Potential of Entomopathogenic Fungi in the Control of Economically Important Spider Mite Species *Tetranychus urticae* Koch and *Tetranychus evansi* Baker & Pritchard

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DECLARATION

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

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DEDICATION

To my big brother, The **Reverend Father Xavier Luheshe Bugeme s.j.**, I dedicate this dissertation, fruit of many months of work and sacrifice. Thanks for your love and support.

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LIST OF ABREVIATIONS AND ACRONYMS

ANOVA	Analysis of variance
BCA	Biological Control Agents
df	Degree of freedom
EPPO	European and Mediterranean Plant Protection Organization
FBCA	Fungal Biological Control Agents
GPS	Global Position System
ICIPE	International Centre of Insect Physiology and Ecology
IPM	Integrated Pest Management
JKUAT	Jomo Kenyatta University of Agriculture and Technology
KARI	Kenya Agriculture Research Institute
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

ABSTRACT

The two-spotted spider mite, *Tetranychus urticae* Koch, and the tomato spider mite, *T. evansi* Baker & Pritchard (Acari: Tetranychidae), are among the most important pest of horticultural crops, such as tomato, beans, cur flowers, eggplants and several other vegetables. While *T. urticae* has been known as a worldwide pest of a wide range of horticultural crops both outdoors and in the greenhouses, the importance of *T. evansi* has dramatically increased during the last decade. In Africa, farmers largely rely on synthetic acaricides to control these two pests. However, due to problems related to the use of synthetic acaricides in controlling *T. urticae* and *T. evansi* (mite resistance and environmental contamination), the control of this pest is still a major problem for farmers and attract a strong attention in the world or researchers. Thus non chemical control measures are being developed as alternatives to synthetic acaricides for the control of the two pests. They include improved crop management, screening for resistance in commercial and wild tomato germplasm and biological control using predatory mites and entomopathogenic fungi.

The aim of this study is to investigate the potential of the entomopathogenic fungi, *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metchnikoff) Sorokin (Ascomycotina: Hypocreales), to control the two-spotted spider mite, *Tetranychus urticae* Koch, and the tomato spider mite, *Tetranychus evansi* Baker & Pritchard (Acari: Tetranychidae), pests of horticultural crops.

Field surveys were carried out in Kerugoya, Kakamega, Machakos, Kitui, Makueni, Kajiado and Taita-Taveta Districts in order to prospect for new fungal isolates for

use in the biological control of spider mites. One isolate of *B. bassiana* and three isolates of *M. anisopliae* were found to be associated with spider mite species in the field in Kakamega and in Kerugoya districts, respectively, during the rainy season.

Twenty-six isolates of entomopathogenic mitosporic fungi *B. bassiana* and *M. anisopliae*, from the ICIPE (International Centre of Insect Physiology and Ecology) culture collection, were tested in the laboratory to determine their pathogenicity to adult *T. urticae* and *T. evansi*. All the fungal isolates were pathogenic to the two spider mite species, causing mortality ranging between 95.2 to 99.0% (*B. bassiana*) and 36.5 to 100% (*M. anisopliae*) in *T. urticae* and between 83.0 to 95.2% (*B. bassiana*) and 30.4 to 90.5 (*M. anisopliae*) in *T. evansi*. The lethal time to 50% mortality (LT_{50}) values varied from 3.0 to 8.3 days with *T. urticae* and from 4.7 to 8.2 days with and *T. evansi*. The radial growth of *B. bassiana* isolates was lower than *M. anisopliae* ones. The radial growth varied from 2 to 2.6 mm day^{-1} for *B. bassiana* isolates and from 3.3 to 5.8 mm day^{-1} for *M. anisopliae* isolates.

The effect of temperature on germination, radial growth and virulence of two isolates of *B. bassiana* and nine of *M. anisopliae* selected during the screening against *T. urticae* and *T. evansi*, was studied in the laboratory. Temperature had significant effects on germination, radial growth and virulence of the various isolates. Over 65.8% of conidia germinated at 20, 25 and 30 ºC while between 15.1 and 84.3% germinated at 35 ºC. Radial growth was slow at 20 and 35 ºC for all isolates, except *M. anisopliae* isolate ICIPE7 at 35 ºC. The optimum temperature for fungal germination was 25 and 30 ºC, while the optimum temperature for fungal

radial growth was 30 ºC. All the *B. bassiana* and *M. anisopliae* isolates were virulent to both *T. urticae* and *T. evansi* at all the temperatures but the virulence varied with isolates and temperatures. Fungal isolates were more effective at 25, 30 and 35 ºC than at 20 ºC.

The susceptibility of *T. urticae* and *T. evansi* developmental stages to infection by *B. bassiana* and *M. anisopliae* was also evaluated in the laboratory. On one hand, the effect of *B. bassiana* (isolate ICIPE279) and the *M. anisopliae* (isolates ICIPE7, ICIPE78 and ICIPE84) were tested against *T. urticae* developmental stages, while on the other hand, the effect of *B. bassiana* (isolates ICIPE278 and ICIPE279) and *M. anisopliae* (isolates ICIPE78 and ICIPE84) were tested against *T. evansi* developmental stages. All stages of *T. urticae* and *T. evansi* were susceptible to infection by *B. bassiana* and *M. anisopliae*. An increment in the concentration reduced egg hatchability and increased mortality in motile stages. The lowest egg hatchability and the highest mortality occurred at the highest concentration of 1 x 10^7 conidia ml^{-1}. However mature stages (Deutonymphs and Adults) were more susceptible to fungal infection than the immature stages (Larvae and Protonymphs). The lethal concentration to 50% mortality (LC_{50}) values varied from 20.8 to 46.3 x 10^7, from 0.3 to 0.7 x 10^7, from 0.2 to 0.4 x 10^7 and from 0.06 to 0.2 x 10^7 conidia ml^{-1} in larvae, protonymphs, deutonymphs and adults, respectively in *T. urticae*, while they varied from 8 to 40.4, from 6.8 to 37.8, from 0.3 to 2.5 and from 0.1 to 0.3 x 10^7 conidia ml^{-1} in larvae, protonymphs, deutonymphs and adults, respectively, in *T. evansi.*

The compatibility between *B. bassiana* (isolate ICIPE279) and *M. anisopliae* (isolate ICIPE78) and synthetic acaricides (three insecticides and three fungicides) was studied in the laboratory. The effect of synthetic pesticides on fungal germination and fungal radial growth was evaluated for this purpose. All the synthetic fungicides inhibited the fungal germination, and are therefore not compatible with the two fungal isolates. The synthetic insecticides, however, showed a high compatibility between them and the fungal isolates, except in the case of *B. bassiana* and Malathion. The latter retarded the *B. bassiana* germination.

The effect of *M. anisopliae* (isolate ICIPE78) and Dynamec® (synthetic acaricide with abamectin as active ingredient) on the *T. urticae* population density and on the *T. urticae*-infested bean production parameters, in the greenhouse, was evaluated. There were significant differences in *T. urticae* population densities between the treatments at postal sampling dates post-spraying, in top and middle leaves. At 3 weeks post-spraying the mite densities were near zero in the treated leaves, compared to control (9.23 and 9.84 mites/cm^2 on top and middle leaves, respectively). At 5 weeks post-treatment, there were no more leaves in the control. There were also significant differences in the number of pods per plant, the number of seeds per pod and the dry weight of seeds per plant between the treatments. Yields were 10.5 and 10.8 more times than the control with fungal and acaricide treatments, respectively.

The effect of *M. anisopliae* (isolate ICIPE78) and the synthetic acaricide (Dynamec) on the population density of *T. urticae* and *T. evansi* in the field was assessed. The

aqueous and emulsifiable fungal formulations reduced the population densities of *T. urticae* and *T. evansi* infesting bean and tomato plants, respectively. The two fungal formulations were as good control agents as the synthetic acaricide in controlling *T. urticae*. In tomato fields, however, the fungus in emulsifiable oil formulation provided a better control of *T. evansi* than the fungus in water formulation.

The results of this study underline the potential of the entomopathogenic fungi *B. bassiana* and *M. anisopliae* as alternative to acaricides for *T. urticae* and *T. evansi* management.

CHAPTER 1: GENERAL INTRODUCTION

1.1 Introduction

The two-spotted spider mite, *Tetranychus urticae* Koch, and the tomato spider mite, *Tetranychus evansi* Baker & Pritchard (Acari: Tetranychidae) are among the most economically important spider mite species (Fasulo and Denmark, 2000; Saunyama and Knapp, 2003). They have a world-wide distribution and attack a wide range of wild and cultivated plants including many fruits, cotton, cereals, legumes, vegetables and many ornamental plants (Jeppson *et al*., 1975; Smith Meyer, 1996; Bolland *et al*., 1998). They feed on the underside of leaves causing speckling and, in severe cases, premature leaf drop, resulting in yield loss and plant death (Jeppson *et al*., 1975; Smith Meyer, 1981, 1996).

In Eastern and Southern Africa, the spider mites, *T. urticae* and *T. evansi*, are regarded as some of the serious pests of vegetables. They cause severe damage and crop losses in tomato (*Lycopersicon esculentum* Mill.), bean (*Phaseolus vulgaris* L.) and other vegetable fields (Smith Meyer, 1996; Bolland *et al*., 1998; Knapp *et al*., 2003a; Saunyama and Knapp, 2003).

Tetranychus urticae is the most important pest species in the family Tetranychidae (Pritchard and Baker, 1955; Bolland *et al*., 1998). It frequently occurs in glasshouse and outdoor crops (van de Vrie, 1985; Kennedy and Store, 2000). It has been recorded on more than 200 host plants, including ornamental plants (arborvitae, azalea, camellia, citrus, evergreens, hollies, ligustrum, pittosporum, pyracantha, viburnum and roses), fruit plants (blackberries, blueberries and strawberries),

vegetables (tomatoes, beans, squash, eggplant and cucumber), as well as wild crops (Jeppson *et al.*, 1975; Smith Meyer, 1996; Bolland *et al.* 1998; Fasulo and Denmark, 2000; Waterhouse and Sands, 2001).

The tomato spider mite, *T. evansi*, is probably of South American origin (Gutierrez & Etienne, 1986) and was first recorded in Africa in Zimbabwe in 1979 (Blair, 1983) from where it spread northwards (Smith Meyer, 1996; Knapp *et al.*, 2003b). More recently, it was also reported from Central, Western and Northern Africa (Bonato, 1999; Kreiter *et al.*, 2002; Duverney *et al.*, 2005). It has also been spreading in Southern Europe since 1991 (Ferreira and Carmona, 1995; Ferragut and Escudero, 1999; Castagnoli *et al.*, 2006). *Tetranychus evansi* is a polyphagous species and has been reported as a destructive pest of Solanaceaous crops which include eggplant, potato, tobacco, nightshade and tomato (Blair, 1983; Moraes and McMurtry, 1985a; Smith Meyer, 1996; Knapp *et al.*, 2003a; Saunyama and Knapp, 2003; Rosa *et al.*, 2005; Fiaboe *et al.*, 2006; Furtado *et al.*, 2006). *Tetranychus evansi* has also been found feeding on crops such as beans, citrus, cotton, castor bean, ornamentals and on many weed species as *Amaranthus*, *Chenopodium*, *Convolvulus*, *Conyza*, *Diplotaxis*, *Hordeum murinum*, *Lavatera*, *Sonchus* (Moraes *et al.*, 1987). In Eastern and Southern Africa, *T. evansi* is considered as the most important dry season pest of tomatoes and cause severe damage and crop losses in tomato fields (Knapp *et al.*, 2003a,b; Saunyama and Knapp, 2003; Varela *et al.*, 2003). When left uncontrolled in tomato field, for example, *T. evansi* can destroy plants within 3-5 weeks under hot and dry conditions (Qureshi *et al.*, 1969) and the farmer can loose his production within a week's time (Keizer and Zuurbier, 2001).

For instance, yield losses of up to 90% due to *T. evansi* damage have been reported in Zimbabwe (Saunyama and Knapp, 2003).

The management of these pests has mainly relied on the use of synthetic acaricides (Jeppson *et al*., 1975; Smith Meyer, 1996; Saunyama and Knapp, 2003; Machini, 2005; Toroitich, 2006). However, due to the excessive use of synthetic acaricides, the environmental and health related problems, and the ability of spider mites to rapidly develop resistance to synthetic acaricides (Cranham and Helle, 1985; van de Vrie, 1985; Hoy, 1998; Jacobson *et al*., 2001; Herron *et al*., 2004), there is an increased demand for alternatives that are sustainable and environmental-friendly. Several cultural techniques such as crop rotation, proper field sanitation, uprooting, burning of old crops, reducing the planting distance, use of resistant varieties can reduce the mite populations (Tindall, 1983; Knapp *et al*., 2003b; Saunyama and Knapp, 2003; Wosula, 2007). In Zimbabwe for instance, the pruning and trellising associated with acaricides was reported to have a strong positive effect on yields and quality of tomatoes as well as the profitability of tomato production in high potential tomato growing areas (Saunyama and Knapp, 2003).

The use of biological control agents constitutes an alternative environmental friendly strategy to control these pests. Traditionally, biocontrol of spider mite species has utilised predatory mites and insects, and recently pathogens (Jeppson *et al*., 1975; Smith Meyer, 1996; Chandler *et al*., 2000; van der Geest *et al*., 2000). For instance, the predatory mite *Phytoseiulus persimilis* Anthias-Henriot (Mesostigmata: Phytoseiidae) has severally been reported in the control of *T. urticae* (Moraes and McMurtry, 1985; Escudero and Ferragut, 2005; Nachman, 2006). Recently, studies

conducted by Collier *et al.* (2007) also showed that *Neoseiulus idaeus* Denmark & Muma (Acari: Phytoseiidae) is a suitable and potential biological control agent of *T. urticae*.

The control of *T. evansi* with the predatory mites, *Neoseiulus californicus* McGregor and *P. persimilis*, was not effective (Moraes and McMurtry, 1985ab, 1986; Escudero and Ferragut, 2005). However, a strain of the predatory mite, *Phytoseiulus longipes* Evans, has shown promising results in laboratory experiments (Ferrero *et al*., 2007; Furtado *et al.*, 2007). *Stethorus tridens* Gordon (Coleoptera: Coccinellidae) has recently been reported to present a higher productive performance when feeding on *T. evansi*, and can maintain the *T. evansi* population under control (Fiaboe *et al*., 2007).

Entomopathogenic fungi are the most common pathogens associated with spider mites in the field and have been widely tested in the laboratory (Chandler *et al*., 2000; van der Geest *et al.*, 2000; Davidson *et al*., 2001; Maniania *et al*., 2008). Although little is known on the association between entomopathogenic fungi and *T. evansi* (Humber *et al*., 1981; Wekesa *et al*., 2005, 2007; Fiaboe, 2007), several reports on the association between entomopathogenic fungi and *T. urticae* have been reported (Carner and Canerday, 1970; Brandenburg and Kennedy, 1981; Tamai *et al*., 2002; Irigaray *et al*., 2003; Chandler *et al*., 2005).

The entomopathogenic fungi *B. bassiana* and *M. anisopliae* have been reported to cause mortality in several mite species (Barreto *et al*., 2004; Oliveira *et al*., 2004; Alves *et al*., 2005; Brooks and Wall, 2005; Rossi-Zalaf and Alves, 2006), including

the two-spotted spider mite, *T. urticae* (Alves *et al*., 2002; Tamai *et al*., 2002; Chandler *et al*., 2005) and the tomato spider mite, *T. evansi* (Wekesa *et al*., 2005).

1.2 Justification

Because of their polyphagous nature, *T. urticae* and *T. evansi* are considered as serious pests of several crops all over the world (Jeppson *et al*., 1975; Smith Meyer, 1996, Bolland *et al*., 1998).

Application of synthetic acaricides is the most common strategy used to control arthropod pests, including spider mites (Jeppson *et al*., 1975; Grantwick, 1992; Saunyama and Knapp, 2003). Synthetic acaricides have been used for several years to control spider mite species damage in the greenhouse and in the fields. However, these mite species not only have developed resistance to most of these synthetic acaricides (van de Vrie, 1985; Hoy, 1998; Jacobson *et al*., 2001; Herron *et al*., 2004), but they can also present negative effects to the environment (destruction of beneficial insects: natural enemies, honey bees and other pollinator insects; water and air pollution; toxic residue accumulation for people and animals), the users (poisoning) and the consumers (presence of residues in the produce destined for consumption). In addition, the cost of these chemical pesticides is high and they cannot be easily afforded by the small-scale farmers.

Entomopathogenic fungi which infect their hosts through the cuticle and do not need to be ingested like the other pathogens (Chandler *et al*., 2000), are considered as alternatives to chemical insecticides/acaricides for the control of mouth-sucking

arthropods such as spider mites (Strasser *et al.*, 2000). In addition, they are effective and can be produced using low-input technology (Prior, 1988). Entomopathogenic fungi are considered safe to mammals and other non-target organisms (Ferron, 1978; Goettel *et al.*, 1990; Strasser *et al.*, 2000; Goettel and Hajek, 2001; Lacey *et al.*, 2001).

On the other hand Acari are anticipated to be good hosts for fungal pathogens because of their soft body and many inhabit environments with humid microclimates which favour infection and disease transmission (Evans, 1992). Thus, entomopathogenic mitosporic fungi, especially *B. bassiana* and *M. anisopliae*, have been suggested as effective biological control agents of the spider mite *T. urticae* and *T. evansi* (Irigaray *et al.*, 2003; Chandler *et al.*, 2000, 2005; Wekesa *et al.*, 2005, 2007)

This study aims, therefore, at investigating the potential of the entomopathogenic fungi *B. bassiana* and *M. anisopliae* to control the two-spotted spider mite, *T. urticae* and and the tomato spider mite, *T. evansi*.

1.3 Objectives

1.3.1 Overall objective

To investigate the potential of entomopathogenic fungi to control the economically important spider mite species, *Tetranychus urticae* and *Tetranychus evansi*.

1.3.2 Specific objectives

a. Bioprospecting for new fungal isolates for use in the biological control of spider mite pests
b. To assess the pathogenicity of *B. bassiana* and *M. anisopliae* isolates to *T. urticae* and *T. evansi* in the laboratory
c. To test the effect of temperature on germination, radial growth and virulence of *B. bassiana* and *M. anisopliae* to *T. urticae* and *T. evansi* in the laboratory
d. To assess the susceptibility of different developmental stages of *T. urticae* and *T. evansi* to infection by *B. bassiana* and *M. anisopliae*
e. To evaluate the effect of chemical pesticides on germination and radial growth of *B. bassiana* and *M. anisopliae*
f. To test the effectiveness of entomopathogenic fungi under greenhouse and field conditions

1.4 Alternative hypotheses

a) The entomopathogenic fungi, *B. bassiana* and *M. anisopliae* are pathogenic to the economically important spider mite species, *T. urticae* and *T. evansi*
b) The germination, radial growth and virulence of the entomopathogenic fungi *B. bassiana* and *M. anisopliae* are influenced by temperature
c) All the different developmental stages of *T. urticae* and *T. evansi* are susceptible to *B. bassiana* and *M. anisopliae* infection
d) The entomopathogenic fungi, *B. bassiana* and *M. anisopliae* germination and radial growth are affected by chemical pesticides

e) The entomopathogenic fungus *M. anisopliae* cause significant mortality/reduction in the *T. urticae* and *T. evansi* populations under greenhouse and field conditions

CHAPTER 2: REVIEW OF LITERATURE

2.1 Spider mite species

2.1.1 Taxonomic position

Mites of agricultural importance belong to five families, namely the Tetranychidae, Tenuipalpidae, Penthaleidae, Tarsonemidae and Eriophyidae (Jeppson *et al*., 1975; Smith Meyer, 1981, 1996). The two-spotted spider mite, *Tetranychus urticae* Koch, and the tomato spider mite, *Tetranychus evansi* Baker & Pritchard, belong to the class Arachnida, super order Actinotrichida, order Acari, suborder Prostigmata, super family Tetranychoidae, and to the family Tetranychidae (Jeppson *et al*., 1975; Smith Meyer, 1996; Bolland *et al*., 1998; Chandler *et al*., 2000). Tetranychid mites form the family of phytophagous Acari with the most severe economic effect on agriculture. Several of these mites are injurious to many crops all over the world (Jeppson *et al*., 1975; Smith Meyer, 1981, 1996; Bolland *et al*., 1998; Migeon and Dorkeld, 2006). Among the genera that belong to this family, *Tetranychus* constitutes the most important with a large number of species (Smith Meyer, 1996; Bolland *et al*., 1998; Migeon and Dorkeld, 2006). From Africa, 40 genera and 319 species of phytophagous tetranychid mites are recognised (Smith Meyer, 1987), and 11 species are known to feed on vegetables in southern Africa (Smith Meyer, 1996; Bolland *et al*., 1998; CAB International, 2000).

2.1.2 Origin, geographic distribution and host plants of *T. urticae* and *T. evansi*

2.1.2.1 *Tetranychus urticae*

The two-spotted spider mite, *T. urticae*, was originally described from European specimens and it is considered to be a temperate zone species. However, it is also found in the subtropical regions (Fasulo and Denmark, 2000). *Tetranychus urticae* is worldwide distributed and has been recorded on more than 200 host plants, including ornamental plants (arborvitae, azalea, camellia, citrus, evergreens, hollies, ligustrum, pittosporum, pyracantha, viburnum and roses), fruit crops (blackberries, blueberries and strawberries), vegetables (tomatoes, beans, squash, eggeplant and cucumber), as well as wild crops (Jeppson *et al*., 1975; Smith Meyer, 1996; Bolland *et al*., 1998; Fasulo and Denmark, 2000; Migeon and Dorkeld, 2006). It frequently occurs in glasshouse and outdoor crops (van de Vrie, 1985; Kennedy and Store, 2000). Wind plays an important role in its dispersal. As a consequence, other crops such as wild plants or weeds can serve as a source of infestation. In the greenhouse this mite is dispersed on clothing and implements. *Tetranychus urticae* mites feed and breed throughout the year, except in areas of extreme cold where they remain quiescent in winter and hibernate as females on the ground under leaves, in cracks, crevices, and other sheltered places. They are most numerous in hot, dry weather, with population often declining after rain (Smith Meyer, 1996).

Figure 2.1: Worldwide distribution of *T. urticae* (Yellow dots) (Source: CAB International 2000).

2.1.2.2 *Tetranychus evansi*

Tetranychus evansi, also known as tobacco spider mite or tomato spider mite, originated from South American and was accidentally introduced into other parts of the world (EPPO, 2004). Found for the first time in Brazil, it was identified as *Tetranychus mariane* McGregor (Moraes *et al*., 1987). An account of the world distribution of these two related species up to the mid 1980s and redescriptions of both was given by Moraes *et al*. (1987). In Africa, *T. evansi* was first recorded from tobacco in Zimbabwe in 1979 (Blair, 1983) from where it spread northwards (Smith Meyer, 1996; Knapp *et al*., 2003b). Since then *T. evansi* has been reported in the Democratic Republic of Congo (DRC), Ethiopia, Gambia, Kenya, Malawi, Maurice isle, Morocco, Mozambique, Namibia, Seychelles, Somalia, South Africa, Tanzania, Tunisia and Zambia (Gutierez and Etienne, 1986; Mingochi and Jensen, 1986; Smith

Meyer, 1996; El-jaouani, 1988; Bolland *et al*., 1998; Bonato, 1999; Kreiter *et al*., 2002; EPPO, 2004; Duverney *et al*., 2005; Migeon and Dorkeld, 2006; Knapp, personal communication). It has also been reported in the USA and Puerto Rico (Moraes *et al*., 1987), Portugal (Ferreira & Carmona, 1995), Spain (Ferragut & Escudero, 1999) and France (Migeon, 2005).

Tetranychus evansi is a polyphagous mite species, but seems to have a strong preference for solanaceous plants. The most frequent host plants are: *Capsicum frutescens* L. (= *annum* L.) (sweet pepper), *Lycopersicon esculentum* Mill. (tomato), *Nicotiana tabacum* L. (tobacco), *Nicotiana galuca* Grah. (tree or wild tobacco), *Solanum tuberosum* L. (potato), *Solanum douglasii* Dun., *Solanum melongena* L. (eggplant = aubergine), *Solanum nigrum* L. (black or deadly nightshade) (Moraes and Flechtmann, 1981; Blair, 1983; Moraes *et al*., 1987; EPPO, 2004; Rosa *et al*. 2005; Migeon and Dorkeld, 2006; Fiaboe, 2007). However, several other studies have reported *T. evansi* from Acanthaceae, Amaranthaceae, Anacardiaceae, Araceae, Aristolochiaceae, Asparagaceae, Asteraceae (Compsitae), Bixaceae, Brassicaceae (cruciferae), Chenopodiaceae, Convolvilaceae, Cupressaceae, Fabaceae (leguminosae), Fumariaceae, Geraniaceae, Hydrophyllaceae, Malvaceae, Myrtaceae, Passifloraceae, Poaceae, Scrophylariaceae, Tiliaceae and Urticaceae families (Bolland *et al*., 1998; Keizer and Zuurbier, 2001; EPPO, 2004; Migeon and Dorkeld, 2006). *Tetranychus evansi* is nowadays one of the major constraints in tomato production in Mozambique, Malawi, Namibia and Zambia (Saunyama and Knapp, 2003), and in Kenya where it was detected in 2001 (Knapp *et al*., 2003b; Varela *et al*., 2003).

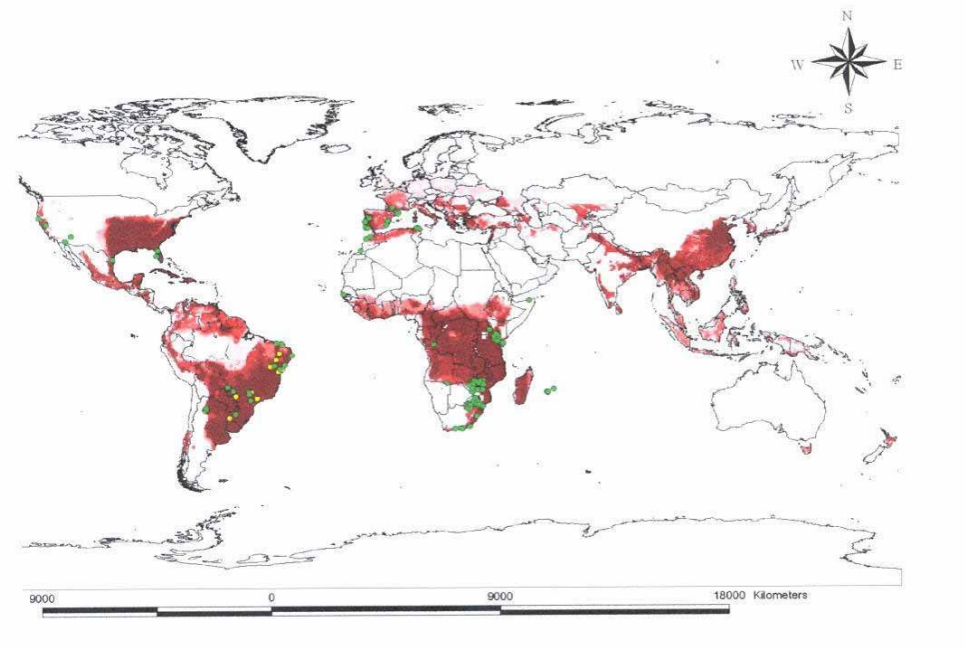


Figure 2.2: Map of the world, illustrating predictive modelling of *Tetranychus evansi* worldwide distribution. Red areas in the map indicate higher probability of potential distribution; the darker the area, the higher the probability (Source: Fiaboe, 2007)

2.1.3 Biology of *T. urticae* and *T. evansi*

The development of both *T. urticae* and *T. evansi* passes through 5 stages, namely egg, lava, protonymph, deutonymph and adult. Males are usually smaller than females and are straw-coloured. The body of females is oval and up to about 0.3-0.5 mm long. Both *T. urticae* and *T. evansi* prefer laying their eggs on the lower leaf surface at the junction of veins. High humidities result in increased oviposition. Apart from their sexual reproduction, arrhenotoky, where unfertilised females give rise to only male progeny, is also common (Smith Meyer, 1996).

Tetranychus urticae mites are oval and up to about 0.5 mm in length. Adult females are brownish red, straw-coloured or green, with a dark blotch on each side. Legs are

pale to yellowish. Larvae and nymphs (protonymphs and deutonymphs) are usually straw-coloured but can vary from light orange to yellow or reddish green, with a dark spot on each side (where the common name of two spotted red spider mite originated). The eggs are pearly-pink, light red or ivory-white and spherical. Under optimal conditions the eggs hatch after 3-5 days. Initially larvae are orange, but turn green after feeding. When 2-3 days old, larvae undergo a short quiescent period and then moult to the protonymphs. After a further 2-3 days and a short period of quiescence the protonymphs moult to the deutonymphs, which after 2 days and a short period of quiescence emerge into adults. Larvae have six legs while protonymphs, deutonymphs and adults are eight-legged. The entire life is completed in 10-14 days, depending on temperature and relative humidity (Smith Meyer, 1996). Males typically mate more than once while for the females only the first mating is effective. The adult females live two to four weeks and are capable of laying several hundred eggs during their life (100 to 150 eggs in 20-30 days) (Smith Meyer, 1996; Fasulo and Denmark, 2000).

Adult females of *T. evansi* are orange-red with reddish legs. Adult males are straw- to orange-coloured. Unlike *T. urticae*, the spots on the prodorsum for *T. evansi* are indistinct. Oviposition begins a day after emergence and females reach their maximum egg-laying capacity on the fourth day. At this time they may oviposit up to 30 eggs per day, after which the rate of oviposition decreases (Qureshi *et al*., 1969; Smith Meyer, 1996). At 23ºC and 49-50% RH, within 3-4 days, the eggs hatch. Larvae are cream-coloured and turn greenish-yellow after feeding. After 2-3 days with a short period of quiescence, the larvae moult into protonymphs, which

after 2-3 days and a short quiescent period moult into deutonymphs. Again after 3 days and short resting period, deutonymphs moult into adults; the whole process, resulting in a shorter life cycle of 9-12 days (Smith Meyer, 1996). *Tetranychus evansi*'s life cycle can be shorter or longer depending on temperature conditions. For instance Bonato (1999) reported developmental times from egg to adult of 13.6, 9.8, 7.8 and 6.3 days at 21, 26, 31 and 36 ºC, respectively. Moraes and McMurtry (1987) reported developmental times between 46.3 and 6.5 days at 15 and 35 ºC, respectively. The longevity of fertilised females is 13-32 days whereas unfertilised females live 27-39 days. Reproduction continues throughout the year, resulting in 24-30 generations per year (Smith Meyer, 1996).

2.1.4 Damage and yield loss due to *T. urticae* and *T. evansi* infestation

Tetranychus urticae and *T. evansi* mites initially feed on the lower surface but as the population increases, they spread over the entire plant. The first symptoms of injury are chlorotic stipples on the leaves; large areas subsequently turn yellow and leaves become convex. In severe infestations the leaves become bronzed, dry out and fall off (Smith Meyer, 1996; ICIPE, 2004).

Consequently, chlorophyll content, photosynthetic activity, CO_2 assimilation and transpiration are reduced. It has been estimated that 18 to 20 cells are destroyed per minute (Fasulo and Denmark, 2000). Disturbance of metabolic process results in decreased growth, flowering and cropping (Mathews and Tanstall, 1994). Mites and their webbing can clearly be seen on the underside of the leaf, and in severe infestations, on the entire plant (Smith Meyer, 1996; Craemer *et al.*, 1998).

Therefore a reduction in yield production is observed. In Zimbabwe, for example, a yield loss of up to 90% in tomato field was attributed to *T. evansi* damage (Knapp *et al.*, 2003a). In bean, however, *T. urticae* has been reported to cause reduction in plant height, flowering, pod number and length, number of seeds per pod and mean seed weight (Papaioannou-Souliotis, 1979).

2.1.5 Control of Tetranychid mites

2.1.5.1 Cultural control

Several cultural techniques such as crop rotation, proper field sanitation, uprooting, burning of old crops, reducing the planting distance, use of resistant varieties can reduce the mite populations (Tindall, 1983). However they vary between production zones and depend on the environmental conditions of production areas. In Zimbabwe for example, the pruning and trellising associated with acaricides was reported to have a strong positive effect on yields and quality of tomatoes as well as the profitability of tomato production in high potential tomato growing areas (Saunyama and Knapp, 2003). Since water stress favours the development of spider mite population, plants should adequately be irrigated during the dry season.

2.1.5.2 Chemical control

Synthetic acaricides are the most common strategies used for control of spider mite species (Cranham and Helle, 1985; Grantwick, 1992; Smith Meyer, 1996; Jacbson *et al.*, 2001; Herron *et al.*, 2004). In Kenya, several synthetic acaricides are used for the control of spider mite species. Among them dicofol, lambda-cyhalothrin,

dimethoate, cypermethrin, bifenthrin, propargite and sulphur are the most used (Machini, 2005; Toroitich, 2006). Spider mites, however, rapidly develop resistance to these pesticides, particularly when they are used for several consecutive seasons (van de Vrie, 1985; Smith Meyer, 1996; Hoy, 1998; Varela *et al.*, 2003).

Tetranychus urticae, for example, has been reported to be resistant to several insecticides/acaricides (Whalon and Mota-Sanchez, 2000) including bifenthrin (Kolmes *et al*., 1994), dicofol (Kolmes *et al*., 1994; Rossi and Conti, 1997; Karban and Zalom, 1998), propargite (Goodwin *et al*., 1995), and dimethoate (Jensen and Mingochi, 1988). Bynum *et al.* (1997) reported that metabolic degradation and target site insensitivity may be involved in mite resistance. General esterase and glutathione- S-Transferases (GST) are related to, and possibly responsible for changes in susceptibility of *T. urticae* to several insecticides particularly the synthetic pyrethroids (Yang *et al*., 2001).

Rotation of acaricides with different chemical compositions is necessary to avoid or delay development of resistance. Preventive application of dosages lower than recommended should be avoided since this may lead to resistance. Use of specific acaricides at appropriate doses and times of application are essential, and use of broad spectrum insecticides should be avoided as much as possible (Smith Meyer, 1996; Varela *et al*., 2003).

Up to now, there are no published data on the effectiveness of the principle acaricides used in the control of *T. evansi*. In Africa, several products in different

formulations have been tested in laboratory in order to determine their effect on the pest (Blair, 1989; Machini, 2005; Toroitich, 2006).

2.1.5.3 Biological control

Natural enemies (predatory mites, insects, spiders and pathogens) are very important agents in reducing and/or regulating populations of plant-feeding mites (Jeppson *et al.*, 1975; Helle and Sabelis, 1985ab; Smith Meyer, 1996) and are being used to control spider mite species.

2.1.5.3.1 Predatory mites

Mites of the family Phytoseiidae are the most effective and widespread predators of injurious plant-feeding mites (Jeppson *et al.*, 1975; Moraes *et al.*, 2004). These predators present a big interest for the integrated pest management, and some of them are effective for the control of mite populations, especially of Tetranychidae and Eriophyidae families (Kreiter & Brian, 1986; McMurtry & Croft, 1997).

The control of *T. urticae* by the Phytoseiidae mites is currently practiced in protected crops in Europe, Asia, Africa, Australia and North America. The common predators used are *P. persimilis* and *N. californicus*. They are used either separately or in association (Gerson *et al.*, 2003; Zhang, 2003). Introduced in the USA for the control of *T. urticae* on strawberry at the end of 1970s, *Phytoseiulus persimilis*'s establishment was successful and gave the satisfactory results in reducing the pest

population (Moraes, 2002). Similar results were obtained in Florida (van de Vrie & Price, 1994). In Brazil, Watanabe *et al*. (1994) demonstrated, under field conditions, the effectiveness of two native species, *N. idaeus* Denmark & Muma and *Phytoseiulus macropilis* (Banques) in reducing *T. urticae* population on strawberry when released at the beginning of the infestation by the pest. These predators have also been reported to be effective in the control of *T. urticae* on vegetables (Pickett and Gilstrap, 1986, Smith Meyer, 1996).

For *T. evansi*, there are no indications that the use of *P. persimilis* can significantly reduce its population (Moraes and McMurtry, 1985b, 1986). However, the association between *T. evansi* and the following predatory mites has been reported in Brazil and in Argentina Furtado (2006). In Brazil: *Euseius citrifolius* Denmark & Muma, *Euseius concordis* (Chant), *Euseius ho* (De Leon), *Euseius inouei* (Ehara & Moraes), *Galendromus annectens* (De Leon), *Neoseiulus californicus* (McGregor), *Neoseiulus idaeus* Denmark & Muma, *Phytoseiulus fragariae* Denmark & Schicha, *Phytoseiulus longipes* Evans, *P. macropilis* (Banks), *Proprioseiopsis mexicanus* (Garman), *Proprioseiopsis ovatus* (Garman), and *Typhlodromalus aripo* De Leon. In Argentina: *Euseius caseariae* De Leon, *E. citrifolius*, *E. concordis*, *N. Californicus*, *N. Idaeus*, *Neoseiulus tunus* (De Leon), *P. Fragariae* and *Proprioseiopsis cannaensis* (Muma). This may explain the reason why the population of *T. evansi* population was low in the field. However, among all these predators found in Brazil and Argentina, *P. longipes* was reported to be the most frequent and the most abundant predator associated with *T. evansi*. An assessment of the acceptance of *T. evansi* as prey for *P. longipes* was then studied in the laboratory and the results

revealed that *P. longipes* presents a higher productive performance than other phytoseiid mites when feeding on *T. evansi*. According to Furtado (2006), *P. longipes* prefers *T. evansi* than *T. urticae*, even when it has been fed by the latter for 77 days, and concluded that *P. longipes* is a potential biological control agent of *T. evansi*.

Predatory mites other than the Phytoseiidae family include certain species in the families Bdellidae, Anystidae, Cheyletidae, Erythreidae, Stigmaeidae, Tarsonemidae and Tydeidae (Jeppson *et al.*, 1975). There are also several known mite predators among the family Stigmaeidae. For instance, *Zetzellia mali* (Ewing) is a predator of *T. urticae, P. ulmi*, the brown mite and other mites on fruit trees in North America, Europe, and Israel (Jeppson *et al*., 1975).

2.1.5.3.2 Spiders

Spiders are almost ubiquitous and have been known to prey on insects. More than 30 species of spiders are known to feed on phytophagous mites in apple orchards in Canada; also as many species of spiders are known in Japan to be mite predators. The small or young spiders feed on mites, but evidence on the use of spiders as biocontrol agents of mites is still lacking (Jeppson *et al*., 1975; Knapp, pers. comm.).

2.1.5.3.3 Insects

Orders Coleoptera, Neuroptera, Hemiptera, and Diptera contain mite predators (Jeppson *et al.*, 1975). *Stethorus* species (Coleoptera: Coccinallidae) have been reported to control mites such as *Eotetranychus sexmaculatus*, *Eutetranychus banksi, Oligonychus pratensis*, *P. ulmi*. For example, *Stethorus japonicus* is considered to be an important predator of *T. urticae* and the Kanzawa spider mite, *T. kanzawai* in apple, citrus, tea, pear, hydrangea and kudzu vine (Gotoh and Gomi, 2000; Kishimoto, 2000). *Stethorus punctillum* is also reported to feed on *T. urticae*, but the prey preference varies with the stage of *S. pinctillum*. The predation appeared to be higher in the adult than in all larval stages of *S. punctillum* (Ragkou *et al*., 2004). The high rate of *T. urticae* consumption by *S. punctillum* adults suggests that this species has certain advantages as a potential biocontrol agent, due to their dispersal characteristics during augmentation releases of this species. *Oligota* species (Coleoptera: Staphylinidae) were also reported as control agents of mites such as *P. citri, P. ulmi,* Apple mites, Subtropical fruit mites, deciduous fruit mites. *Amthocorus musculus* Say (Hemiptera: Anthocoridae) was reported to feed on *P. ulmi* (Koch) in Nova Scotia, while *A. nemorum* L. on *T. utricae* living on beans in England. Recently Fiaboe (2007) found *Stethorus tridens* Gordon associated with *T. evansi* in the field, and reported the insect as a promising potential biological control agent of the mite species.

2.1.5.3.4 Pathogens

Pathogens have been reported to reduce populations of some mites including hibernating populations of *T. urticae* (Jeppson *et al*., 1975). A bacterial toxin, thuringiensin, a toxin produced by *Bacillus thuringiensis* Berliner was tested against *T. urticae* and was found to have potential as an acaricide (Royalty *et al*., 1990). The toxic effect of thuringiensin on reduction of oviposition of *T. urticae* was also demonstrated by Guo *et al*. (1993).

Viral diseases are known in only a few mites, namely, the European red mite, *P. ulmi* and the citrus red mite, *P. citri* (van der Geest *et al*., 2000) where they play an important role in the regulation of mite populations in citrus and peach orchads. Although the first viral disease in mites was reported by Muma (1955), the application of viruses as inundative biological control agents does not seem practicable.

Until now, there are yet no reports on the association of microsporidia with T. *urticae* and *T. evansi*. However, protozoa associated with mites are found in the phylum Apicomplexa, in the classes Gregarinia and Coccidia, and the phylum Microspora (Purrin *et al*., 1979).

Entomopathogenic fungi are the most common pathogens infecting mites naturally in the field. One of the first observations of a fungus infecting Tetranychid mites dates form 1951 by Fisher in Florida. Reviews on entomopathogenic fungi on spider

mites are the ones by Chandler *et al*. (2000) and van der Geest *et al*. (2000). More recently, Maniania *et al*. (2008) reviewed the role of entomopathogenic fungi in the control of *T. urticae* and *T. evansi*. Chandler *et al*. (2000) divided the fungal pathogens into three functional groups as follows: Acari-specific pathogens (some of which are important natural regulations of phytophagous mites); "No-specialist" species of fungi which infect a range of insects and Acari; and finally "Minor" species which are rarely reported as pathogens of Acari and not been studied for biological control.

2.2 Entomopathogenic fungi

The characteristics that are needed before an entomopathogenic fungus can be considered as a potential microbial pesticide include: high virulence, rapid mode of action, a broad host range, stability in culture and storage, amenability to submerged fermentation, amenability to quantitative bioassay, and safety to workers (McCoy, 1990). Acari are anticipated to be good hosts for fungal pathogens because they are soft bodied and many inhabit environments with humid microclimates which favour infection and disease transmission (Evans, 1992). About 750 species of entomopathogenic fungi, attacking arthropods are distributed approximately throughout the whole kingdom of fungi (Hawksworth *et al*., 1995). In spite of this great number, only about 90 species have received more attention and have been studied intensively for use against important agricultural pests and medical vectors (Gillespie and Moorhouse, 1990). Entomopathogenic fungi, in common with other insect natural enemies, can be employed under broad biological control strategies,

namely classical biological control, augmentation and conservation (Goettel *et al*., 2001; Shah and Pell, 2003).

2.2.1 Abiotic factors affecting the efficacy of entomopathogenic fungi

2.2.1.1 Temperature

Temperature not only regulates the physiology of the fungus and insect, but also the ability of the fungus to infect the host. It is one of the principal factors affecting the effectiveness of entomopathogenic fungi. It also affects the progression of disease and the time of death (Benz, 1987; Inglis *et al*., 2001). For example, Ekesi *et al*. (1999) assessed the effect of four temperatures (15, 20, 25 and 30 ºC) on germination, radial growth and virulence of fungal isolates of *M. anisopliae* and *B. bassiana* on the legume flower thrips, *Megalurothrips sjostedti* (Trybom). They found that germination, radial growth and the virulence does not vary only with isolates but also with the temperature, fungal isolates being most effective at 25 and 30 ºC. Dimbi *et al*. (2004) also reported that the susceptibility of the three species of African tephritid fruit flies, *Ceratitis corysa* (Walker), *C. capitata* (Wiedemann) and *C. fsciventris* (Bezzi) to *M. anisopliae* infection varies with temperature. In general, optimum temperatures for the germination, growth, sporulation and virulence of entomopathogenic mitosporic fungi range between 20-30 ºC (Ferron, 1978; Hall and Papierock, 1982).

2.2.1.2 Relative humidity

High humidity is essential for spores of entomopathogenic fungi to germinate, penetrate the cuticle and sporulate on cadavers (Benz, 1987; Hajek *et al.*, 1990; Inglis *et al.*, 2001). However, Fargues *et al.* (1997) reported that *M. anisopliae* var. *acridum* can infect the desert locust at relative humidities as low as 13% and the fungus can even produce spores within cadavers under dry weather conditions. Water, either liquid or vapour, has been recognised as essential for the germination of spores of most fungi, and furthermore, high atmospheric humidities are known to favour development of epizootic mycosis (Ferron, 1978). However, many studies on the effect of the relative humidity on the virulence of fungal strains against arthropod pests have not yet been conducted (Maniania, personal communication).

2.2.1.3 Solar radiation and light

Solar radiation constitutes one of the most important factors affecting propagules persistence in the environment. Conidia of entomopathogenic fungi are susceptible to solar radiation, especially ultra-violet radiation (Inglis *et al.*, 2001). For instance, exposure of conidia formulated in oil for 2 hours to radiation below 320 nm reduced germination from 99.0% to 37.5% after incubation for 48 hours (Moore *et al.*, 1993) However, in spite of the detrimental effects of ultra-violet radiation, light has been reported to stimulate mycelial growth, intensity of sporulation and germination of spores of *B. bassiana* (Benz, 1987). Tang and Hou (2001) reported that the virulence of *N. rileyi* against the larval stage of *H. armigera* was higher when incubated under full (24 hours) and half-light (12 hours) than under full darkness.

2.2.2 Biotic factors affecting the efficacy of entomopathogenic fungi

2.2.2.1 The pathogen properties

Major biological properties of a pathogen involved in causing diseases are method of infection, virulence, pathogenicity (infectivity), and replication. Pathogen properties, however, vary with different types of pathogens (viruses, bacteria, fungi, protozoa, and nematodes). Virulence and infectivity are the most important properties and are essential elements in the selection of a suitable candidate for microbial control (Tanada and Fuxa, 1987).

2.2.2.2 The host population

The susceptibility of arthropods to entomopathogenic fungi can be influenced by different factors such as the population density, behaviour, age, genetics, exposure to injury and nutrition (Tanada and Fuxa, 1987; Inglis *et al*., 2001). Maniania *et al*. (1998) reported that when reared on different cultivars of *Sorghum bicolor* (Linnaeus), the time to 50% mortality (LT_{50}) of larvae of the stem borer, *Chilo partellus* (Swinhoe), when infested by *M. anisopliae* varies significantly. All the developmental stages of the host are not, at all time, equally susceptible to entomopathogenic fungi. For example, Wekesa *et al*. (2006) reported that adults and deutonymphs of *T. evansi* are more susceptible to *B. bassiana* and *M. anisopliae* than larvae and protonymphs. Similar phenomenon was observed in the cassava

green mite, *Mononychellus tanajoa* Bondar, when infested by *Neozygites floridana* (Oduor, 1995).

2.2.3 Infection Process

Pathogenic fungi infect their host through the external integument (cuticle). Some Hyphomycetes have also been reported to infect their host through the digestive tract and the respiratory system (Ferron, 1978; Goettel and Inglis, 1997; Inglis and Goettel, 2001; Maniania *et al*., 2002). Three phases are recognised in the development of fungal infection process and disease development: adhesion and germination, penetration of the host integument and intra-haemocoelian development of the fungus (Ferron, 1978; Inglis and Goettel, 2001).

2.3 Safety of entomopathogenic fungi

Several studies have shown that Fungal Biological Control Agents (FBCAs) can be used effectively and safely to control invertebrate pests with minimal adverse effects on non-target organisms. They have reduced inputs of harmful synthetic chemical pesticides in agricultural, horticultural and forest systems. They are in most of cases host specific and do not secrete copious metabolites in the environment (Strasser *et al*., 2000; Goettel and Hajek, 2001; Vestergaard *et al*., 2003; Zimmermann, 2007). Although FBCAs are considered to be environmentally-friendly, they could potentially pose some risks in the form of toxicity, allergies or direct infection to non-target organisms (humans, domestic animals and wildlife), and to the environment (Vestergaard *et al*., 2003). For instance, a human patient with

empyema caused by *B. bassiana* was recently reported (Gürcan *et al*., 2006). Consequently, even if risks are limited and are not well known, care should be taken into account, when applying FBCAs in crop protection programmes.

CHAPTER 3: GENERAL MATERIALS AND METHODS

3.1 Field surveys

Field surveys were conducted in Kerugoya, Kakamega, Kitui, Machakos, Makueni, Kajiado, and Taita-Taveta Districts (Figure 3.1) for collection of spider mite species. Mites were collected from tomato, bean and any other alternative host plant of *T. urticae* and *T. evansi*.

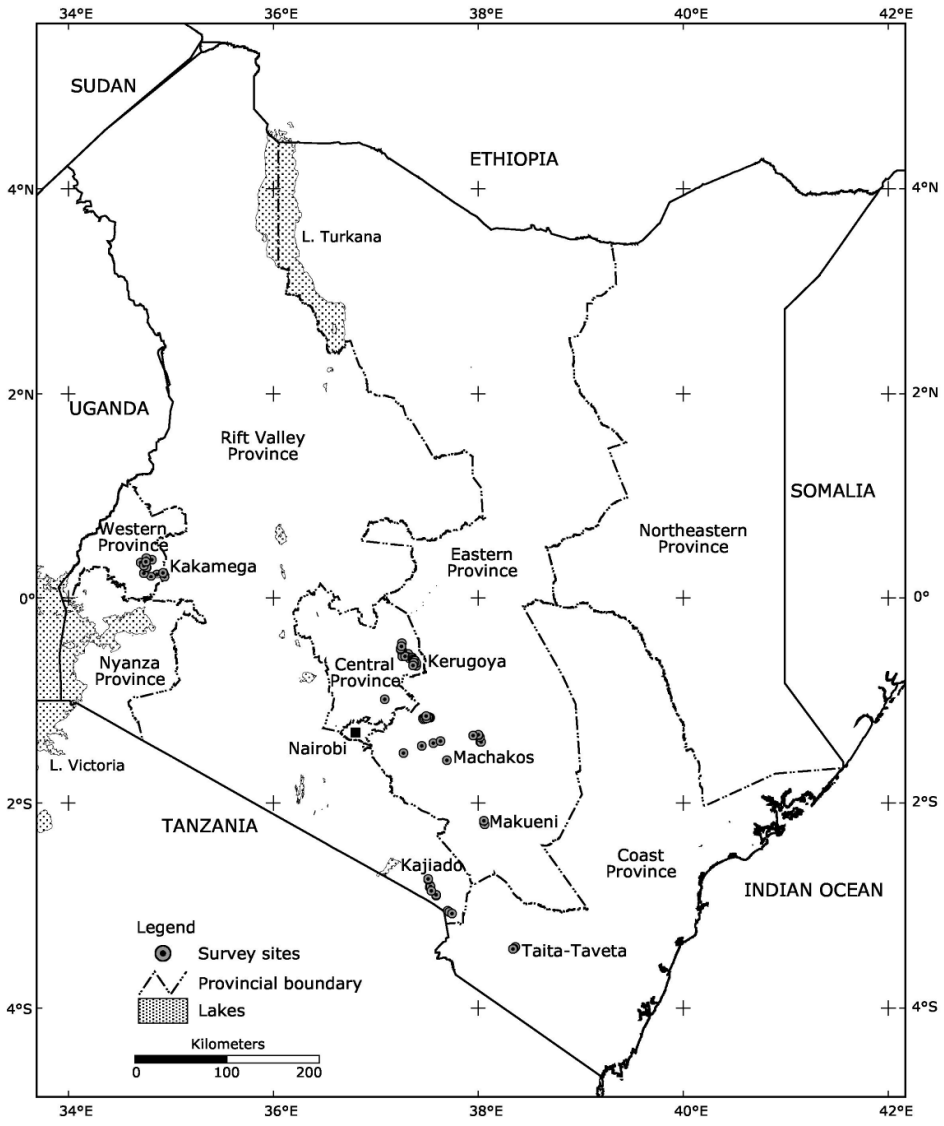


Figure 3.1: Map of Kenya showing the locations of the survey sites.

3.2 Test crops and Study sites

Bean, *Phaseolus vulgaris* L. and Tomato, *Lycopersicon esculentum* Mill., were grown in the greenhouse at the International Centre of Insect Physiology and Ecology (ICIPE), Headquarters, Duduville, Nairobi, and used for rearing *T. urticae* and *T. evansi*, respectively. Laboratory and greenhouse experiments were conducted

at the ICIPE Headquarters while field experiments were conducted at the Kenya Agriculture Research Institute (KARI), Thika station, Thika, Kenya.

3.3 Spider mite and fungal cultures

Stock cultures of *T. urticae* and *T. evansi* were maintained at room temperature of 23-30 ºC and 40-70% RH under a photoperiod of L:D 12:12. They were established in the laboratory at the ICIPE Headquarters.

The fungal isolates of *B. bassiana* and *M. anisopliae* used in the different experiments were sourced from the ICIPE Arthropod Germplasm centre where they were preserved at -20 ºC. Their accession number, host/substrate, locality and year of isolation are given in Table 5.1 (see Chapter 5). The fungal isolates were then maintained on Sabouraud Dextrose Agar (SDA) in Petri dishes at room temperature of 23-30 ºC and 40-70 % RH after they were retrieved from freezer (Figure 3.2).

3.4 Preparation of conidial suspensions

Conidia were harvested from 3 week-old cultures by scrapping the surface using a sterile rubber. Spores were suspended in 20 ml sterile 0.05% Triton X-100 water solution in universal glass bottles containing 3 mm glass beads. Bottles were then vortexed for 5 min to produce a homogenous conidial suspension. Conidial concentration was determined by using a haemocytometer and the desired concentration was obtained by serial dilutions. Viability of conidia was determined before every bioassay by spread-plating 0.1 ml of conidial suspension (titrated to 3×10^6 conidia ml^{-1}) on SDA plates. A sterile cover slip was placed on each plate and

incubated at 26 ± 2 ºC. The percentage germination was determined after 15 hours from 100-spore counts on each plate, using a compound microscope at 400× magnification.

3.5 Bioassays

3.5.1 Pathogenicity tests

A single dose of 1.0×10^7 conidia ml^{-1} was used to evaluate the pathogenicity of the 26 fungal strains on the adult stage of *T. urticae* and *T. evansi*. Ten (10) ml of conidial suspension was sprayed onto both sides of bean and tomato leaf discs (25 mm diameter) using Burgerjon's spray tower (Burgerjon, 1956) (Figure 3.3). Leaf discs were allowed to dry for 20 minutes and transferred to Petri dishes. Mites (20) were then introduced to the treated leaf discs (Figure 3.4). Petri dishes were sealed with Parafilm membrane and incubated at 26 ± 2 ºC (Figure 3.4). Controls were treated with sterile distilled water containing 0.05 % Triton X-100. Mortality was recorded daily for 10 days (see Chapter 5).

3.5.2 Fungal radial growth

A conidia suspension of 1×10^7 conidia ml^{-1} was spread-plated on SDA plates. Plates were then incubated at 26 ± 2 ºC for 3 days in order to obtain mycelial mats. The unsporulated mycelial mats were cut from culture plates into round agar plugs using an 8-mm diameter cork borer (Rapilly, 1968). Each agar plug was then transferred singly onto the centre of a fresh SDA plate. Plates were sealed with

Parafilm membrane and incubated in complete darkness at 26 ± 2 ºC. Radial growth was then recorded daily for 10 days (see Chapter 6).

3.5.3 Susceptibility of *T. urticae* and *T. evansi* stages to fungal infection

The susceptibility of *T. urticae* and *T. evansi* developmental stages (eggs, larvae, protonymphs, deutonymphs and adults) to infection by selected isolates of *B. bassiana* (isolates ICIPE278, ICIPE279) and *M. anisopliae* (isolates ICIPE7, ICIPE78, ICIPE84) was assessed. These isolates were chosen because of their virulence to *T. urticae* and *T. evansi* at broad range of temperature. The treatments consisted of four concentrations: 3×10^5, 1×10^6, 3×10^6 and 1×10^7 conidia ml^{-1}. Controls were treated with sterile distilled water. Egg hatchability and mortality in motile stages were monitored daily for 7 and 10 days, respectively (see Chapter 7).

3.6 Effect of chemical pesticides on fungal germination and radial growth

The effect of synthetic pesticides on germination and radial growth of two fungal isolates (*B. bassiana* isolate ICIPE279 and *M. anisopliae* isolate ICIPE78) was studied in the laboratory (see Chapter 8).

3.7 Greenhouse and field experiments

3.7.1 Greenhouse experiment

Potted-bean plants (Figure 3.5) were artificially infested with *T. urticae.* The *M. anisopliae* isolate ICIPE78 in aqueous and emulsifiable (oil) formulations and an acaricide solution were sprayed on infested plants. A concentration of 1×10^8 conidia ml^{-1} was used for both fungal formulations (Wekesa et al., 2005). The *T.*

urticae population densities on top and middle leaves, was evaluated 2 days before, and weekly after the first spraying. Spraying was done 3 times at the interval of 10 days. The production parameters (number of pods per plant, number of seeds per pod and weight of dry seeds per plant) were also evaluated (see chapter 9).

3.7.2 Field experiments

The procedure for field experiments was similar to that of the greenhouse experiment. The only difference is that there were two different fields, one with bean plants, infested by *T. urticae*, and another one with tomato plants infested by *T. evansi* (Figure 3.6). For more details, see chapter 10.

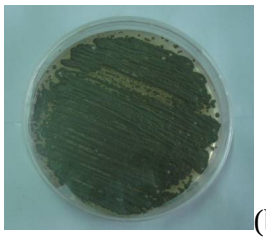

(a)

(b)

Figure 3.2: (a) *Beauveria bassiana* and (b) *Metarhizium anisopliae* pure cultures

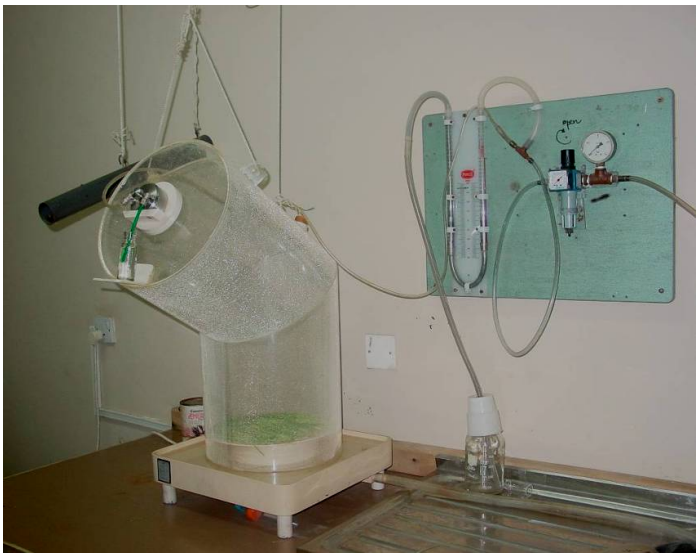
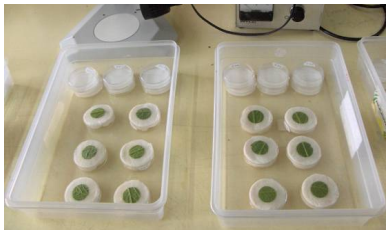


Figure 3.3: Burgerjon's spray tower



(a) (b)

(c)

Figure 3.4: (a) set up of leaf disc on soaked-cotton wool in Petri dish (b) placed in plastic box (c) and placed in an incubator for a laboratory pathogenicity test.

(a)

(b)

Figure 3.5: Potted bean plants in the greenhouse

(a)

(b)

Figure 3.6: (a) Bean and (b) Tomato fields

CHAPTER 4: PROSPECTING FOR DISCOVERY OF NEW FUNGAL ISOLATES FOR BIOLOGICAL CONTROL OF SPIDER MITE SPECIES

4.1 Introduction

Entomopathogenic fungi are promising alternatives to chemical insecticides. There is significant interest in developing fungal biological control agents (FBCAs) for use in crop protection programmes (Strasser *et al*., 2000). Fungi have one of the widest host ranges among the pathogens of arthropods (Inglis *et al*., 2001). They exhibit a very wide range of host specificity. Some species are very host-specific (Chandler *et al*., 2000; Strasser *et al*., 2000; Goettel *et al*., 2001), while others are generalists and are known from a wide range of hosts (Goettel *et al*., 2001). Fungal BCAs have reduced inputs of harmful synthetic chemical pesticides in agricultural, horticultural and forest systems. Considerable progress has been made in the development of fungal BCAs for the control of pests (arthropods, nematodes), weeds and plant diseases (Butt *et al*., 1999; Goettel *et al*., 2001). Butt *et al*. (1999) reported that most fungal BCAs are found world-wide and exotic strains can be efficacious for indigenous pests.

Mycopesticides have features that provide ecologically sound pest control compared to chemical pesticides (Moore and Prior, 1993). They are safe to humans and other non-targets, selective to varying degrees, often suitable for integrated pest control programmes, may provide extended periods of control by remaining in the environment or even establish permanently and are biodegradable (Goettel and Johnson, 1992; Lacey *et al*., 2001). Strasser *et al*. (2000) reported that BCAs do not pose a health risk because they are host specific and do not secrete copious

metabolites in the environment. Acari are anticipated to be good hosts for fungal pathogens because they are soft bodied and many inhabit environments with humid microclimates which favour infection and disease transmission (Evans, 1992; Hajek & St Leger, 1994; Chandler *et al.*, 2000).

Although few field trials have been undertaken to evaluate the effect of entomopathogenic fungi against spider mite species, several reports have been published on natural incidence of entomopathogenic fungi on tetranychid mites (Humber *et al*., 1981; Brandenburg and Kennedy, 1981; Smitley *et al*., 1986; Chandler *et al.*, 2000; van der Geest *et al*., 2000; Fiaboe 2007; Furtado *et al*., 2007; Maniania *et al.*, 2008). The aim of this study was therefore to prospect for new fungal isolates, potential natural enemies of spider mite species, for the use in their biological control; and the reasons of prospecting for new isolates were due to the fact that it has generally been reported that fungal strains isolated from specific hosts are more virulent to the same hosts than to the others (Maniania 1992; Pena *et al*., 1996; Meikle *et al.*, 2005), and can therefore be used in an IPM programs for the pest ("specific hosts") control.

4.2 Materials and methods

4.2.1 Field surveys and collection of spider mite species

Field surveys were conducted in Kerugoya, Kakamega, Machakos, Kitui, Makueni, Kajiado and Taita-Taveta Districts (Figure 3.1) for collection of spider mite species. Collections were focused on tomato and bean growing farms though this was

extended to other alternative spider mite host plants, such as *Amaranthus* spp., water melon, cassava, potatoes, eggplants, Cowpea, roses and several weeds. Mites were collected from 10 plants, randomly chosen per field. Both live and dead spider mites found on leaves were collected and kept in brown paper bags, which were then transferred to separate plastic paper bags and kept in a cool box (containing ice blocs) to minimise stress on the organisms and retard unwanted saprophytic growth until examination in the laboratory (Lacey and Brooks, 1997). On each brown paper bag, there was a label on which the name of the host plant, the name of the location/sub location and the GPS coordinates (plus altitude) were indicated. Samples were later kept in a cold room at ICIPE. The field trips were organised at random, but it happened that in some places, it was raining, and in some other places it was dry.

4.2.2 Isolation and purification of fungal isolates in the laboratory

In the laboratory, dead mites were collected and transferred in Petri dishes (90 mm diameter) lined with damp filter paper. Petri dishes were sealed by Parafilm membrane and maintained in an incubator at 26 ± 2ºC for 14 days in order to determine the cause of the spider mite death. Mortality due to fungal infection was determined and confirmed by microscopic examination of the fungal growth (mycosis) on the cadavers. Conidia from mycosed mites were isolated and transferred onto *Beauveria* (2% oatmeal infusion, 2% agar, 550 µg/ml dodine (*N*-dodecylguanidine monoacetane, 5 µg/ml chlortetracycline and 10 µg/ml crystal violet) (Chase *et al.*, 1986) and *Metarhizium* (1% glucose, 1% peptone, 1.5% oxgall,

3.5% agar, 10 µg/ml dodine (*N*-dodecylguanidine monoacetate, 250 µg/ml cycloheximide (actidione) and 500 µg/ml chloramphenicol) (Veen and Ferron, 1966) selective media, which inhibits saprophytic fungi, and then incubated at 28 ± 2 ºC for minimum two weeks.

4.2.3 Identification of isolates

Pure cultures of isolated fungal isolates were subcultured on SDA medium for identification, using an improved identification technique (Humber, 1997). Sterile cover slips were inserted into the media at an angle of 45º to allow growth of aerial hyphae on the slide and to maintain morphological characteristics of the fungi for identification. The plates were sealed by Parafilm membrane and incubated at 26 ± 2 ºC. After 72 hours, fungi were identified by the observation of both conidia and conidiogenous cells (Humber, 1997) using a compound microscope. The examination was conducted after every 24 hours until sporulation.

4.3 Results

Spider mite species were collected from 80 different sites (Table 4.1). In the laboratory, mites were collected from 182 leaves. Among all the 80 sites surveyed, spider mite species were found to be associated with fungal species in only 4 sites (Table 4.2). A *B. bassiana* isolate was isolated from *Tetranychus* spp. In Shinyalu (Kakamega District) and given the accession number of ICIPE318 (Figure 4.1). Three *M. anisopliae* isolates, named ICIPE315, and ICIPE316 and ICIPE317 (Figure 4.1), were isolated from *T. urticae* and *Tetranychus* spp. in Mwea, Kirinyaga

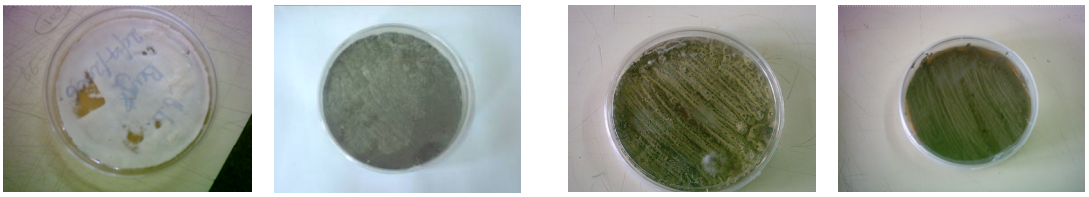
and Kutus, respectively (Table 4.2). The three latter sites are situated in Kerugoya District. Although spider mites were collected in the areas surveyed during dry season, no fungal strain was isolated from the collected mites.

Table 4.1: Survey sites, GPS coordinates, Number of plants sampled per site

			GPS coordinates			
No	Districts	Localities	Altitude (in meters)	Latitude	Longitude	NS
1	Kerugoya	Kutus	1339	S00º 32.770'	E037º 18.806'	2
2		Kutus	1281	S00º 34.860'	E037º 19.439'	1*
3		Kutus	1268	S00º 35.213'	E037º 19.727'	1
4		Kutus	1271	S00º 35.382'	E037º 19.468'	2
5		Kanyei	1516	S00º 31.064'	E037º 15.313'	1
6		Kanyei	1475	S00º 31.522'	E037º 15.137'	3
7		Kathare	1427	S00º 32.268'	E037º 15.312'	3
8		Kianjege	1380	S00º 33.520'	E037º 15.983'	4
9		Kiaja	1302	S00º 34.328'	E037º 15.496'	1
10		Kanyei	1321	S00º 34.192'	E037º 16.909'	2
11		Kirinyaga	1598	S00º 30.296'	E037º 14.488'	3*
12		Kirunda	1641	S00º 29.803'	E037º 14.546'	1
13		Waigir	1841	S00º 26.505'	E037º 15.235'	1
14		Waigiri	1690	S00º 28.558'	E037º 14.873'	2
15		Mwea	1187	S00º 38.320'	E037º 22.349'	3
16		Mwea	1174	S00º 38.984'	E037º 22.884'	1
17		Mwea	1160	S00º 39.766'	E037º 23.425'	2*
18		Nyangate	1280	S00º 35.078'	E037º 21.216'	2
19		Nyangate	1251	S00º 35.042'	E037º 21.373'	1
20		Nyangate	1234	S00º 36.632'	E037º 21.647'	1
21		Nyangate	1223	S00º 36.809'	E037º 21.992'	2
22		Guchui	1211	S00º 36.611'	E037º 22.553'	2
23		Guchui	1205	S00º 36.837'	E037º 22.906'	3
24		Kirundiro	1180	S00º 38.750'	E037º 22.647'	1
25		Guchui	1161	S00º 38.153'	E037º 23.526'	1
26		Muthangauka	1187	S00º 38.342'	E037º 22.338'	1
27		Muthangauka	1197	S00º 37. 887'	E037º 22.148'	1
28		Kiarukungu	1178	S00º 39.621'	E037º 21.898'	2
29		Kiarukungu	1174	S00º 39.631'	E037º 21.624'	5
30	Kakamega	Virembe	1627	N00º 14.093'	E034º 51.788'	4
31		Ikuya	1650	N00º 12.441'	E034º 55.381'	3
32		Chikovani	1636	N00º 15.143'	E034º 44.207'	1
33		Shasaba	1577	N00º 12.771'	E034º 48.273'	3*
34		Shasaba	1494	N00º 14.889'	E034º 44.207'	2
35		Ingotsi	1516	N00º 21.117'	E034º 42.171'	1
36		Lurambi	1549	N00º 18.127'	E034º 45.895'	3
37		Shikangania	1532	N00º 18.344'	E034º 45.536'	2
38		Shikangania	1503	N00º 19.300'	E034º 44.536'	2
39		Simuli	1501	N00º 22.900'	E034º 44.211'	2
40		Lukume	1540	N00º 23.792'	E034º 48.764'	2
41		Matia	1544	N00º 21.648'	E034º 45.469'	4
42	Machakos	Masii	1349	S01º 26.788'	E037º 26.690'	3
43		Ikasala	1275	S01º 25.122'	E037º 33.443'	1
44		Ikasala	1253	S01º 35.163'	E037º 41.416'	1

45		Unkown	1063	S01º 23.805'	E037º 37.758'	3
46		Mach Town	1641	S01º 30.922'	E034º 16.162'	1
47		Matuu	1273	S01º 10.053'	E034º 31.680'	2
48		Matuu	1272	S01º 10.011'	E037º 31.245'	1
49		Kithimani	1267	S01º 09.823'	E037º 30.640'	2
50		Kithimani	1275	S01º 10.202'	E037º 30.531'	5
51		Kithimani	1257	S01º 10.531'	E037º 27.483'	1
52		Kithimani	1267	S01º 10.984'	E037º 27.801'	3
53		Kithimani	1270	S01º 10.915'	E037º 28.730'	1
54		Kithimani	1277	S01º 10.561'	E037º 28.730'	2
55		Kithendu	1231	S01º 09.111'	E037º 29.318'	2
56	Kitui	Kyanika	1116	S01º 24.256'	E038º 00.973'	4
57		Kyanika	1078	S01º 24.272'	E038º 01.617'	2
58		Kyagwuthye	1129	S01º 21.578'	E038º 00.718'	3
59		Kyahwithye	1143	S01º 20.473'	E038º 00.186'	1
60		Munuvemuma	1151	S01º 20.212'	E037º 59.782'	1
61		Kyagwuthye	1139	S01º 20.638'	E037º 56.949'	5
62	Makueni	Ngulu	708	S02º 11.416'	E038º 03.241'	4
63		Ngulu	712	S02º 12.501'	E038º 03.596'	5
64		Athi	724	S02º 10.523'	E038º 03.205'	2
65	Kajiado	Rombo	1154	S03º 03.172'	E037º 41.947'	1
66		Rombo	1101	S03º 03.850'	E037º 42.902'	1
67		Rombo	1106	S03º 04.015'	E037º 42.805'	2
68		Rombo	1044	S03º 04.854'	E037º 44.805'	1
69		Kimana	1338	S02º 49.335'	E037º 31.539'	5
70		Kimana	1302	S02º 48.485'	E037º 31.803'	1
71		Kimana	1308	S02º 48.999'	E037º 32.010'	2
72		Kimana	1227	S02º 44.893'	E037º 30.568'	6
73		Kimana	1232	S02º 44.640'	E037º 30.479'	2
74		Kuku	1475	S02º 54.700'	E037º 34.845'	5
75		Kuku	1450	S02º 54.332'	E037º 35.208'	5
76		Kuku	1440	S02º 53.882'	E038º 35.259'	1
77		Kimana	1438	S02º 51.565'	E037º 32.284'	3
78	Taita-Taveta	Wundanyi	1387	S03º 24.281'	E038º 21.623'	1
79		Wundanyi	1562	S03º 25.277'	E038º 20.533'	5
80		Wundanyi	1629	S03º 25.546'	E038º 20.142'	2

No= Number of sites surveyed; **NS**= Number of leaves where mites were collected from in the laboratory; *Location where fungus were found to be associated with spider mites.



(a) **(b)** **(c)** **(d)**

Figure 4.1: (a) *Beuaveria bassiana* isolate from *Tetranychus* sp.

(b) Metarhizium anisopliae isolate from Tetranychus urticae and (c) and (d) from M. anisopliae isolates Tetranychus sp.

The characteristics followed for identification of *B. bassiana* and *M. anisopliae* are as follows:

Baeuveria bassiana: Dense white and covering exoskeleton; conidiogenous cells usually dense clustered (or whorled or solidary), colorless, with globose or flask-like base and dentriculate apical extension bearing one conidium per denticle; Conidia are separate.

Metarhizium anisopliae: Usualy mummify/cover the whole host, conidiophores in compact patches, individual conidiophore broadly branched (candelabrum-like), densely intertwined; conidiogenous cells with rounded to conidial apices, arranged in dense hymenium; conidia aseparate, cylindrical or ovoid, columns or solid mass parallel chains, pale to bright green, to yellow green, olivaceous.

Table 4.2: New fungal isolates recovered from a survey of Western, Central and Eastern Kenya

Isolates	Species	Host	Host plant	Location	District	GPS Coordinates	Altitude
ICIPE318	*Beauveria bassiana*	*Tetranychus* spp.	Unknown weed	Shinyalu	Kakamega	N00º12.771'E034º48.273'	1579m
ICIPE315	*Metarhizium anisopliae*	*Tetranychus urticae*	*Phaseolus vulgaris* L.	Mwea	Kerugoya	S00º39.766'E037º23.425'	1161m
ICIPE317	*Metarhizium anisopliae*	*Tetranychus* spp.	*Amaranthus* spp.	Kirinyaga	Kerugoya	S00º29.803'E037º14.546	1641m
ICIPE316	*Metarhizium anisopliae*	*Tetranychus* spp.	*Lycopersicon esculentum* Mill.	Kutus	Kerugoya	S00º34.86'E037º19.439'	1281m

4.4 Discussion

The surveys yielded one isolate of *B. bassiana* from *Tetranychus* spp. and three isolates of *M. anisopliae*, two from *Tetranychus* spp. and one from *Tetranychus urticae*. These isolates were found in the areas surveyed during rainy season (Kakamega and Kerugoya). However mites were found in all the sites surveyed. , they Although mites were in Kakamega and Kerugoya, they did not present a severe level of infestation, and this may be attributed not only to the pesticides used by farmers, but also to the associated natural enemies, including entomopathogenic fungi, that maintain their populations below economic damage levels. Rainfall has been also reported to reduce mite population in the field (Humber *et al.*, 1981; Fiaboe 2007). Studies indicate that the pathogenic effect of the fungus together with the direct effect of the rain play an important role in reducing mite populations. We therefore suspect that this might have been the case in Kakamega and Kerugoya districts where we observed low populations of mite.

It is generally admitted that fungal strains isolated from a host are more virulent than that isolated from non-host (Maniania 1992, Pena *et al.*, 1996; Meikle *et al.*, 2005). For example, Maniania (1992) found a strain of *M. anisopliae* on the maize stem borer, *Busseola fusca* Fuller, which was more virulent to *B. fusca* than those originating from other sources. Similar observations were reported by Meikle *et al.* (2005) who found that *Isaria fumosorosea* (= *Paecilomyces fumosoroseus*) (Holmsk.) Fr. isolate obtained from the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, was highly virulent to this termite species than the commercial strain of *M. anisopliae*, BioBlast®, isolated from another host.

Natural association between entomopathogenic fungi and tetranychid mites has been reported in several studies (Carner and Canerday, 1970; Humber *et al*., 1981; van der Geest, 1985; Chandler *et al*., 2000; Fiaboe, 2007). For instance Carner and Canerday (1970) observed epizootics caused by *Entomophthora* sp. in the two spotted spider mite, *T. urticae.* Natural association between *T. urticae* with several other entomopathogenic fungi such as *Basidiobolus* sp. (van der Geest, 1985), *Conidiobolus obscurus* (Andreeva and Shternshis, 1995), *Neozygites floridana* (Weiser and Muma, 1966; Kenneth *et al.* 1972, Smitley *et al*. 1986; Mietkiewski *et al.* 1993), *N.* sp. near *floridana* (Ramaseshiah, 1971), *N. tetranychi* (Weiser, 1968) has been reported. However, little information is available on the interaction between tetranychid mites and the entomopathogenic fungi, *B. bassiana* and *M. anisopliae* under natural conditions (Chandler *et al*. 2000; Maniania *et al*., 2008). Nevertheless Wright and Kennedy (1996) reported the association between *B. bassiana* and *T. urticae* in the field. Although many samples of mites were collected during the surveys, no association could be found between mites and entomopathogenic fungi. For *T. evansi*, Humber *et al*. (1981) and Fiaboe (2007) reported the natural infection of the said spider mite by *Neozugites* sp. and *N. floridana*, respectively. Apart from these two reports, there is no other report on the natural infection of *T. evansi* by entomopathogenic fungi in the field.

The new isolates found are promising biological control agents of spider mite species and can therefore be incorporated in spider mites IPM strategies. However, further studies are important for their assessment. We suggest that they are tested at different weather conditions levels and different concentrations. Nevertheless results

of this study highlight the importance and interest of prospecting for new fungal isolates and their use as biological control agents of spider mite pests.

CHAPTER 5: PATHOGENICITY OF *BEAUVERIA BASSIANA* AND *METARHIZIUM ANISOPLIAE* TO ADULT *TETRANYCHUS URTICAE* AND *TETRANYCHUS EVANSI*

5.1 Introduction

The two-spotted spider mite, *Tetranychus urticae* Koch and the tomato spider mite, *T. evansi* Baker & Pritchard (Acari: Tetranychidae) are among the most economically important spider mite species (Jeppson *et al*., 1975; Fasulo and Denmark, 2000; Saunyama and Knapp, 2003). They have a world-wide distribution and attack a wide range of wild and cultivated plants including many fruits, cotton, cereals, legumes, vegetables and ornamental plants (Jeppson *et al*., 1975; Smith Meyer, 1996; Bolland *et al*., 1998). The management of these pests has mainly relied on the use of synthetic acaricides (Meyer, 1996; Knapp *et al*., 2003b). However, due to the excessive use of synthetic acaricides and the related problems of synthetic acaricide resistance (Cranham and Helle, 1985; Hoy, 1998; Herron *et al*., 2004) and environmental contamination (van de Vrie, 1985), there is an increased demand for alternatives that are sustainable and environmental-friendly. Several studies have reported predatory mites as potential biological control agents of *T. urticae* (Moraes and McMurtry, 1985a; Escudero and Ferragut, 2005; Oliveira *et al*., 2007). Entomopathogenic fungi are the most common pathogens associated with *T. urticae* and *T. evansi* in the field (Brandenburg and Kennedy, 1981; Humber *et al*., 1981; Maniania *et al*., 2008) and have been widely tested in the laboratory (Gardener *et al*., 1982; Tamai *et al*., 2002; Chandler *et al*., 2005; Wekesa *et al*., 2005, 2007; Maniania *et al*., 2008). They could be therefore used in biological control programme of *T. urticae* and *T. evansi* either as a stand-alone solution in

replacement of synthetic acaricides that are currently in use or as a component of integrated mite management. The objective of this study was to assess the pathogenicity of different isolates of *B. bassiana* and *M. anisopliae* to adults of the two spider mite species, *T. urticae* and *T. evansi* in order to select the most pathogenic fungal isolates for further studies.

5.2 Materials and Methods

5.2.1 Mite cultures

The spider mite species, *T. urticae* and *T. evansi* were reared on bean, *Phaseolus vulgaris* L. variety GLP2, and tomato, *Lycopersicon esculentum* Mill. Variety Cal-J, respectively, in a rearing room maintained at 25 ± 2 ºC, 60-70% RH and a photoperiod 12:12 L:D at the International Centre of Insect Physiology and Ecology (ICIPE) Headquarters, Nairobi, Kenya. The initial cultures of *T. urticae* and *T. evansi* originated from mites collected on rose plants in a screenhouse in Naivasha, Kenya, in 2004 and from mites collected on tomato plants in Mwea Irrigation Scheme, Kenya, in 2001, respectively. Quiescent deutonymps of *T. urticae* and *T. evansi* were collected from the mite cultures using a fine camel hairbrush and transferred to bean and tomato leaf discs, respectively. After two days, newly emerged adult female mites (1-2 day-old) were selected and used for the experiments.

5.2.2 Fungal Isolates

Three isolates of *B. bassiana* and 23 isolates of *M. anisopliae*, originating from different hosts were obtained from the *icipe* Arthropod Germplasm Centre (Table 5.1). From the 26 isolates, 2 (ICIPE315 and ICIPE316) were isolated from mites collected from the field (see Chapter 4), and the other 23 originated from the ICIPE germplasm. Conidia were harvested by scrapping the surface of 3 week-old sporulating cultures grown on Sabouraud Dextrose Agar (SDA) in Petri dishes at 26 ± 2 ºC and 40-70 % RH. Conidia were suspended in 20ml sterile distilled water containing 0.05% Triton X-100 in universal bottles containing glass beads. The suspension was vortexed for 5 minutes to produce homogenous conidial suspension. The viability of conidia was then determined by spread-platting 0.1ml of the suspension (titrated to 3.0×10^6 conidia ml^{-1}) on SDA plates. Sterile microscope cover slip was placed on each plate. Plates were incubated at 26 ± 2 ºC and examined after 24 hours. Percentage germination was determined by counting 100 spores for each plate.

5.2.3 Bioassays

Ten millilitres (10 ml) of standard concentration of 1.0×10^7 conidia ml^{-1} was sprayed on both sides of bean and tomato leaf discs (25 mm diameter) using the Burgerjon's spray tower (Burgerjon, 1956) (INRA, Dijon, France). In the control treatments, mites were sprayed with sterile distilled water containing 0.05 % Triton X-100. The leaf discs were then air-dried under the laminar flow cabinet for 20 minutes and placed on wet cotton wool in Petri dishes (60 mm diameter). Twenty

young adult females of *T. urticae* and *T. evansi* (1-2 day-old) were then placed onto the treated bean and tomato leaf discs, respectively, and maintained for 4 to 5 days, after which they were transferred to untreated leaves. Test-mites were maintained in an incubator at 26 ± 2 ºC. Mortality was recorded daily for 10 days. Dead mites were transferred to Petri dishes lined with moist filter paper to allow the growth of fungus on the surface of the cadaver. Mortality caused by fungus was confirmed by microscopic examination. Each treatment was replicated six times.

5.2.4 Radial growth

Conidial suspension of 1×10^7 conidia ml^{-1} was spread-plated on SDA plates (90 mm diameter), which were then incubated at 26 ± 2 ºC for 3 days in order to obtain mycelial mats. The unsporulated mycelial mats were cut from culture plates into round agar plugs using an 8-mm diameter cork borer (Rapilly, 1968). Each agar plug was then transferred singly onto the centre of a fresh SDA plate. Plates were sealed with Parafilm M and incubated in complete darkness at 26 ± 2 ºC. Radial growth was then recorded daily for 10 days by measuring colony cardinal diameters, through two orthogonal axes previously drawn, using a simple plastic ruler (15 cm long), on the bottom of each Petri dish to serve as a reference. The experiment was replicated six times.

5.2.5 Statistical analysis

Mortality data was corrected for natural mortality (Abbott, 1925) and arcsine-transformed to normalize the data before ANOVA analysis (SAS, 1999-2001).

Means were separated by Student-Newman-Keuls test at p=0.05. Probit analysis was used to estimate the lethal time to 50% mortality (LT_{50}) (SAS, 1999-2001). Correlation between the fungal radial growths and LT_{50} *T. urticae* and *T. evansi* mortality was established using SAS (SAS, 1999-2001).

5.3 Results

5.3.1 Pathogenicity of fungal isolates against *T. urticae* and *T. evansi*

In the viability tests, 86.9-96.3 % of spores germinated (Table 5.1). All the fungal isolates tested were pathogenic to the adult females of *T. urticae* (Table 5.2) and *T. evansi* (Table 5.3). However, mortality depended on isolate/species. *Metarhizium anisopliae* isolates ICIPE25, ICIPE7 and ICIPE315 were the most virulent to *T. urticae* causing mortality of 100.0%, while *M. anisopliae* isolate ICIPE43 was the least virulent causing 36.5% (F = 51.38; df = 26, 135; $P < 0.0001$) (Table 5.2). In the case of *T. evansi*, *B. bassiana* isolate ICIPE279 was the most virulent inducing mortality of 95.2 % whilst *M. anisopliae* isolate ICIPE20 was the least virulent causing mortality 30.4% (F = 20.49; df = 26, 135; $P < 0.0001$) (Table 5.3). The LT_{50} values ranged from 3.0 to 8.3 for *T. urticae* (Table 5.2) and from 4.7 to 8.3 days for *T. evansi* (Table 5.3).

Among the 4 new isolates (see chapter 4), 2 (ICIPE315 and ICIPE316) were tested for their pathogenicity against *T. urticae* and *T. evansi*. They caused mortality of 100 and 71.8% in *T. urticae* (5.2) and 54.6 and 54% in *T. evansi* (Table 5.3). However, *T. evansi* was more susceptible to the two fungal isolates than *T. urticae*.

Table 5.1. Fungal isolates tested against adult females of *T. urticae* and *T. evansi* (% viability ± SE)

Species	Isolates	Year of isolation	Host/Substrate	Locality	Country	%Viability (± S.E)
Beauveria bassiana	ICIPE279	1996	Soil	Mombasa	Kenya	96.3 ± 0.7
	ICIPE278	2005	*Cyclocephala* sp. (Coleoptera)	Kericho	Kenya	93.8 ± 0.6
	ICIPE273	2004	Soil	Mbita	Kenya	91.5 ± 0.9
Metarhizium anisopliae	ICIPE18	1989	Soil	Mbita	Kenya	92.0 ± 0.4
	ICIPE20	1989	Soil	Migori	Kenya	90.9 ± 1.0
	ICIPE21	1999	*Lacusta gregaria*	Port-Sudan	Sudan	86.9 ± 1.0
	ICIPE30	1989	*Busseola fusca* Fuller (Lepidoptera)	Mbita	Kenya	92.9 ± 0.7
	ICIPE41	1990	Soil	Lemba	DR Congo	94.4 ± 0.4
	ICIPE49	2005	Soil	Mount Kenya	Kenya	92.3 ± 0.5
	ICIPE62	1990	Soil	Matete	DR Congo	90.2 ± 0.6
	ICIPE69	1990	Soil	Kinshasa	DR Congo	94.3 ± 0.4
	ICIPE78	1990	*Temnoschoita nigroplagiata* (Curculionidae)	Ungoye	Kenya	90.0 ± 0.5
	ICIPE84	2003	*Ornithacris turbidda* cavroisi	Kaffraine	Senegal	94.0 ± 0.4
	ICIPE315*	2005	*Tetranychus urticae*	Mwea	Kenya	96.0 ± 0.4
	ICIPE316*	2005	*Tetranychus* spp.	Kutus	Kenya	90.9 ± 0.9
	ICIPE55	2005	Soil	Embu	Kenya	93.2 ± 0.6
	ICIPE51	2005	Soil	Embu	Kenya	94.6 ± 0.4
	ICIPE24	1999	Soil	Kitui	Kenya	92.0 ± 0.4
	ICIPE25	1999	Sandy Soil	Kitui	Kenya	91.3 ± 0.3
	ICIPE43	2005	Soil	Meru	Kenya	96.2 ± 0.5
	ICIPE48	2005	Unknown	Unknown	Kenya	93.6 ± 0.8
	ICIPE7	1996	*Amblyomma variegatum*	Homabay	Kenya	92.5 ± 0.5
	ICIPE97	2005	Unknown	Unknown	Kenya	94.0 ± 0.4
	ICIPE95	2005	Soil	Unknown	Kenya	90.7 ± 1.2
	ICIPE8	1990	*Galleria melonella* (Linnaeus) (Lepidoptera)	Matete	DR Congo	92.6 ± 0.4
	ICIPE59	2005	Cartepillar	Nairobi	Kenya	88.5 ± 1.2

*Isolates recovered in this study (see chapter 4)

Table 5.2. Percent mortality, LT_{50} and correlation between the fungal radial growth and LT_{50} values of adult female *T. urticae* 10 days post-treatment at 26 ± 2 ºC

Fungal species	Isolates	% mortality ± S.E	LT_{50} (day) (95% Fiducial limits)	Correlation RG-LT_{50} (r-value)
Beauveria bassiana	ICIPE278	99.0 ± 1.0a	4.7 (4.6-4.8)	-0.25149
	ICIPE273	95.5 ± 1.6a	5.2 (5.1-5.3)	-0.791811
	ICIPE279	95.2 ± 3.9a	4.5 (4.4-4.6)	0.01168
Metarhizium anisopliae	ICIPE25	100.0 ± 0.0a	6.1 (6.0-6.1)	0.62792
	ICIPE7	100.0 ± 0.0a	3.1 (3.0-3.2)	0.46487
	ICIPE315	100.0 ± 0.0a	3.0 (2.9-3.2)	0.07714
	ICIPE24	99.1 ± 0.9a	5.5 (5.4-5.6)	0.27801
	ICIPE8	98.3 ± 1.0a	3.2 (3.1-3.3)	-0.35654
	ICIPE51	98.2 ± 1.1a	3.6 (3.5-3.7)	0.35293
	ICIPE78	97.2 ± 1.9a	5.3 (5.2-5.4)	-0.49737
	ICIPE84	95.6 ± 1.6a	3.2 (3.1-3.4)	0.01448
	ICIPE20	95.2 ± 6.1a	4.8 (4.7-4.9)	0.17774
	ICIPE62	95.1 ± 2.8a	4.5 (4.4-4.7)	0.74176
	ICIPE59	92.9 ± 3.8a	3.2 (3.1-3.4)	-0.12450
	ICIPE48	92.8 ± 3.0a	3.5 (3.4-3.6)	0.03130
	ICIPE49	92.0 ± 4.4a	4.5 (4.5-4.7)	0.25188
	ICIPE41	91.2 ± 3.7a	4.7 (4.6-4.8)	-0.08317
	ICIPE21	87.3 ± 3.9ab	5.5 (5.4-5.6)	0.06280
	ICIPE18	85.6 ± 3.6ab	5.4 (5.3-5.5)	0.20294
	ICIPE316	71.8 ± 4.1bc	7.4 (7.3-8.0)	0.91279*
	ICIPE30	63.4 ± 8.8cd	7.9 (7.8-8.0)	0.69389
	ICIPE97	52.0 ± 6.2de	8.3 (8.1-8.5)	-0.59453
	ICIPE55	43.3 ± 13.0e	-	-
	ICIPE95	38.6 ± 8.1e	-	-
	ICIPE69	37.7 ± 12.7e	-	-
	ICIPE43	36.5 ± 8.1e	-	-
	CONTROL	11.5 ± 8.1f	-	-

Means with the same letter are not significantly different ($P = 0.05$); * Significant difference ($P < 0.05$). (–) LT_{50} was not determined because mortality was lesser than 50%

Table 5.3. Percent mortality, LT_{50} and correlation between the fungal radial growth and LT_{50} values of adult female *T. evansi* 10 days post-treatment at 26 ± 2 ºC

Fungal species	Isolates	% mortality ± S.E	LT_{50} (day) (95% Fiducial limits)	Correlation RG-LT_{50} (r-value)
Beauveria bassiana	ICIPE279	95.2 ± 2.3a	4.7 (4.6-4.8)	-0.01978
	ICIPE273	83.3 ± 5.6abcd	6.6 (6.5-6.7)	-0.12461
	ICIPE278	83.0 ± 7.7abcd	5.8 (5.7-5.9)	-0.30263
Metarhizium anisopliae	ICIPE24	90.5 ± 3.8ab	6.4 (6.3-6.5)	0.42003
	ICIPE84	89.4 ± 3.7abc	6.6 (6.5-6.6)	0.41343
	ICIPE78	86.8 ± 8.8abc	5.9 (5.8-6.0)	-0.37699
	ICIPE43	84.8 ± 5.7abcd	6.4 (6.3-6.6)	0.24519
	ICIPE55	81.7 ± 9.2abcd	6.3 (6.2-6.4)	0.12728
	ICIPE59	79.9 ± 7.1abcd	7.0 (7.0-7.2)	-0.51871
	ICIPE8	79.3 ± 8.8abcd	6.9 (6.7-7.0)	0.52012
	ICIPE51	73.0 ± 7.2abcde	7.7 (7.6-7.8)	0.04288
	ICIPE7	70.9 ± 6.3abcdef	8.0 (7.9-8.0)	-0.26565
	ICIPE25	68.4 ± 8.0abcdef	7.4 (7.3-7.6)	-0.51871
	ICIPE48	60.1 ± 7.9bcdefg	7.8 (7.7-8.0)	-0.46221
	ICIPE49	57.9 ± 9.0cdefg	8.3 (8.1-8.6)	-0.21817
	ICIPE315	54.6 ± 6.3defg	7.6 (7.4-7.8)	0.71150
	ICIPE316	54.0 ± 10.5defg	8.2 (8.1-8.4)	0.46961
	ICIPE95	44.3 ± 11.5efg	-	-
	ICIPE62	43.8 ± 10.4efg	-	-
	ICIPE21	43.7 ± 8.2efg	-	-
	ICIPE30	42.4 ± 12.5efg	-	-
	ICIPE41	40.6 ± 3.5fg	-	-
	ICIPE18	37.0 ± 9.4gh	-	-
	ICIPE97	35.8 ± 7.3gh	-	-
	ICIPE69	35.0 ± 4.3gh	-	-
	ICIPE20	30.4 ± 4.5gh	-	-
	CONTROL	12.9 ± 0.9h	-	-

Means with the same letter are not significantly different ($P = 0.05$). (–) LT_{50} was not determined because mortality was lesser than 50%

5.3.2 Radial growth

There were significant differences in radial growth between the fungal isolates (F = 15.18; df = 25, 130; $P < 0.0001$) (Table 5.4). *Metarhizium anisopliae* isolates grew faster than *B. bassiana* isolates. The mean radial growth recorded after 10 days varied between 2.0 mm day^{-1} for the lowest (*B. bassiana* isolate ICIPE279) and 5.8 mm day^{-1} for the fastest (*M. anisopliae* isolate ICIPE25). Positive and negative correlations were observed between fungal radial growth and the LT_{50} values (Table 5.2 and 5.3). However, the significant difference was only observed in the correlation between the *M. anisopliae* isolate ICIPE316 and the LT_{50} *T. urticae* mortality ($P < 0.05$) (Table 5.2).

Table 5.4: Fungal mean radial growth (mm day^{-1} ± SE) at 26 ± 2ºC

Fungal species	Isolates	Mean radial growth ± S.E
Metarhizium anisopliae	ICIPE25	5.8 ± 0.2a
	ICIPE51	5.6 ± 0.3ab
	ICIPE48	5.6 ± 0.0abc
	ICIPE316	5.4 ± 0.8abcd
	ICIPE43	5.1 ± 0.0abcde
	ICIPE24	4.8 ± 0.0abcdef
	ICIPE62	4.7 ± 0.0bcdefg
	ICIPE21	4.6 ± 0.1cdefgh
	ICIPE7	4.4 ± 0.1defghi
	ICIPE30	4.3 ± 0.4defghi
	ICIPE59	4.2 ± 0.4efghi
	ICIPE95	4.2 ± 0.2efghi
	ICIPE20	4.2 ± 0.6efghi
	ICIPE49	4.1 ± 0.1efghi
	ICIPE8	4.1 ± 0.2efghi
	ICIPE41	4.0 ± 0.2efghi
	ICIPE69	4.0 ± 0.0efghi
	ICIPE55	4.0 ± 0.1efghi
	ICIPE18	3.9 ± 0.3fghi
	ICIPE315	3.6 ± 0.3ghi
	ICIPE97	3.5 ± 0.2ghij
	ICIPE78	3.4 ± 0.2hij
	ICIPE84	3.3 ± 0.2ij
Beauveria bassiana	ICIPE273	2.6 ± 0.1jk
	ICIPE278	2.1 ± 0.1k
	ICIPE279	2.0 ± 0.0k

Means with the same letter are not significantly different ($P = 0.05$)

5.4 Discussion

All the 26 isolates tested were pathogenic to *T. urticae* and *T. evansi* in the laboratory, but there were significant variations between the isolates. *Beauveria bassiana* and *M. anisopliae* have previously been reported to be pathogenic to other mite species (Tamai *et al*., 2002; Barreto *et al.*, 2004; Oliveira *et al.*, 2004; Alves *et al.*, 2005; Brooks and Wall, 2005; Chandler *et al*., 2005; Rossi-Zalaf and Alves, 2006). Intranspecific differences in pathogenic activity of fungal isolates have been reported against many arthropods (Fargues and Remaudière, 1977; Maniania, 1992; Vestergaard *et al*., 1995; Ekesi *et al*., 1998, 2002; Dimbi *et al*., 2003; Davidson and Chandler, 2005; Lecuona *et al*., 2005b; Wekesa *et al*., 2005; Marannino *et al*., 2006). This factor is very important and has to be taken into account in strain selection (Soper and Ward, 1981; Ekesi, 1999). The pathogenicity of *B. bassiana* and *M. anisopliae* has already been reported in *Tetranychus urticae* (Alves *et al*., 2002; Tamai *et al*., 2002; Irigaray *et al*., 2003; Chandler *et al*., 2005) and *T. evansi* (Wekesa *et al*., 2005). The pathogenicity of other fungal species has also been reported in *T. urticae* (Carner and Canerday, 1970; Brandenburg and Kennedy, 1981; Rosas-Acevedo *et al*., 2003) and in *T. evansi* (Humber *et al*., 1981; Fiaboe, 2007; Wekesa *et al*., 2007).

It is generally admitted that the most virulent fungal isolates are the ones isolated from the host. For example, Pena *et al*. (1996) showed that fungal isolates originating from *Polyphagotarsonemus latus* Banks were more pathogenic to this mite species than those isolated from other hosts. This was demonstrated in this study where the *M. anisopliae* isolate ICIPE315 that originated from *T. urticae* was

highly pathogenic to *T. urticae* causing 100% mortality and had a the shortest lethal time to 50% mortality (LT_{50}) of 3 days. However, this was not the case for *T. evansi* where the most pathogenic isolates did not originate from acarine hosts.

All the fungal isolates grew at 26 ºC, but the radial growth varied with isolates. Compared to *M. anisopliae* isolates, *B. bassiana* isolates showed a low radial growth rate. Based on the coefficients of correlation values, the fungal radial growth appeared to have no relationship with fungal infection in the two spider mite species. For example, *B. bassiana* isolate ICIPE279 had the slowest growth (2.0 mm/day) but caused 95.5% mortality with a LT_{50} of 4.7 days, while *M. anisopliae* ICIPE43 with a growth rate of 5.1 mm/day could only achieve mortality of 36.5% in *T. urticae*. However the same isolate (*M. anisopliae* isolate ICIPE43) caused up to 84.8% mortality in *T. evansi* with a LT_{50} of 6.4 days. The lack of correlation between fungal growth and infection has been reported in other insects such as *Spodoptera littoralis* (Boisduval) with isolates of *I. fumosorosea* (Wize) (Maniania and Fargues, 1992). Moreover, the optimum temperature for fungal growth is not necessarily the same as that for fungal infection of arthropods (Fargues *et al*., 1992). The findings of this study confirm the pathogenicity of *B. bassiana* and *M. anisopliae* against *T. evansi* and *T. urticae* as reported by many other workers (Tamai *et al*. 2002; Chandler *et al*. 2005; Wekesa *et al*., 2005).

Tetranychus urticae appeared to be more susceptible to infection by *B. bassiana* and *M. anisopliae* than *T. evansi*. This may be attributed to either their physico-

morphological features (e.g., cuticle composition) or to their physiological characteristics.

This study highlights the importance of strain selection and confirms the potential of the entomopathogenic fungi *B. bassiana* and *M. anisopliae* for possible use in biological control programmes of the two spider mite species. Based on LT_{50} values of the 26 isolates, 11 isolates were selected for bioassays on the effect of temperature on germination, radial growth and virulence.

CHAPTER 6: EFFECT OF TEMPERATURE ON GERMINATION, RADIAL GROWTH AND VIRULENCE OF *BEAUVERIA BASSIANA* AND *METARHIZIUM ANISOPLIAE* TO *TETRANYCHUS URTICAE* AND *TETRANYCHUS EVANSI*

6.1 Introduction

Tetranychid mites form the family of phytophagous Acari with the most severe economic effect on agriculture and several of these mites are injurious to many crops all over the world (Jeppson *et al*., 1975; Smith Meyer, 1996; Bolland *et al*., 1998). They attack both wild and cultivated crops. In eastern and southern Africa, the spider mites *T. urticae* and *T. evansi* are regarded as one of the most serious enemies of vegetables. They cause severe damage and crop losses in tomato and other vegetable fields (Smith Meyer, 1996; Saunyama and Knapp, 2003).

Due to the problems related to the excessive use of the chemical acaricides, biological control agents, including entomopathogenic fungi, are being developed as possible alternatives to chemical pesticides for the control of Tetranychid mites (Oduor, 1995; Chandler *et al*., 2000, 2005; Davidson *et al*., 2001; Irigaray *et al*., 2002; Delalibera and Hajek, 2004; Wekesa *et al*., 2005). However, entomopathogenic fungi are exposed to a number of biotic and abiotic factors that influence their survival and capability to cause mortality (Benz, 1987; Goettel and Inglis, 1997; Inglis *et al*., 2001). Temperature, humidity and solar radiation are probably the most severe environmental factors that affect entomopathogenic fungi (Inglis et al., 2001).

Temperature affects the germination, growth, survival, pathogenicity of the pathogen, the host susceptibility and the host-pathogen interactions (Fargues *et al*., 1992; Maniania and Fargues, 1992; Ekesi *et al*., 1999; Dimbi *et a*l., 2004; Cuthberson *et al*., 2005; Devi *et al*., 2005; Lecuona *et al*., 2005a; Kiewnick, 2006). In general, optimum temperatures for germination, growth, sporulation and pathogenicity of entomopathogenic fungi range between 20-30 ºC (Ferron, 1978; Hall and Papierock, 1982; Ekesi *et al*., 1999; Tefera and Pringle, 2003; Dimbi *et al*., 2004; Kiewnick, 2006). However, variation in temperature tolerance within an isolate can be significant (Fargues *et al*., 1992; Ekesi *et al*., 1999; Tefera and Pringle, 2003; Dimbi *et al*., 2004; Cuthberson *et al*., 2005; Lecuona *et al*., 2005a). This study aimed therefore at evaluating the effect of temperature on germination, radial growth and virulence of selected isolates of *B. bassiana* and *M. anisopliae* to the two spider mite species, *T. urticae* and *T. evansi* in order to select isolates with broad temperature range.

6.2 Materials and Methods

6.2.1 Mite cultures

Stock cultures of *T. urticae* and *T. evansi* were established as described in chapter five (section 5.2.1). One to 2-day old female adults were used for this experiment.

6.2.2 Fungal cultures

Based on mortality and the LT_{50} values from the test of pathogenicity of the 26 isolates against *T. urticae* and *T. evansi* (see chapter 5), 15 isolates (2 *B. bassiana*

isolates and 13 *M. anisopliae* isolates) were selected for the assessment of temperature on their germination and radial growth. However, only 11 isolates were tested for their virulence against the two spider mite species. The selected isolates were tested at 20, 25, 30 and 35 ºC. Germination, radial growth and virulence were observed at all temperatures tested, but varied with isolate and temperature. Two isolates of *B. bassiana* (ICIPE278 and ICIPE279) and 13 of *M. anisopliae* (ICIPE7, ICIPE8, ICIPE24, ICIPE25, ICIPE43, ICIPE48, ICIPE49, ICIPE55, ICIPE59, ICIPE62, ICIPE78, ICIPE84 and ICIPE315) Isolates were maintained as outlined in Chapter 5 (section 5.2.2).

6.2.3 Effect of temperature on germination

Conidial suspension (0.1 ml of 3×10^6 conidia ml^{-1}) was spread on SDA plates in Petri dishes (90 mm diameter). Sterile microscope cover slips were placed on each plate and the inoculated plates were sealed with Parafilm membrane and incubated at 20, 25, 30 and 35 ºC in complete darkness. At 24 hours post inoculation, 1 ml formaldehyde (0.5%) was transferred onto each plate to halt germination. Percentage germination was then determined from 100-spore counts for each plate at × 40 magnification. Each treatment was replicated four times.

6.2.4 Effect of temperature on radial growth

A conidia suspension of 1×10^7 conidia ml^{-1} was spread-plated on SDA plates (90 mm diameter). Plates were then incubated at 26 ± 2 ºC for 3 days in order to obtain mycelial mats. The unsporulated mycelial mats were cut from culture plates into

round agar plugs using an 8-mm diameter cork borer (Rapilly, 1968). Each agar plug was then transferred singly onto the centre of a fresh SDA plate. Plates were sealed with Parafilm M and incubated in complete darkness at 20, 25, 30 and 35 ºC. Radial growth was then recorded daily for 10 days. The experiment was replicated four times (Dimbi *et al*., 2004).

6.2.5 Effect of temperature on virulence

The procedures are similar to that described in section 5.2.3 of chapter 5, but the only difference is that mites were maintained at different temperatures (20, 25, 30 and 35 ºC) and the treatment was replicated four times.

6.2.6 Statistical analysis

Germination and growth data were analysed by using ANOVA procedures of SAS (SAS Institute, 1999-2001) after arcsine transformation to normalize the data (Gomez & Gomez, 1984). Mortality data was corrected for natural mortality (Abbott, 1925) and arcsine-transformed to normalize the data before ANOVA procedure of SAS. Means were separated by Student-Newman-Keuls test at p=0.05. Probit analysis was used to estimate the lethal time to 50 % mortality (LT_{50}) and the lethal time to 90 % mortality (LT_{90}) (SAS, 1999-2001). Parallelism X was used to determine whether a common slope was adequate for isolates within species. The effect of temperature on germination, radial growth and virulence was analysed using ANOVA procedures of SAS (SAS, 1999-2001).

6.3 Results

6.3.1 Effect of temperature on germination

Significant differences in germination between fungal isolates were observed at 20 ºC ($F = 22.45$; df = 14, 45; $P < 0.0001$), 25 ºC ($F = 30.18$; df = 14, 45; $P < 0.0001$), 30 ºC ($F = 20.66$; df = 14, 45; $P < 0.0001$) and at 35 ºC ($F = 98.85$; df = 14, 45; $P < 0.0001$). The germination for all isolates was above 50% at the four temperatures except at 35 ºC where *B. bassiana* isolates ICIPE279 and ICIPE278 were very low (20.6 and 15.1%, respectively) (Table 6.1).

The optimum temperature for germination was between 25 and 30 ºC for all the isolates.

Table 6.1: Effect of temperature on germination (%) of *Beauveria bassiana* and *Metarhizium anisopliae* isolates

Species	Isolates	Germination (Mean % ± SE)			
		20ºC	25ºC	30ºC	35ºC
Beauveria bassiana	ICIPE279	86.2 ± 3.2aB	93.5 ± 0.6abAB	96.9 ± 0.3aA	20.6 ± 3.5cC
	ICIPE278	67.7 ± 1.1cdB	95.0 ± 1.0aA	95.0 ± 1.3abA	15.1 ± 1.7cC
Metarhizium anisopliae	ICIPE24	84.8 ± 1.4abB	91.8 ± 0.5abcA	93.0 ± 0.3abcdA	81.4 ± 1.2aC
	ICIPE59	82.9 ± 1.4abA	88.2 ± 1.0bcA	87.5 ± 1.7cdefA	77.2 ± 1.5aB
	ICIPE7	73.3 ± 2.0bcdB	86.3 ± 0.9cA	81.7 ± 2.1fA	81.1 ± 1.0aA
	ICIPE48	74.0 ± 0.7abcdB	91.1 ± 0.9abcA	69.2 ± 2.1gC	75.5 ± 1.2aB
	ICIPE55	76.1 ± 2.5abcC	91.5 ± 0.4abcA	88.6 ± 1.3bcdeA	81.0 ± 0.9aB
	ICIPE84	79.0 ± 3.5abcdB	86.9 ± 1.4cB	93.6 ± 1.6abcA	84.2 ± 1.3aB
	ICIPE78	74.7 ± 1.4abC	91.4 ± 1.4abcA	89.6 ± 1.6abcdA	84.2 ± 1.1aB
	ICIPE8	65.8 ± 0.7dC	91.4 ± 1.6abcA	81.2 ± 2.1fB	81.6 ± 1.3aB
	ICIPE49	84.0 ± 3.0abBC	89.6 ± 2.2abcAB	95.6 ± 0.3abA	80.6 ± 1.3aC
	ICIPE43	75.2 ± 3.0abcdB	74.9 ± 1.6dB	86.1 ± 3.9defA	85.6 ± 0.3aA
	ICIPE62	35.6 ± 4.6eC	71.9 ± 2.0dB	82.4 ± 0.2efA	67.9 ± 1.4bB
	ICIPE25	73.2 ± 1.4bcdC	92.0 ± 0.2abcA	91.0 ± 0.5abcdA	81.7 ± 1.4aB
	ICIPE315	79.9 ± 3.2abA	95.0 ± 0.9aA	95.4 ± 0.6abA	78.9 ± 6.7aA

Means (SE) within column followed by the lower case letter and within row bearing the same upper case letter are not significantly different (Student-Newman-Keuls test, P=0.05)

6.3.2 Effect of temperature on radial growth

All isolates grew at all temperatures but for most isolates, the radial growth rate was slower at 20 and 35 ºC than at 25 and 30 ºC. The two isolates of *B. bassiana* recorded the least radial growth. There were significant differences in radial growth between the isolates at 20 ºC ($F = 41.18$; df = 14, 45; $P < 0.0001$), 25 ºC ($F = 30.58$; df = 14, 45; $P < 0.0001$), 30 ºC ($F = 16.70$; df = 14, 45; $P < 0.0001$) and at 35 ºC ($F = 32.42$; df = 14, 45; $P < 0.0001$) (Table 6.2). The optimum temperature for fungal radial growth was 30 ºC.

Table 6.2: Effect of temperature on radial growth of *Beauveria bassiana* and *Metarhizium anisopliae* isolates

		Fungal radial growth (mm/day ± SE)			
Fungal Species	Isolates	20ºC	25ºC	30ºC	35ºC
Beauveria bassiana	ICIPE279	0.6 ± 0.0dC	1.2 ± 0.1hB	1.6 ± 0.0ghA	0.7 ± 0.1eC
	ICIPE278	0.8 ± 0.1dC	1.5 ± 0.1ghA	1.2 ± 0.1hB	0.8 ± 0.1eC
Metarhizium anisopliae	ICIPE315	2.3 ± 0.2aB	3.6 ± 0.1aA	2.7 ± 0.3defB	1.5 ± 0.0bcdC
	ICIPE55	2.1 ± 0.1abA	2.4 ± 0.1cdA	2.3 ± 0.1fgA	2.2 ± 0.0aA
	ICIPE49	1.3 ± 0.1cC	2.1 ± 0.1cdefB	3.5 ± 0.3bcdA	1.3 ± 0.1dC
	ICIPE59	1.2 ± 0.1cC	2.0 ± 0.0cdefB	4.3 ± 0.1aA	1.8 ± 0.1bB
	ICIPE62	1.5 ± 0.0cD	2.3 ± 0.0cB	3.0 ± 0.1cdefA	1.7 ± 0.0bcC
	ICIPE78	1.2 ± 0.1cB	1.9 ± 0.1defgB	3.3 ± 0.5bcdeA	1.4 ± 0.1cdB
	ICIPE84	1.3 ± 0.0cB	1.7 ± 0.1fgB	3.9 ± 0.3abcA	1.4 ± 0.1cdB
	ICIPE24	1.3 ± 0.0cC	2.1 ± 0.1cdeB	2.6 ± 0.1defA	1.3 ± 0.2dC
	ICIPE25	1.4 ± 0.0cC	1.9 ± 0.0defgB	2.4 ± 0.2fgA	1.3 ± 0.0dC
	ICIPE48	1.9 ± 0.2bBC	3.0 ± 0.2bA	2.3 ± 0.3fgB	1.5 ± 0.0cdC
	ICIPE8	1.5 ± 0.1cC	2.4 ± 0.1cdB	3.2 ± 0.2bvdeA	2.3 ± 0.1aB
	ICIPE43	1.3 ± 0.0cC	2.4 ± 0.2cB	4.0 ± 0.3abA	1.7 ± 0.1bcdC
	ICIPE7	1.1 ± 0.2cC	1.8 ± 0.1efgB	2.5 ± 0.1efA	2.2 ± 0.1aA

Means (± SE) within column followed by the lower case letter and within row bearing the same upper case letter are not significantly different (Student-Newman-Keuls test, $P = 0.05$)

6.3.3 Effect of temperature on virulence

Effect of temperature on virulence of fungal isolates to *T. urticae*

All the 11 fungal isolates tested were virulent to the two-spotted spider mite species at all temperatures, but mortality varied with fungal isolate and temperature (Table 6.3). All the isolates performed well at 30 and 35 ºC. There was no significant difference in virulence between fungal isolates at 30 ºC ($F = 1.87$; $df = 10, 33$; $P = 0.0861$) and at 35 ºC ($F = 2.07$; $df = 10, 33$; $P = 0.0574$). Significant differences were, however, observed at 20 ºC ($F = 2.17$; $df = 10, 33$; $P = 0.0463$) and 25 ºC ($F = 3.36$; $df = 10, 33$; $P = 0.0041$). Mortality occurred at all temperatures, but it was generally lower at 20 ºC (Table 6.3). At all the temperatures, mortality was less than 15% in the controls. The LT_{50} ranged from 6.7 to 9.9, 4.2 to 6.9, 2.1 to 4.6 and 1.6 to 3.0 for 20, 25, 30 and 35ºC, respectively (Table 6.4). The LT_{90}, however, ranged from 10.2 to 16.3, 6.8 to 11.1, 5.0 to 8.3 and 3.6 to 7.0 for 20, 25, 30 and 35ºC, respectively (Table 6.5).

Table 6.3: Effect of temperature on virulence of *Beauveria bassiana* and *Metarhizium anisopliae* isolates on *Tetranychus urticae* (% Mean ± SE)

		Mortality (Mean % ± SE)			
Species	Isolates	20 ºC	25 ºC	30 ºC	35 ºC
Beauveria	ICIPE279	80.0 ± 7.2abB	98.5 ± 1.5aA	95.7 ± 2.8aA	100aA
bassiana	ICIPE278	54.7 ± 13.4abB	92.8 ± 2.7abA	89.7 ± 6.9aA	100aA
Metarhizium	ICIPE315	58.1 ± 11.0abB	91.6 ± 2.8abA	93.4 ± 2.4aA	100aA
anisopliae	ICIPE49	57.8 ± 9.2abB	95.6 ± 2.8aA	91.5 ± 5.3aA	89.7 ± 4.4aA
	ICIPE62	57.8 ± 7.6abB	93.3 ± 2.5abA	100aA	91.1 ± 5.1aA
	ICIPE78	84.2 ± 5.6aAB	71.5 ± 8.3bB	100aA	97.2 ± 2.8aA
	ICIPE84	68.5 ± 8.1abB	93.0 ± 5.2abA	100aA	97.2 ± 2.8aA
	ICIPE25	54.0 ± 6.9abB	77.4 ± 10.4abA	88.9 ± 3.8aA	100aA
	ICIPE48	38.8 ± 11.4bC	77.1 ± 0.4abB	97.1 ± 1.7aA	100aA
	ICIPE8	62.5 ± 11.0abB	91.4 ± 3.8abA	100aA	98.5 ± 1.5aA
	ICIPE7	77.8± 2.8abB	84.4 ±2.5abB	98.5 ± 1.5aA	97.1± 2.9aA

Means (± SE) within column followed by the lower case letter and within row bearing the same upper case letter are not significantly different (Student-Newman-Keuls test, $P = 0.05$)

Table 6.4: Effect of temperature on virulence of *Beauveria bassiana* and *Metarhizium anisopliae* to the two-spotted spider mite, *Tetranychus urticae*: Lethal time to 50% mortality (in days) (95% fiducial limits), Slope (± SE)

	20ºC		25ºC		30ºC		35ºC	
Isolates	LT_{50}	Slope (± SE)	LT_{50}	Slope (± SE)	LT_{50}	Slope (± SE)	LT_{50}	Slope (± SE)
Beauveria bassiana								
ICIPE279	7.3 (7.1-7.4)	0.28 ± 0.01	4.2 (4.0-4.3)	0.38 ± 0.01	2.1 (3.8-4.1)	0.35 ± 0.01	2.3 (2.2-2.4)	0.27 ± 0.01
ICIPE278	9.8 (9.5-10.1)	0.26 ± 0.01	5.4 (5.3-5.6)	0.40 ± 0.01	3.5 (3.4-3.7)	0.45 ± 0.01	2.5 (2.4-2.6)	0.32 ± 0.01
Metarhizium anisopliae								
ICIPE49	8.4 (8.2-8.6)	0.37 ± 0.01	5.6 (5.5-5.7)	0.31 ± 0.01	4.6 (4.5-4.7)	0.51 ± 0.02	2.3 (2.1-2.5)	0.55 ± 0.02
ICIPE62	8.2 (8.0-8.4)	0.29 ± 0.01	5.6 (5.5-5.7)	0.35 ± 0.01	4.0 (3.9-4.1)	0.61 ± 0.02	2.8 (2.6-3.0)	0.70 ± 0.02
ICIPE78	6.7 (6.6-6.9)	0.24 ± 0.01	6.9 (6.8-7.1)	0.31 ± 0.01	3.1 (3.0-3.2)	0.61 ± 0.01	2.3 (2.2-2.4)	0.72 ± 0.02
ICIPE84	8.6 (8.4-8.8)	0.37 ± 0.01	5.1 (4.9-5.2)	0.48 ± 0.01	3.3 (3.2-3.4)	0.42 ± 0.01	2.7 (2.6-2.8)	0.87 ± 0.03
ICIPE315	8.4 (8.2-8.7)	0.28 ± 0.01	5.1 (5.0-5.3)	0.36 ± 0.01	3.9 (3.8-4.1)	0.38 ± 0.01	3.0 (2.9-3.1)	1.14 ± 0.04
ICIPE25	8.5 (8.3-8.8)	0.24 ± 0.01	5.7 (5.5-5.8)	0.24 ± 0.01	4.2 (4.0-4.3)	0.30 ± 0.01	2.5 (2.4-2.6)	0.96 ± 0.04
ICIPE48	9.9 (9.5-10.3)	0.20 ± 0.01	6.3 (6.1-6.4)	0.28 ± 0.01	2.8 (2.6-2.9)	0.32 ± 0.01	2.3 (2.3-2.4)	0.96 ± 0.04
ICIPE8	8.6 (8.4-8.8)	0.38 ± 0.01	5.3 (5.2-5.4)	0.35 ± 0.01	3.4 (3.3-3.5)	0.82 ± 0.03	1.6 (1.4-1.7)	0.49 ± 0.02
ICIPE7	7.4 (7.3-7.5)	0.40 ± 0.01	5.9 (5.8-6.1)	0.33 ± 0.01	3.3 (3.1-3.4)	0.53 ± 0.02	2.8 (2.6-2.9)	0.49 ± 0.01

Table 6.5: Effect of temperature on virulence of *Beauveria bassiana* and *Metarhizium anisopliae* isolates on adult *Tetranychus urticae*: Lethal time to 90% mortality (LT_{90}) (in days) (95% fiducial limits).

Isolates	20ºC	25ºC	30ºC	35ºC
Beauveria bassiana				
ICIPE279	10.7 (10.5-11.0)	6.8 (6.7-7.0)	5.5 (5.3-5.7)	3.8 (3.7-3.9)
ICIPE278	14.4 (13.8-15.1)	9.0 (8.8-9.3)	7.8 (7.6-8.1)	3.6 (3.5-3.7)
Metarhizium anisopliae				
ICIPE315	13.9 (13.3-14.5)	9.3 (9.0-9.6)	7.0 (6.8-7.2)	4.8 (4.7-4.9)
ICIPE49	13.0 (12.6-13.5)	9.0 (8.8-9.2)	8.3 (8.1-8.5)	7.0 (6.7-7.2)
ICIPE62	13.1 (12.7-13.7)	8.9 (8.7-9.1)	6.8 (6.7-7.0)	6.8 (6.6-7.0)
ICIPE78	10.2 (10.0-10.5)	11.1 (10.8-11.4)	5.6 (5.5-5.8)	4.6 (4.5-4.8)
ICIPE84	12.9 (12.5-13.4)	8.8 (8.6-9.0)	5.4 (5.3-5.6)	4.5 (4.4-4.6)
ICIPE25	13.8 (13.3-14.4)	10.9 (10.6-11.3)	8.1 (7.9-8.4)	3.8 (3.7-3.9)
ICIPE48	16.3 (15.4-17.2)	10.8 (10.5-11.2)	6.0 (5.9-6.2)	3.7 (3.6-3.8)
ICIPE8	12.0 (11.7-12.4)	8.9 (8.7-9.2)	5.0 (4.8-5.1)	4.2 (4.0-4.3)
ICIPE7	10.6 (10.4-10.8)	9.7 (9.5-10.0)	5.7 (5.5-5.9)	5.4 (5.3-5.6)

Effect of temperature on virulence of fungal isolates to *T. evansi*

All the 11 fungal isolates tested were virulent to the tomato spider mite species at all temperatures, but mortality varied with fungal isolate and temperature (Table 6.6). There was no significant difference in mortality between fungal isolates at 30 ºC ($F = 0.98$; df = 10, 33; $P = 0.4762$) and 35 ºC ($F = 1.15$; df = 10, 33; $P = 0.3607$). However, significant differences were observed at 20 ºC ($F = 2.43$; df = 10, 33; $P = 0.0267$) and 25 ºC ($F = 2.62$; df = 10, 33; $P = 0.0181$). *B. bassiana* isolate ICIPE279 was the best in causing mortality at 20 ºC while *M. anisopliae* isolate ICIPE84 was the best at 25 ºC (Table 6.6). In the controls, at all the temperatures, mortlity was lesser than 13%.

The LT_{50} values ranged from 7.3 to 12.6, 5.5 to 9.5, 3.0 to 6.0 and 1.5 to 3.8 days for 20, 25, 30 and 35ºC, respectively (Table 6.7). The LT_{90} values, however, ranged

from 10.9 to 25.6, 9.0 to 13.6, 5.5 to 8.7 and 3.5 to 7.7 days, respectively for 20, 25, 30 and 35ºC (Table 6.8)

Table 6.6: Effect of temperature on virulence of the selected fungal isolates on *Tetranychus evansi* (Mean % ± SE)

		Temperature			
Species	Isolates	20ºC	25ºC	30ºC	35ºC
Beauveria	ICIPE279	72.5 ± 15.1aA	88.5 ± 4.3abA	100aA	98.5 ± 1.5aA
bassiana	ICIPE278	50.5 ± 15.8abB	90.4 ± 4.1abA	98.5 ± 4.4aA	97.1 ± 1.7aA
Metarhizium	ICIPE55	26.3 ± 10.1abC	54.4 ± 9.8bB	100aA	97.2 ± 1.6aA
anisopliae	ICIPE59	8.8 ± 2.4bB	75.0 ± 10.2bA	93.8 ± 4.4aA	91.5 ± 3.7aA
	ICIPE78	50.9 ± 12.1abB	72.7 ± 7.8abAB	100aA	100aA
	ICIPE84	37.8 ± 12.1abB	97.1 ± 1.7aA	100aA	86.8 ± 11.4aA
	ICIPE24	43.8 ± 6.6abB	77.3 ± 13.3abA	100aA	93.8 ± 4.4aA
	ICIPE25	21.3 ± 8.8abB	90.3 ± 6.2abA	93.8 ± 6.3aA	100aA
	ICIPE8	42.5 ± 10.6abC	66.5 ± 6.5abB	100aA	100aA
	ICIPE43	30.4 ± 12.3abB	65.4 ± 12.3ab	98.6 ± 1.4aA	100aA
	ICIPE7	64.8 ± 13.4abB	83.7 ± 2.8abAB	98.6 ± 1.4aA	91.4 ± 5.0aAB

Means (±SE) within column followed by the lower case letter and within row bearing the same upper case letter are not significantly different (Student-Newman-Keuls test, $P = 0.05$)

Table 6.7: Effect of temperature on virulence of *Beauveria bassiana* and *Metarhizium anisopliae* to the tomato spider mite, *Tetranychus evansi*: Lethal time to 50% mortality (LT_{50}) (in days) (95% fiducial limits), Slope (± SE)

	20ºC		25ºC		30ºC		35ºC	
Isolates	LT_{50}	Slope (± SE)	LT_{50}	Slope (± SE)	LT_{50}	Slope (± SE)	LT_{50}	Slope (± SE)
Beauveria bassiana								
ICIPE279	7.3 (7.1-7.4)	0.35 ± 0.01	7.6 (7.5-7.7)	0.51 ± 0.02	4.3 (4.2-4.4)	0.70 ± 0.02	1.5 (1.3-1.7)	0.37 ± 0.01
ICIPE278	9.0 (8.8-9.1)	0.27 ± 0.01	5.5 (7.5-7.8)	0.37 ± 0.00	3.0 (2.9-3.2)	0.50 ± 0.02	1.9 (1.7-2.0)	0.42 ± 0.01
Metarhizium anisopliae								
ICIPE59	18.1 (16.1-21.2)	0.17 ± 0.02	7.5 (7.3-7.6)	0.37 ± 0.11	6.0 (5.8-6.1)	0.47 ± 0.01	3.8 (3.7-3.9)	0.40 ± 0.01
ICIPE78	8.8 (8.6-9.1)	0.26 ± 0.00	8.5 (8.4-8.7)	0.34 ± 0.01	4.5 (4.4-4.6)	0.72 ± 0.20	2.8 (2.7-2.9)	0.73 ± 0.02
ICIPE84	10.3 (10.1-10.6)	0.36 ± 0.02	6.4 (6.3-6.6)	0.46 ± 0.01	4.6 (4.5-4.7)	0.60 ± 0.02	3.7 (3.6-3.9)	0.32 ± 0.00
ICIPE55	11.6 (11.2-12.2)	0.43 ± 0.03	9.5 (9.3-9.8)	0.32 ± 0.01	3.9 (3.9-4.0)	0.82 ± 0.23	3.1 (3.0-3.2)	0.55 ± 0.16
ICIPE24	10.4 (10.0-10.9)	0.19 ± 0.00	7.6 (7.5-7.8)	0.39 ± 0.01	4.6 (4.5-4.7)	0.63 ± 0.02	3.3 (3.1-3.4)	0.44 ± 0.01
ICIPE25	12.8 (12.2-13.6)	0.31 ± 0.02	6.6 (6.5-6.7)	0.45 ± 0.01	5.5 (5.4-5.6)	0.48 ± 0.01	3.1 (3.0-3.2)	1.04 ± 0.04
ICIPE8	10.2 (9.8-10.5)	0.24 ± 0.01	8.2 (8.0-8.4)	0.31 ± 0.01	4.7 (4.6-4.7)	0.66 ± 0.02	2.5 (2.5-2.6)	0.84 ± 0.03
ICIPE43	12.6 (12.0-13.4)	0.35 ± 0.03	8.9 (8.7-9.0)	0.42 ± 0.02	3.8 (3.7-3.8)	0.62 ± 0.02	3.0 (2.9-3.0)	2.26 ± 0.12
ICIPE7	7.8 (7.7-8.0)	0.33 ± 0.31	7.1 (6.1-7.2)	0.46 ± 0.01	3.8 (3.7-3.9)	0.65 ± 0.02	3.5 (3.4-3.7)	0.34 ± 0.01

Table 6.8: Effect of temperature on virulence of *Beauveria bassiana* and *Metarhizium anisopliae* isolates on adult *Tetranychus evansi*: Lethal time to 90% mortality (LT_{90}) (in days) (95% fiducial limits)

Isolates	20ºC	25ºC	30ºC	35ºC
Beauveria bassiana				
ICIPE279	10.9 (10.6-11.2)	10.1 (9.9-10.3)	6.1 (6.0-6.3)	5.0 (4.8-5.2)
ICIPE278	13.8 (13.3-14.4)	9.0 (8.7-9.2)	5.6 (5.4-5.8)	5.0 (4.8-5.1)
Metarhizium anisopliae				
ICIPE55	14.6 (13.9-15.6)	13.6 (13.1-14.1)	5.5 (5.4-5.6)	5.5 (5.3-5.6)
ICIPE59	25.6 (22.3-30.9)	10.9 (10.7-11.2)	8.7 (8.5-8.9)	7.0 (6.8-7.1)
ICIPE78	13.8 (13.3-14.4)	12.4 (12.0-12.8)	6.3 (6.2-6.4)	4.5 (4.4-4.7)
ICIPE84	13.9 (13.4-14.5)	9.2 (9.0-9.4)	6.7 (6.6-6.9)	7.7 (7.5-7.9)
ICIPE24	17.3 (16.4-18.5)	10.9 (10.7-11.2)	6.6 (6.5-6.7)	6.2 (6.0-6.4)
ICIPE25	17.0 (15.8-18.5)	9.5 (9.3-9.7)	8.2 (8.0-8.4)	4.3 (4.2-4.4)
ICIPE8	15.6 (14.9-16.5)	12.3 (11.9-12.3)	6.6 (6.5-6.8)	4.1 (3.9-4.2)
ICIPE43	16.2 (15.1-17.8)	11.9 (11.6-12.2)	5.8 (5.7-6.0)	3.5 (3.4-3.6)
ICIPE7	11.7 (11.4-12.1)	9.9 (9.7-10.1)	5.8 (5.7-5.9)	7.3 (7.1-7.6)

6.4 Discussion

Conidia of both *B. bassiana* and *M. anisopliae* isolates germinated at all temperatures, but the optimum temperature of germination for most of the isolates was 25 and 30 ºC. This is in accordance with the results of many workers who found that the optimum temperature for germination of *B. bassiana* and *M. anisopliae* isolates varies between 25 and 30 ºC (Fargues *et al.*, 1997; Ekesi *et al*., 1999; Tefera and Pringle, 2003; Dimbi *et al*., 2004; Kienwnick, 2006). Compared to *M. anisopliae* isolates, germination of *B. bassiana* isolates was low at 35 ºC (Table 6.1).

Although all the fungal isolates grew at all temperatures, the optimum temperature of most isolates was 30 ºC. Similar results have been reported in a study conducted

by Tefera and Pringle (2003). However, Ekesi *et al*. (1999) and Dimbi *et al*. (2004) reported that the optimum temperature for radial growth of most *B. bassiana* and *M. anisopliae* isolates used in their studies lie between 25 and 30 ºC. Ouedraogo et al. (1997) reported that the optimum temperature for vegetative growth of *M. anisopliae* isolates is between 25 and 32 ºC, but with 25 ºC for most of isolates. The growth rate of fungal isolates did not necessarily relate to virulence. An isolate could grow faster than others but did not necessarily cause higher mortality. For example, the *B. bassiana* isolate ICIPE279 that had a radial growth rate of 0.6 mm/day at 20 ºC caused 72.5% mortality in *T. evansi* while the *M. anisopliae* isolate ICIPE55 with a higher radial growth rate (2.1 mm/day) induced 26.3% mortality in the same population of *T. evansi* at the same temperature.

All the selected isolates were pathogenic to the two spider mite species, but mortality varied with isolate and temperature. The susceptibility of *T. urticae* and *T. evansi* to *B. bassiana* and *M. anisopliae* infection increased as temperature increased. Most fungal isolates were more pathogenic at 25, 30 and 35 ºC than at 20 ºC. Several fungal isolates have been reported to be more pathogenic to arthropod pests at a temperature range of 25 to 35 ºC (Hsiao *et al*., 1992; Fargues *et al*., 1997; Thomas and Jenkins, 1997; Ekesi *et al*., 1999; Milner *et al*., 2003; Dimbi *et al*., 2004; Cuthbertson *et al*., 2005). Dimbi *et al*. (2004) reported similar results with a higher pathogenicity of *M. anisopliae* isolates in three species of African tephritid fruit flies, *Ceratitis capitata*, *C. cosyra* and *C. fasciventris* at 25, 30 and 35 ºC. However, Ekesi *et al*. (1999) showed that *B. bassiana* and *M. anisopliae* isolates were highly pathogenic at 20 ºC to the legume flower thrips, *Megalurothrips*

sjostedti. For instance the *M. anisopliae* isolate ICIPE62 that was among the more pathogenic to the two-spotted spider mite *T. urticae* at 25, 30 and 35 ºC in this study, has already been reported to be more pathogenic to the three species of African tephritid fruit flies, *C. capitata, C. cosyra* and *C. fasciventris* at the same temperatures (Dimbi *et al*., 2004), while Ekesi *et al*. (1999) reported that the same *M. anisopliae* isolate ICIPE62 was highly pathogenic to *M. sjostedti* at 15 and 20 ºC.

From our findings, we selected one isolate of *B. bassiana* (ICIPE279) and three *M. anisopliae* isolates (ICIPE7, ICIPE78 and ICIPE84) as potential biological control agents for *T. urticae*, and on the other hand we selected two *B. bassiana* isolates (ICIPE278 and ICIPE279) and two isolates of *M. anisopliae* (ICIPE7 and ICIPE78) as potential biological control agents of *T. evansi*. These isolates were selected because their ability to infect and cause a high rate of mortality between 20 and 35 ºC, which is the range of temperature where the pest is found.

CHAPTER 7: SUSCEPTIBILITY OF *TETRANYCHUS URTICAE* AND *TETRANYCHUS EVANSI* DEVELOPMENTAL STAGES TO INFECTION BY *BEAUVERIA BASSIANA* AND *METARHIZIUM ANISOPLIAE*

7.1 Introduction

The two-spotted spider mite, *Tetranychus urticae* Koch and the tomato spider mite, *Tetranychus evansi* Baker & Pritchard are polyphagous and attack a large number of cultivated and wild plants (Smith Meyer, 1996; Bolland *et al*., 1998). In southern Africa, *T. urticae* has been reported on more than 200 host plants (Smith Meyer 1996). In Kenya, *T. urticae* is considered as the second-most important mite pest of tomatoes after *T. evansi* (ICIPE, 2004; Varela *et al*., 2003). *Tetranychus evansi*, however, has been reported on several vegetables and ornamental crops such as roses as well as on many weeds, but with preference to Solanaceous (Bolland *et al*., 1998; Keizer and Zuurbier, 2001; EPPO, 2004). The control of these pests relies on the use of synthetic acaricides. However, a problem in controlling these spider mite species is their ability to rapidly develop resistance to acaricides after only a few applications. This has prompted research for alternative control measures with an emphasis on biological control (Escudero and Ferragut, 2005; Collier *et al*., 2007; Oliveira *et al*., 2007), including the use of entomopathogenic fungi (Irigaray *et al*., 2003; Chandler *et al*., 2005; Wekesa *et al*., 2005; Maniania et al., 2008).

The entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae* have been reported to be pathogenic to various mites, and are being developed as components of integrated pest management (IPM) programmes (Chandler *et al*.,

2000, 2005; Tamai *et al*., 2002; Davidson *et al*., 2001; Barreto *et al*., 2004; Oleivera *et al*., 2004; Alves *et al*., 2005; Wekesa *et al*., 2005).

The susceptibility of arthropods to entomopathogenic fungi is influenced by several factors such as the pathogen and host population properties, as well as environmental conditions (Benz, 1987; Inglis *et al*., 2001). Among the host factors, developmental stage has mostly been reported to influence arthropod susceptibility to the entomopathogenic fungi (Ferron, 1985; Feng *et al*., 1985; Ekesi and Maniania; 2000; Samish *et al*., 2001; Dimbi *et a*l., 2003; Wekesa *et al*., 2006). Oduor (1995) found that immature stages of the cassava green mite, *Mononychellus tanajoa* Bondar were more susceptible to fungal infection than mature stages. Similar results were reported in *T. urticae* (Irigaray *et al*., 2003) and in *T. evansi* (Wekesa *et al*., 2006). It is therefore important, in an assessment of the biological control potential of an entomopathogenic fungus, to test it against more than one developmental stage of its host because according to Oduor (1995), a pathogen that attacks and kills more than one stage effectuates better control on the host population. This study is aimed at studying the susceptibility of different developmental stages of *T. urticae* and *T. evansi* (eggs, larvae, protonymphs, deutonymphs and adults) to infection by *B. bassiana* and *M. anisopliae* and their implication in the control of these pests.

7.2 Materials and Methods

7.2.1 Mite cultures

The developmental stages (eggs, larvae, protonymphs, deutonymphs and adults) were obtained from *T. urticae* and *T. evansi* colonies, reared in the laboratory as described in chapter 3 (section 3.3).

7.2.2 Entomopathogenic fungal isolates

In this experiment *B. bassiana* isolate ICIPE279 and *M. anisopliae* isolates ICIPE7, ICIPE78 and ICIPE84 were used against *T. urticae*; while *B. bassiana* isolates ICIPE279 and ICIPE278, and *M. anisopliae* isolates ICIPE7 and ICIPE78 were used against *T. evansi*. These isolates were selected because of their virulence to *T. urticae* and *T. evansi*, respectively, at broad range of temperature. The fungi were grown for 2-3 weeks on Sabouraud Dextrose Agar (SDA) plates at 26 ± 2ºC. Viability of conidia was determined before carrying out bioassays as described in chapter 3 (section 3.4). Conidial suspensions were prepared as described in the same chapter 3 (section 3.4).

7.2.3 Inoculation of eggs

Twenty freshly laid eggs (24 hours old) of *T. urticae* and *T. evansi* were transferred to bean and tomato leaf discs (25 mm diameter), respectively, in Petri dishes (60 mm diameter) lined with wet cotton wool. Ten (10) ml of conidial suspensions was then sprayed on the leaf discs with eggs using Burgerjon's spray tower (Burgerjon,

1956). Four concentrations (3×10^5, 1×10^6, 3×10^6 and 1×10^7 conidia ml^{-1}) were used. Controls were treated with sterile distilled water containing 0.05% Triton X-100. Petri dishes containing eggs were kept in an incubator at 26 ± 2ºC at 60-70% RH in a completely randomised design. Egg hatchability was observed daily for 7 days. The experiment was replicated four times.

7.2.4 Inoculation of larvae, protonymphs, deutonymphs and adults

Fresh bean and tomato leaf discs (25 mm diameter) were placed in a 60-mm diameter Petri dish lined with wet cotton wool and were sprayed with 10 ml of conidial suspensions using Burgerjon's spray tower as described in chapter 5 (section 5.2.3). Twenty *T. urticae* and *T. evansi* mites were then transferred to the treated bean and tomato leaf discs, respectively, using a fine camel hairbrush. Concentrations of 3×10^5, 1×10^6, 3×10^6 and 1×10^7 conidia ml^{-1} were used on each developmental stage. Control lots were treated with sterile distilled water containing 0.05 Triton X-100. Mites were exposed to treated bean and tomato leaf discs for 5 days (*T. urticae*) and 4 days (*T. evansi*); after which they were removed and replaced with fresh, and untreated ones. Mites were maintained in an incubator at 26 ± 2ºC and 80-95% RH in a completely randomised design. Mortality was recorded daily for 10 days. Treatments were replicated four times.

7.2.5 Statistical Analysis

Percentage egg hatchability and percentage mortality in the motile stages were corrected for natural mortality (Abbott, 1925) and arcsine-transformed to normalize

the data before being subjected to ANOVA (SAS, 1999-2001). Means were separated by Student-Newman-Keuls test at p=0.05. Probit analysis was used to estimate the lethal concentration causing 50% mortality (LC_{50}) and the lethal concentration inducing 90% mortality (LC_{90}) (SAS, 1999-2001).

7.3 Results

7.3.1 Effect of fungal infection on *T. urticae* and *T. evansi* egg hatchability

In both species, all the eggs (100%) hatched in the controls. However, the percentage of egg hatchability reduced with increased fungal concentration and fungal isolate. The lowest egg hatchability for the two spider mite species occurred at the highest concentration of 1×10^7 conidia ml^{-1} at 7 days post treatment (Figures 7.1 and 7.2).

In *T. urticae*, significant differences in egg hatchability were observed between isolates at the concentrations of 3×10^5 conidia ml^{-1} ($F=3.32$; $df= 3, 12$; $P= 0.0567$), 3×10^6 conidia ml^{-1} ($F=12.19$; $df=3, 12$; $P=0.0006$) and 1×10^7 conidia ml^{-1} ($F=5.60$; $df=3, 12$; $P=0.0123$). However there was no significant difference between isolates at the concentration of 1×10^6 conidia ml^{-1} ($F=0.61$; $df= 3, 12$; $P=0.6214$) (Figure 7.1). Significant differences were also observed between concentrations in all isolates: ICIPE279 ($F=343.42$; $df=4, 15$; $P< 0.0001$); ICIPE78 ($F=590.96$; $df=4, 15$; $P< 0.0001$); ICIPE84 ($F=186.33$; $df=4, 15$; $P< 0.0001$) and ICIPE7 ($F=220.86$; $df=4, 15$; $P< 0.0001$) (Figure 7.1).

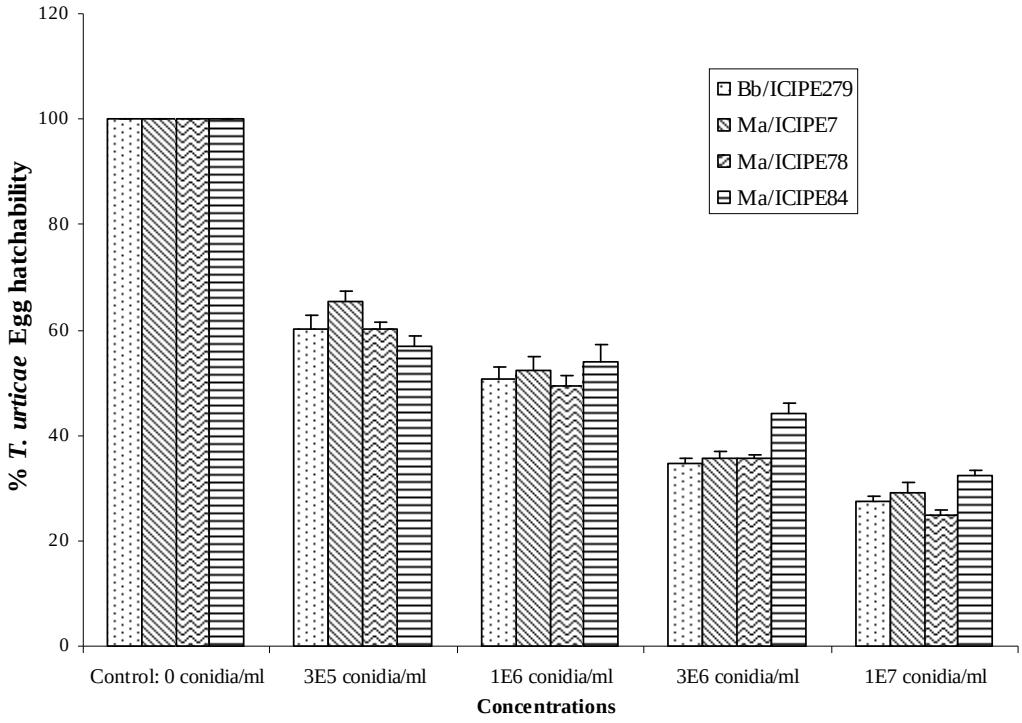


Figure 7.1: Effect of *B. bassiana* (Bb) and *M. anisopliae* (Ma) infection on *T. urticae* egg hatchability

In *T. evansi*, there were also significant differences in egg hatchability among isolates at all the concentrations: 3×10^5 conidia ml^{-1} ($F=20.44$; $df=3, 12$; $P< 0.0001$); 1×10^6 conidia ml^{-1} ($F=4.96$; $df=3, 12$; $P= 0.0182$); 3×10^6 conidia ml^{-1} ($F=6.65$; $df=3, 12$; $P= 0.0068$) and 1×10^7 conidia ml^{-1} ($F=9.16$; $df=3, 12$; $P< 0.0001$) (Figure 7.2). Lower hatchability was observed in the highest fungal concentrations. There were also significant differences in *T. evansi* egg hatchability between concentrations in all isolates: ICIPE279 ($F=965.54$; $df=4, 15$; $P < 0.0001$); ICIPE78 ($F=430.71$; $df=4, 15$; $P< 0.0001$); ICIPE278 ($F=419.45$; $df= 4, 15$; $P < 0.0001$) and ICIPE7 ($F=570.73$; $df=4, 15$; $P< 0.0001$) (Figure 7.2).

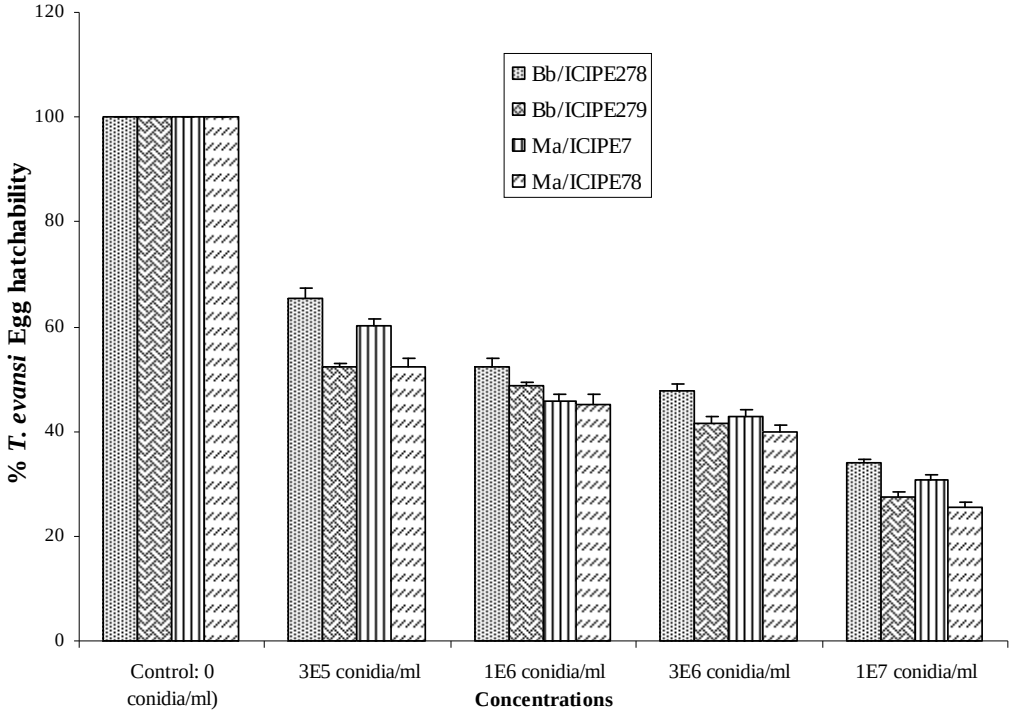


Figure 7.2: Effect of *B. bassiana* (Bb) and *M. anisopliae* (Ma) infection on *T. evansi* egg hatchability

7.3.3 Effect of fungal infection on *T. urticae* and *T. evansi* motile stages

Mortality in the control treatments was 3.8, 3.8, 2.5 and 8.8% in larvae, protonymphs, deutonymphs and adults of *T. urticae*, respectively; and 2.5, 2.5, 5.0 and 8.8% in larvae, protonymphs, deutonymphs and adults of *T. evansi*, respectively. Mortality of motile stages in fungus treatments increased with increase in concentration in both *T. urticae* and *T. evansi* (Tables 7.1 and 7.4). The highest *T. urticae* and *T. evansi* mortality occurred at the highest concentration of 1×10^7 conidia ml^{-1} at 10 days post treatment. However, deutonymphs and adults were more susceptible to fungal infection than larvae and protonymphs. In both species, larvae

were the least susceptible to fungal infection than all the other motile stages (Tables 7.1 and 7.4).

Mortality was dose-dependent within all developmental stages with the highest concentration causing the highest mortality. There were significant differences in mortality between concentrations in all developmental stages, namely larvae ($F=24.39$; $df=16, 51$; $P < 0.0001$), protonymphs ($F=140.64$; $df=16, 51$; $P < 0.0001$), deutonymphs ($F=133.88$; $df=16, 51$; $P < 0.0001$) and adults ($F=85.84$; $df=16, 51$; $P < 0.0001$) of *T. urticae* (Table 7.1). The LC_{50} values varied from 20.8 to 46.3 × 10^7 conidia ml^{-1}, from 0.3 to 0.7 × 10^7 conidia ml^{-1}, from 0.2 to 0.4 × 10^7 conidia ml^{-1} and from 0.06 to 0.2 × 10^7 conidia ml^{-1} in larvae, protonymphs, deutonymphs and adults, respectively (Table 7.2). LC_{90} values varied from 2.11 to 25.50 × 10^8 conidia ml^{-1} in larvae, from 0.66 to 4.10 × 10^8 conidia ml^{-1} in protonymphs, from 0.40 to 3.77 × 10^8 conidia ml^{-1} in deutonymphs and from 0.08 to 5.24 × 10^8 conidia ml^{-1} in adults (Table 7.3).

Table 7.1: Susceptibility of *T. urticae* motile stages to infection by *Metarhizium anisopliae* and *Beauveria bassiana* 10 days after treatment at 26 ± 2 ºC (% mortality ± SE)

			Percent mortality (± SE)			
Fungal species	Isolates	Concentrations (conidia ml^{-1})	Larvae	Protonymphs	Deutonymphs	Adults
Beauveria bassiana	ICIPE279	3×10^5	9.6 ± 1.5efB	19.5 ± 1.3fAB	24.3 ± 1.1hijA	26.0 ± 2.3gA
		1×10^6	17.9 ± 2.8bcdB	35.1 ± 1.2dA	34.6 ± 1.1fgA	39.6 ± 1.6fA
		3×10^6	25.2 ± 3.2abB	54.6 ± 2.0cA	51.3 ± 2.2dA	57.2 ± 3.8cdeA
		1×10^7	29.2 ± 2.4aC	63.6 ± 3.3bB	73.2 ± 3.7bAB	76.8 ± 3.8bA
Metarhizium anisopliae	ICIPE7	3×10^5	5.3 ± 0.0fC	15.5 ± 1.9fB	19.1 ± 2.2jB	27.2 ± 2.5gA
		1×10^6	14.4 ± 1.9cdeC	24.6 ± 2.2eB	29.5 ± 1.6ghB	39.6 ± 1.6fA
		3×10^6	23.3 ± 2.8abcC	35.1 ± 1.7dB	53.8 ± 2.2dA	50.7 ± 2.1deA
		1×10^7	27.1 ± 2.1abB	57.2 ± 0.7cA	65.4 ± 3.8cA	62.9 ± 2.0cdA
	ICIPE78	3×10^5	8.2 ± 1.7efC	14.2 ± 2.3fC	25.6 ± 1.8hijB	40.0 ± 4.0fA
		1×10^6	17.6 ± 0.0bcdC	16.9 ± 1.4fC	37.2 ± 1.0fB	49.2 ± 4.0efA
		3×10^6	27.1 ± 2.1abD	38.9 ± 1.3dC	53.8 ± 3.9dB	68.5 ± 2.7cA
		1×10^7	29.2 ± 2.4aD	71.4 ± 1.7aC	82.0 ± 1.5aB	100.0 ± 0.0aA
	ICIPE84	3×10^5	6.7 ± 1.5fB	5.2 ± 0.1gB	21.8 ± 1.1ijA	23.1 ± 2.1gA
		1×10^6	11.3 ± 2.5defC	25.9 ± 1.8eB	26.9 ± 1.1hjB	39.8 ± 4.2fA
		3×10^6	19.5 ± 1.8abcD	33.8 ± 2.5dC	43.6 ± 0.8eB	53.4 ± 2.3deA
		1×10^7	25.2 ± 3.2abC	51.9 ± 1.6cB	60.3 ± 1.1cA	65.7 ± 1.1cdA
Control	Control	0	3.8 ± 1.3gA	3.8 ± 1.3hA	2.5 ± 1.4kA	8.8 ± 2.4hA

Means (± SE) within column followed by the lower case letter and within row bearing the same upper case letter are not significantly different (SNK test, $P = 0.05$)

Table 7.2: Susceptibility of *T. urticae* motile stages to infection by *Metarhizium anisopliae* and *Beauveria bassiana* 10 days after treatment at 26 ± 2 ºC: Lethal concentration to 50% mortality (LC_{50}) (95% fiducial limits)

Species/Isolates	*T. urticae* Stages	LC_{50} (95% fiducial limits) conidia ml^{-1}	Slope ± SE	Intercept	Chi-Square (χ^2)	*P* values
Beauveria bassiana						
ICIPE279	Larvae	46.3 (41.8-53.2) $\times 10^7$	0.25 ± 0.03	-4.99	7.52	< 0.0001
	Protonymphs	0.3 (0.2-0.3) $\times 10^7$	0.55 ± 0.03	-8.08	15.86	< 0.0001
	Deutonymphs	0.2 (0.2-0.3) $\times 10^7$	0.59 ± 0.05	-8.63	12.79	< 0.0001
	Adults	0.1 (0.1-0.3) $\times 10^7$	0.58 ± 0.06	-8.14	9.36	< 0.0001
Metarhizium anisopliae						
ICIPE7	Larvae	20.8 (10.3-42.3) $\times 10^7$	0.33 ± 0.03	-6.28	10.71	< 0.0001
	Protonymphs	0.6 (0.5-0.8) $\times 10^7$	0.52 ± 0.03	-8.13	15.11	< 0.0001
	Deutonymphs	0.3 (0.2-0.3) $\times 10^7$	0.62 ± 0.04	-9.24	14.58	< 0.0001
	Adults	0.2 (0.2-0.3) $\times 10^7$	0.38 ± 0.02	-5.50	19.70	< 0.0001
ICIPE78	Larvae	25.5 (20.9-31.1) $\times 10^7$	0.28 ± 0.01	-5.42	21.34	< 0.0001
	Protonymphs	0.4 (0.2-0.6) $\times 10^7$	0.78 ± 0.01	-11.86	55.85	< 0.0001
	Deutonymphs	0.2 (0.2-0.2) $\times 10^7$	0.69 ± 0.05	-9.86	14.59	< 0.0001
	Adults	0.06 (0.05-0.07) $\times 10^7$	0.82 ± 0.04	-10.91	18.83	< 0.0001
ICIPE84	Larvae	39.5 (17.3-90.2) $\times 10^7$	0.30 ± 0.02	-5.98	12.33	< 0.0001
	Protonymphs	0.7 (0.6-0.8) $\times 10^7$	0.59 ± 0.02	-9.39	29.26	< 0.0001
	Deutonymphs	0.4 (0.4-0.5) $\times 10^7$	0.49 ± 0.02	-7.55	23.29	< 0.0001
	Adults	0.2 (0.1-0.2) $\times 10^7$	0.47 ± 0.02	-6.68	21.05	< 0.0001

Table 7.3: Susceptibility of *T. urticae* motile stages to infection by *Metarhizium anisopliae* and *Beauveria bassiana* 10 days after treatment at 26 ± 2 ºC: Lethal concentration to 90% mortality (LC_{90}) (95% fiducial limits)

Fungal species	Isolates	*T. urticae* stages	LC_{90} (95% fiducial limits) conidia ml^{-1}
Beauveria bassiana	ICIPE279	Larvae	6.93 (6.87-7.10) $\times 10^8$
		Protonymphs	1.35 (0.80-2.30) $\times 10^8$
		Deutonymphs	0.86 (0.40-1.85) $\times 10^8$
		Adults	0.54 (0.25-1.17) $\times 10^8$
Metarhizium anisopliae	ICIPE7	Larvae	25.50 (24.34-26.79) $\times 10^8$
		Protonymphs	4.10 (1.99-8.47) $\times 10^8$
		Deutonymphs	0.95 (0.55-1.63) $\times 10^8$
		Adults	5.24 (2.77-9.92) $\times 10^8$
	ICIPE78	Larvae	2.11 (2.10-2.15) $\times 10^8$
		Protonymphs	0.66 (0.58-0.75) $\times 10^8$
		Deutonymphs	0.40 (0.25-0.65) $\times 10^8$
		Adults	0.08 (0.07-0.10) $\times 10^8$
	ICIPE84	Larvae	7.72 (7.70-7.74) $\times 10^8$
		Protonymphs	2.84 (2.09-3.84) $\times 10^8$
		Deutonymphs	3.77 (2.58-5.50) $\times 10^8$
		Adults	1.86 (1.09-3.17) $\times 10^8$

Mortality was also dose-dependent within each developmental stage of *T. evansi* with high mortality recorded at the higher concentrations. There were significant differences between concentrations in all the motile stages: larvae ($F=23.27$; df=16, 51; $P <0.0001$), protonymphs ($F=24.36$; df=16, 51; $P< 0.0001$), deutonymphs ($F=51.52$; df=16, 51; $P< 0.0001$) and adults ($F=70.48$; df=16, 51; $P< 0.0001$) (Table 7.4). The LC_{50} values ranged from 8.0 to 40.4 $\times 10^7$ conidia ml^{-1}, from 6.8 to 37.8 $\times 10^7$ conidia ml^{-1}, from 0.3 to 2.5 $\times 10^7$ conidia $ml^{-1}$and from 0.1 to 0.3 $\times 10^7$ conidia $ml^{-1}$for larvae, protonymphs, deutonymphs and adults, respectively (Table 7.5). LC_{90} values, however, ranged from 1.82 to 18.6 $\times 10^{10}$ conidia ml^{-1}in larvae, from 1.88 to 29.7 $\times 10^{10}$ conidia ml^{-1} in protonymphs, from 0.009 to 0.78 $\times 10^{10}$ conidia ml^{-1} in deutonymphs, and from 0.0009 to 0.03 $\times 10^{10}$ conidia ml^{-1} in adults (Table 7.6).

Table 7.4: Susceptibility of *T. evansi* motile stages to infection by *Metarhizium anisopliae* and *Beauveria bassiana* 10 days after treatment at 26 ± 2 ºC (% mortality ± SE)

			Percent mortality in motile stages (± SE)			
Species	Isolates	Concentrations (conidia ml^{-1})	Larvae	Protonymphs	Deutonymphs	Adults
B. bassiana	ICIPE278	3×10^5	11.5 ± 1.2eA	15.8 ± 1.5fA	16.4 ± 1.6hA	17.2 ± 2.5iA
		1×10^6	15.3 ± 1.9deB	24.4 ± 1.0bcdeA	24.6 ± 2.3gA	25.9 ± 1.7hA
		3×10^6	20.9 ± 1.1cdC	23.1 ± 1.5cdeC	28.0 ± 2.7fgB	53.2 ± 1.7cA
		1×10^7	26.7 ± 1.9abcD	32.0 ± 1.1abC	43.7 ± 2.3dB	80.5 ± 1.1bA
	ICIPE279	3×10^5	14.0 ± 2.3deB	21.6 ± 1.2defA	22.3 ± 1.1gA	24.0 ± 0.9hA
		1×10^6	24.3 ± 2.1bcB	31.9 ± 1.9abA	33.3 ± 1.6efA	38.3 ± 1.4fA
		3×10^6	26.7 ± 1.9abcC	29.5 ± 1.6bcC	54.1 ± 1.3cB	76.9 ± 0.9bcA
		1×10^7	33.6 ± 1.6aC	37.2 ± 2.4aC	68.4 ± 1.4aB	90.6 ± 1.7aA
M. anisopliae	ICIPE7	3×10^5	14.0 ±2.3deB	20.6 ± 2.4defA	24.4 ± 1.0gA	26.8 ± 1.7ghA
		1×10^6	23.8 ± 2.3bcB	25.6 ± 1.8bcdAB	32.5 ± 1.0efA	32.3 ± 2.5gA
		3×10^6	25.8 ± 1.6bcD	32.0 ± 2.1abC	52.2 ± 1.4cB	73.0 ± 1.5cdA
		1×10^7	33.6 ± 1.1aD	38.5 ± 1.6aC	67.3 ± 1.3aB	86.5 ± 0.7aA
	ICIPE78	3×10^5	10.2 ± 2.0eC	17.3 ± 1.3efB	23.4 ± 0.0gA	23.9 ± 0.2hA
		1×10^6	15.3 ± 2.7deB	21.8 ± 1.8defAB	28.1 ± 1.8fgA	27.4 ± 3.4ghA
		3×10^6	23.9 ± 1.8bcC	25.5 ± 2.6bcdC	34.1 ± 2.4eB	54.4 ± 1.4eA
		1×10^7	31.2 ± 0.7abC	32.0 ± 2.1abC	58.5 ± 1.5bB	70.0 ± 1.2dA
Control	Control	0	2.5 ± 1.4fB	2.5 ± 1.4gB	5.0 ± 0.0iB	8.8 ± 1.3jA

Means (± SE) within column followed by the lower case letter and within row bearing the same upper case letter are not significantly different (Student-Newman-Keuls test, $P = 0.05$)

Table 7.5: Susceptibility of *T. evansi* motile stages to infection by *Metarhizium anisopliae* and *Beauveria bassiana* 10 days after treatment at 26 ± 2 ºC: Lethal concentration to 50% mortality (LC_{50}) and 95% fiducial limits

Fungal species	Isolates	Stages	LC_{50} (95% fiducial limits) conidia ml^{-1}	Slope ± SE	Intercept	Chi-square (χ^2)	P values
Beauveria bassiana	ICIPE278	Larvae	40.4 (36.1-47.6) $\times 10^{7}$	0.39 ± 0.07	-3.3210	34.19	< 0.0001
		Protonymphs	37.8 (34.4-41.4) $\times 10^{7}$	0.31 ± 0.06	-2.6487	24.92	< 0.0001
		Deutonymphs	2.5 (1.5-5.9) $\times 10^{7}$	0.52 ± 0.06	-3.8155	71.45	< 0.0001
		Adults	0.3 (0.2-0.3) $\times 10^{7}$	1.23 ± 0.06	-7.8464	363.82	< 0.0001
	ICIPE279	Larvae	10.4 (3.9-19.2) $\times 10^{7}$	0.40 ± 0.07	-3.1686	40.48	< 0.0001
		Protonymphs	19.6 (4.3-36.1) $\times 10^{7}$	0.26 ± 0.06	-2.1330	19.03	< 0.0001
		Deutonymphs	0.3 (0.2-0.3) $\times 10^{7}$	0.85 ± 0.06	-5.4372	201.16	< 0.0001
		Adults	0.1 (0.1-0.1) $\times 10^{7}$	1.42 ± 0.07	-8.5814	431.80	< 0.0001
Metarhizium anisopliae	ICIPE7	Larvae	8.0 (3.7-27.4) $\times 10^{7}$	0.52 ± 0.07	-4.1463	63.02	< 0.0001
		Protonymphs	35.8 (7.7-63.9) $\times 10^{7}$	0.31 ± 0.06	-2.6314	24.95	< 0.0001
		Deutonymphs	0.7 (0.5-1.1) $\times 10^{7}$	0.61 ± 0.06	-4.1557	105.77	< 0.0001
		Adults	0.3 (0.2-0.3) $\times 10^{7}$	0.88 ± 0.06	-5.6551	215.24	< 0.0001
	ICIPE78	Larvae	10.9 (4.0-68.3) $\times 10^{7}$	0.40 ± 0.06	-3.1878	40.59	< 0.0001
		Protonymphs	6.8 (2.6-42.7) $\times 10^{7}$	0.35 ± 0.06	-2.7545	34.87	< 0.0001
		Deutonymphs	0.3 (0.2-0.3) $\times 10^{7}$	0.78 ± 0.06	-5.0449	175.26	< 0.0001
		Adults	0.1 (0.1-0.1) $\times 10^{7}$	1.24 ± 0.06	-7.5728	369.62	< 0.0001

Table 7.6: Susceptibility of *T. evansi* motile stages to infection by *Metarhizium anisopliae* and *Beauveria bassiana* 10 days after treatment at 26 ± 2 ºC: Lethal concentration to 50% mortality (LC_{50}) (95% fiducial limits)

Fungal species	Isolates	Stages	LC_{90} (95% fiducial limits) conidia ml^{-1}
Beauveria bassiana	ICIPE278	Larvae	8.47 (3.76-12.23) $\times 10^{10}$
		Protonymphs	5.34 (3.57-7.88) $\times 10^{10}$
		Deutonymphs	0.78 (0.2-0.97) $\times 10^{10}$
		Adults	0.03 (0.02-0.04) $\times 10^{10}$
	ICIPE279	Larvae	1.82 (1.2-2.99) $\times 10^{10}$
		Protonymphs	1.88 (1.12-2.27) $\times 10^{10}$
		Deutonymphs	0.009 (0.005-0.02) $\times 10^{10}$
		Adults	0.0009 (0.0008-0.001) $\times 10^{10}$
Metarhizium anisopliae	ICIPE7	Larvae	2.2 (0.4-4.4) $\times 10^{10}$
		Protonymphs	5.25 (2.98-7.62) $\times 10^{10}$
		Deutonymphs	0.09 (0.03-0.4) $\times 10^{10}$
		Adults	0.008 (0.005-0.01) $\times 10^{10}$
	ICIPE78	Larvae	18.6 (1.2-3052.6) $\times 10^{10}$
		Protonymphs	29.7 (1.5-47.2) $\times 10^{10}$
		Deutonymphs	0.01 (0.006-0.03) $\times 10^{10}$
		Adults	0.001 (0.001-0.002) $\times 10^{10}$

7.4 Discussion

The reduction in egg hatchability of *T. urticae* and *T. evansi* following infection with entomopathogenic fungi observed in the present study is similar to the one reported by other authors in *T. urticae* (Irigaray *et al*., 2003), *T. cinnabarinus* (Shi and Feng, 2004) and *T. evansi* (Wekesa *et al*., 2006). Infection by *B bassiana* and *M. anisopliae* has also been reported to reduce egg hatchability in other arthropods such as the tick *Amblyomma variegatum* Koch (Kaaya *et al*., 1996), *M. sjostedti* (Ekesi and Maniania, 2000) and *Chilo partellus* (Maniania, 1991).

Altough all the different developmental stages of *T. urticae* and *T. evansi* were susceptible to *B. bassiana* and *M. anisopliae* infection, deutonymphs and adults were more susceptible to fungal infection than the others. Similar results have been reported in the cassava green mite, *Mononychellus tanajoa* (Barreto *et al*., 2004), the citrus rust mite *Phyllocoptruta oleivera* (Alves *et al*., 2005), the cattle tick, *Boophilus annulatus* (Pirali-Kheirabadi *et al*., 2006), in *T. urticae* (Irigaray *et al*., 2003) and in *T. evansi* (Wekesa *et al*., 2006). The mentioned results may explain why mature stages are more susceptible to fungal infection that younger (immature) ones and epizootics are generally only observed in mature stage. For example, Carner and Canerday (1970) observed over 50% infection of field-collected deutonymphs and adult *T. urticae* by *Entomophthora* sp. compared to 1.6 and 25.6% of larvae and protonymphs, respectively. Similarly, Oduor (1995) reported that deutonymphs and adults of the cassava green mite, *M. tanajoa* were more susceptible to infection by *Neozygites floridana* than larvae and protonymphs. Ekesi and Maniania (2000) reported that adults of the legume flower thrips *Megalurothrips sjostedti* (Trybom) were more susceptible to *M. anisopliae* infection than larvae. The differences in susceptibility of the different developmental stages of arthropods to fungal infection may be attributed to the characteristics of their cuticle (Samish *et al*., 2001), the interaction between the arthropod integument being penetrated by the fungus, and to the moulting. It has been demonstrated that when the time interval between consecutive moulting is short, moulting plays a significant role in arthropod resistance to fungal infectivity (Vey and Fargues, 1977). Oduor (1995) speculated that the differences in susceptibility of different mite developmental stages to fungal infection may also be attributed to the smaller size

and more limited movement of the younger stages of mites, which reduce the encounters with the conidia.

Although, *B. bassiana* and *M. anisopliae* are reported to be pathogenic to many arthropod pests, the LC_{50} values vary with the fungal species, fungal isolate as well as the host properties. In this study, the LC_{50} values also varied with isolates and developmental stages, which is in agreement with our results (Table 7.1 and 7.4). Mortality was dose-dependent in all the motile stages, with the highest concentration producing the highest mortality. Similar results have been reported by many workers (Feng and Curruthers, 1985; Fransen *et al.*, 1987; Feng *et al*., 1990; Ekesi *et al*., 1998; Ekesi, 1999; Ekesi and Maniania, 2000; Ekesi *et al*., 2000, 2002; Barreto *et al.*, 2004; Alves *et al.*, 2005; Pirali-Kheirabadi *et al.*, 2006; Wekesa *et al.*, 2006; Yaginuma *et al*., 2006). For example, Wekesa *et al*. (2006) reported that the highest concentration of *B. bassiana* and *M. anisopliae* induced the highest mortality of motile stages of *T. evansi* than the lower concentration. According to the results of this study, it is advisable to target eggs, deutonymphs and adults while applying the fungi *B. bassiana* and *M. anisopliae* for the control of these two mite species.

CHAPTER 8: EFFECTS OF CHEMICAL PESTICIDES ON GERMINATION AND RADIAL GROWTH OF *BEAUVERIA BASSIANA* AND *METARHIZIUM ANISOPLIAE*

8.1 Introduction

Although entomopathogenic fungi are reported to be effective for the management of various arthropod pests, their use will not surpass the need for synthetic pesticides in all commercial production systems but can, under several instances, be applied in conjunction with synthetic pesticides in integrated pest management (IPM) programmes (Maniania et al., 2008). Regardless of the way an entomopathogenic fungus is to be introduced in an environment as part of an IPM programme, it is very important to know how it might be affected by synthetic pesticides frequently used in the same environment in order to determine whether the pesticide application need to be temporarily or spatially separated from the most susceptible stages of the fungal pathogen (Pell *et al*., 2001) Unfortunately, in most cases, interactions between the different control methods are not taken into account before their application. The need to obtain information on the compatibility of specific fungal isolates to be used for the control of a specific pest with the agrochemicals used in the same agro-ecosystem can not be underestimated as such information is important in the development of application strategies for both the fungi and the chemical pesticides.

All classes of agrochemicals have been reported as potentially inhibitory to entomopathogenic hyphomycetes, including herbicides, insecticides and fungicides (Inglis *et al*., 2001). However, the inhibitory effects of chemical pesticides on fungal

germination and growth vary among fungal species and isolates within a species, and also among chemical pesticides (Anderson *et al.*, 1989; Li and Holdom, 1994; Poprawski and Majchrocwicz, 1995; Dimbi, 2003). The aim of this research was, therefore, to evaluate the effects of chemical pesticides on germination and radial growth of the entomopathogenic fungi, *B. bassiana* isolate ICIPE279 and *M. anisopliae* isolate ICIPE78, reported earlier as the most virulent isolates to the two-spotted spider mite *T. urticae* and the tomato spider mite *T. evansi* (see chapter 6 and chapter 7).

8.2 Materials and Methods

8.2.1 Fungi

One *B. bassiana* isolate (ICIPE279) and one *M. anisopliae* isolate (ICIPE78) were used for this study. They were selected earlier as potential candidates for the control of *T. urticae* and *T. evansi* (See Chapter 6)

8.2.2 Chemical pesticides

Three insecticides (Dimethoate®, Karate® and Malathion®) and three fungicides (Antraco®l, Mancozeb® and Milraz®) were used in this experiment (Table 8.1). The three insecticides are commonly used by small-scale farmers for the control of insect pests, while the three fungicides are generally used for the control of fungal diseases in tomatoes and other vegetable crops where *T. urticae* and *T. evansi* are serious pests. All the synthetic pesticides were used at their recommended field concentrations (Table 8.1). The insecticide concentrations were 1.5 ml/litre, 0.9

gram/litre and 1.5 ml/litre of water for Dimethoate, Karate and Malathion, respectively. For the fungicides, concentrations were 2.25 grams/litre, 2.5 grams/litre and 2 grams/litre of water for Antracol, Mancozeb and Milraz, respectively.

Table 8.1: Chemical pesticides used to test effects on fungal germination and growth

Pesticides	Active ingredient content	formulation	Field rate (in 1 litre of water)	Manufacturer
Insecticides				
Dimethoate (DANADIM)	Dimethoate 39% Cyclohexanone 43% Xylene 13% Emulsifiers 5%	EC	1.5-2.0 ml	Cheminova Agro A/s DK-7620 Lemvin, Denmark
Karate	25g/kg lambdacyhalothrin	WG	0.8-1.0 gram	Syngenta Crop Protection AG, Basle, Switzerland
Malathion	Malathion 50% Inert ingredients 50%	EC	1.0-2.0 ml	Southern Agricultural Insecticides, Inc., USA
Fungicides				
Antracol	Propineb (70%) 700g/kg Hexamethylenetetramine 1% Others (≈ 30%)	WP	2.0-2.5 grams	Bayer CropScience, AG, Germany
Mancozeb (DITHANE* M-45)	Manganese 16% Zinc 2% Ethylenebisdithiocabarmate ion 62% Inert ingredients 20%	WP	2.5 grams	Dow AgroSciences, Switzerland
Milraz	Propineb 700g/kg Cymoxanil 60g/kg	WP	1.5-2.5 grams	Bayer CropScience, AG, Germany

EC= Emulsifiable Concentration; WP= Wettable Powder; WG= Water dispersible Granules

8.2.3 Media preparation

The methods described by Li and Holdom (1994) were used to evaluate the compatibility of fungi and pesticides. Sabouraud Dextrose Agar medium (SDA) was prepared and each 500 ml portion was dispensed into 1000ml medium bottles. The media was then autoclaved at 121 ºC for 20 minutes and allowed to cool at about 50 ºC. The pesticides were filter sterilized using a 0.2 µm syringe and then added into each bottle to make the field recommended concentrations. The bottle containing the mixture was shaken to allow uniform distribution and poured into 90 mm Petri dishes. Control consisted of SDA without pesticide (Dimbi, 2003).

8.2.4 Effect of chemical pesticides on fungal germination

The effect of pesticides on germination was evaluated by spread-plating 0.1 ml of conidial suspension containing 3×10^6 conidia ml^{-1} onto SDA plates containing the pesticides and pesticide-free SDA plates as control. Sterile microscope cover slips were placed on each plate as described earlier (See chapter 3). Inoculated plates were sealed with Parafilm membrane and incubated at room temperature (25 ± 2º C). At 20 hours post inoculation, germination was halted by adding 1 ml of formaldehyde (0.5%) onto each plate and percentage germination was determined by counting 100 spores for each plate at 200 × magnification. The treatment was replicated four times, and the experiment was repeated twice.

8.2.5 Effect of chemical pesticides on fungal radial growth

A conidial suspension of 1×10^7 conidia ml^{-1} was spread-plated on SDA plates. The plates were sealed with Parafilm membrane and then incubated at 25 ºC 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 centre of the SDA agar plates containing the different pesticides (each at its recommended field concentration), as well as in the control plates. Each plate was sealed with Parafilm membrane and incubated at room temperature (23-28 ºC). Radial growth was then recorded daily for 10 days. Treatments were replicated 4 times, and the whole experiment was repeated twice.

8.2.6 Data analysis

Germination and vegetative growth data were subjected to one-way analysis of variance (ANOVA) for a completely randomized design using the ANOVA procedures of SAS (SAS Institute, 1999-2001) after arcsine transformation of the percentage data. Means were separated using the SNK test.

8.3 Results

8.3.1 Effect of chemical pesticides on fungal germination

All the insecticides had an effect on fungal germination, but germination varied with insecticide and fungal species. *Metarhizium anisopliae* germination was greater or equal to 87.3% in all the insecticide treatments. However, *B. bassiana* germination

was considerably affected by the insecticides, except Karate (Table 8.2). Dimethoate and Malathion reduced the germination of *B. bassiana* by up to 80 and 90%, respectively. On the other hand, all the fungicides (Antracol, Mancozeb and Milraz) inhibited the germination of *M. anisopliae. Beauveria bassiana* germination was inhibited by Mancozeb and Milraz, but not by Antracol, However, germination of *B. bassiana* in the latter treatment was very low (19.0%)

Table 8.2: Effect of pesticides on fungal germination (% viability ± SE)

	Metarhizium anisopliae isolate ICIPE78	*Beauveria bassiana* isolate ICIPE279
Control	89.9 ± 0.6a	92.3 ± 0.1a
Insecticides		
Dimethoate	87.3 ± 1.3a	20.1 ± 2.8b
Karate	87.8 ± 0.6a	90.8 ± 0.7a
Malathion	88.2 ± 1.0a	6.2 ± 0.5c
Fungicides		
Antracol	No germination	19.0 ± 3.0b
Mancozeb	No germination	No germination
Milraz	No germination	No germination

Means (± SE) within column followed by the same lower case letter and within row bearing the same upper case letter are not significantly different (Student-Newman-Keuls test, $P = 0.05$).

8.3.2 Effect of chemical pesticides on fungal radial growth

The two fungal isolates (ICIPE78 and ICIPE279) grew in all insecticide treatments, but their growth was lower compared to the control. However, no fungal growth was recorded in fungicide treatments, except in Antracol treatment where fungal growth occurred (Table 8.3).

Table 8.3: Effect of pesticides on fungal radial growth (mm/day)

	Metarhizium anisopliae isolate ICIPE78	*Beauveria bassiana* isolate ICIPE279
Control	5.0 ± 0.3a	2.3 ± 0.1a
Insecticides		
Dimethoate	2.7 ± 0.1b	1.5 ± 0.1b
Karate	1.9 ± 0.2b	1.5 ± 0.0b
Malathion	2.3 ± 0.2b	0.9 ± 0.3b
Fungicides		
Antracol	No growth	1.1 ± 0.1b
Mancozeb	No growth	No growth
Milraz	No growth	No growth

Means (± SE) followed by the same letter are not significantly different (Student-Newman-Keuls test, $P = 0.05$).

8.4 Discussion

Considering germination as a compatibility parameter, as suggested by Nevez *et al.* (2001), generally all the insecticides in this study were compatible with the *M. anisopliae* strain ICIPE78, but not with the *B. bassiana* strain ICIPE279. The latter was only compatible with Karate. Many studies have been devoted to the compatibility between entomopathogenic fungi (Brandenburg and Kennedy, 1983; Boyklin *et al.*, 1994; Smitley *et al.*, 1986; Tamai *et al.*, 2002b; Irigaray *et al.*, 2003; Wenzel *et al.*, 2004; Shi *et al.*, 2005; Klingen and Westrum, 2007). For instance, Tamai *et al.* (2002b) found a large variability in the toxicity of 36 fungicides and 54 insecticides/acaricides to *B. bassiana*. Three of the 36 fungicides (propamocarb hydrochloride, sulphur and kasugamycin) and 24 out 54 insecticides/acaricides (including those with the following active ingredients: abamectin, acephate, acetamiprid, betacyfluthrin, bifenthrin, ciromazine, deltamethin, diafenthriuron, diflubenzuron, dimethoate, fenpropathrin, fenpyroximate, fenvalerate, imidacloprid,

metamidophos, propargite and tebufenozide etriclorfon) were compatible with *B. bassiana*. Wenzel *et al*. (2004) reported that the insecticides Provado (imidachloprid) and Trigard700PM (cyromazine) were compatible with *Lecanicillium* (=*Verticillium*) *lecanii* in terms of vegetative growth, sporulation, conidial viability and pathogenicity against *T. urticae*. Comparing the effect of different fungicides, insecticides and acaricides used in strawberry crops, Klingen and Westrum (2007) found that all the fungicides tested (tolyfluanid, fenhexamid, cyprodinil + fludioxonil) were harmful to *N. floridana*, while all the insecticides and acaricides were compatible. Dara and Hountondji (2001) reported that reduction in fungal germination results concomitantly in decrease of fungal infectivity on arthropod pests. However, they showed that the effect of pesticides on fungal effectiveness varies with the pesticide concentration used. For example, they demonstrated that when Imidacloprid (an insecticide) is combined with *Hirsutella thompsonii* (a spider mite pathogen) for mite control, at a concentration of 50 ppm, the insecticide significantly reduced the fungal germination. However, when used at a concentration of 100 ppm and above, the insecticide increased the *H. thompsonni* germination. This had been reported earlier by Anderson and Roberts (1983), who pointed out that insecticide formulation is more important than the active ingredient, and that wettable powder insecticide formulations usually do not cause inhibition but may increase the number of *B. bassiana* conidia. Synergic effect between wettable powder insecticides and *M. anisopliae* has also been reported (Duarte *et al*., 1992). However, compatibility between water dispersible granule formulations with *B. bassiana* and *M. anisopliae* has also been confirmed (Moino and Alves, 1998). A formula for determination of an *in vitro* compatibility between pesticides and

entomopathogenic fungi was proposed by Alves *et al.* (1998). In the said formula, fungal growth and conidia production are the key factors used to determine the levels of compatibility between pesticides and entomopathogenic fungi. Nonetheless, this formula was later challenged by Neves *et al.* (2001). They suggested that *in vitro* compatibility tests should consider inhibition of conidia germination when mixed with formulations since that is an important factor at field conditions. Additionally, since the long term exposure of conidia to pesticide formulations can have adverse effects on the effectiveness of the fungi, the mode of preparation and application should be taken into account before.

In the present study, all the three fungicides tested (Antracol, Mancozeb and Milraz) were toxic to the *M. anisopliae* isolate ICIPE78. In the case of the *B. bassiana* strain ICIPE279, only Antracol was not toxic to it. It can be therefore concluded that on one hand, Dimethoate, Karate and Malathion have no effect on the *M. anisopliae* strain ICIPE78 germination, and on the other hand, Karate does not have an effect on the *B. bassiana* strain ICIPE279 germination. Therefore, in an IPM program, the above pesticides can be recommended for pest control in the same agro-ecosystems where these entomopathogenic fungal strains are used. However, it known that some other factors such as mode of preparation of formulations, mode of application of IPM since long exposure of conidia in formulations may have detrimental effects on fungus. Therefore, all these factors should be taken into account while studying the compatibility between fungi and synthetic pesticides.

CHAPTER 9: EFFICACY OF THE *METARHIZIUM ANISOPLIAE* STRAIN ICIPE78 ON *TETRANYCHUS URTICAE* INFESTATION ON BEAN PLANTS GROWN IN THE GREENHOUSE

9.1 Introduction

The two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae), is one of the most important pests of many crops in temperate and tropical climates. It has been recorded on more than 200 hosts of some economic value throughout the world (Jeppson *et al.*, 1975; Smith Meyer, 1996; Bolland *et al.*, 1998). It is actually a serious pest of greenhouse crops. Chemical control is the main method of fighting this spider mite, but it is nowadays less preferred due to the development of resistance as well as environmental problems related to the use of chemical pesticides (Young-Joon *et al.*, 1993; Tamai *et al.*, 2002; Herron *et al.*, 2004). Biological control has been tried as a rational alternative method for chemical control. Several species of natural enemies, including entomopathogenic fungi have been reported to prey on/kill and maintain *T. urticae* populations under control (Chandler *et al*., 2000, 2005; Maniania *et al*., 2008). Predatory mites constitute the most natural enemies studied and used for the control of this pest (Escudero and Ferragut, 2005; Opit *et al.*, 2005; Oliveira *et al.*, 2007). On the other hand, entomopathogenic fungi have been reported as good biological control agents of *T. urticae* (Tamai *et al*., 2002; Simova and Draganova, 2003; Chandler *et al*., 2005, Paz *et al*., 2007) and can induce a high mortality in this mite at a range of temperatures between 20 and 35 ºC in the laboratory (see chapter 6). Therefore, the objective of this study was to evaluate the effect of the *Metarhizium anisopliae*

isolate ICIPE78 on the population density of *T. urticae* infesting common bean (*Phaseolus vulgaris* L.) plants in the greenhouse.

9.2 Materials and methods

9.2.1 Mite culture

The *T. urticae* stock culture was established in the laboratory at the ICIPE Headquarters, Nairobi, Kenya. The initial culture originated from mites collected from rose plants in a greenhouse in Naivasha, Kenya, in 2004. The mites were reared on common bean, *Phaseolus vulgaris* L. variety GLP-2 at 25 ± 2 ºC, 60-70 % RH and a photoperiod of 12:12 L:D.

9.2.2 Fungal pathogen

An isolate of *M. anisopliae* (ICIPE78) was used for this experiment. It was selected because of its virulence against *T. urticae* over a broad temperature range (see chapter 6, Table 6.3 and 6.6) and its virulence on all the developmental stages of *T. urticae* (see chapter 7, Table 7.1 and 7.4).

9.2.3 Production of fungal inoculum formulation

The *M. anisopliae* isolate ICIPE78 was produced on long white rice. Three kilograms of rice was weighed into polyethylene bags and autoclaved for 40 minutes at 121 ºC. Erlenmeyer flasks containing 250 ml of Sabouraud dextrose medium were inoculated with conidial suspension of the fungus and incubated for 3 days in a

shaker incubator at 28 ºC and 100 rpm. The aerated rice bags were inoculated with the broth and maintained in an incubation room (23-27 ºC, 35-60% RH) for three weeks (21 days). The substrate containing conidia/mycelia was transferred into plastic basins and allowed to dry for 5 days at room temperature before the conidia were harvested by sieving. The conidia were stored for two weeks in the refrigerator (4-6 ºC) before being used for the greenhouse experiments (Wekesa *et al*., 2005). After 24 hours on SDA, 97.5 to 99.5 of conidia germinated.

Conidial suspension titrated at 1×10^8 conidia ml^{-1} was formulated in two different formulations: water formulation at the ratio of 0.25:99.75 (0.25 ml of Silwet-L77 and 99.75 ml of water) and emulfsiable formulation at the ratio of 0.25:6:93.75 (0.25 ml of Silwet-L77, 6ml of oil and 93.75 of water).

9.2.4 Greenhouse experiment

9.2.4.1 Effect of fungal formulations on *T. urticae* population density

Sixty potted bean plants (*Phaseolus vulgaris* L., variety GLP-2) were artificially infested with *T. urticae* by placing three or four infested leaflets from the mite stock culture on each plant. The mites were given two weeks to establish and multiply before the treatments were applied. Ten plants were randomly assigned to each treatment and mite density was estimated two days before treatment by picking two leaves per bean plant (one from the top and one from the middle). All the motile developmental stages (larvae, protonymphs, deutonymphs and adults) of spider mites were counted in the laboratory using a dissecting microscope. Leaf area was

determined with a leaf area meter (Li-3100, Li-Cor, Lincoln, Nebraska, USA) to establish the density of mites per square centimetre

9.2.4.2 Application of treatments

In this study six treatments were used and they consisted of: **T1** (untreated control), **T2** (water+Silwet-L77), **T3** (water+oil+Silwet-L77), **T4** (Fungus+Silwet-L77+Water), **T5** (Fungus+Silwet-L77+Oil+Water) and **T6** (Synthetic acaricide with abamectin as active ingredient, Dynamec®). The latter was used at the field recommended rate (1 ml in 2 litres of water) in order to compare its effect and fungal formulation effects on *T. urticae*. Treatments were sprayed using a 1.5-litres hand sprayer (Spraying Systems Co., Wheaton, IL, USA) outside the greenhouse to avoid contamination of neighbouring plants. Fungal formulations and the chemical acaricide were applied 3 times after every 10 days. Each treatment consisted of two bean plants replicated five times in completely randomised blocks. Population densities of *T. urticae* were again estimated at 1, 2, 3, 4 and 5 weeks after spraying. The mean daily temperature and relative humidity in the greenhouse was 25.62 ºC and 60.72 %, respectively.

9.2.5 Data analysis

Population counts were log transformed to normalize the means and then subjected to analysis of variance (ANOVA) using the procedures of SAS (SAS Institute, 1999-2001). Production parameters data were also log transformed before being subjected to ANOVA. Means were separated by Student Newman-Keuls (SNK) test at P=0.05.

9.3 Results

9.3.1 Efficacy of fungal application on *T. urticae* population density

Application of fungal formulations and the acaricide reduced *T. urticae* population densities as compared to controls. There were however significant differences in *T. urticae* population densities between the treatments at sampling dates post-spraying in top and middle leaves (Figure 9.1A). At 3 week post-spraying for example, the mite densities were near zero in the treated leaves compared to control (9.23 and 9.84 mites/cm^2 on top and middle leaves, respectively). At 5 week post-treatment, there were no more leaves left in the control as they had all fallen off.

The *T. urticae* population densities in the top leaves varied significantly with the treatments: before spray application ($F=2.56$; $df=5, 54$; $P=0.0376$), 1 week ($F=23.44$; $df=5, 54$; $P < 0.0001$), 2 weeks ($F=29.53$; $df=5, 54$; $P < 0.0001$), 3 weeks ($F=45.24$; $df=5, 54$; $P < 0.0001$), 4 weeks ($F=44.21$; $df=4, 45$; $P < 0.0001$) and at 5 weeks post-spray applications ($F=28.53$; $d=4, 45$; $P < 0.0001$) (Figure 9.1A).

In the middle leaves, there was no significant difference between the treatments before spray applications ($F=1.99$; $df=5, 54$; $P=0.0950$); however, there were significant differences between the treatments at 1 week ($F=35.46$; $df=5, 54$; $P < 0.0001$), 2 weeks ($F=18.02$; $df=5, 54$; $P < 0.0001$), 3 weeks ($F=239.23$; $df=5, 54$; $P < 0.0001$), 4 weeks ($F=54.88$; $df= 4, 45$; $P < 0.0001$) and at 5 weeks post-spraying ($F=31.27$; $df=4, 45$; $P < 0.0001$) (Figure 9.1B).

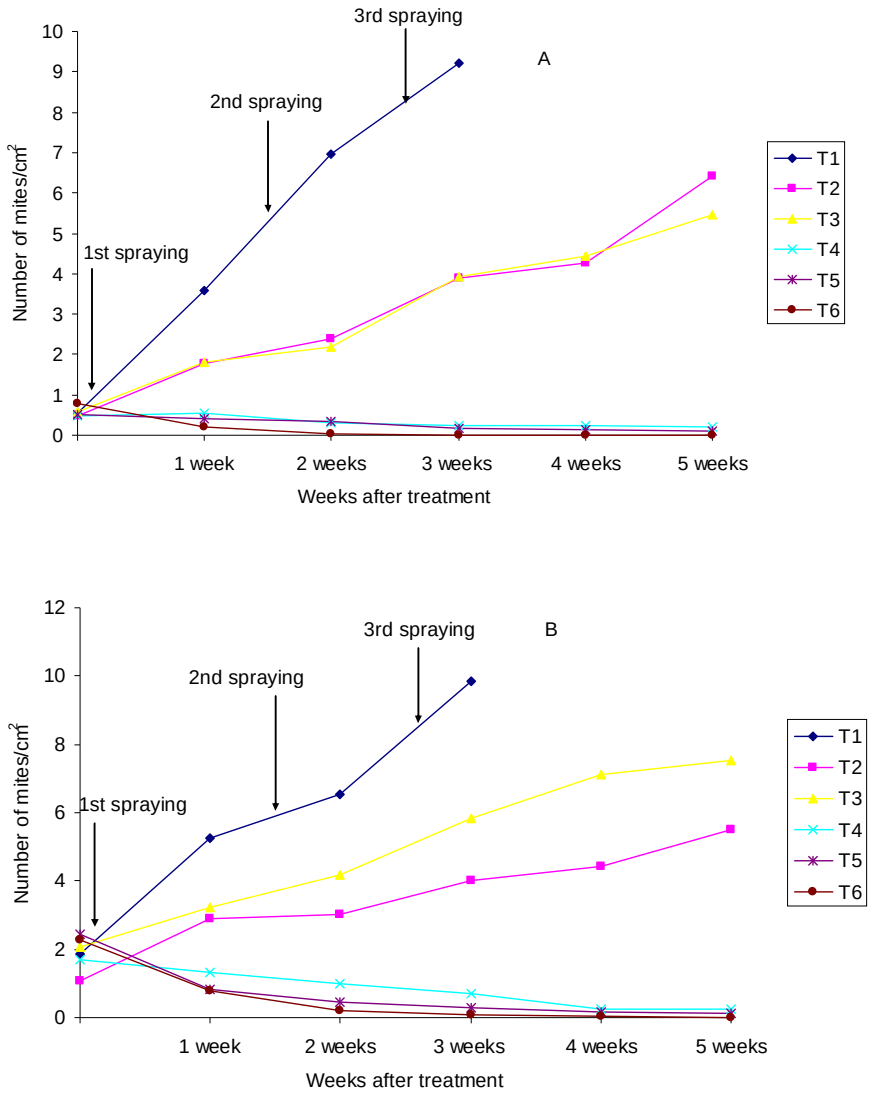


Figure 9.1: Effect of fungal infection on *T. urticae* population densities (A) on top and (B) middle of bean (*P. vulgaris*) leaves. (The arrow indicates the spraying time).

Legend:
T1 = Untreated control
T2 = Silwet-L77+Water
T3 = Silwet-L77+Oil+Water
T4 = ICIPE78+Silwet-L77+Water (water formulation) (0.25:99.75)
T5 = ICIPE78+Silwet-L77 +Oil+Water (Oil formulation) (0.25:6:93.75)
T6 = Chemical acaricide (Dynamec®) (0.5ml in 1 litre of water)

9.3.2 Efficacy of fungal application on *T. urticae*-infested *P. vulgaris* production parameters

There were significant differences between treatments in number of pods produced per plant (F=6.48; df=5, 54; P < 0.0001), number of seeds per pod (F=12.80; df=5, 54; P < 0.0001) and in dry weight of bean seeds per plant (F=28.10; 5, 54; P < 0.0001) (Table 9.1). Yields were 10.5 and 10.8 more times than the control with fungal and acaricide treatments, respectively (Table 9.1).

Table 9.1: Effect of fungal formulations on bean (*P. vulgaris*) production parameters

Treatments	Number of pods/plant	Number of seeds/pod	Weight of dry beans/plant (in grams)
Untreated Control (T1)	5.05 ± 0.59bc	1.20 ± 0.36b	0.80 ± 0.07d
Silwet/Water (T2)	5.60 ± 0.36bc	4.80 ± 0.36a	4.40 ± 0.57c
Silwet/Oil/Water (T3)	4.60 ± 0.45c	3.60 ± 0.48a	3.90 ± 0.64c
Fungus/Silwet/Water (T4)	6.15 ± 0.37bc	4.30 ± 0.30a	6.23 ± 0.70b
Fungus/Silwet/Oil/Water (T5)	8.05 ± 0.58a	4.60 ± 0.40a	8.40 ± 0.62a
Chemical acaricide (T6)	6.70 ± 0.52ab	4.90 ± 0.43a	8.60 ± 0.51a

Means within columns bearing the same letters are not significantly different (SNK-test, P= 0.05)

9.4 Discussion

The *M. anisopliae* isolate ICIPE78 formulated in water and oil formulations had a significant effect on the population density of *T. urticae* on common bean plants, as was the chemical acaricide treatments over time. However, there was no significant difference between the two control agents. Alves *et al*. (1998) reported that fungal formulations of *B. bassiana* performed better than chemical pesticides against *T. urticae* on chrysanthemum in a semi-field trial. Similar results were reported by Tamai *et al*. (2002). In another study, spray application of *B. bassiana*, *Hirsutella*

thompsoni, *M. anisopliae* and *Verticillium lecanii* resulted in reduction of *T. urticae* populations in the greenhouse (Chandler *et al*., 2005). A reduction of up to 97% of T. urticae was achieved by Naturalis-L, a *B. bassiana* commercial formulation. However, unsuccessful results were observed in a greenhouse trial where Andreeva and Shternshis (1995) used entomopathogenic fungi to control *T. urticae*.

Conidia formulated in oil performed better than the ones in water. Recently, Wekesa *et al*. (2005) used two different fungal formulations (water and oil formulations) to treat the tomato spider mite *T. evansi* infesting tomatoes in the greenhouse. They found that at 1 week post-application, the number of *T. evansi* on both tomato top and middle leaves in the untreated and treated controls was higher than in the fungal formulations-treated plants. However, they reported that conidia of fungal isolates formulated as emulsifiable oil, performed better than the ones formulated in water and Silwet L-77. This was not the case in the current study in terms of mite population density evaluation. We only found that there was a significant difference in the effectiveness of the two different fungal formulations on *T. urticae* population density on bean middle leaves at 1 week post-spraying.

The reduction of mite populations resulted in the increase of number of pods per plant, number of seeds per pod and the weight of dry bean seeds per plant. The lowest number of pods per plant and the lowest weight of dry bean seeds per plant were observed in the untreated control. This may have been due to the fact that seeds obtained from the treated controls were smaller than the ones obtained from the

other treatments. Seeds obtained from the untreated controls were very small and of a very bad quality.

Considering the undesirable effects chemical pesticides may have on the environment (destruction of beneficial insects: natural enemies and other pollinator insects; water and air pollution; toxic residue accumulation for people and animals), on the handlers (poising) and the consumers (presence of residues in the produce destined for consumption), this fungal isolate can be incorporated in the *T. urticae* IPM programs, and as an alternative to acaricides in *T. urticae* control in the greenhouse. Further studies are required to evaluate its efficacy in an open field.

CHAPTER 10: FIELD EVALUATION OF TWO FORMULATIONS OF THE *METARHIZIUM ANISOPLIAE* ISOLATE ICIPE78 FOR CONTROL OF *TETRANYCHUS URTICAE* AND *TETRANYCHUS EVANSI*

10.1 Introduction

The tomato spider mite, *Tetranychus evansi* Baker & Pritchard and the two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae) are polyphagous pests that attack a large number of cultivated and wild plants (Jeppson *et al.*, 1975; Smith Meyer, 1996; Bolland *et al*., 1998). *Tetranychus evansi* has been reported on several vegetables, ornamental crops such as roses and on many weeds but with preference to the Solanaceae (Bolland *et al*., 1998; Keizer and Zuurbier, 2001; EPPO, 2004; Rosa *et al.*, 2005). It is currently one of the major constraints to tomato production in Malawi, Mozambique, Namibia, Zambia and Zimbabwe (Saunyama and Knapp, 2003). In Kenya, it was detected in 2001 and is currently the most important spider mite pest of tomato (Varela *et al*., 2003; ICIPE, 2004). *Tetranychus urticae* has also been reported on more than 200 host plants (Smith Meyer, 1996; Bolland *et al*., 1998), and in the semi-arid areas of Kenya, it is considered the second-most important spider mite pest of tomato after *T. evansi* (Knapp *et al*., 2003a; Varela *et al.*, 2003).

In eastern and southern Africa, the management of these pests is largely based on the use of chemical pesticides which are often inefficient (Sibanda *et al.*, 2000; Saunyama and Knapp, 2003; Varela *et al*., 2003). Additionally, due to the capacity of spider mites to rapidly develop resistance to acaricides and the growing concern

regarding environmental and health risks related to pesticide use, alternatives to chemical control are important.

Several fungal species have been reported as potential biological control agents of the two spider mite species *T. urticae* and *T. evansi* (Carner and Canerday, 1970; Brandenburg and Kennedy, 1981; Humber *et al*., 1981; Tamai *et al*., 2002; Irigaray e*t al.*, 2003; Chandler *et al.*, 2005; Wekesa *et al.*, 2005, 2007; Maniania *et a*l., 2008). Therefore, this study was initiated to evaluate the efficacy of the entomopathogenic fungus *Metarhizium anisopliae* isolate ICIPE78 for suppression of the population of *T. urticae* and *T. evansi* infesting bean and tomato plants, respectively, in field conditions.

10.2 Materials and Methods

10.2.1 Mite cultures, fungal isolate and laboratory bioassays

Mite cultures were reared as described earlier (see chapter 3) and the *M. anisopliae* strain ICIPE78 was used. Laboratory bioassays were done as described in chapter 9.

10.2.2 Study site and experimental conditions

The experiments were conducted from April to July 2007 and from September 2007 to January 2008, at field sites located at Thika within the Kenya Agriculture Research Institute (KARI) station (S00º59' E037º04', altitude 1548 m, with an annual mean rainfall of 983 mm). The mean monthly minimum and maximum temperatures vary from 12.2 to 15.5 ºC and from 22.5 to 27.2 ºC respectively.

10.2.2.1 Bean and Tomato fields

10.2.2.1.1 Tomato Nursery

The nursery for tomato (*Lycopersicon esculentum* Mill., variety Cal-J) was maintained from 17th March to 15th April 2007 at the International Centre of Insect Physiology and Ecology (*icipe*) Headquarters, Kasarani, Nairobi, Kenya. Tomato seedlings (4 weeks old) were then transferred to the field at KARI.

10.2.2.1.2 Bean and Tomato field experiments

The field experiments were conducted in a randomized complete block design with four replications in each of 6 plots, each plot representing one treatment (T1, T2, T3, T4, T5 and T6) and repeated twice. The first season was from April 2007 to July 2007, and the second from September 2007 to January 2008. The bean field experimental plot sizes were 2 m x 2 m, with inter- and intra row spacing of 0.4 m x 0.2 m respectively. The tomato field experimental plot sizes were 5 m x 5 m, with inter- and intra row spacing of 0.6 m x 0.6 m respectively. The distance between the blocks and plots, for both the bean and tomato experiments, was 1.5 m and 1 m, respectively.

Three weeks after transplanting tomatoes and planting bean, bean and tomato plants were artificially infested with *T. urticae* and tomato with *T. evansi*, respectively, and mites then allowed two to three weeks to multiply. Two days before the fungal treatments were applied *T. urticae* and *T. evansi* initial populations were estimated by picking two leaves per plant; one from the top and another one from the middle,

on 10 plants randomly chosen. After spraying, the mite population densities were evaluated weekly until harvest time. The mite density was determined by direct counting in the laboratory using a dissecting microscope. The treatments were as follows: T1 (untreated control), T2 and T3 (Water/Silwet-L77 and Water/Oil/Silwet-L77 treated controls), T4 and T5 (fungus in Water, and emulsifiable oil formulations) and T6 (synthetic acaricide Dynamec®).

Bean production parameters were also established by determining the number of pods per plant, number of seeds per pod, dry weight of 100 seeds and yield in metric tons per hectare. For tomatoes, the number of fruits per plant and weight of tomato fruits (in metric ton per hectare) were established.

During the first trial, the daily mean minimum and maximum temperatures were 9.4 ºC and 31.2 ºC, respectively. During the second trial, the temperatures were 9.2 ºC and 31.5 ºC, respectively.

10.2.3 Statistical analysis

Population counts were log transformed to normalize the means and then subjected to analysis of variance (ANOVA) (SAS, 1999-2001). Means were separated by Student Newman-Keuls (SNK) test at P = 0.05. The production parameters data were analysed using ANOVA procedures of SAS (SAS, 1999-2001)

10.3 Results

10.3.1 Bean field experiments

10.3.1.1 Effect of fungal formulations on *T. urticae* population densities on top and middle bean leaves

No significant differences in *T. urticae* population density were observed on top leaves between the treatments before spraying (F=0.11; df=5, 18; P=0.9885), at one week (F=1.47; df=5, 18; P=0.2473), two weeks (F=1.47; df=5, 18; P=0.2473) and 3 weeks post-spraying (F=1.44; df=5, 18; P=0.2567) during the first season (Figure 10.1A). A decrease in *T. urticae* population density was observed in all the treatments, including the controls. The number of mites per bean leaflet was almost the same in all the treatments at all the sampling dates. In the middle leaves, however, while there were no significant differences in *T. urticae* population density before spraying (F=1.12; df=5, 18; P=0.9851) and at one week post-spraying (F=2.60; df=5, 18; P=0.0614), significant differences were observed at two and three weeks post spraying (F=4.21; df=5, 18; P=0.0104) and (F=4.91; df=5, 18; P=0.0052) respectively (Figure 10.1B).

During the second season, there were no significant differences in *T. urticae* populations between the treatments before spray applications on top leaves (F=1.20; df=5, 18; P=0.3498). However, significant differences were observed on top leaves at one week (F=15.24; df=5, 18; P < 0.0001) and two weeks post-spraying (F=24.02; df=5, 18; P< 0.0001) (Figure 10.2A). At one and two weeks post spraying, significant decrease of mites was recorded in the fungal and the acaricide treatments,

while there was an increase of mites per bean leaflet in the controls. *Tetranychus urticae* population density did not differ significantly between the different treatments before spraying ($F=0.70$; $df=5, 18$; $P=0.6285$) in the middle leaves. The number of mites per bean leaflet was lower in fungal and the acaricide treatments than in the controls. However, significant differences were observed between treatments at one week ($F=10.48$; $df=5, 18$; $P < 0.0001$) and at two weeks ($F=15.92$; $df=5, 18$; $P < 0.0001$) post-spraying (Figure 10.2B).

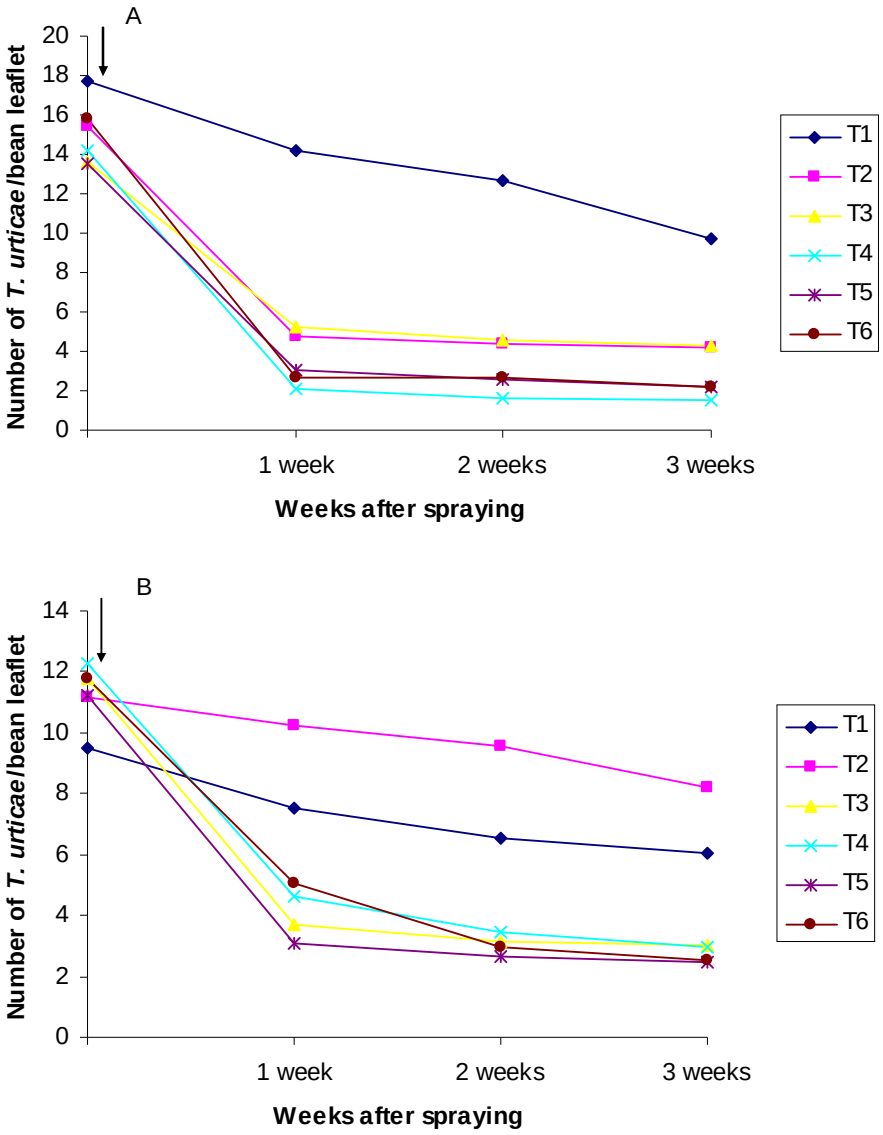


Figure 10.1: Effect of different formulations of *M. anisopliae* and acaricide on the population density of *T. urticae* infesting bean (A) top leaves and(B) middle leaves during season I. (The arrow indicates the spraying time)

Legend:
T1 = Untreated control (mites only)
T2 = Silwet-L77+Water
T3 = Oil+Silwet-L77+Water
T4 = ICIPE78+Silwet-L77+Water
T5 = ICIPE78+Oil+Silwet-L77+Water
T6 = Acaricide (abamectin active ingredient)

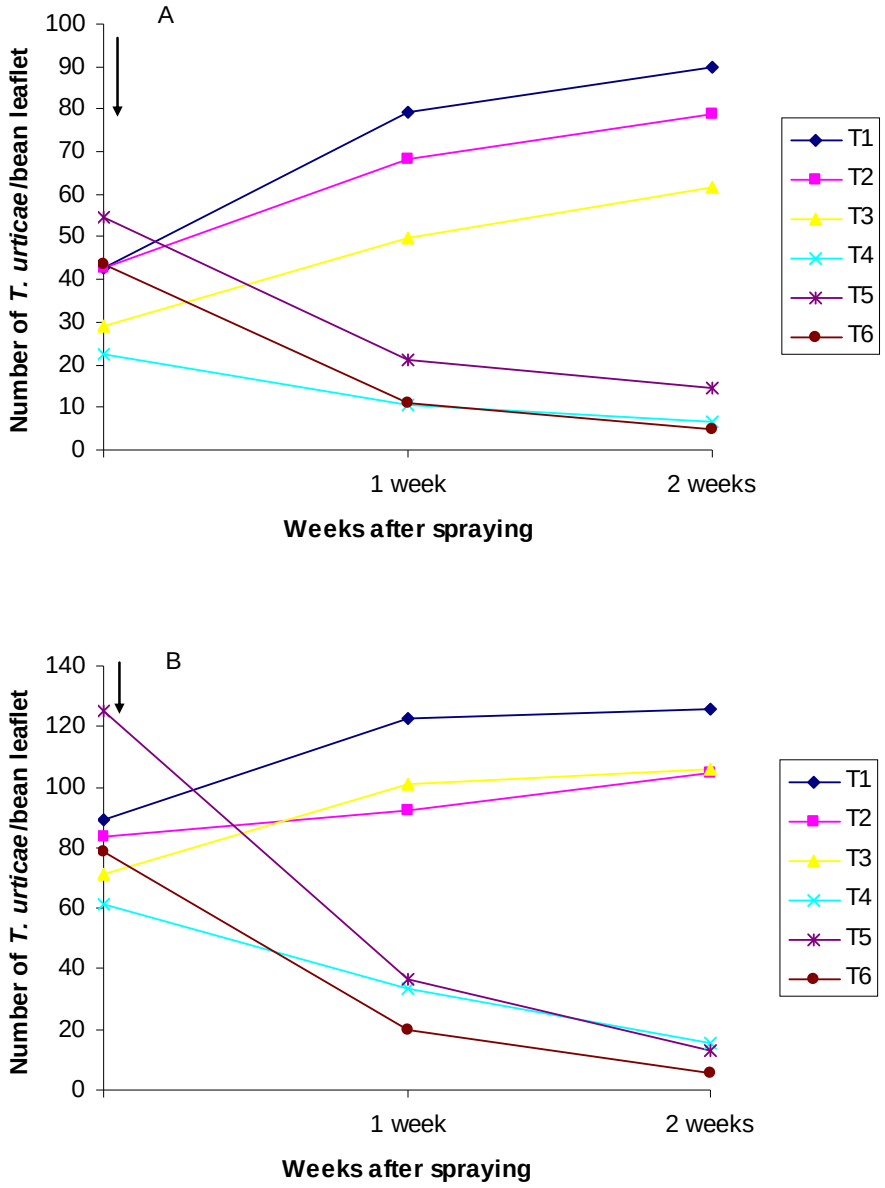


Figure 10.2: Effect of formulations of *M. anisopliae* and acaricide on the population density of *T. urticae* infesting bean (A) top leaves and (B) middle leaves during season II. (The arrow indicates the spraying time)

Legend:
T1 = Untreated control (mites only)
T2 = Silwet-L77+Water
T3 = Oil+Silwet-L77+Water
T4 = ICIPE78+Silwet-L77+Water
T5 = ICIPE78+Oil+Silwet-L77+Water
T6 = Acaricide (abamectin active ingredient)

10.3.1.2 Effect of fungal treatments on bean production parameters

During season I, there were no significant differences between the treatments in number of pods per plant (F=0.31; df=5, 18; P=0.9007) and in yield in metric ton per hectare (F=0.88; df=5, 18; P=0.5128). However, there were significant differences in number of seeds per pod (F=7.31; df=5, 18; P=0.0008) and dry weight of 100 seeds (F=5.32; df=5, 18; P=0.0036) (Table 10.1). During season II, there were no significant differences between the treatments in all the bean production parameters: number of pods per plant (F=0.46; df=5, 18; P=0.8016), number of seeds per pod (F=0.46; df=5, 18; P=0.4705), dry weight of 100 seeds (F=0.51; df=5, 18; P=0.7646) and dry weight of beans in metric tons per hectare (F=0.23; df=5, 18; P=9454) (Table 10.1).

Table 10.1: Effect of different treatments on *T. urticae*-infested common bean (*P. vulgaris* L.) production parameters (number of pods/plant, number of seeds/pod, weight of dry 100 seeds and weight in metric ton/Ha) during season I and during season II

Treatments	Season I				Season II			
	Number of pods/plant	Number of seeds/pod	Weight of 100 seeds (in grams)	Weight (in metric ton/Ha	Number of pods/plant	Number of seeds/pod	Weight of 100 seeds (in grams)	Weight (in metric ton/Ha
T1	8.45 ± 1.27a	3.25 ± 0.21b	45.13 ± 0.21bc	2.51 ± 0.31a	9.08 ± 1.11a	3.60 ± 0.33a	39.30 ± 3.07a	1.64 ± 0.24a
T2	7.50 ± 0.82a	3.55 ± 0.24b	43.78 ± 0.47c	1.84 ± 0.81a	8.33 ± 0.74a	3.68 ± 0.20a	39.65 ± 1.55a	1.38 ± 0.29a
T3	6.83 ± 1.05a	3.30 ± 0.33b	45.18 ± 1.28bc	1.77 ± 0.28a	8.65 ± 0.54a	3.33 ± 0.09a	39.58 ± 1.11a	1.58 ± 0.17a
T4	7.58 ± 0.71a	3.85 ± 0.16ab	45.45 ± 1.06bc	2.38 ± 0.52a	7.75 ± 0.36a	3.20 ± 0.22a	37.58 ± 0.83a	1.42 ± 0.21a
T5	8.15 ± 0.59a	4.63 ± 0.09a	49.70 ± 0.93ab	2.28 ± 0.38a	8.30 ± 0.81a	3.63 ± 0.21a	40.48 ± 1.84a	1.64 ± 0.27a
T6	8.08 ± 1.54a	4.40 ± 0.19a	50.40 ± 2.14a	2.36 ± 0.20a	9.58 ± 1.59a	3.75 ± 0.22a	41.15 ± 0.34a	1.48 ± 0.22a

Means in the same columns followed by the same letters are not significantly different (SNK-Test, P = 0.05)

Legend:
T1 = Untreated control (mites only)
T2 = Silwet-L77+Water
T3 = Oil+Silwet-L77+Water
T4 = Fungus+Silwet-L77+Water
T5 = Fungus+Oil+Silwet-L77+Water
T6 = Acaricide (abamectin active ingredient)

10.3.2 Tomato field experiments

10.3.2.1 Effect of fungal application on *T. evansi* population densities in tomato

During season I, there were no significant differences between the treatments in the density of *T. evansi* on top leaves before spraying (F=0.06; df=5, 18; P=0.9967) and at one week post-treatment (F=1.88; df=5, 18; P=0.1481). However significant differences were observed between the treatments at two weeks (F=10.55; df=5, 18; P < 0.0001) and at three weeks (F=9.74; df=5, 18; P=0.0001) post-spraying (Figure 10.3A). At two and three weeks post-spraying, the number of *T. evansi* on tomato leaflet was higher in the controls than in the fungal fand the acaricide treatments. In the middle leaves, there were also no significant differences between the treatments in *T. evansi* population density before spraying (F=0.09; df=5, 18; P=0.9937) and at one week post-treatment (F=1.53; df=5, 18; P=0.2308). Significant differences were, however, observed at two (F=5.38; df=5, 18; P=0.0034) and at three (F=6.11; df=5, 18; P=0.0018) weeks post-spraying (Figure 10.3B). As in the case of top leaves, the number of mites per tomato leaflet was lower in the fungal and acaricide treatments than in the controls.

During the second season, significant differences were observed in *T. evansi* population density in the top leaves between the treatments before spraying (F=3.44; df=5, 18; P=0.0235), at one week (F=5.21; df=5, 18; P=0.0039), two weeks (F=9.92; df=5, 18; P=0.0001), three weeks (F=10.71; df=5, 18; P < 0.0001), four weeks (F=15.32; df=5, 18; P < 0.0001) and at five weeks post-spraying (F=21.68; df=5, 18; P < 0.0001) (Figure 10.4A). In the middle leaves, however, no significant

differences were observed between the treatments in *T. evansi* population density before spraying (F=1.80; df=5, 18; P=0.1637). However, significant differences were observed in T. evansi density on middle leaves at one week (F=7.98; df=5, 18; P=0.004), two weeks (F=11.95; df=5, 18; P < 0.0001), three weeks (F=11.70; df=5, 18; P < 0.0001), four weeks (F= 16.47; df=5, 18; P < 0.0001) and five weeks (F=14.74; df=5, 18; P < 0.0001) post-spraying (Figure 10.4B). In both canopy levels, the number of *T. evansi* was lower in the fungal formulations and the acaricide treatments than in the controls. However, emulsifiable formulation of the fungus performed better than the aqueous formulation.

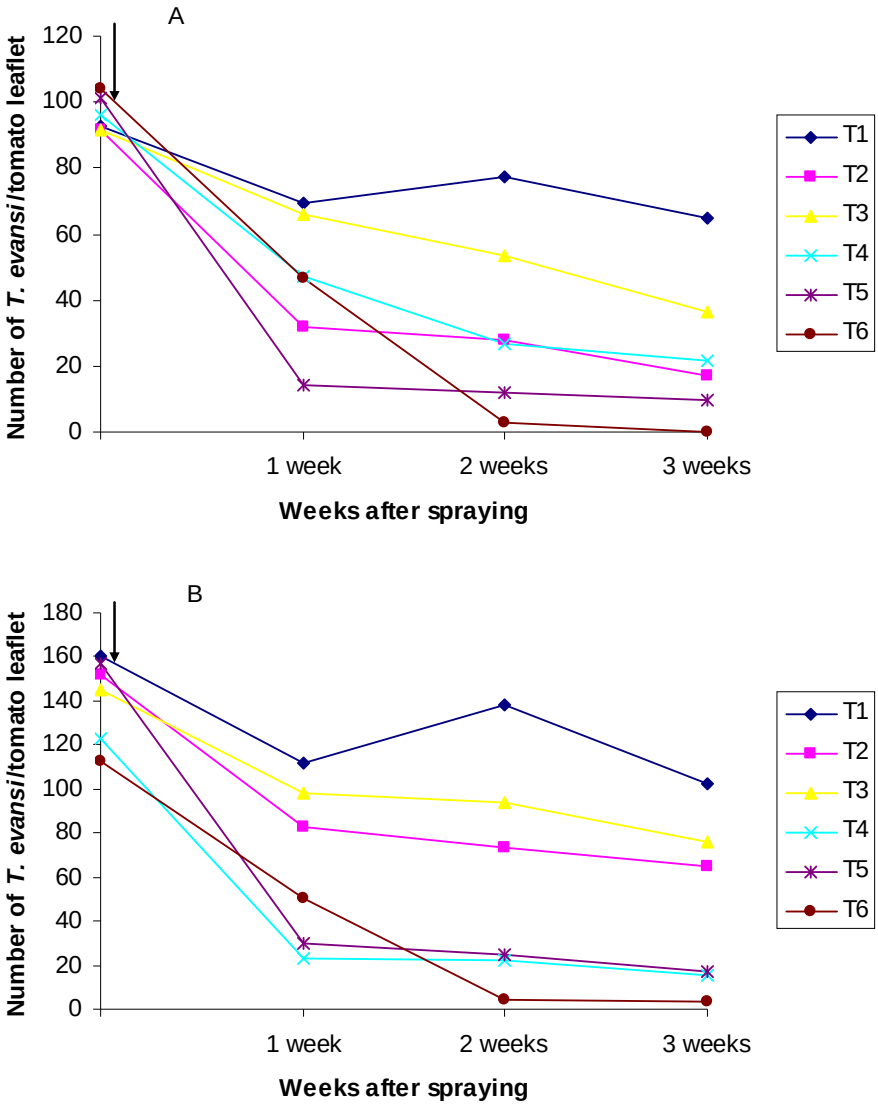


Figure 10.3: Effect of formulations of *M. anisopliae* and acaricide *T. evansi* population density on tomato (A) top and (B) middle leaves during season I

Legend:
T1 = Untreated control (mites only)
T2 = Silwet-L77+Water
T3 = Oil+Silwet-L77+Water
T4 = ICIPE78+Silwet-L77+Water
T5 = ICIPE78+Oil+Silwet-L77+Water
T6 = Acaricide (abamectin active ingredient)

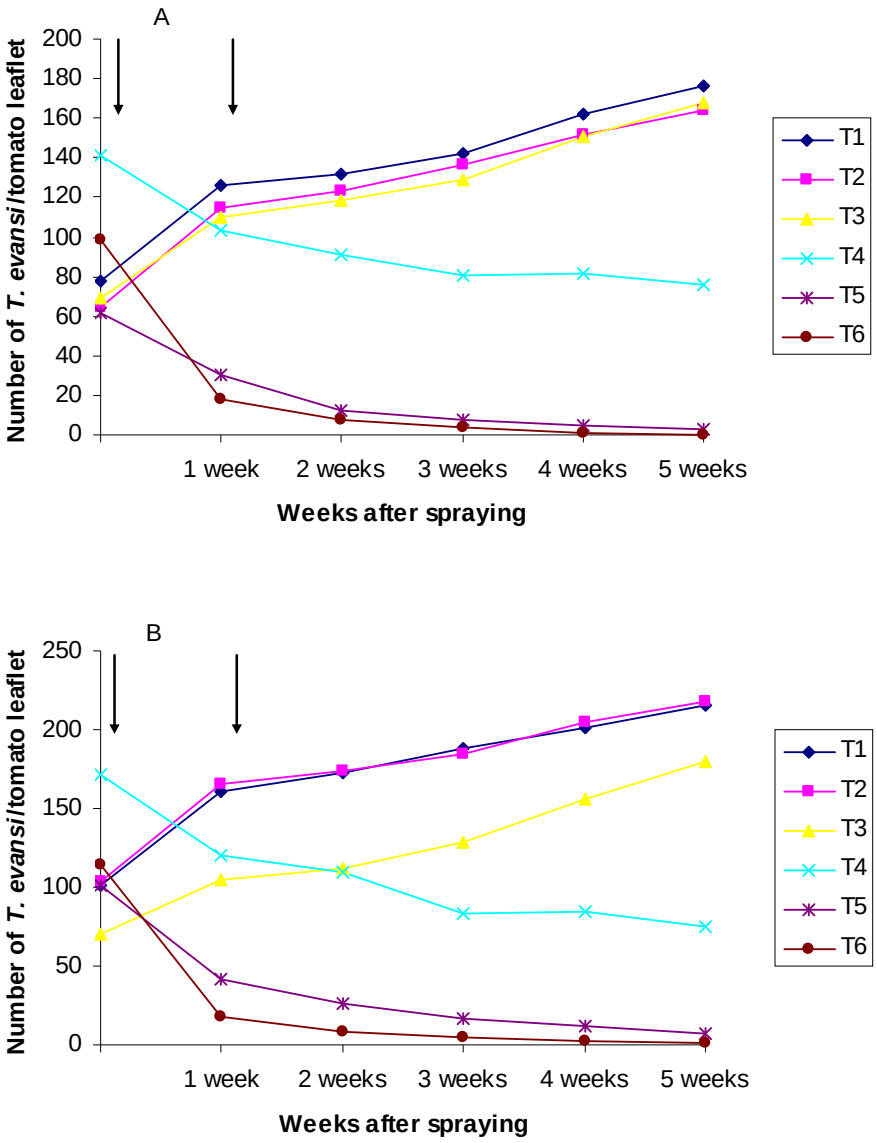


Figure 10.4: Effect of formulations of *M. anisopliae* and acaricide *T. evansi* population density on tomato (A) top and (B) middle leaves during season II

Legend:
T1 = Untreated control (mites only)
T2 = Silwet-L77+Water
T3 = Oil+Silwet-L77+Water
T4 = ICIPE78+Silwet-L77+Water
T5 = ICIPE78+Oil+Silwet-L77+Water
T6 = Acaricide (abamectin active ingredient)

10.3.2.2 Effect of fungal formulations on tomato production parameters

In season I, there were no significant differences between the treatments in number of fruits per plant (F=0.53; df=5, 18; P=0.7542) and in yield in metric ton per hectare (F=1.39; df=5, 18; P=0.2754) (Table 10.2). During season II, the number of fruits per plant (0.58; df=5, 18; P=0.7160) did not differ significantly; however differences were observed for weight of tomatoes in metric ton per hectare (F=22.47; df=5, 18; P < 0.0001) (Table 10.2). The weight of tomatoes (in metric tons per hectare) was very high in oil formulated fungus and the acaricide treatments, followed by the aqueous formulation, and finally in the controls where it was very low. In this experiment, there were no significant diffence in weight of tomatoes between the oil formulated fungus and the acaricide treatments.

Table 10.2: Effect of different treatments on *T. evansi*-infested *L. esculentum* production parameters (number of tomatoes/plant and weight of tomatoes/Ha) during season I and season II

Treatments	Season I		Season II	
	Number of tomatoes/plant	Weight of tomatoes/Ha	Number of tomatoes/plant	Weight of tomatoes/Ha
T1	26.83 ± 4.71a	5.99 ± 1.10a	14.70 ± 2.68a	1.62 ± 0.35c
T2	25.27 ± 1.27a	5.45 ± 4.10a	10.95 ± 3.08a	2.17 ± 0.23c
T3	25.35 ± 3.48a	5.47 ± 6.29a	14.60 ± 3.46a	2.24 ± 0.29c
T4	22.15 ± 2.05a	5.38 ± 0.39a	13.75 ± 4.92a	4.24 ± 0.60b
T5	25.25 ± 4.29a	4.87 ± 1.21a	9.90 ± 1.62a	6.48 ± 0.45a
T6	30.20 ± 4.51a	7.72 ± 0.94a	15.75 ± 0.64a	6.92 ± 0.78a

Means within columns bearing the same letters are not significantly different (SNK test, P=0.05). **Legend: T1** = Untreated control (mites only), **T2** = Silwet-L77+Water, **T3** = Oil+Silwet-L77+Water, **T4** = ICIPE78+Silwet-L77+Water, **T5** = ICIPE78+Oil+Silwet-L77+Water and **T6** = Acaricide (abamectin active ingredient)

10.4 Discussion

Although mites decreased in fungal and acaricide treatments during the first season, a decrease in mites in the control treatments was also observed on both crops. This decrease might have been due to the prevailing weather conditions. Rainfall and low temperatures are known to render the establishment and multiplication of mites difficult (Wermelinger *et al*., 1990, 1992; Sarr, 2003; Knapp *et al*., 2006). The daily minimum temperature during the experimental period was 9.4 ºC, which is suboptimal for the development of spider mite species, including *T. urticae* and *T. evansi* (Moraes & McMurtry 1987; Bonato, 1999). This was not the case during the second season. The reduction of *T. urticae* and *T. evansi* population densities can therefore be attributed to the *M. anisopliae* and the acaricide treatments in both bean and tomato fields.

Reduction of populations of *T. urticae* and *T. evansi* by entomopathogenic fungi has previously been reported. For instance, Dresner (1949) obtained mortality of 71% of *T. urticae* following application of a dust formulation of conidia containing 0.5% spores of *B. bassiana*. Alves *et al*. (1998) reported the reduction of *T. urticae* population density on chrysanthemum by *B. bassiana*. Similar results were also reported by Tamai *et al*. (2002) who obtained a reduction in *T. urticae* population density treated with *B. bassiana*.

Entomopathogenic fungi have also been reported to reduce the population of many other arthropods. For instance, Maniania (1993) used *B. bassiana* formulations for the control of the spotted stem borer, *C. partellus* resulting in a substantial increase

in grain yield. Godonou *et al*. (2000) reported that *B. bassiana* can play an important role in the management of the banana weevil, *Cosmopolites sordidus* Germar, on plantain. Benjamin *et al*. (2002) recorded a reduction of more than 50% in adult *Ixodes scapularis* Say following application of *M. anisopliae*. McCoy *et al*. (1971) applied large quantities of mycelia of *Hirsutlla thompsonii* as foliar spray to control the citrus rust mite, *Phyllocoptruta oleivora* Ashmead, infesting orange trees. They found that the mean number of mites per leaf decreased and the rate of mite infection increased at 1 week post-treatment. Mite populations were maintained at low levels for 10 to 14 weeks. Therefore field application of the most lethal dosage of *M. anisopliae* from this study to manage spider mites in beans and tomatoes could be a feasible and sustainable management strategy. There are several documented cases of successful field application of entomopathogens. For instance in 1998-1999, *Neozygites tanajoa* (=*N. floridana*) Delalibera, Hajek and Humber was introduced into Benin for the control of the cassava green mite, *Mononychellus tanajoa* Bondar, where it successfully established.

Formulation of entomopathogenic fungi for pest control has a big influence on the rate of success in the field. In Israel, an emulsifiable formulation of *M. anisopliae* sprayed at a concentration of 5×10^6 conidia/ml caused 25.9 to 90.6% mortalities in *Tetranychus cinnabarinus* (Boisduval) on eggplants (Batta, 2003). Shi and Feng (2006) reported an excellent control of the citrus red mite *Panonychus citri* (McGregor) by an emulsifiable formulation of *B. bassiana*. Shi *et al.* (2008) tested four formulations of *B. bassiana* and *M. anisopliae* for control of cotton spider mites, mainly *T. truncates* (Ehara) and *T. turkestani* (Ugarov et Nikolski), and

observed a significant reduction in cotton mite population densities. They then concluded that the two fungal species would be of high potential for practical use in the management of the cotton spider mite species, including *T. urticae.* In this study, oil formulation was generally more effective than the aqueous formulation. It has been reported that conidia formulated in oil persist longer than the ones formulated in water (Inglis *et al*., 1995; Thompson *et al*., 2006), and this may be the reason of the differences in efficacy between the different types of formulations. Shi and Feng (2006) and Shi *et al*. (2008) reported a high efficacy of *B. bassiana*, and *M. anisopliae* and *B. bassiana* formulated in oil in the control of the citrus red mite *P. citri* and the cotton mites, respectively, in the field.

There were no significant differences between both bean and tomato production parameters in the first season. This could be explained by the fact that, due to bad weather conditions, artificial infestation failed in two occasions and only succeeded when the plants were at the pod formation stage. At this stage mites could no longer have an impact on yields. Therefore, although the yields data were taken, only the effect of the treatments on mite density was the most important parameter to be considered. During the second season, although the fungal formulations and the acaricide had a positive effect on *T. urticae* population density, *T. urticae* did not affect the bean yield because of the delay in artificial infestation. Although, there were significant differences in tomato yields between the treatments, the yields were very low in all the treatments as compared to the literature (Anonymous, 1991; Varela *et al*., 2003). This could be explained by the presence of other pests and diseases such as fruitworms (*Helicoverpa armigera* Hübner) larvae, bacterial spot

(caused by *Xanthomonas campestris* pv. *vesicatoria* Dye) blossom-end root (caused by calcium deficiency), sunscald (caused by the exposure of fruits to the sun) that were observed during the fruiting stage.

CHAPTER 11: GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

11.1 General discussion and conclusions

Tetranychus urticae and *T. evansi* are among the most economically important spider mite species. They are distributed worldwide and known as pests of a large range of crops, including ornamental crops, fruit crops, cereals, legumes and vegetables. They initially feed on the underside of leaves causing speckling and, in severe cases they feed on the entire plant causing premature leaf drop, resulting in yield loss and plant death. The management of these pests has mainly relied on synthetic acaricides. However, a problem in controlling these spider mites is their ability to rapidly develop resistance to acaricides after only a few applications. In addition, the improper use of the synthetic acaricides can be harmful to the environment, users and the consumers. Due to the excessive use and the misuse of synthetic acaricides and to the all related problems, there has been a shift of emphasis towards biological control, including the use of entomopathogenic fungi. The aim of this study was therefore to investigate the potential of the entomopathogenic fungi *B. bassiana* and *M. anisopliae* for the control of the two spider mite species, *T. urticae* and *T. evansi*.

To achieve the objective of this study, the following steps were undertaken:

1. Field surveys were conducted in different districts of Kenya in order to find out whether spider mites could be associated with entomopathogenic fungi in the field;

2. Bioassyas were carried out (i) to screen 23 isolates of *M. anisopliae* and 3 isolates of *B. bassiana* for their pathogenicity against *T. urticae* and *T. evansi* in order to select the most virulent isolates; (ii) to evaluate the effect of temperature on germination, radial growth and virulence of 13 isolates of *M. anisopliae* and two of *B. bassiana* to the two spider mite species; (iii) to study the susceptibility of different developmental stages of *T. urticae* and *T. evansi* to infection by three isolates of *M. anisopliae* and two isolates of *B. bassiana*; (iv) to evaluate the compatibility between the entomopathogenic fungi *B. bassiana* (isolate ICIPE279) and *M. anisopliae* (isolate ICIPE78) and chemical pesticides. Results from these studies led to the selection of M. anisopliae isolate ICIPE78 as the best isolate for greenhouse and field trials;
3. Trials were conducted in order to evaluate the efficacy of two different fungal formulations (acqueous and emulsifiable formulations) and an acaricide with abamectin active ingredient, on one hand on *T. urticae* infesting bean plants in the greenhouse, and on the other hand on *T. urticae* and *T. evansi* infesting bean and tomato plants, respectively, in the field.

The results presented in this study confirm the virulence of the entomopathogenic fungi *B. bassiana* and *M. anisopliae* against *T. urticae* and *T. evansi*, and the prospects of these pathogens for the management of these mite pests in horticulture. This study confirms the infection of spider mites by entomopathogenic in the field which has been earlier confirmed by several authors (Carner and Canerday, 1970; Humber *et al*., 1981; Chandler *et al*., 2005; Fiaboe, 2007). The infection depends on the environmental conditions such as temperature, relative humidity and rainfall

(Smitley *et al*., 1986; Klubertanz *et al*., 1991; Fiaboe, 2007). Several studies have also reported the pathogencicity of *B. bassiana* and *M. anisopliae* against *T. urticae* (Alves *et al*., 2002; Tamai *et al*., 2002; Chandler *et al*., 2005). However this study is the second to report the pathogenicity of *B. bassiana* and *M. anisopliae* towards *T. evansi* in the laboratory, which agrees with the results of the first studies (Wekesa *et al.* 2005, 2006). The study is also the first to report the effect of *M. anisopliae* formulations against *T. urticae* and *T. evansi* infesting bean and tomato plants, respectively, in the field.

Regarding the results obtained from the laboratory, greenhouse and field trials, we concluded that:

1. Entomopathogenic fungal isolates can be isolated from spider mite species in the field in Mwea, Kirinyaga, Kutus (Kerugoya district) and in Shinyalu (Kakamega district) during rainy seasons;
2. The entomopathogenic fungi *B. bassiana* and *M. anisopliae* are pathogenic to the two spider mite species *T. urticae* and *T. evansi* in the laboratory;
3. Although isolates of *B. bassiana* and *M. anisopliae* germinate, grow and cause mortality in *T. urticae* and *T. evansi* at 20, 25, 30 and 35 ºC, their germination, radial growth and virulence depend on temperature and on the isolate. *Beauveria bassiana* and the *M. anisopliae* are able to cause mortality in *T. urticae* and *T. evansi* at 20, 25, 30 and 35 ºC;
4. *Beauveria bassiana* and *M. anisopliae* infection reduces *T. urticae* and *T. evansi* egg hatchability and cause mortality in all their motile developmental stages (larvae, protonymphs, deutonymphs and adults). However, egg

hatchability and motile stage mortality depend on isolate and fungal concentration. The higher the concentration, the lower the egg hatchability and the higher the mortality in motile stages.

5. *Tetranychus urticae* and *T. evansi* mature stages (deutonymphs and adults) are more susceptible to fungal infection than the immature stages (larvae and protonymphs);
6. The synthetic insecticides (Dimethoate, Karate and Malathion) at the recommended field rate do not inhibit *M. anisopliae* (isolate ICIPE78) germination and are therefore compatible with the said isolate. On the other hand, only Dimethoate and Karate are compatible with *B. bassiana* (isolate ICIPE279), while Malathion is not. The latter, at the recommended field rate, do not inhibit the *B. bassiana* isolate ICIPE279 germination. The synthetic fungicides (Antracol, Mancozeb and Milraz), at the recommended field rate, inhibit the two fungal strains germination, and therefore are not compatible with them. However, since long term of exposure of conidia to chemical pesticides amy have detrimental effects of fungus, compatibility between chemical pesticides and fungi will depend on the (i) mode of preparation of formulations, (ii) mode of application of IPM and the (iii) time between the use of the two control methods.
7. The aqueous and emulsifiable formulations of the *M. anisopliae* isolate ICIPE78 reduce the population density of *T. urticae* infesting bean plants in the greenhouse. Fungal formulations and synthetic acaricide (Dynamec®) are equally suitable in controlling *T. urticae* infesting bean plants in the

greenhouse. However, emulsifiable formulation performs better than the acqueous one;

8. The aqueous and emulsifiable formulations of the *Metarhizium anisopliae* isolate ICIPE78 reduce the population densities of *T. urticae* and *T. evansi* infesting bean and tomato plants, respectively, in the field. The two fungal formulations are as effectice as the synthetic acaricide (Dynamec®) in controlling *T. urticae* infesting bean plants in the field. In tomato field, however, the fungus in oil formulation provides a better control of *T. evansi* than the fungus in water formulation.

11.2 Recommendations

1. Surveys should be repeated in order to confirm the infection of spider mites by entomopathogenic fungi. The surveys should also be conducted in other Kenyan districts which were not covered in this study, and on the other side surveys should be conducted during both rainy and dry seasons
2. Studies should be done on the effect of the fungal strains isolated from spider mite species under various temperatures and relative humidities, on mites fed on different host plants and at different concentrations
3. Test the effect of different relative humidities on the effectiveness of the isolates used in the study of the effect of temperature on fungal effectiveness
4. To confirm the compatibility between fungi and all synthetic acaricides used in the same agro-ecosystems for pest control before any combination of the two pest control methods, for a better IPM program planning

5. To test the compatibility between *B. bassiana* and *M. anisopliae* with the predatory mites

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