed with Parafilm and then incubated at 25°C in total darkness for three days in order to obtain mycelial mats. The mycelial mats were then cut from the culture plates into round agar plugs using 8 mm-diameter cork borer. Each agar plug was transferred singly onto the center of the SDA agar plates containing different acaricides (each at the recommended field concentration, 10^{-1} and 10^{-2}) and control plates. The treatments were replicated five times and the experiment was repeated two times to ensure consistency of the results. Each plate was sealed with Parafilm membrane and incubated at room temperature. Radial growth was measured with a school ruler at the four cardinal points from the plug and measurements were recorded daily for 10 days.

3.2.5 Data analysis

Percent germination and the mean values of radial growth were normalized through angular transformation. Germination and radial growth values were subjected to analysis of variance (ANOVA) and means were separated by Tukey test, at 5% probability using ANOVA procedure of SAS.

3.3 Results

3.3.1 Effect of acaricides on fungal germination

The results of the effects of acaricides on fungal germination are presented in Table 2. The data shows that all the acaricides tested caused different levels of inhibition on the germination of *M. anisopliae*. These inhibitory levels were mainly dependent on the

chemical nature of the compounds as well as on the concentrations used. No significant differences were observed between fungal isolate and Abamectin treatments in germination at all three concentrations; second concentration of Propargite and third concentration of Fenpyroximate ($F = 104.45$; $DF = 12, 62$; $P = < 0.0001$). Abamectin allowed greater conidial germination at second concentration than the control. However, reduced conidial germination was observed at all three concentrations of Sulphur, first concentration of Fenpyroximate and first and third concentration of Propargite as compared to the fungus, *M. anisopliae* ICIPE 78. First concentration of Propargite significantly had the highest germination inhibition followed by third concentration of Sulphur than Abamectin and the control.

Table 3: Percentage germination of *M. anisopliae* after exposure to three concentrations of acaricides

Treatments	% Germination \pm SE		
	10^0	10^{-1}	10^{-2}
<i>M. anisopliae</i> IC 78	77.6 \pm 0.5a	77.6 \pm 0.5a	77.6 \pm 0.5a
IC78+Abamectin	77.9 \pm 0.5a	78.2 \pm 0.4a	77.3 \pm 0.7a
IC78+Propargite	52.5 \pm 1.4c	76.7 \pm 0.5a	69.8 \pm 0.6b
IC78+Fenpyroximate	70.0 \pm 0.8b	75.0 \pm 0.3a	76.0 \pm 0.5a
IC78+ Sulphur	71.0 \pm 0.4b	70.0 \pm 0.7b	67.0 \pm 0.4c

Means with the same letter to the control are not significantly different (Tukey test, $P > 0.05$). 10^0 - Recommended field dose, 10^{-1} - Ten times less recommended field dose, - 10^{-2} - Hundred times less recommended field dose

3.3.2 Effect of acaricides on fungal growth

Table 3 shows the mean growth/ day of *M. anisopliae* after exposure to acaricides. Mean growth of *M. anisopliae* after exposure to acaricides ranged between 1.9 to 3.2 mm/day.

In general, all the acaricides tested induced low levels of inhibition on radial growth of *M. anisopliae*, but this depended on the acaricide concentrations. Significant differences were observed between the control and Propargite at all three concentrations; and Abamectin at the first and second concentration ($F = 17.19$; $DF = 6, 68$; $P = < 0.0001$). No significant differences were observed between fungal isolate and Fenpyroximate and Sulphur treatments in radial growth at all the three concentrations ($F = 28.86$; $DF = 4, 2$; $P = < 0.0001$). When a product presents compatibility with radial growth of a given fungus it almost will never show the same compatibility level with conidia germination. In this case, the formulations with active ingredient Sulphur induced high levels of inhibition on germination but relatively low level of inhibition on radial growth.

Table 4: Mean growth (mm/day) of *M. anisopliae* after exposure to three concentrations of acaricides

Treatments	Radial growth \pm SE		
	10^0	10^{-1}	10^{-2}
<i>M. anisopliae</i> IC78)	3.0 \pm 0.1a	3.0 \pm 0.1a	3.0 \pm 0.1a
IC78+Abamectin	2.4 \pm 0.1b	2.5 \pm 0.1b	3.2 \pm 0.2a
IC78+ Propargite	1.9 \pm 0.1b	2.4 \pm 0.1b	2.4 \pm 0.1b
IC78+Fenpyroximate	2.9 \pm 0.1a	3.0 \pm 0.1a	2.9 \pm 0.9a
IC78+Sulphur	2.6 \pm 0.1a	2.7 \pm 0.2a	2.9 \pm 0.0a

Means followed by similar letters to the control are not significantly different from the control (Tukey test, $P < 0.05$) 10^0 - Standard concentration, 10^{-1} - Ten times diluted concentration and 10^{-2} - hundred times diluted concentration

3.4 Discussion

The results of this study showed that conidial germination was significantly affected by some of the acaricides which were used, irrespective of the concentrations used. Stimulating effect on conidial germination was observed with Dynamec (Abamectin) at the second level of the concentration (78.2%) in relation to the control (77.6%).

The findings agree with the manufacturer's recommendation that abamectin is compatible with other pest control products at the manufacturer's recommended field rate of 37.5ml/20L of water. The results of this study also support those obtained by Oliveira and Neves (2004) who reported that Abamectin and Acrinathrin presented lower toxicity than the other formulations on different developmental stages of *Beauveria bassiana*, mainly when conidial germination was considered. The results also concur with the findings of Oliveira *et al.*, (2004) who reported that Abamectin has no significant anti-fungal activity. The findings are also in agreement with those obtained by Wekesa *et al.*, (2008) who reported less effects of Abamectin on entomopathogenic fungus *Neozygites floridana*.

Sulphur is an inorganic fungicide with fumigant effect. It interferes with electron transport along the cytochromes, reducing to hydrogen sulphite, which is toxic to most cellular proteins (Nollet, 2000). This could have contributed to inhibition of conidial germination observed in the study.

Inhibition of conidial germination which was observed could be attributed to the presence of chemical products that block conidia metabolic functions. Metabolic blockage in phytopathogenic fungi conidia is due to ions accumulation on the surface of the cellular membrane. Oliveira and Neves (2004) reported that, molecules analogous to prosthetic groups diffuse to the cytoplasm where they bind to specific receivers affecting membrane permeability and enzymatic synthesis, consequently affecting metabolic processes. The same mechanism of inhibition was probably responsible for the drastic reduction on *M.*

anisopliae conidia germination and vegetative growth observed in this study. Another possible explanation for inhibition of conidia germination is the accumulation of chemical compounds against a concentration gradient, starting from diluted solutions for phytopathogenic fungi. In this process the compound molecules get in contact with conidia and slowly diffuse to the inner cytoplasm allowing fungal germination before achieving a lethal dose.

The findings of this study showed that the radial growth decreased with increase in concentrations of the acaricides. All the acaricides tested showed mild inhibitory effects on radial growth of *M. anisopliae*. The results concurred with those of Júnior *et al.*, (2008). They reported that the use of combined applications of *M. anisopliae* with the neonicotinoid insecticide imidacloprid did not inhibit colony growth for *M. anisopliae* in the media containing this insecticide. Dimbi (2003) have reported that growth of isolates is dose-dependant and this is probably because high concentrations are more toxic. The results of this study demonstrated that propargite (Omite), fenpyroximate (Ortus) and Sulphur (Thiovit) are not compatible with *M. anisopliae* because they caused strong inhibition in its development. The high toxicity of a product *in vitro* does not always mean that the same will occur in the field, but is evidence that it is possible to occur (Oliveira *et al.*, 2003). Only abamectin (Dynamec) formulation could be used simultaneously with this in the entomopathogenic fungus in integrated pest management. Data obtained in this study may guide future recommendations of these active ingredients in IPM programs where *M. anisopliae* is intended to be used as a control agent for a given mite through inundative strategy.

CHAPTER 4

4. EFFECTS OF ABAMECTIN ON THE PATHOGENICITY OF THE ENTOMOPATHOGENIC FUNGUS *Metarhizium anisopliae*, AGAINST *Tetranychus urticae* UNDER LABORATORY CONDITIONS

4.1 Introduction

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is a pest of many cultivated crops, and it often reaches pest population levels following pesticide treatments (Klingen & Westrum, 2007). The two-spotted spider mite, *Tetranychus urticae* Koch, has been recorded on more than 150 hosts of some economic value throughout the world (Irigaray *et al.*, 2002). It often reaches pest population levels because of the continuous use of acaricides. Complete reliance on chemical acaricides for pest control is not the answer to increased and sustainable agricultural productivity (Ekesei, 2001). Excessive use of conventional chemical insecticides has resulted in pest resistance, elimination of economically beneficial insects, residue persistence in the environment, toxicity to humans and wildlife, and high crop production costs (Júnior *et al.*, 2008).

In developing effective replacements for toxic chemicals, one possible medium- and long-term solution to minimize this effect is the use of integrated management as economical and ecological options (Mochi *et al.*, 2005). Entomopathogenic fungi offer an environmentally benign alternative to chemical insecticides and adoption of a biological control agents, such as *Metarhizium anisopliae*, in the integrated pest management (IPM) often results in overall reduction in the total amount of pesticide applied (Júnior *et al.*, 2008). One strategy of the integrated management is the combined use of selective chemical products and entomopathogens (Neves *et al.*, 2001). However, the use of incompatible pesticides may inhibit the development and reproduction of these pathogens,

affecting IPM. In some cases, compatible products may enhance pathogenicity of entomopathogenic fungi, increasing the control efficiency, decreasing the amount of insecticides required and minimizing the risks of environmental contamination and pest resistance (Neves *et al.*, 2001). The current study aimed at evaluating the effects of Abamectin on the pathogenicity of *Metarhizium anisopliae* to control *Tetranychus urticae*.

4.2 Materials and methods

4.2.1 Mite and fungal cultures

Adult female mites were obtained from *T. urticae* stock culture established at ICIPE. The initial culture was originally obtained from Naivasha on mites collected from roses, in 2004. The two-spotted spider mite colonies were reared on common beans, *P. vulgaris* L. variety, Rose coco and maintained at a room temperature of 25°C±2, 65% relative humidity and a photoperiod of 12:12 Light: Darkness. *M. anisopliae* ICIPE78 isolate was obtained from ICIPE Germplasm Centre. Its selection was due to its virulence against *T. urticae* at all the developmental stages and over a broad temperature ranges (Bugeme, 2008).

4.2.2 Bioassay

Ten milliliter of a single dose of 1.0×10^7 and 1.0×10^6 conidia ml⁻¹ of *M. anisopliae* ICIPE 78 and three different concentrations viz:17.75ppm i.e. thousand times less, 1.775ppm i.e. ten thousand times less and 0.1775ppm i.e. hundred thousand times less recommended concentrations of Abamectin were prepared. Sterile distilled water with 0.02% Triton X-100 was used as control. French bean leaf discs (25mm diameter)

prepared by cutting fresh French bean leaves by a leaf cutter were dipped into the prepared solutions for about 10 seconds. The leaf discs were then air-dried under laminar flow cabinet for 20 minutes. The bioassay was done with 1-2 days old female *T. urticae* and a soft camel hairbrush was used for transferring the mites. Twenty adult *T. urticae* females were transferred onto the treated inverted French bean leaf discs, placed on damp absorbent cotton wool in Petri dishes, and incubated at 25°C at L: D, 16:8h for the duration of the bioassay (Plate 2). The mites were exposed to the treated discs for 4 days after which they were then transferred to untreated leaf discs. Saturating the cotton wool with water helped to confine the mites to the discs and prevent desiccation of the leaf disc. Controls consisted of sterile distilled water and 0.02% Triton X-100. Each treatment was repeated five times. The experiment was arranged in a randomized complete block design. Mortality of mites was recorded daily for 10 days and dead mites were transferred to Petri dishes lined with moist filter paper for 14 days to confirm mycosis by microscopic examination of spores on the surface of the mites.

4.2.2 Data analysis

The cumulative mortality data under laboratory condition were subjected to analysis of variance to evaluate the relative efficiency of the tested acaricide (Abamectin), the isolate (*M. anisopliae* ICIPE78) and when the two are combined against *T. urticae*. Means were separated by SNK (Student-Newman-Keuls) test at 5% probability using ANOVA procedure of SAS (SAS Institute, 1999). Percent mortality was corrected for control mortality using Abbott's formula (Abbott, 1925). Data were subjected to probit analysis (logit transformation) to generate lethal time (LT) response.



Plate 2: Sets of leaf discs on water soaked-cotton wool in Petri dishes placed in plastic boxes in an incubator

4.3 Results

The results of the effects of Abamectin on the pathogenicity of *M. anisopliae* against *T. urticae* are as shown in Table 4. The results show no significant interactions between *M. anisopliae* and Abamectin. High mortality percentage of mite (99%) was observed from the combination of *M. anisopliae* at 1×10^7 with Abamectin at 10^{-3} . The percentage of mite mortality resulting from acaricide and *M. anisopliae* combinations was not significantly different from the acaricide alone ($F = 41.89$; $DF = 3, 47$; $P < 0.0001$). Mortality was also not significantly higher in the acaricide and *M. anisopliae* combinations than the isolate alone. There was also no significant difference among the three concentrations of Abamectin.

There was no significant interaction between *M. anisopliae* and acaricides with their concentrations. The percentage of mite mortality resulting from the combination of Abamectin (Dynamec) with *M. anisopliae* ICIPE 78 at different concentrations was not

significantly different from those obtained from the acaricide and that of the isolate when tested alone. These results indicate that Abamectin (Dynamec) had an antagonist effect on *M. anisopliae*.

Table 5: Percent mortality of adult female *T. urticae* after 10 days following exposure to French bean leaf discs treated with *Metarhizium anisopliae* ICIPE 78 and Abamectin at different concentrations

Treatments	% Mortality \pm SE
Control	23.0 \pm 1.2a
<i>M. anisopliae</i> (1×10^6)	94.0 \pm 2.9b
<i>M. anisopliae</i> (1×10^7)	95.0 \pm 3.8b
<i>M. anisopliae</i> + Abamectin ($1 \times 10^6 + 10^{-3}$)	97.0 \pm 3.0b
<i>M. anisopliae</i> + Abamectin ($1 \times 10^6 + 10^{-4}$)	94.0 \pm 2.9b
<i>M. anisopliae</i> + Abamectin ($1 \times 10^6 + 10^{-5}$)	73.0 \pm 5.4bc
<i>M. anisopliae</i> + Abamectin ($1 \times 10^7 + 10^{-3}$)	99.0 \pm 1.0b
<i>M. anisopliae</i> + Abamectin ($1 \times 10^7 + 10^{-4}$)	93.0 \pm 3.7b
<i>M. anisopliae</i> + Abamectin ($1 \times 10^7 + 10^{-5}$)	89.0 \pm 4.8bc
Abamectin (10^{-3})	97.0 \pm 2.0b
Abamectin (10^{-4})	97.0 \pm 1.2b
Abamectin (10^{-5})	89.0 \pm 3.3bc

Means within column followed by the same letter are not significantly different (Student-Newman-Keuls' test, $P > 0.05$). 10^{-3} - one thousand times diluted Abamectin concentration, 10^{-4} - ten thousand times diluted Abamectin concentration, 10^{-5} - hundred thousand times diluted Abamectin concentration

4.4 Discussion

Compatibility with agrochemicals is required whenever a fungus is used together with pesticides. In the laboratory, Abamectin was effective to the adult female mites at all concentrations; causing mortality of between 89-97 percent. The fungal isolate was pathogenic to the *T. urticae* mites at both concentrations (1×10^6 and 1×10^7). Mortality of the mites was significantly higher from *M. anisopliae* ICIPE78, when used alone than from the control. No synergism was found between the fungus and abamectin and their combination was as effective as the fungus alone on mortality of the mites. Significantly

lower mortalities were found in the control throughout the experiment. The findings confirm the pathogenicity of *M. anisopliae* against *Tetranychus urticae* as reported previously by other authors (Wekesa *et al.*, 2005; Bugeme, 2008). The results of this study have demonstrated that a combination of Abamectin and *M. anisopliae* fungus is not economical. This could be attributed to the low concentration rate of Abamectin used (17.75ppm), which did not improve the pathogenicity of *M. anisopliae*. Several other studies have showed negative or positive interactions between entomopathogenic fungi and pesticides used in the same environment for controlling mite populations (Maniania *et al.*, 2008). For example, use of fungi together with sub-lethal doses of imidacloprid has led to enhanced levels of infection and mortality in several pest insects, while the field efficacy of *Beauveria bassiana* was increased against grasshoppers when used with diflubenzuron (Brownbridge *et al.*, 1994). The results obtained in this study suggest that *M. anisopliae* could provide a novel alternative to chemical acaricides for the management of *T. urticae*.

CHAPTER 5

5. EFFICACY OF ABAMECTIN AND THE ENTOMOPATHOGENIC FUNGUS *M. anisopliae*, ON *T. urticae* UNDER FIELD AND SEMI-FIELD CONDITIONS

5.1 Introduction

The two-spotted spider mite, *Tetranychus urticae* is a serious pest of a variety of agricultural crops and ornamentals in both greenhouse and in the field. High numbers and high reproductive rates make the management of the two-spotted spider mites population difficult. When mites begin to feed on a plant, they produce webbing. This webbing can protect both motile and egg stages from the acaricides (Ashley, 2003). In Africa, farmers rely heavily on chemical pesticides for the management of *T. urticae* (Ramasubramanian *et al.*, 2005). The great reliance on chemical pesticides has its serious drawbacks, manifested in resistance problems and high residue levels in food products (fruits, vegetables, grains and seeds) that may hinder its marketing. Such undesirable consequences have led to alienating effects on the irrational use of chemical agents; hence foster other approaches that capitalize on safe natural products (El Kady *et al.*, 2007). Other factors for the lack of success with acaricide applications includes reduced efficacy of the product in hot weather, poor plant coverage by grower application, lack of toxicity of acaricides to eggs and poor timing of the initial application. The study was conducted to determine the efficacy of low application rate of Abamectin and *M. anisopliae* strain ICIPE 78 on the two-spotted spider mites on French beans.

5.2 Materials and methods

5.2.1 Production of fungal inoculum

5.2.1.1 Preparation of nutrient broth

The liquid broth was prepared by mixing 30g of glucose, 30g of yeast extract and 10g of peptone in 1000ml of distilled water. The 50ml broth was dispensed into 250ml Erlenmeyer flasks and autoclaved at 121⁰C for 20 minutes. The broth was inoculated with 1ml of start culture of *Metarhizium anisopliae* isolate ICIPE 78 using 1ml micropipette and then incubated for three days in a shaker incubator at 28⁰C and 100 rpm. 200ml of sterile distilled water was added to the inoculum during inoculation of substrate to distribute the spores on the substrate.

5.2.1.2 Fungal inoculum

Metarhizium anisopliae isolate ICIPE 78 was produced on long white rice. Two kilograms of rice was weighed, washed properly three to four times. The rice was pre-cooked by soaking it in 90-100⁰C water for 15 minutes. The substrate (rice) was placed into polyethylene bags and autoclaved for 60 minutes at 121⁰C. The substrate was cooled to about 40-45⁰C. The aerated rice bags were inoculated with the broth by making a small opening on one corner of the bag using sterile scissor, sealed and incubated at room temperature for 12 hours. After 12 hours, the bags were shaken manually and incubated again for one week. A week later, the bags were slightly shaken manually to enhance aeration and incubated for another two weeks. The substrate containing conidia was transferred into plastic basins and allowed to dry in desiccators by using silica gel for 12 hours before the conidia were harvested by sieving. The conidia were stored in sealed

paper bags in the refrigerator at 4°C for up to six months without affecting the viability before being used in field and greenhouse experiments.

5.2.2 Field experiment

A field trial was conducted from January to May, 2009 at Athi River, Kenya (S1°24' 6.36' latitude, E37°1'36.35' longitude) with mean rainfall of 82mm. The mean monthly minimum and maximum temperatures vary from 14 to 16°C and from 23 to 26°C respectively (Appendix 1). French bean, cv. Alexandria seeds were sown in flat bed plots at a spacing of 30x15cm (Plate 3). The seeds were sown in plots of 3m x 3m in a randomized complete block design with three replications. There were 1m alleys between plots and replications. Kraal manure was applied at 9kg/9m² plot. Water was applied twice a week while weeding was carried out as weeds appear. The experiment consisted of four treatments: **T1** (Untreated control), **T2** (TritonX-100 + water), **T3** (Abamectin) and **T4** (Emulsifiable *M. anisopliae* ICIPE 78 isolate). Fungal formulation was mixed with TritonX-100 as a wetting agent to increase the stability.

Plants were inspected for mite infestation at weekly interval after emergence. *In situ* sampling was done to confirm the presence of mites. Sampling was started when fifteen mites per plant were observed and continued weekly prior to spraying. Spraying was done every second week after sampling. Motile stages of two-spotted mites were counted from the leaves of 10 randomly selected plants per plot to determine mites' population density. From each plant, three leaflets were collected, one from the bottom, the middle and the top third of the plant. Leaf samples were collected and placed in a cool box and

taken to the laboratory for counting the mites under the dissecting microscope. The count was carried out within 24 h after sampling. The leaf damage was assessed by using a visual density scale of 1-5 where: 1= 1-15%, 2= 20-30%, 3= 35-50%, 4=55-70% and 5= 80-100% (Modified after Smith Meyer, 1996). Production parameters included overall yield per plot per treatment, number of pods per plant for ten plants. French bean pods were harvested as soon as they reached extra fine grade (very tender, seedless and no strings). Harvesting was spread for a total of three weeks. Population counts, damage scores and production parameters were log transformed and subjected to ANOVA.

5.2.3 Screen house experiment

In addition to field experiment a greenhouse experiment was also conducted evaluating the same treatments. This was done from March to May, 2009 at ICIPE, Nairobi, Kenya. The experiment aimed to determine the efficacy of the reduced recommended field dose of Abamectin and *M. anisopliae* isolate ICIPE 78 on the two-spotted mites in French beans. French bean, cv. Alexandria seeds were sown in pots. The potted plants were arranged in a completely randomized design with four replications and four pots in each experimental unit (Plate 4&5). The mite population was established artificially by placing three heavily infested leaflets on each potted plant and allowing the mites to establish for three weeks. Adult female mites were obtained from *T. urticae* stock culture established at ICIPE. The initial culture was originally obtained from Naivasha on mites collected from roses, in 2004. The two-spotted spider mite colonies were reared on common beans, *P. vulgaris* L. variety, Rose coco and maintained at room temperature of $25^{\circ}\text{C}\pm 2$, 65% relative humidity and a photoperiod of 12:12 L:D.

There were four treatments: T1 (Untreated control, T2 (TritonX-100 + Water), T3 (Abamectin) and T4 (Emulsifiable *M. anisopliae* ICIPE 78 isolate). Scouting of mites, mite sampling and counting was carried out the same way as with the field experiment. Spraying of each treatment was done outside greenhouse to avoid spray drift due to limited space inside the greenhouse. Production parameters assessed included overall yield per plot per treatment, number of pods per plant for ten plants. French bean pods were harvested as soon as they reached fine grade (small seeds, short with strings). Harvesting was spread for a total of three weeks. Population counts, damage scores and production parameters were log transformed and subjected to ANOVA.



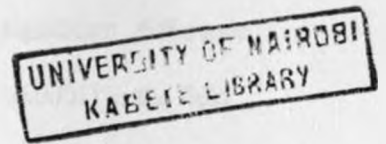
Plate 3: French bean field at Athi River, Kenya



Plate 4: Potted bean plants in the screen house



Plate 5: Screen house at ICIPE, Kenya



5.3 Results

Mite population trends are shown in figures 1 and 2. Mite population samples taken before spray application ranged between 24.5–26.37/ plant in the field and 62.86–63.71/ plant in the screen house respectively. Mite densities were reduced in both the acaricide and *M. anisopliae* treatments as compared with the controls (Triton X-100 and untreated) after spray applications in both the field and screen house experiments. The reduction in mite population density was greater in the *M. anisopliae* treatment plots than in the acaricide-treated plots at 21 days after treatment (DAT) ($F=752.29$; $DF=3, 7$; $P<0.0001$), and 28 DAT ($F=608.41$; $DF=3, 7$; $P<0.0001$) in the field and screen house trial respectively. There was no significant difference in the number of mites during 0 DAT in both the field and screen house trials. However, there was a significant difference between the first 42 DAT and 49 DAT ($F=184.57$; $DF=7, 2528$; $P<0.0001$) in the field.

A drastic reduction in mite populations was observed during 49 DAT in all the treatments in the field experiment. Application of both Abamectin (Dynamec) and *M. anisopliae* significantly reduced the severity of damage by the two-spotted mites on beans as recorded by visual scores during both trials: field trial ($F=98.06$; $DF=3, 928$; $P<0.0001$)(Table 6), screen house trial ($F=101.10$; $DF=3, 928$; $P<0.0001$) (Table 7). There was gradual increase in leaf damage in the untreated plots over time, while leaf damage scores were not significantly different in the treated plots in both the field and screen house experiments. A significant difference was observed in the yield of French beans between treated plots and untreated plots from the different treatments in the field experiment ($F=59.67$; $DF=3, 15$; $P<0.0001$) (Table 8).

However, the *M. anisopliae* treated plots gave better yields compared with Abamectin treated plots and untreated controls in the field experiment. There was a significant difference in the number of pods/plant among treatments in the field trial ($F=86.16$; $DF=3, 355$; $P<0.0001$). A high number of pods were recorded from the *M. anisopliae* treated plots.

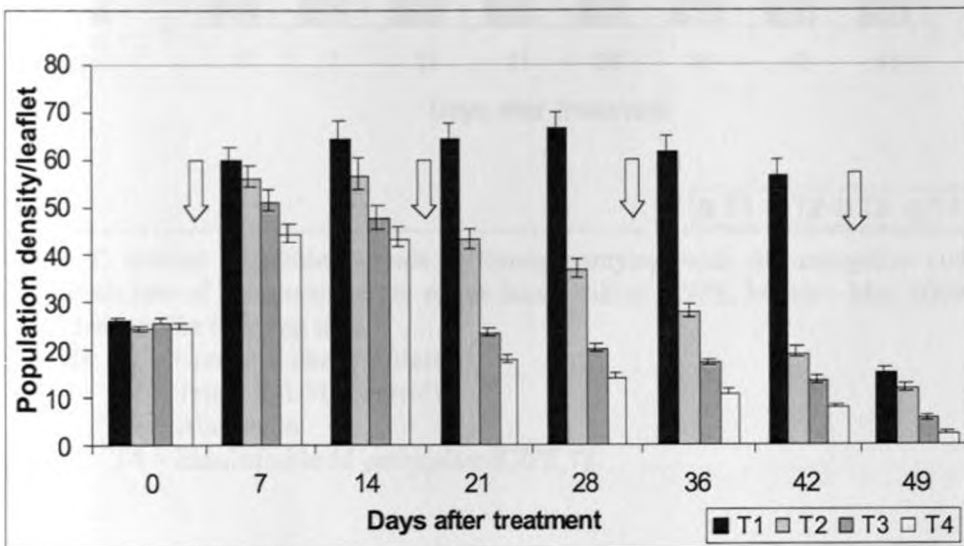


Fig.1: *T. urticae* population trends following spraying with *M. anisopliae* and low application rate of Abamectin during the field trial at Athi River, January– May 2009. The arrow denotes the spraying time

- Legends: T1 = Untreated check (Control)
 T2 = Triton X-100 (Control)
 T3 = Abamectin
 T4 = Emulsifiable *M. anisopliae* ICIPE 78

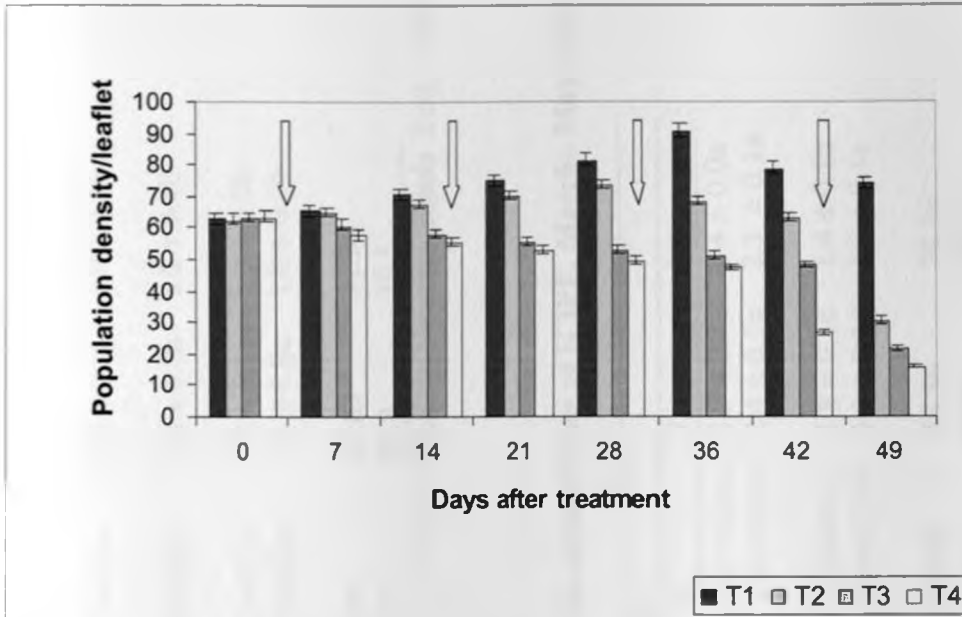


Fig.2: *T. urticae* population trends following spraying with *M. anisopliae* and low application rate of Abamectin in the screen house trial at ICIPE, March – May 2009. The arrow denotes the spraying time

- Legends: T1 = Untreated check (Control)
 T2 = Triton X-100 (Control)
 T3 = Abamectin
 T4 = Emulsifiable *M. anisopliae* ICIPE 78

Table 6: *T. urticae* damage scores on French beans under different treatments in the field at Athi River, January– May 2009

Treatment	Damage score for 8 weeks ± SE							
	1	2	3	4	5	6	7	8
Untreated control	1.0 ± 0.0a	1.2 ± 0.0a	2.5 ± 0.1a	3.0 ± 0.1a	3.3 ± 0.1a	2.9 ± 0.1a	2.4 ± 0.1a	1.9 ± 0.1a
Triton X-100 (control)	1.0 ± 0.0a	1.1 ± 0.0a	2.3 ± 0.0a	2.6 ± 0.0b	2.4 ± 0.0b	2.0 ± 0.1b	1.6 ± 0.1b	1.2 ± 0.0b
Abamectin	1.0 ± 0.0a	1.1 ± 0.0a	1.9 ± 0.1b	1.6 ± 0.1c	1.4 ± 0.1c	1.2 ± 0.1c	1.2 ± 0.1c	1.0 ± 0.0b
<i>M.anisopliae</i> ICIPE 78	1.0 ± 0.0a	1.1 ± 0.0a	1.4 ± 0.1c	1.2 ± 0.1d	1.2 ± 0.1c	1.1 ± 0.1c	1.1 ± 0.0c	1.0 ± 0.0b
F-value	0.0	0.57	15.51	64.87	97.91	77.60	37.83	31.36
CV%	0.0	30.2	32.2	26.3	25.8	29.2	33.9	30.5

Within column means followed by the same letter are not significantly different at $P=0.05$ (Student-Newman Keuls Test), SE-Standard Error

Table 7: *T. urticae* damage scores on French beans under different treatments in the screen house at ICIPE, March– May 2009

Treatment	Damage score for 8 weeks ± SE							
	1	2	3	4	5	6	7	8
Untreated control	1.0 ± 0.0a	1.3 ± 0.1a	1.6 ± 0.1a	1.9 ± 0.1a	2.7 ± 0.1a	3.1 ± 0.1a	2.9 ± 0.1a	2.4 ± 0.0a
Triton X-100 (control)	1.0 ± 0.0a	1.2 ± 0.0ab	1.4 ± 0.1b	1.9 ± 0.1a	2.5 ± 0.1a	2.8 ± 0.1b	2.4 ± 0.1b	2.3 ± 0.1a
Abamectin	1.1 ± 0.0a	1.1 ± 0.0b	1.2 ± 0.1b	1.7 ± 0.1ab	2.3 ± 0.1b	1.9 ± 0.1c	1.8 ± 0.1c	1.4 ± 0.1b
<i>M.anisopliae</i> ICIPE 78	1.1 ± 0.0a	1.1 ± 0.0b	1.2 ± 0.1b	1.5 ± 0.1b	1.6 ± 0.1c	1.3 ± 0.1d	1.3 ± 0.1d	1.1 ± 0.1c
F-value	0.14	3.19	7.82	5.90	27.40	88.49	85.56	74.36
CV%	23.0	30.8	33.3	31.9	26.0	23.5	22.5	25.7

Within column means followed by the same letter are not significantly different at $P=0.05$ (Student-Newman Keuls Test), SE-Standard Error

Table 8: Yields of French beans under different *T. urticae* management regimes in the field at Athi river and screen house at ICIPE

Treatments	Total yield/treatment in g ± SE	
	Field experiment	Screen house experiment
Untreated control	984.44 ± 28.97c	936.11 ± 37.95c
Triton X-100 (control)	997.78 ± 37.89c	957.78 ± 38.40c
Abamectin	1509 ± 94.64b	1403.89 ± 88.91b
<i>M. anisopliae</i> IC78	2163.89 ± 128.64a	2174.44 ± 165.40a
F value	59.67	48.53
CV%	2.0	2.3

Within column means followed with the same letter are not significantly different at $P = 0.05$ (Student-Newman Keuls test), SE- Standard Error

5.4 Discussion

There were great variations in the numbers of mites between the field and screen house trials. High number was recorded from the screen house trial. This could have been due to artificial infestation which was carried out to establish the mite population. Mite population established naturally in the field trial. Mite population densities and their damage to French beans were substantially reduced by the application of *M. anisopliae* in both experiments. The concentration of inoculum of 1.0×10^8 conidia ml^{-1} used in the present study was also used by Bugeme (2008) to control the two-spotted spider mites on common beans. The same concentration resulted in the reduced number of mites, which in turn, resulted to increased numbers of pods per plant, number of seeds per pod and the weight of dry bean seeds per plant. The concentration of 1.0×10^8 conidia ml^{-1} could, therefore, be considered as economical and effective in the management of the two-spotted spider mites.

Application of *M. anisopliae* every second week was effective in reducing mite populations on French beans in the present study. The two-spotted mites usually infest underside of the leaves, spray treatments were directed to this part of the plant which is protected from direct sunlight, thus, providing protection to conidia of *M. anisopliae* from excess ultraviolet radiation. Oil formulated conidia of *M. anisopliae* persist longer than the aqueous formulated ones (Bugeme, 2008). The oil formulated conidia used in this study could be responsible for the efficacy of *M. anisopliae* observed from this study.

There was a drastic reduction in mite populations during the seventh week following spray applications in all the plots in the field trial. This decrease in the mite population might have been due to the rainfall which was received during the same week. Generally, rainfall reduces densities of mites by washing them off the plant and down to the soil. Population of *T. urticae* often declines after the rains and is usually high during hot and dry weather (Smith Meyer, 1996).

The use of the reduced application rate of Abamectin resulted in less deleterious effect on the mite populations. This could probably have been due to the sub lethal concentration which was used could have been too weak to cause death. Overall, results obtained from this study suggest that *M. anisopliae* has the potential use as a biological control agent against *T. urticae*. However, further studies are required to confirm its efficacy in the field.

There were significant differences in yields of the beans between treatments in field experiments. The fungal treated plots gave high yields compare to the acaricide treated and untreated plots. However, yields were very low as compared to the earlier recorded

yields, French bean yields of 9-12t/ha (HCDA, 2008). Low yields obtained during this study could have resulted from other factors such as water stress which was experienced during the pod formation during the experiment. These low yield levels can therefore not be solely attributed to the treatments.

CHAPTER 6

6. GENERAL DISCUSSIONS, CONCLUSIONS AND RECOMMENDATIONS

6.1 Discussions

Results obtained from the laboratory experiments showed that Abamectin (Dymanec) which was compatible with *M. anisopliae* isolate ICIP78 in terms of conidial germination caused moderate level of inhibition on radial growth. These agree with the manufacturer's recommendation that the fungus is compatible with other pest products. Abamectin belongs to the prosthetic chemical group of Avermectins, which are known to enhance fungal activity. However, propargite (Omite), fenpyroximate (Ortus) and Sulphur (Thiovit) are not compatible since they cause strong inhibition on conidial germination and radial growth.

Laboratory evaluation of the pathogenicity of *M. anisopliae* ICIP78 against *T. urticae* was demonstrated in this study and the results which were obtained agree with those reported previously by Wekesa *et al.*, (2005) and Bugeme, (2008). Synergistic interaction of acaricides with fungal agents in insect control has been reported previously (Alizahed *et al.*, 2007). In this study, it was demonstrated that combination of Abamectin and the fungus did not enhance efficacy and the potential insecticidal activity of the fungus. The most cost-effective use of Abamectin in the presence of *M. anisopliae* is therefore to apply them in rotation.

The efficacy of entomopathogenic fungus *M. anisopliae* on the *T. urticae* in the field and in the screen house was confirmed in this study. In the present study, application of *M. anisopliae* was more effective than chemical acaricide in reducing mite populations on beans in the present study. The results from this study agree with findings by Bugeme, (2008) who reported a reduction in *T. urticae* population density after treatment with *M. anisopliae*.

Less deleterious effects of reduced application rate of Abamectin on mite populations were observed. The factor that could lead to inability of the acaricide to effectively control the mites may be because the sub-lethal concentration used was too weak to cause death.

6.2 Conclusions

Abamectin (Dymanec) is compatible with *M. anisopliae* isolate ICIPE78 in terms of conidial germination, but caused moderate level of inhibition on radial growth. Propargite (Omite), fenpyroximate (Ortus) and Sulphur (Thiovit) are not compatible because they cause strong inhibition on conidial germination and radial growth. The efficiency of *M. anisopliae* ICIPE 78 did not improve when applied together with Abamectin. Therefore, application of Abamectin in the presence of *M. anisopliae* ICIPE 78 is most cost-effective when applied in rotation. Application of *M. anisopliae* causes reduction of mite populations on beans in both the field and screen house experiments. Sub-lethal concentration of Abamectin caused less deleterious effects on mites.

6.3 Recommendations

From this study, the following can be recommended:

1. Propargite (Omite), fenpyroximate (Ortus) and Sulphur (Thiovit) should not be used simultaneously with entomopathogenic fungus, *M. anisopliae* in the integrated pest management because of their antagonistic effect on the survival of the fungus.
2. Combination of Abamectin and *M. anisopliae* does not support synergism in the management of *T. urticae* and therefore should not be used in IPM and can be applied separately or in rotation in the management of *T. urticae*.
3. Further studies to confirm the efficacy of *M. anisopliae* in the field and semi-field conditions are required.
4. Economic analysis of the use of this fungus against other methods (e.g. acaricides or cultural methods) needs to be done.

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APPENDIX

Table 9: Weather report during the field experiment at Athi River, Kenya

Month	Mean maximum temperature (°C)	Mean minimum temperature (°C)	Precipitation (mm)	Relative humidity (%)
January	25	14	45	69
February	26	14	40	59
March	26	15	65	67
April	24	16	150	78
May	23	15	110	77

Source: Nairobi Airport Weather (<http://www.weatheronline.co.uk/weather/maps/city>)

