EFFECT OF TEMPERATURE ON THE EFFICACY OF *Metarhizium anisopliae* (Metchnikoff) Sorokin IN THE CONTROL OF WESTERN FLOWER THRIPS IN FRENCH BEANS

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DECLARATION

I declare that this is my original work and has not been presented for any award of a degree in any other university

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DEDICATION

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LIST OF ACRONYMS AND ABBREVIATIONS

AIC	-	Akaike Information Criterion
ANOVA	-	Analysis of Variance
DAP	-	Diammonium Phosphate
DAT	-	Days after treatment
EU	-	European Union
GLOBALGAP	-	Global Good Agricultural Practices
HCDA	-	Horticultural Crops Development Authority
icipe	-	International Centre of Insect Physiology and Ecology
ILCYM	-	Insect Life Cycle Modeling Software
INSV	-	Impatiens Necrotic Spot Virus
IPM	-	Integrated Pest Management
MOA	-	Ministry of Agriculture
MRLS	-	Maximum Residue Levels
SDA	-	Sabouraud Dextrose Agar
SNK	-	Student-Newman-Keuls means separation test
TSWV	-	Tomato Spotted Wilt Virus
WFT	-	Western Flower Thrips

ABSTRACT

Metarhizium anisopliae isolate ICIPE69 is a highly virulent fungal pathogen against several thrips species such as western flower thrips (*Frankliniella occidentalis*), bean flower thrips (*Megalurothrips sjostedti*), onion thrips (*Thrips tabaci*) and others infesting key vegetable crops in Africa. Recently the isolate has been commercialized for pest control under the trade name, Campaign ®. The efficacy of the entomopathogenic fungi is known to be influenced by the prevailing climatic conditions with temperature being a key climate driver. This study aimed to predict the efficacy of Campaign® for thrips control in different Agro ecological zones (AEZ) in Kenya. To achieve this, effects of different temperatures on spore germination, radial growth, sporulation and virulence of the test fungus against *F. occidentalis* on French beans were evaluated and a cubic model was used to establish the efficacy of entomopathogenic fungus in relation to temperature. Further this model was run on a temperature geographic information system and a map was developed to predict the efficacy of entomopathogenic fungus at geospatial scale.

The test fungus was exposed to different temperatures; 10, 15, 20, 25, 30, 35 and 40ºC in the laboratory to assess impacts on spore germination, radial growth and sporulation. French beans pods were sprayed with incremental concentrations of the test fungus at; 10^6, 10^7 and 10^8 conidia ml^{-1} to check the mortality of the adult and second instars of *F. occidentalis* and were incubated at above temperatures. A field experiment was also conducted at different AEZs (low, mid and high); where the fungus was tested at higher concentrations of 10^{12} and

10^{13} in comparison with an insecticide (Imidacloprid 0.125 g/L a.i) on two French bean varieties.

Temperatures ranging from 20 to 35ºC were found to be suitable for spore germination, radial growth and sporulation of the fungus. At 25 and 30ºC, the isolate induced 100% mortality to adult *F. occidentalis* in six days. The adult thrips were more susceptible to the test fungus than the second instars. Field application of *M. anisopliae at* 10^{13} conidia/ha on French beans at weekly intervals was equally effective as the application of Imidacloprid. There was a significant difference ($p < 0.001$) in the yield of French beans between fungus treated and untreated plots.

The findings of this study highlight the importance of temperature influences on the activity of *M. anisopliae* ICIPE69. The optimum temperature for the fungal activity both in the field and laboratory was 25 and 30ºC. *Metarhizium anisopliae* has a broad temperature range, thus increasing the potential for its usage in various regions. The test fungus was not affected by the AEZs since most the temperatures at the three locations were favourable for the activity of the fungus. The cubic model could be used as a support tool to predict the efficacy of ICIPE69 in relation to temperature.

CHAPTER ONE

INTRODUCTION

1.1 Background information

French bean (*Phaseolus vulgaris* L.) is grown as a cash crop by large and smallholder farmers in Kenya. It is ranked first among Kenya's vegetables grown for export (HCDA, 2012). It contributes more than 55% of the value of vegetable exports and rank second, after cut flowers, in volume and value among export crops (MOA, 2006). The main importers of Kenya's French beans are the European Union (EU) countries, which include; Germany, United Kingdom, France and the Netherlands. Other markets are South Africa and United Arab Emirates (HCDA, 2012).

The most common varieties of French beans grown in Kenya are Samantha, Teresa, Julia, Amy, Monei, Morgan, Paulista, Cupvert, Gloria, Pekera, Bakara, Serengeti, Super Monel, Coby and Tokai (HCDA, 2012). French bean grows well at an optimum temperature of 20 - 25ºC in regions with altitude ranging between 1,000 - 2,100 m above sea level. Well distributed high rainfall of 900 – 1200 mm per annum is adequate for rain fed cultivation. However in most cases they are cultivated as irrigated crop. The optimum soil pH is 6.5 - 7.5, but plants can tolerate up to a soil pH of 4.5. French beans grow best on friable, medium loam soils that are well drained (HCDA, 2011).

French bean production is constrained by several factors. They include high cost of seed for farmers, market fluctuations and biotic factors including insect pests and diseases such as rust, anthracnose, powdery mildew and *Fusarium* root rot during crop growth (Monda *et al*., 2003).

The key insect pests include bean stem maggots, flower thrips, pod borers, pollen beetles, foliage beetles, aphids and Tetranychid mites (Nderitu *et al*., 1996). Among these pests, thrips are reported to be the primary pest causing serious constraints to French bean production (MOA, 2006; Waiganjo *et al*., 2006). Western Flower Thrips (WFT) *Frankliniella occidentalis* (Pergande) are reported to have developed resistance to chemical insecticides in Kenya (Nderitu *et al*., 2001). A lot of research has been done to develop alternative management options. Entomopathogenic fungi, which infect by penetrating directly through the insect integument, are the most promising bio-control agents (Niassy *et al*., 2009). Selected entomopathogenic fungi have been successfully applied as bio insecticides towards management of bean flower thrips, *Megalurothrips sjostedti* (Trybom) (Ekesi *et al*., 1998; Ngakou *et al*., 2008) and *Thrips tabaci* (Lindeman) (Maniania *et al*., 2003b).

1.2. Problem statement

French bean is an economically important export vegetable crop in Kenya (HCDA, 2012). Several biotic constraints including pest and diseases affect the productivity of this crop, among which thrips rank as the primary pest of French beans in Kenya (MOA, 2006; Nderitu *et al*., 2008; Nyasani *et al*. 2012; 2013). They are reported to cause 63 - 68% yield losses to French beans at the farm level mainly through abscission of buds, flower abortion and pod malformation making them unfit for the export market (Nyasani *et al*., 2012). Seif *et al* (2001) reported that *M. sjostedti* is an indigenous legume pest in Kenya and *F. occidentalis* had established itself as the main pest since its advent twenty years ago. Management of *F. occidentalis* has posed challenges due its resistance to most insecticides used by local farmers (Kasina *et al*., 2006; Nderitu *et al*., 2007).

Many French beans farmers in Kenya frequently use chemicals to manage thrips (Nderitu *et al*., 2001) which has resulted in pest resurgence and resistance, environmental contamination and lethal effects on non-target organisms. French beans farmers are reported to spray pesticides prior to harvest without observing the Pre-harvest interval (PHI) resulting in residues on/in the produce (Monda *et al*., 2003). The Good Agricultural Practices standards, such as, GLOBALGAP have imposed stringent export requirements for Maximum Residue Levels (MRLS) mainly in the EU markets (Seif *et al*., 2001). The pesticides residue that exceed default levels of 0.01mg/kg outlined by the EU market standards for fruits and vegetables limits access to export markets (Monda *et al*., 2003). The residues also pose danger to the health of the consumers as they are perceived to be a health risk (Monda *et al*., 2003). Additionally, increasing cost of chemical insecticides is making them inaccessible to farmers; particularly in developing countries (Nderitu *et al*., 2007).

1.3 Justification

Frankliniella occidentalis has developed resistance to all major classes of chemical insecticides; carbamates, organophosphate and pyrethroid used for its management in East Africa (Kasina *et al*., 2006). Hence, alternatively ecologically sustainable thrips management options are under development to overcome problems associated with the use of chemical insecticides such as adverse effects on non target organisms, environmental contamination, and the demand for pesticide-free foods (Nderitu *et al*., 2007).

Entomopathogenic fungi have been identified as an effective alternative management option (Maniania *et al*., 2001). Butt and Brownbridge (1997) reported that fungi are mostly associated with thrips in nature thus have a role to play as microbial insecticides for thrips

control. Some of these fungi are entomopathogens and possesses the necessary enzymes to penetrate the insect's cuticle during its feeding (Gillespie *et al*., 1988).

Fungal products have some desirable traits, for example, they leave no toxic residues, are generally harmless to beneficial insects (Ekesi *et al*., 1998) and pose minimal risk to the environment and humans (Goettel *et al*., 2001). Formulations based on *Beauveria bassiana, Metarhizium anisopliae* and *Lecanicillium muscarium* significantly reduced thrips populations in greenhouse vegetable and floral crops under research conditions (Ugine *et al*., 2006; Gouli *et al*., 2008). Fungal bio-control agents are promising because they act by contact and do not need ingestion, they can be mass produced very easily and are host specific (Shahid *et al*., 2012). *Metarhizum anisopliae* isolate ICIPE69 has been commercialized for thrips, mealy bugs and fruit fly and *Bactrocera invadens* management in several sub-Saharan African countries (Ekesi *et al*., 2009)

Resistance of *F. occidentalis* to major classes of chemical insecticides has led to an urgent need for alternatives in order to continue accessing the market. Entomopathogenic fungi have been identified as an effective alternative (Ekesi *et al.,* 2008). Advantages of using entomopathogenic fungi includes safety to humans and other non-target organisms, minimizing pesticide residues in food and increasing biodiversity in managed ecosystems (Shahid *et al*., 2012). However, the field efficacy of these entomopathogenic fungi is highly influenced by climatic factors such as temperature and humidity and there is a need to understand these influences at geographical scales to predict where and how effective the fungus can be used for the management of thrips.

However, most of the entomopathogens are sensitive to environmental factors such as humidity and temperature (Ignoffo, 1992; Ekesi *et al*., 2003) and this greatly determines the effectiveness of the fungus in different agro-ecological regions. Hence this study aims at understanding the impact of temperature on the efficacy of the *M. anisopliae*, and further develops geospatial predictions to identify regions where high and low efficacy with the fungus could be expected for better implementation of EPF as a management option for thrips.

1.4 Objectives

1.4.1 Major objective

To determine the influence of temperature on the growth of *M. anisopliae* and its effect on WFT, *F. occidentalis* and validate the efficacy of *M. anisopliae* in different agro ecological zones

1.4.2 Specific objectives

1) To assess the effect of temperatures changes on spore germination, radial growth and sporulation of *M. anisopliae* isolate ICIPE69.

2) To find most effective rate of *M. anisopliae* isolate ICIPE69 on WFT at different temperatures and develop temperature based models on pathogenicity of the fungus.

3) To assess the efficacy of *M. anisopliae* isolate ICIPE69 in the management of WFT infesting French beans at three agro-ecological zones.

CHAPTER TWO

LITERATURE REVIEW

This study aimed at investigating the effect of temperature on the efficacy of *Metarhizium anisopliae on* Western flower thrips (WFT), *Frankliniella occidentalis* (Pergande). This was achieved through laboratory and field experiments. The review has tackled the biology and ecology of WFT, economic importance of WFT, management of WFT, factors affecting pathogenicity of entomopathogenic fungi and modeling the efficacy of *M. anisopliae* in relation to temperature.

2.1 Biology and ecology of thrips infesting French beans

Western Flower Thrips (WFT), *F. occidentalis*; Bean Flower thrips, *Megalurothrips sjostedti* Trybom; Yellow flower thrips, *Frankliniella schultzei* Trybom and *Hydatothrips adolfifriderici* Karny, (Thysanoptera: Thripidae) are the common thrips species found on French beans in Kenya (Nyasani *et al*., 2012; Moritz *et al*., 2013). Thrips have piercing and sucking mouthparts (Harrewijin *et al*., 1996). Damage due to thrips occurs on rapidly growing tissues in plants such as flowers, buds and young leaves (Abate and Ampofo, 1995). Thrips damage through feeding reducing the quantity and quality of produce and further results in transmission of tospoviruses in some host plants (Dreistadt *et al*., 2007).

Western Flower Thrips, *F. occidentalis* is native to North America but has spread to other continents including Europe, Australia, Africa, Asia and South America via transport of infested plant material and has become a major worldwide pest of horticultural crops over the last few decades (Mound, 1997; Kirk, 2002; Kirk and Terry, 2003; Morse and Hoddle, 2006).

F. occidentalis was initially introduced in Kenya in 1989 (Kedera and Kuria, 2003). It is a polyphagous insect feeding on a broad variety of more than 500 different species of host plants ranging from field, vegetable and ornamental crops (Yudin *et al*., 1986; Moritz *et al*., 2004). Some of the key vegetable crops attacked by WFT in Kenya include beans (*Phaseolus vulgaris*), Tomato (*Solanum lycopersicum* L.), Capsicum (*Capsicum annuum* L.) and ornamentals such as rose, carnations *etc* (Moritz *et al*., 2013).

Western Flower Thrips is a tiny, slender and narrow fringe winged insect (Morse and Hoddle, 2006). The life cycle consists of egg, two nymph stages, two pupae stages, and an adult (Morse and Hoddle, 2006). A female can lay 20 - 40 eggs with unmated females producing only males. Hatching of the egg takes an average of 3 days (Reitz, 2009). Nymphs (larvae) are yellowish-white (Morse and Hoddle, 2006). The second stadium is longer than the first one and pupation takes place in the soil (Reitz, 2009). The larval stage is followed by short transitional pro pupa followed by pupae stages which do not feed. Winged adults emerge after 1 - 3 days. Males are 1.2 mm long, pale yellow, with a narrow abdomen at the end. Females are 1.6 - 1.7 mm long, yellow to brown with a more rounded abdomen with a saw-like ovipositor. Different developmental stages are typically found in different parts of plants. Eggs are found on leaves, flower tissue and fruits; nymphs on leaves, in buds and flowers and on emerging pods, pupae in soil or in hiding places on host plants such as the bases of leaves and adults are found on leaves, in buds and flowers (Gillespie, 2010).

The development rates, fecundity and longevity of *F. occidentalis* are affected by many factors, including temperature, day length, and the plant species it is feeding on (Deligeorgidis *et al.,* 2006; Soria and Mollemam, 1992; Gaum *et al*., 1994; Brødsgaard, 1994; Katayama, 1997). Development occurs whenever temperatures exceed 8 - 10ºC (Katayama, 1997;

McDonald *et al*. 1999). At the most favorable temperatures of 25 - 30ºC, the development period from egg to adult is the shortest between 9 - 13 days (Lublinkhof and Foster, 1977; Robb, 1995; Gaum *et al*. 1994; Katayama, 1997; Reitz, 2009). The presence of pollen as food source for *F. occidentalis* enhances the various growth parameters (Trichilo and Leigh, 1988).

Bean flower thrips (BFT), *M. sjostedti* is a common, polyphagous and widespread pest feeding mainly on legumes in Africa (Palmer, 1987; Tamo *et al*., 1993; Kyamaywa *et al*., 1996). *Megalurothips sjostedti* is prevalent during cold and rainy periods (Gitonga *et al.*, 2002). Total developmental time ranges from 33.1 days at 14 °C to 19.2 days at 26 °C (Ekesi *et al*., 1999). Egg, larval and pupal stages requires 94.3, 97.1 and 105.3 deg-days (DD) above a threshold of 8.2, 9.1 and 10.4 °C, respectively to complete development (Ekesi *et al*., 1999).Total developmental cycle was completed at 163.9 DD above a threshold of 12.6 °C (Ekesi *et al*., 1999). It breeds rapidly and oviposits into leaf petioles before development of inflorescences (Tamo *et al*., 1993). Development from egg to adult takes 19 days at 29º C and 58 % relative humidity; adults live for 23 days (Salifu, 1992).

Yellow flower thrips (YFT), *F. schultzei* is a polyphagous species feeding on flowering plant parts of at least twelve dicotyledonous plant families (Vierbergen and Mantel, 1991). It feeds on various ornamental and vegetable hosts in different parts of the world (Milne *et al*,. 1996). It has been recorded from 83 species of plants among 35 families (Palmer, 1990). The major hosts of *F. schultzei* are cotton, groundnut, beans and pigeon pea. However, due to its polyphagous feeding behavior, *F. schultzei* also attacks tomato, sweet potato, coffee, sorghum, chillies, onion and sunflower (Hill, 1975). *F. schultzei* is one among the key thrips species infesting French bean (Lohr and Michalik, 1995; Nyasani *et al.,* 2012). There are two instars and two inactive and non-feeding stages in the life cycle. Females of *F. schultzei* insert

their eggs in flower tissue. Pinent *et al*., (2008) in Brazil studied the life cycle of this thrips at 24.5°C and reported that a complete generation takes around 12.6 days. The embryonic stage lasts for four days and the 1st and 2nd larval instars, pre-pupa and pupa take an average of 2.5, 2.5, 1.2, and 2.1 days, respectively. The adult female and male longevity is approximately 13 days.

Hydatothrips adolfifriderici is an oligophagous insect. Among the four thrips species commonly found on French beans; it is the least dominant (Nyasani *et al*., 2012). *Hydatothrips adolfifriderici* feeds on leaves of legumes most of the time. The mean development time from eggs to adult at 19°C is 42.0 days. At 19°C, eggs hatched after 20 days. First - stage larvae were reported to be delicate and cream-colored, and they molted within 2 – 4 days. Second - stage larvae are yellow and robust. The combined larval period is 13.1 days. Pre-pupa period was 3.2 days (Ananthakrishnan, 1984).

2.2 Economic importance of *Frankliniella occidentalis*

Western flower thrips have become a global threat to horticultural crops production due to the expanding world trade in fresh horticultural produce and changes in production and marketing systems (Loomans and van Lanteren, 1995). Among arthropod pests of French beans, *F. occidentalis* (Thysanoptera: Thripidae), is ranked as a major pest in Kenya (Nderitu *et al*., 2009; Nyasani *et al*. 2012, 2013). It attacks the crop before budding, and causes the flower buds to dry and brown, progressively aborting to leave dark red scars on the plant (Childers and Achor, 1995). The feeding of the WFT on the pollen and buds leads to deformation of flowers and pods (Trichilo and Leigh 1988; Childers, 1997). The feeding also results to flower abscission, open flower peduncles curling and malformation of pods leading to

quantitative and qualitative yield losses (Kibata and Anyango, 1996). Nyasani *et al*., (2010) reported pod losses of 63 - 68% in French beans. Nuessly and Nagata (1995) reported that losses caused by *F. occidentalis* and *Thrips palmi* in 1993 in Florida exceeded $10 million. Annual losses of up to $75,000 per hectare have been reported due to direct damage to cucumbers in a UK glasshouse due to these thrips (Zhang *et al*., 2007).

Twenty Tospovirus species have been identified globally along with 14 thrips species in the family Thripidae that can serve as vectors (Ullman *et al*., 1997; Jones, 2005; Pappu *et al*., 2009; Ciuffo *et al*., 2010; Hassani-Mehraban *et al*. 2010). *Frankliniella occidentalis* is the most studied vector of Tospovirus. Apart from the direct damage, WFT transmits tospovirus diseases (Funderburk *et al*., 2009). The most important of which are Impatiens Necrotic Spot Virus (INSV) and Tomato Spotted Wilt Virus (TSWV) (German *et al*., 1992; Ullman *et al*., 1997; Cloyd, 2009). Tospovirus infections can lead to total crop losses (Kyamaywa and Kuo, 1996). Over 80% potential yield losses of TSWV were reported by tomato farmers in Nakuru County (Wangai *et al*., 2001). Hausbeck *et al*. (1992) reported that an infection by TSWV and INSV virus caused $675,000 in losses in Pennsylvania in 1990. As an accurate value of loss is difficult to obtain, an estimate that TSWV alone causes over $1 billion in losses annually to various crops has been reported (Goldbach and Peters, 1994).

2.3 Management of western flower thrips

2.3.1 Cultural control

Cultural control methods include sanitation practices like rouging old infected plants and alternate hosts and cultivation in clean field (Cloyd, 2009). Weeds from the Compositae family; dandelion and sowthistle and Solanaceae family; silverstar, with yellow flowers not only attract WFT adults, but also serve as reservoirs for the tospoviruses vectored by WFT

(Yudin *et al*. 1986; Chatzivassiliou *et al*. 2001; Kahn *et al*. 2005). Mulching with black polythene paper (Hajek *et al.,* 2003) suppressed weeds and kept the soil moist and warm for rapid plant growth It also hinders the life stages of the thrips especially pre-pupae and pupae from attack during susceptible stages therefore, the plants are able to evade thrips. Over-head irrigation also creates less favorable conditions for thrips development and decreases WFT population (Lindquist *et al*. 1987). Use of lure or trap crops such as, yellow flowering chrysanthemums and eggplants may also help in managing WFT by attracting WFT away from the main crop (Hoyle and Saynor, 1993; Pow *et al*. 1998; Bennison *et al*. 2001). The trap crops may be removed from the field, sprayed with an insecticide, or inoculated with biological control agents such as predatory bugs or predatory mites that will feed on the nymph and adult stages residing in the flowers (Bennison *et al*. 2001). Intercropping of French beans with baby corn compromises the yield but the marketable yield is improved due to reduced damage on the French beans pods (Nyasani *et al*., 2012). Plots with French beans alone had about 1.4 times higher yields compared to intercropped plots of French beans with baby corn. The percentage of pods that could get rejected on the market due to thrips damage was highest on plots with French beans alones (68 and 63%) and lowest on plots with French beans and baby corn (35 and 37%) in the first and second seasons, respectively (Nyasani *et al*., 2012). Nderitu *et al* (2007) reported that French bean cultivar "Impala", tolerant to thrips supported the lowest thrips infestation while J12 was the most tolerant with high count of thrips but low pods damage score.

2.3.2. Chemical control

Most farmers in Kenya use insecticides such as Karate 1.75% EC (Lambda cyhalothrin), Mesurol 500 SC (Methiocarb), Tracer 480 SC (Spinosad), Regent 50 SC (Fipronil) and DC

Tron (Petroleum spray oil) for controlling thrips (Nderitu *et al*., 2007, 2008). Effective chemical control can be achieved by using insecticides from different classes and with different mode of action to impede the onset of resistance time after time (Loughner *et al*., 2005). Appropriate use of chemical sprays results in high yields of unblemished pods (Nderitu *et al*., 2008). However, the French bean farmers in Kenya depend heavily on pesticides and apply up to 15 sprays per season to be able to produce unblemished pods (Nderitu *et al*., 1996). This has resulted in the residue problem on French beans (Lohr, 1996).

Sustained use, abuse usage and overuse of pesticides can result in high resistance of insect to pesticides (Cloyd, 2009). Spinosad has been an effective pesticide in thrips management since it was developed in 1985 (Nayak *et al*., 2005). Due to its high efficacy for thrips control, spinosad had become almost the only insecticide used against thrips in some areas. Some growers have applied more than 10 applications of spinosad on crops per growing cycle (Bielza *et al*., 2007). The high application rates leads to thrips resistance. Loughner *et al*. (2005) reported thrips were resistant to spinosad when spinosad was applied up to 8 times a year. However, repeated use of spinosad has rendered it ineffective due to resistance development.

Due to prolific spray applications, the residue exceeds the minimum requirement of 0.02 parts per million (ppm) set by the importing countries of the EU (Pesticide Residue committee, 2009). This hinders smallholder farmers from accessing the export market (Nderitu *et al*., 2007). Further insecticides have many disadvantages including; high cost, residues in the environment, effect on human and animal health (Kasina *et al*., 2006). Recently the ban of dimethoate by the EU markets has further limited French bean production and exports from Kenya (www.nation.co.ke/business/.French-beans-use/index.html date accessed). Continuous

use of insecticides has resulted in resistance of *F. occidentalis* to major class of insecticides (Robb *et al*., 1995).

2.3.3 Biological control

2.3.3.1 Use of predators and parasitoids

Most beneficial organisms work to suppress thrips and pose no risk to humans and the environment (Desneux and O'Neil, 2008). Pirate bugs, *Orius* spp (Hemiptera: Anthocoridae) are the principal predacious insects associated with WFT population (Nderitu *et al*., 2009) and have been used as biological control agents against *F. occidentalis* in beans (Xu *et al*., 2006), sweet pepper and greenhouse cucumber (Messelink *et al*., 2006). *Orius insidious* (Say) (Hemiptera: Heteroptera: Anthocoridae) is an aggressive predator that seeks out thrips even in closely protected areas like deeper parts of flowers (Silveria *et al*., 2004). In Kenya, Gitonga (1999) reported that *Orius albidipennis* (Reuter) (Hemiptera: Anthocoridae) inflicted predation levels of 15 - 20% *M. sjostedti* and 11 - 30% *F. occidentalis.*

Sabelis and Van Rijin (1997) recorded that *Amblyseius cucumeris* (Oudemans) (Phytoseiidae: Acari) and *Amblyseius barkeri* (Hughes) (Acari: Phytoseiidae) predate some stages of *F. occidentalis.* Release of *A. cucumeris* in ornamental plants reduced the number of pesticide applications needed to control *F. occidentalis* (Gill, 1994). Parasitoids *Ceranisus menes* (Walker) and *Ceranisus americensis* (Girault) have a minor role in reducing thrips population. The two have longer developmental time than that of *F. occidentalis* and their level of parasitism ranged from 3.5% to 9.5%, respectively (Loomans *et al*., 2006).

2.4 Use of entomopathogens

2.4.1 Types of entomopathogens

Entomopathogens include entomopathogenic fungi, nematodes, bacteria and viruses (Evans, 2008). They are wide spread in the natural environment and cause infections to many pest species (Bale *et al*., 2008). They can be mass produced, formulated and applied to pest population and are released either inundatively or as classical biological control agents (Dent, 2000).

Entomopathogenic nematodes (Bale *et al*., 2008) and baculoviruses (Hajek, 2004) are obligate pathogens of insects while, entomopathogenic bacteria and fungus are facultative and can be multiplied in synthetic media (Romeis *et al*., 2006). Infection of insects due to entomopathogenic nematodes; Heterorhabditidae and Steinernematidae (Hajek, 2004) occur through the cuticle while infection due to baculoviruses; Nuclear polyhedrosis virus occur through ingestion (Dent, 2000).

Bacteria; *Bacillus thuringiensis* infects insects through ingestion (Shelton *et al*., 2002). It is the most widely used biological control of caterpillars, beetles and small haematophagous fleas (Romeis *et al*., 2006). In nature, Bt are widespread in the soils and are lethal pathogens of insects belonging to a range of insect orders. Examples of entomopathogenic fungi; *Metarhizium, Beauveria, Verticillium* which infect insects through the cuticle (Goettel *et al*., 2001).They are important biocontrol agents among the various pathogens because of the route of pathogenicity, broad host range and the ability to control both sap sucking pests such as mosquitoes and aphids as well as pests with chewing mouthparts (Hoffmann and Frodsham, 1993; Charnley *et al*., 1997; Goettel *et al*., 2001; Khan *et al*., 2012).

Entomopathogenic fungi are found in the divisions of Zygomycota, Ascomycota and Deuteromycota (Bischoff *et al*., 2009). Many genera of entomopathogenic fungi under research belong either to the order entomophthorales in the class zygomycota or hyphomycetes in the deutromycota (Shahid *et al*., 2012). About 750 species of fungi in 56 genera are known to be pathogens of arthropods (Hawksworth *et al*., 1995). Fungi of the class hyphomycetes have global distributions and can be readily mass produced (Chandler *et al*., 2000). Genera of fungus such as *Metarhizium*, *Beauveria*, *Verticillium*, *Nomuraea*, *Entomophthora* and *Neozygites* commonly include entomopathogens (Deshpande, 1999). Mitosporic entomopathogens such as *B. bassiana* and *M. anisopliae* have high potential for control of sucking insect pests (Chandler *et al*., 2000; Shi *et al*., 2007, Humber, 2008). Over 15 mycopesticides, formulated from the mitosporic genera are available commercially for the management of a range of pests in the orders; Coleoptera, Diptera, Homoptera, Lepidoptera and Orthoptera (Shah and Goettel, 1999, Bischoff *et al*., 2009).

Entomopathogenic fungi are very specific to insects, often to particular species and they have considerable epizootic potential and can spread quickly through an insect population and cause its collapse (Hoffmann and Frodsham, 1993). Several fungal species have potential as microbial insecticides and, in some countries, are commercially available in formulations that can be applied using conventional spray equipment (Hoffmann and Frodsham, 1993). The use of entomopathogenic fungi as microbial insecticides has been developed and increased with the discovery of new strains and genetic improvement of others in the last decade (Lacey and Goettel, 1995; Butt and Brownbridge, 1997). They have been reported to infect a range of insects including thrips, lepidopterous larvae and aphids which are of great importance in agriculture worldwide (Roberts and Humber, 1981).

There is less information available on pathogenic fungi infecting thrips, but fungi are invariably the most common microbes' recovered (Tanada, 1993; Butt and Brownbridge, 1997). Several isolates of *Beauveria bassiana* (Balsamo) and *Metarhizium anisopliae* (Metschnikoff) have been reported infective to *F. occidentalis* (Niassy *et al*., 2012). Among the isolates tested for pathogenicity, *M. anisopliae* isolate ICIPE69 is most potent against *Megalurothrips sjostedti* (Trybom, F), *F. occidentalis* (Pergande) and *Thrips tabaci* (Lindeman).

2.4.2 Mode of action of entomopathogenic fungi

Virulence of entomopathogens involves adhesion to, germination and penetration of the host integument (Cloyd, 1999; Mustafa and Kaur 2008, Sandhu *et al*., 2012, Shahid *et al*., 2012). After contact with a potential host and if host recognition is positive, infective spores of entomopathogenic fungi adhere to the insect cuticle (Al-Aidroos and Roberts, 1978) through both physical and chemical interactions (Fargues, 1984). Some compounds that are found in the epicuticle such as fatty acids, amino acids and glucosamines determine the specificity and pathogenicity of entomopathogenic fungi (Boucias and Pendland, 1984; Kerwin, 1983; Smith and Grula, 1982; Woods and Grula, 1984; Shahid *et al*., 2012).

Spore germination is highly dependent on temperature and moisture content, and probably requires free water (Mullens *et al*., 1987; Newman and Carner, 1975; Roberts and Campbell 1977; Kim and Kim 2008), but this requirement may be met by moisture conditions of the microclimate in the absence of measureable precipitation (Kramer, 1980; Mullens *et al*., 1987; Kim and Kim 2008).

Entomopathogenic fungi penetrate the host cuticle shortly after germination or after limited hyphae growth between 24 to 48 hours under favorable conditions (Kim and Kim 2008). Upon germination, appresoria are formed at the end, sub terminally or side branches of short germ tube which facilitates host penetration through formation of narrow penetration peg (Ment *et al*., 2010; Khan *et al*., 2012). The penetration sites are observed as dark lesions in the epicuticle indicative of enzymatic action and mechanical pressure (Shahid *et al*., 2012). Death occurs usually within three to ten days after infection due to water loss, nutrient deprivation, gross mechanical damage and action of toxins (Cloyd, 1999). Under favourable conditions, the fungus sporulates extensively on the cadaver (Chandler *et al*., 2000).

Vegetative growth allows the entomopathogen to disperse rapidly and colonize the insect's circulatory system and increases the fungal surface area that is in contact with the nutrient medium. Vegetative growth is characterized by discrete yeast-like structures or hyphae bodies (Shahid *et al*., 2012). Disease development during the vegetative phase and its incubation period is typically temperature-dependant. Shortly after host death, the fungal hyphae penetrate the cuticle from within and terminate in the formation of sporophores that yield asexual spores which function as dispersive and infective units for secondary infection and spread (Shahid *et al*., 2012).

2.4.3 Use of *Metarhizium anisopliae* in managing crop pests

Metarhizium anisopliae has been reported to infect a very wide range of insects including Lepidopteran (Freed *et al*., 2012), beetles (Milner, 1991), locusts (Lomer *et al*., 2001), dipterans (Migiro *et al*., 2010), aphids (Sahayaraj and Borgio, 2010) and thrips (Ekesi *et al.* 1999; Maniania *et al.* 2001, 2003b) which are of great concern in agriculture worldwide.

Metarhizium anisopliae is highly infective on the larval stages of lepidopterans (Sahayaraj and Borgio, 2010), adult and larval stages of beetles (Sahayaraj and Borgio, 2010), adults and pupal stages of dipterans, nymphs and adults of locust (Lomer *et al*., 2001) and adult and larval stages of thrips (Niassy *et al*., 2012).

The potential of the entomopathogenic fungus, *M. anisopliae* for control of the legume flower thrips (*Megalurothrips sjostedti* (Trybom) on cowpea was demonstrated by Ekesi *et al* (1998). The *M. anisopliae* isolate ICIPE69 was virulent on both the adult and larval stages of *F. occidentalis in* French bean, legume, onion and chrysanthemum production (Ekesi *et al.* 1999; Maniania *et al.* 2001, 2003; Niassy *et al*. 2012). *M. anisopliae* can be grown on starch-rich substrates like cereal grains such as rice and wheat (Cloyd, 2009). The development of methods and successful mass culture of *M. anisopliae* has led to the commercial development of this fungus as a microbial insecticide and as an ideal candidate for augmentative biological control (Cloyd, 2009).

2.4.4 Factors affecting pathogenicity of the entomopathogenic fungi

Pathogenicity of entomopathogenic fungi is controlled by host, pathogen and environmental factors. Host factors include population size and developmental stages; Pathogen factors include inoculum dose, virulence and nutritional requirements and the environmental factors include host plants, moisture, light, temperature, relative humidity and type of soil (Goettel and Inglis, 1997).

All the stages of *M. sjostedti* were susceptible to *Metarhizium anisopliae* isolate ICIPE69, although the pupae and larval stages were less susceptible to infection than the adult stages (Ekesi *et al*., 2000). The differential susceptibility at various life stages can be attributed to

interaction between the fungus and ecdysis of larval and pupae stages (Ekesi *et al*., 2000). Ecdysis is known to be an important factor in insect resistance to fungal infection particularly when the time interval between successive ecdysis is short (Vey and Fargues, 1977). Ekesi *et al* (2000) found that although mortality at the larval stage was reduced, adult thrips surviving infection as larvae produced fewer numbers of eggs and showed reduced egg hatchability and longevity than untreated insects. The host body temperature is an important factor in the efficacy of the fungus (Blanford and Thomas, 2000). Nutrition influenced growth, sporulation and virulence of the insect pathogenic fungus, *M. anisopliae*. Virulent conidia were produced on susceptible insect hosts, 1% yeast extract, 2% peptone, osmotic stress medium (OSM) and CN 10:1 medium (Shah *et al*., 2005).

The effectiveness of the entomopathogenic fungi is highly variable with environmental temperature and host thermoregulatory behaviour critically determining the pattern and extent of mortality after applications (Klass *et al*., 2007a). Ekesi *et al*., (2000) studied the effect of temperature on the virulence of *M. anisopliae* and *B. bassiana* isolates on *M. sjostedti* in the laboratory. Isolate ICIPE69 was found to have a broad temperature range of pathogenic activity against the pest. The study showed that the fungus was most virulent to *M. sjostedti* at temperatures between 25 and 30ºC. Soil temperature and moisture are among the most important factors influencing the response of host insect to entomopathogenic fungal infection (McCoy *et al*., 1992). Survival of conidia in the soil is not much affected because this environment provides shelter from environmental extremes, therefore, increasing their persistence and recycling (Ekesi *et al*., 2003).

Relative humidity is crucial for microbial biocontrol agents to germinate, spread, and infect their insect hosts. For example, lower humidity usually inhibits the ability of *M. anisopliae* to control the rice green leafhopper, *Nephotettix virescens* (Tseng *et al*., 2011). The findings of Fargues and Luz (1998) highlight that fungal entomopathogens may require a period of rehydration under high humidity prior to the initiation of sporulation, and this requirement may prolong interruptions in sporulation under fluctuating moisture conditions.

More recent studies have shown that *M. anisopliae* conidia exposed to the full spectrum of solar UV radiation for 4 h (weighted dosage, equivalent to ca. 7 to 9 kJ m^{-2}) have reduced relative cultivability by approximately 30% for strain ARSEF 324 and 100% for strains ARSEF 23 and 2575 (Braga *et al*., 2001a). UV- A exposure also exerts negative effects on the relative cultivability of conidia and conidial germination, but it is not as pronounced as exposure to the full-spectrum solar radiation (Braga *et al*., 2001a; Braga *et al*., 2001b). Studies on UV-B tolerance suggest that with increasing exposure time, the rate of conidial germination declines (Mustafa and Kaur, 2009). The desert locust and other insects could offset, retard, or eradicate conidial germination and infection of entomopathogens through basking or fever behaviours (Butt *et al*., 2001).

2.4.5 Modeling the efficacy of EPF in relation to temperature

Non-linear models are important in understanding and predicting fungal development at fluctuating temperatures (Xu, 1996). The Sharpe and DeMichele (1977) model of poikilotherm development has been used to analyze the effect of temperature on the mortality rate of two spotted spider mites, *Tetranychus urticae*, infected with the entomophthoralean fungus *Neozygites floridana* (Smitley *et al*.1986). Using the Sharpe and DeMichele (1977)

model, Davidson *et al* (2003) identified that thermal requirements of the isolates examined against *Varroa destructor* are well matched to the temperatures in the broodless areas of honey bee colonies, and a proportion of isolates. The model could be utilized in the selection of isolates for microbial control prior to screening for infectivity and could help in predicting the activity of a fungal control agent of *Varroa destructor* under fluctuating temperature conditions (Davidson *et al*., 2003). The non-linear model proposed by Lactin *et al*.(1995) could provide a useful tool to assist in interpreting effectiveness of control operations, develop improved application strategies to optimize the performance of the bio-pesticide and identify appropriate target species and environments (Klass *et al*., 2007a,b). Lactin model is a useful tool, and currently is being used to explore and evaluate the performance of a fungal pathogen being used in several locust and grasshopper biocontrol programs throughout the world. Lactin model has a role in interpreting patterns of mortality after application, provision of real-time guidance as to when to expect substantial levels of mortality to locust control officers, and for prospective analysis to identify candidate targets for biocontrol and optimize use strategies (Klass *et al*., 2007a, b)

CHAPTER THREE

MATERIALS AND METHODS

This first part of the study aimed to understand the effects of temperature on spore germination, radial growth, and sporulation of *Metarhizium anisopliae* isolate, ICIPE69, henceforth called as ICIPE69. In the second series of experiments, influence of temperature on the efficacy of ICIPE69 against western flower thrips was assessed. Based on the outcome of the laboratory efficacy trials, temperature based models for the pathogenicity of *M. anisopliae* ICIPE69 on *Frankliniella occidentalis* were developed and run on a geospatial temperature data layer to develop efficacy maps. The third set of experiments were a series of field studies conducted to evaluate the efficacy of ICIPE69 on WFT infesting French beans at different agro ecologies. This chapter outlines the general and specific methods adopted for the various experiments and analysis outlined above.

3.1 General methods

3.1.1 Insect rearing

A clean colony of *F. occidentalis* was obtained from the Animal Rearing and Containment Unit, ICIPE where colonies of different thrips species are maintained. The stock culture was established in laboratory with 20 adult females introduced in plastic vials containing French beans (*Phaseolus vulgaris* L.) pods var Samantha. Female thrips were allowed to oviposit for 2 days, after which the pods were removed and transferred to another vial. First instars emerged on the surface of the pods within 2-3 days. Larvae were reared on the pods until they were about to pupate. Adults emerged within 4-5 days. Three day - old adults were used in the experiment. Thrips cultures were maintained in a controlled room at 25ºC±2, 60 - 80%

relative humidity and a photoperiod of 12/12 h light/darkness as indicated by Goettel and Inglis (1997).

3.1.2 Culturing of *Metarhizium anisopliae* isolate ICIPE69

Metarhizium anisopliae isolate ICIPE69 was obtained from ICIPE's Arthropod Germplasm Centre. Sabouraud Dextrose Agar (SDA) medium was prepared by mixing 65 grams of the commercial agar media with 1 litre of distilled water. The agar media was suspended in the water by stirring and then autoclaved at 121ºC for 20 minutes and allowed to cool to 50ºC. The SDA medium was then dispensed into sterile Petri dishes in the laminar flow hood (Sterilgard class II, type A/B 3, Sunford, Germany) and allowed to solidify. Using sterile wire loop, spores were transferred from the agar slants onto the SDA plates.

3.1.3 Spore suspension preparation

The fungal isolate was cultured on SDA media in 9 cm Petri dishes and incubated at 25±2ºC in complete darkness for 21 days after which conidial suspension was harvested by flooding culture surface with sterile distilled water and scraping with a sterile wire loop. The conidia were suspended in 10 ml sterile distilled water containing 0.05% Triton X-100 in universal bottles containing 3 mm glass beads. The conidial suspension was vortexed for 15 minutes to produce a homogenous suspension by breaking spore chains. Spore concentration was determined by counting the spores using a Neubauer haemocytometer under a compound microscope (Laborlux K, Leitz, Sunford, Germany) at 40×. The final working concentration was obtained using the formula by (Goettel and Inglis, 1997) N/V x D where N= number of conidia, V= volume of the chamber (constant of 2.5×10^5), D= dilution factor.

3.1.4 Mass production of *Metarhizium anisopliae*

Metarhizium anisopliae isolate ICIPE69 inoculum for the field experiments was produced on long white rice substrate (Maniania *et al*., 2003a). Two kilograms of rice were weighed, washed thoroughly three to four times. The rice was pre-cooked by soaking it in 90 - 100ºC water for 15 minutes. The pre cooked rice was placed into polythene bags and autoclaved for 60 minutes at 121ºC. This was cooled to about 40 - 45 ºC. Blastospores were produced in shake flasks by liquid fermentation followed by surface conidiation on solid substrate. The aerated rice bags were inoculate with a 3-day old culture of blastospores (50ml) by making a small opening on one corner of the bag using sterile scissor, sealed and incubated at room temperature for 12 hours. After 12 hours, the bags were shaken manually and incubated again for one week. A week later, the bags were slightly shaken manually to enhance aeration and incubated for another two weeks. The substrate with the fungus was transferred into plastic basins and allowed to dry for 5 days at room temperature before the conidia were harvested by sieving through a 295 µm mesh size. The conidia were stored in sealed paper bags in the refrigerator at 4 ºC for use in the field as inoculum.

3.2 Effect of temperature on spore germination, radial growth and sporulation of ICIPE69

3.2.1 Spore germination

Conidial suspension (0.1 ml) containing 3 X 10^6 conidia ml^{-1} of *M. anisopliae* was spread on SDA plates. Three sterile microscope cover slips were placed on the surface of the agar media in each plate. Then inoculated plates were sealed with Parafilm and incubated at 10, 15, 20, 25, 30, 35 and 40º C in complete darkness. Four plates were used for each temperature and the

experiment was laid out as complete randomized design. At 24 hours post inoculation, 1 ml of 0.5% lacto phenol blue was dropped into each plate to halt spore germination. Germination of conidia was observed using a compound microscope (Laborlux K, Leitz) at 40 × magnification and the number of germinated and un-germinated conidia was determined by counting every 100 conidia from each cover slip using a tally counter. Percentage spore germination was then determined by dividing the number of germinated spores with the total number of spores counted in a specified field and multiplied 100.

3.2.2 Radial growth and sporulation

0.1 ml conidial suspension of 1 x 10^7 spores ml^{-1} were spread plate on SDA plates. The plates were then sealed with Parafilm and incubated at 26±1°C for 72 hours in complete darkness to obtain mycelial growth. Mycelial plugs were cut from the culture plates using 8 mm diameter cork borer and each agar plug was singly transferred onto the centre of a freshly prepared SDA plate. The plates were then sealed with Parafilm and incubated for 11 days at 10, 15, 20, 25, 30 35 and 40°C in complete darkness. Five plates were prepared for each temperature and complete randomized design was used for the experiment and the experiment was repeated three times to ensure consistency of the results. The diameter of the resulting colonies was measured with a ruler at four cardinal points from the plug daily for 11 days on a pre-marked line. After completion of data cultures were kept in the cold room until when required.

Spores from the plates incubated at 10, 15, 20, 25, 30 35 and 40°C were harvested in sterile distilled water by scraping the surface of the cultures with a sterile camel hair brush into a 500 ml glass beaker containing 50 ml sterile distilled water containing a drop of Tween 80 for the

sporulation experiment. The conidial suspension was uniformly mixed using a magnetic stirrer. The concentration of conidia in the suspension harvested from each plate was determined using a Neubauer haemocytometer as described earlier in section 3.1.2.

3.2.3 Effect of temperature on the pathogenicity of *Metarhizium anisopliae* to adults and second instars of *Frankliniella occidentalis*

The concentrations of spore suspension were adjusted to 1×10^6, 1×10^7, and 1×10^8 conidia ml^{-1} and 10 ml of each of the three concentrations were sprayed on pods of French beans using a Burgerjon spray tower (Burgerjon, 1956). Pods were allowed to dry for five minutes after which they were transferred into 9 cm Petri dishes. For the control treatment the pods were sprayed with sterile distilled water containing 0.05% Triton X-100. Adult WFT were immobilized by chilling them for 25 seconds, twenty adults of WFT were introduced gently into each Petri dish for the first experiment. In the second experiment, 20 second instars of WFT were gently introduced into each Petri dish and were incubated for ten days in the incubator (Heraeus BB6220 C U Co_2) at fixed temperature of 10, 15, 20, 25, 30, 35 and 40ºC. Each spore concentration; 10^6, 10^7 and 10^8 and temperature level treatment was replicated four times for both the adults and second instar experiments in a completely randomized design. The number of dead insects in both the adults and second instar WFT experiments was recorded daily for ten days. The dead insects were transferred in Petri dishes lined with a moist filter paper for 14 days to confirm infectivity with the fungus by microscopic examination of spores on the surface of the insects. Mortality data of the adult thrips over days at a dose of 1×10^8 for each temperature were subjected to probit analysis (logit transformation) (R Development Core Team, 2010) to generate lethal time (LT) response for achieving 50 and 90% mortality.

3.3 Development of temperature based model for the pathogenicity of *Metarhizium anisopliae* ICIPE69 on *Frankliniella occidentalis*

Relationship between temperature and mean proportional mortality of adult thrips across different fungal spore concentrations were obtained by fitting non-linear regression models such as Cubic model and Lactin 1 model (Klass *et al*., 2007a). The cubic model is represented by the equation as outlined below

$$Y = b0 + b1*T + b2*T\text{^}2 + b3*T\text{^}3$$

Where Y represents the mean predicted mortality of adult thrips in relation to temperature T. While b0, b1, b2, b3 are parameter constants.

Similarly the Lactin 1 model is represented by the equation as outlined below

$$Y = e^{\rho T} - e^{\left(\rho.T_{\max} - \left(\frac{T_{\max} - T}{\Delta}\right)\right)}$$

Where Y represents the mean predicted mortality of adult thrips in relation to temperature T. ρ is the rate of increase at optimal temperature, Δ is the difference between optimal and upper temperature threshold. T_{max} is the upper temperature threshold constant.

The best fitting parameters for both the Cubic model (b0, b1, b2 and b3) and Lactin 1 model (ρ, Δ, T_{max}) were estimated using the library (relimp) procedure which facilitates inference on the relative importance of predictors in a regression model (Turner and Firth, 2011). The Akaike information criterion (AIC) for both the Cubic and the Lactin 1 models were also estimated for model comparison.

3.3.1 Spatial simulation and mapping of the fungus efficacy

The temperature data used for spatial simulations are available at 4 different spatial resolutions; from 30 seconds (0.93 x 0.93 = 0.86 km^2 at the equator) to 2.5, 5 and 10 minutes (18.6 x 18.6 = 344 km^2 at the equator. The temperature data was obtained from WorldClim available at http://www.worldclim.org/ at a resolution of 10 minutes spatial resolution. Simulations for predicting the Y (mortality rate) for each grid were based on (daily) minimum and maximum temperatures and used a 15 minutes time step length for considering the within-day temperature variability as described by Kroschel *et al*. (2013). For the simulated Y (mortality rate) values for each grid a new matrix (Longitude = column and Latitude = row) with dimensions similar to the temperature simulations at 10 min spatial resolution was created, which was thereafter converted to American Standard Code for Information Interchange (ASCII) files; this refers to a "text" file that is readable by the naked eye (it only contains the letters a-z, numbers, carriage returns, and punctuation marks) and transferred to Q GIS (version 1.8.0) for mapping the estimated Y (mortality rate) values as an indicator for efficacy of the fungus.

3.4 Efficacy of *Metarhizium anisopliae* isolate ICIPE69 on western flower thrips of French beans in different agro ecological zones

3.4.1 The Study sites

On-farm field experiments were carried out between February to August 2012 at low, mid and high altitude areas in Kibwezi, Mwea and Naivasha, respectively. The experimental site at Kibwezi (2º 25'S: 37º 58'E) was located at an altitude of 913m above sea level (msl) with an average annual rainfall of 345 mm and mean temperature of 32 ºC. In Mwea (0º 37'S: 37º

20’E), the site was located at an altitude of 1159 msl with an average annual rainfall of 1159 mm and mean temperature of 27 ºC. In Naivasha (0º 43’S: 36º 25’E), the site was located at an altitude of 2085m above sea level with an average annual rainfall of 2000 mm and temperature of 18 ºC (Jaetzold, *et al*., 2006). During the first crop season, the mean temperature and relative humidity at Mwea, Naivasha and Kibwezi was (22.4; 59.5%), (22.8; 52.4%) and (25.5; 36.4%) respectively while, during the second crop season; the mean temperature and relative humidity at Mwea, Naivasha and Kibwezi was (20.57ºC; 70.5%), (20.1 ºC; 67.3%) and (22.67 ºC; 57.6) respectively.

3.4.2 Experimental design and layout

Two French beans varieties Tana and Serengeti were sown in 3 x 3 m flat bed plots at a spacing of 30 x 15 cm with 1 m alleys between plots. At sowing, diammonium phosphate (DAP; 18% nitrogen and 46% phosphate) fertilizer was applied at the rate of 200 kg/ha and was mixed well with the soil before planting while calcium ammonium nitrate (CAN; 26% nitrogen) was applied at the rate of 200 kg/ha three weeks after crop emergence or at first trifoliate leaf stage. The crop was irrigated twice per week in Kibwezi and once per week in Mwea and Naivasha. Weeding was done as the weeds appeared. Spores of *M. anisopliae* were weighed in the laboratory then stored at 4 ºC in a mobile refrigerator that was carried to the field. For spray applications, 0.1 g conidia of *M. anisopliae* were weighed in the laboratory and the spore concentrations of 1×10^{12} and 1×10^{13} calculated. From these spore concentrations; it was possible to calculate the amount of spores to be weighed to give two spore concentrations to be used in the field. The weighed conidia for the specific spore concentrations were suspended in water containing 0.05% Triton and 30 ml of corn oil as a protectant for every litre of formulation (Maniania, 1993). The concentration of inoculums in

the formulation was determined with a Neuber haemocytometer. The fungus was applied at two rates of 1 x 10^{12} and 1 x 10^{13} conidia/ml and Imidacloprid at the recommended rate of 0.125 g/L active ingredient. The fungus and Imidacloprid were applied after every week for four weeks with a different CP 15 s knapsack sprayer (Cooper Pegler and Co. Ltd, Sussex, England) at an output of 350 L/ha. The control treatment was sprayed with water containing 0.05% Triton, corn oil. Spray applications were performed in the evenings between 17:00 h and 18:30h to lessen the adverse effects of ultraviolet radiation (Moore and Prior, 1993). Each of the treatments was replicated four times and arranged in a split plot arrangement. The French Tana and Serengeti varieties constituted the main plots while the *M. anisopliae* spore concentration/chemical constituted the sub-plots. Data collected included thrips population, thrips damage on pods and pod yield.

3.4.3 Assessment of thrips population, damage and yield

Thrips population was determined by destructive and non-destructive sampling of flowers (Steiner, 1990) once per week for a period of four weeks. Before flowering non-destructive sampling which involved tapping of the whole plant on a white enamel tray and counting the number of thrips. Ten plants were sampled per plot. At flowering destructive sampling was adopted and three flowers per plant were picked from the top, middle and bottom of the plant canopy. The flowers were then dipped in vials containing 70% alcohol. The thrips were extracted from the flowers and identified using a dissecting microscope. Both sampling methods were performed concurrently from the flowering phase of the crop. Pod damage by thrips was also assessed from samples of ten pods per plot harvested weekly after their formation. The damage was rated on a scale of 1-5 where 1 = no damage, 2 = 1-25%, 3 = 26-

50%, 4 = 51-75% and 5= over 76% damage (Nderitu *et al*., 2007). The crop was harvested three times during each cropping season. Pod yield was determined by weighing the marketable beans and each harvest. The weight of marketable beans at each harvest was summed up to estimate the yield per plot. The yield of the French beans was extrapolated into kilograms per hectare as follows: Y= (W x 10,000) ÷ A, where, Y is the yield in Kg per hectare; W is the total weight in kg of harvested French beans and A is the plot size in m^2. The damage and yield data was taken during harvest.

3.5 Data analysis

Percentage spore germination values were normalized through angular transformation; (Little and Hills 1978) y= arcsine √ p where p is the proportion and y is the result of the transformation. The transformed values were subjected to Analysis of Variance (ANOVA) procedure using the R software and means were separated by Student- Newman-Keuls (SNK) test at 5 % probability. Radial growth and sporulation mean values were subjected to ANOVA procedure of R for a complete randomized design and means were separated by SNK test at 5 % probability (R Development Core Team, 2010). The mean values for the cumulative insect mortality data of both the adults and second larvae were subjected to ANOVA procedure of R and means separated by SNK test at 5 %. The mean mortality values for the adult thrips at different temperatures over dose of fungus treatment were used to parameterize the Lactin 1 and cubic models. For field experiment data were analyzed as split plot arrangement where the French beans varieties were in the main plot and the three treatments as the sub plots (R Development Core Team, 2010).

CHAPTER FOUR

RESULTS

Results presented below include the outcomes of laboratory test on spore germination, radial growth, and sporulation of *M. anisopliae* at different temperatures; mortality of the adult and second instars of western flower thrips exposed to different temperatures and different concentrations of ICIPE69, modelling of ICIPE69's pathogenicity and mapping the potential efficacy of the fungus and the evaluation of field efficacy of ICIPE69 on French beans for thrips control.

4.1.1 Effect of temperature on spore germination

Spores of *Metarhizium anisopliae* germinated at 15, 20, 25, 30 and 35ºC except at 10 and 40ºC after 24 hours of incubation (Table 1). Germination increased significantly with increasing temperatures from 15 to 30ºC, but started to decline thereafter. Spore germination was highest at 25ºC (100%) and the lowest at 15ºC (4.2%) (Table 1). Spore germination was significantly higher at 25 and 30ºC compared to both lower and higher temperatures. At 35ºC the spore germination declined sharply to 7.3% ($F_{6, 56} = 2883.8$; $P < .001$) (Table 1).

4.1.2 Effect of temperature on radial growth of *Metarhizium anisopliae*

Radial growth of the fungus occurred at 15, 20, 25, 30 and 35 ºC but no growth occurred at 10 and 40ºC (Table 1). Radial growth increased significantly with temperatures from 15 to 30ºC, thereafter it declined. After 11 days of incubation, the highest radial growth was (4.3 mm) at 25ºC and the lowest (1.2 mm) at 35ºC (Table 1). Radial growth was significantly higher at

optimal temperature range of 25 and 30ºC as compared to both lower and higher temperatures ($F_{6, 56} = 2569.8$; $P < .001$) (Table 1).

4.1.3 Effect of temperature on sporulation of *Metarhizium anisopliae*

The fungus sporulated at 15, 20, 25, 30 and 35ºC except at 10 and 40ºC (Table 1). The highest sporulation of 100 x 10^8 conidia/ml occurred at 25ºC, while the lowest sporulation 10 x 10^8 conidia/ml occurred at 15ºC (Table 1). Significant differences were observed in the sporulation of the fungus at different temperatures, 25 and 30ºC proved to be the optimum temperature range with significantly higher sporulation than other temperature ranges ($F_{6, 56} = 48.14$; $P < .001$) (Table 1). Temperature significantly ($p< 0.001$) affected the sporulation; 25 and 30ºC was observed to be the optimum range with higher sporulation than the other temperatures.

Table 1: Effect of temperature on spore germination, radial growth and sporulation of *Metarhizium anisopliae* ICIPE69

Growth parameters	Temperature(ºC)							LSD	CV%
	10	15	20	25	30	35	40		
Spore germination (%)	0.0±0.0e	4.2±1.2d	70.0±1.2b	100.0±0.0a	98.0±1.5a	7.3±2.2c	0.0±0.0e	2.5	6.6
Radial growth (mm)	0.0±0.0e	1.2±0.1d	2.8±0.2b	4.3±0.1a	4.1±0.2a	1.8±0.1c	0.0±0.0e	0.1	3.8
Conidia yield $x10^8$ conidia ml^{-1}	0.0±0.0d	10.0±0.01c	30.0±0.01b	100.0±18.0a	100.0±17.0a	10.0±0.0c	0.0±0.0d	3.7	31

Means within rows followed by the same letter do not differ significantly by Student- Newman- Keul's test ($P<0.05$)

4.2 Effect of temperature on the pathogenicity of *M. anisopliae* on western flower thrips

4.2.1 Second instar

Mortality in the controls was 5% at 20 ºC, 10% at 25 and 30 ºC, and 65% at 40ºC. Higher temperature was detrimental to the insects. No mortality was recorded at 10 and 40 ºC. However, mortality increased significantly with increased spore concentration across the different temperature regimes ($F_{6, 42}$ = 92.05; P<0.001) (Table 2). Mortality was significantly high ($F_{6, 42}$ = 84.87; P<0.001) at 25 and 30ºC as compared to 20 and 35 ºC (Table 2). 36.67%, 50% and 66.7% were recorded with spore concentrations of 10^6 ($F_{6, 42}$= 115.7; P<0.0001), 10^7 ($F_{6, 42}$ = 12.2; P<0.001) and 10^8 ($F_{6, 42}$ = 59.8; P<0.001), respectively.

4.2.2 Adult thrips

Mortality in the controls was. Higher temperature was detrimental to the insects. Mortality of the adults was higher than of the second instars (Table 3). Mortality of the adults increased significantly with increased spore concentration across the different temperature regimes ($F_{6, 42}$ = 824.18; P<0.001) (Table 3). In all the three concentrations tested, the mortality of adults increased with temperature from 10ºC up to an optimum temperature range of 25 - 30ºC, where the mortalities were significantly higher than the other temperatures tested. Thereafter, the mortality decreased at 35ºC with no mortality at 40ºC ($F_{6, 42}$ = 463.16; P<0.001). At optimal temperatures of 25 and 30 ºC, 35%, 80% and 100% were recorded with spore concentrations of 10^6 ($F_{6,14}$= 41.67; P<0.001), 10^7 ($F_{6, 14}$ = 114.79; P<0.001) and 10^8 ($F_{6,14}$ = 1949.2; P<0.001), respectively (Table 3). The median lethal time to cause 50 and 90% mortalities decreased as temperatures increased from 15 to 25ºC, beyond which the time increased until 35ºC. However, there was no significant difference in the lethal time to cause 50 and 90% mortality at 25 and 30ºC (Table 4). At 25ºC, the shortest LT_{50} and LT_{90} values of 1.9 and 3.3 days, respectively (Table 4).

Table 2: Percentage mortality percentage of second instars of *F. occidentalis* at different temperatures and spore concentrations of *M. anisopliae*

Spore concentration (conidia/ml)	Temperature (ºC)							LSD	CV%
	10	15	20	25	30	35	40		
Control	0±0d	0±0d	0±0d	5.0±0.2c	10.0±1.1b	10.0±1.1b	65a	2.1	14.1
10^6	0±0d	11.7±1.7c	16.7±1.7b	33.3±1.6a	36.7±1.7a	20.7±1.7b	0d	4.6	11.3
10^7	0±0d	21.7±1.7c	36.7±9.3b	50.0±2.9a	50.0±2.9a	35.0±2.9b	0d	13.9	23.1
10^8	0±0d	33.3±3.3c	50.0±2.9b	66.7±1.6a	66.7±1.6a	50.0±2.8b	0d	7.5	8.9
LSD	0	8.2	4.7	2.7	3.8	5	15		
CV %	0	15	23.5	12.3	17.2	7.5	0		

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

Table 3: Percentage mortality of adult *F. occicdentalis* at different temperatures and different spore concentrations of *M. anisopliae*

Concentration (conidia/ml)	Temperature (°C)							LSD	CV%
	10	15	20	25	30	35	40		
Control	0±0d	5.0±1.1c	5.0±1.1c	5.0±1.1c	5.0±0.9c	10.0±0.9b	100a	3.5	13.1
10^6	8.0±1.6e	15.0±0d	21.7±1.6c	28.3±1.7b	35.0±2.9a	15.0±0d	0±0f	4.6	15.2
10^7	15.0±0d	25.0±2.8c	60.7±1.6b	73.3±1.7a	80.0±0a	53.3±1.7b	0±0e	11.6	5.6
10^8	28.3±1.6c	71.6±1.7b	100.0±0a	100.0±0a	100.0±0a	68.3±1.6b	0±0d	3.3	2.8
LSD	5.6	24.7	18.7	22.5	18.3	13	15		
CV %	13.7	6.5	5.2	11.6	4	6.4	0		

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

Table 4: Median lethal time of 50% and 90% mortality of the adult thrips at $1x10^8$ conidia/ml at different temperatures

Temperature (ºC)	LT_{50} (days) (95% CI)*	LT_{90} (days) (95% CI)*
10	8.1 (7.4 - 9.5)	11.0 (9.7 - 14)
15	4.8 (4.5 - 5.1)	7.9 (7.9 – 8.8)
20	2.8 (2.6 – 2.9)	4.2 (3.8 - 4.4)
25	1.9 (1.7 – 2.2)	3.3 (3.1 – 3.8)
30	2.2 (1.9 – 2.5)	4.5 (2.5 – 4.9)
35	3.4 (2.8 – 3.9)	8.5 (7.5 – 10.2)

4.3 Estimation of pathogenicity of *M. anisopliae* ICIPE69 on western flower thrips using both cubic and Lactin 1 model.

The relationship between temperature and mean mortality proportions of adult thrips across different doses of the test fungus 10^6, 10^7 and 10^8 was modeled using non-linear regression models namely cubic and Lactin 1. The best fitting cubic model (Figure 1) and the Lactin 1 model (Figure 2) and parameter estimates for both the models are presented in Table 5 and Table 6, respectively.

Both cubic and Lactin 1 model predicted 25 – 30ºC to be the optimum temperature for maximum efficacy of the fungus (Figure 1 and 2). Beyond the optimum temperature range of 25 – 30ºC both the models predicted a sharp decline in the efficacy of the fungus and at the upper threshold of 39 - 40ºC, the fungus was predicted not to be effective. The models differed in the prediction of lower thresholds for fungus efficacy. The cubic model predicted a

sharp decline in efficacy with a lower threshold close to 5ºC. However the Lactin 1 model predicted a gradual decline in efficacy which was asymptotic in nature to the temperature axis (Figure 1 and 2).

The Akaike Information Criterion estimates for both the Cubic Model and Lactin 1 model were -64.36 and -32.95, respectively (Table 5 and Table 6).

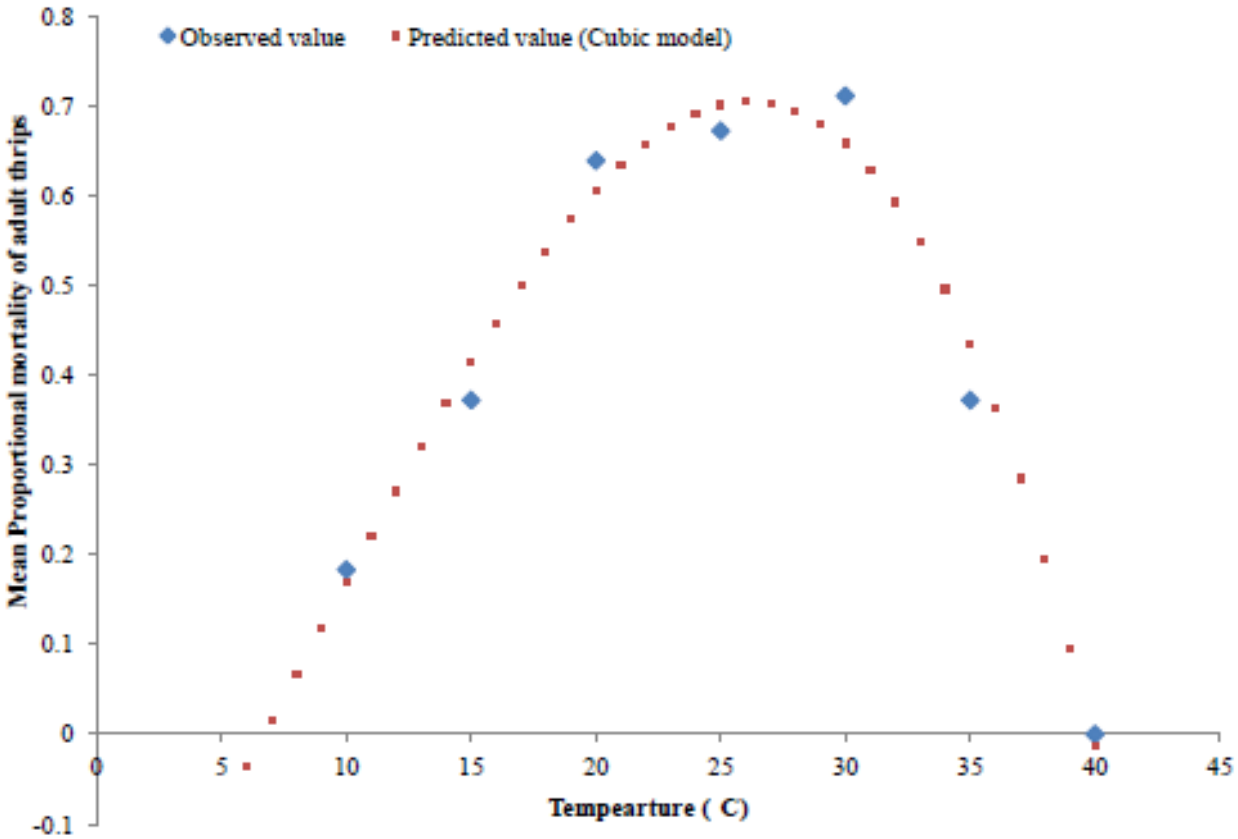

Figure 1: Observed and Predicted mortality of adult thrips across different concentrations of the fungus in relation to temperature using Cubic Model

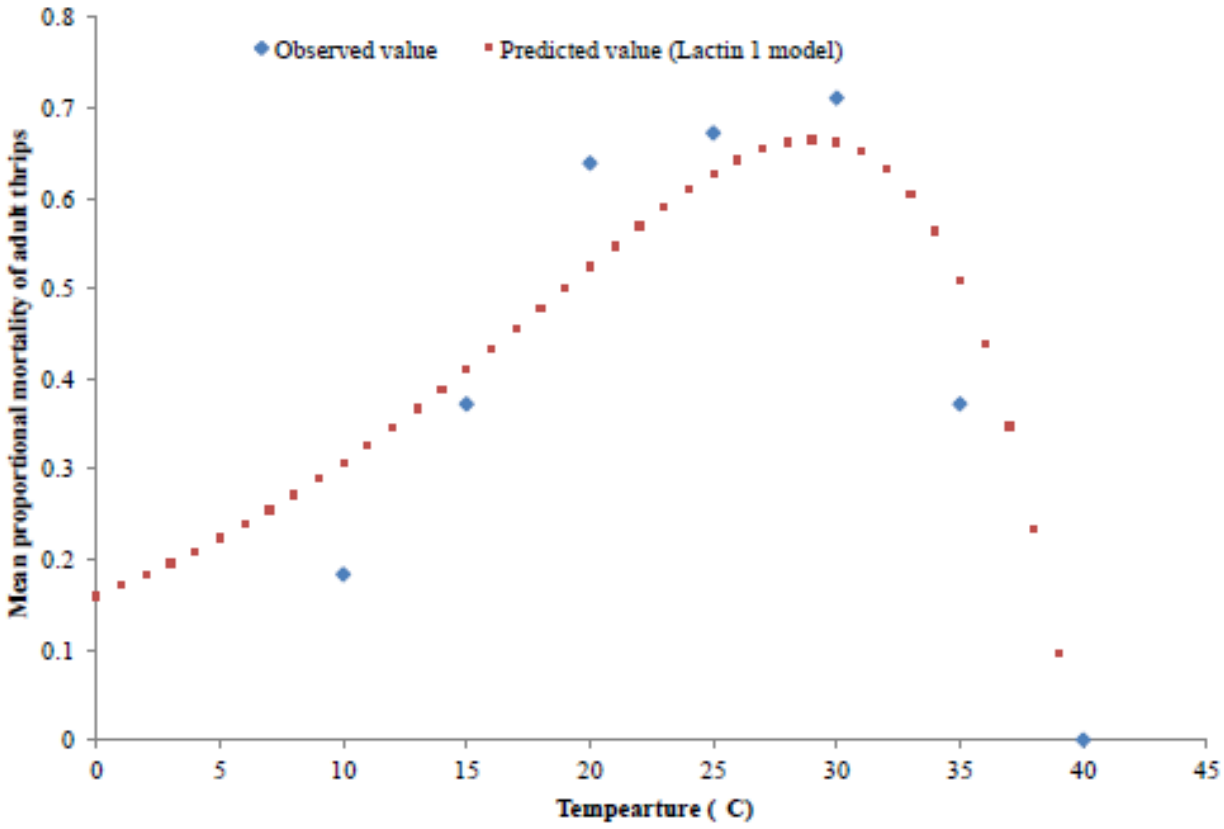

Figure 2: Observed and Predicted mortality of adult thrips across different concentrations of the fungus in relation to temperature using Lactin 1 Model

Table 5: Parameter estimates for the best fitting Cubic model for predicting the mortality of adult thrips across different temperatures

Parameters	Estimates	P (≥t)
B0	-0.3099 ± 0.18	0.09439
B1	0.03867 ± 0.03	0.14266
B2	0.001487 ± 0.0011	0.18861
B3	-5.679e-05 ± 1.44e-05	0.00104
Akaike Information Criterion	-64.36	

B0, B1, B2 and B3 are parameter constants

Table 6: Parameter estimates for the best fitting Lactin 1 model for predicting the mortality of adult thrips across different temperatures

Parameters	Estimates	P (≥t)
ρ	0.08999 ± 0.00686	1.19e-10
T_{max}	39.74671 ± 0.31602	< 2e-16
Δ	10.57151 ± 0.67886	6.88e-12
Akaike Information Criterion	-32.95	

ρ is the rate of increase at optimal temperature, T_{max} is the upper temperature threshold and Δ is the difference between optimal and T_{max}

4.4 Spatial simulation and mapping of the fungus efficacy

Spatial simulation and mapping of the fungus efficacy revealed that both the Cubic and the Lactin 1 model predicted higher efficacy of the fungus in the tropics as compared to the temperate climates (Figure 1 and Figure 2). The fungus was predicted to be ineffective in the Tropical deserts of North Africa and Asia and in the temperate zones during the cold season. In East Africa, the fungus was predicted to be effective with higher mortality of adult thrips in most of the mid and low altitude zones, while they might not be effective in the high altitude regions in the Rift Valley, Mt. Kenya in Kenya and Mt. Kilimanjaro in Tanzania (Figure 1 and Figure 2).

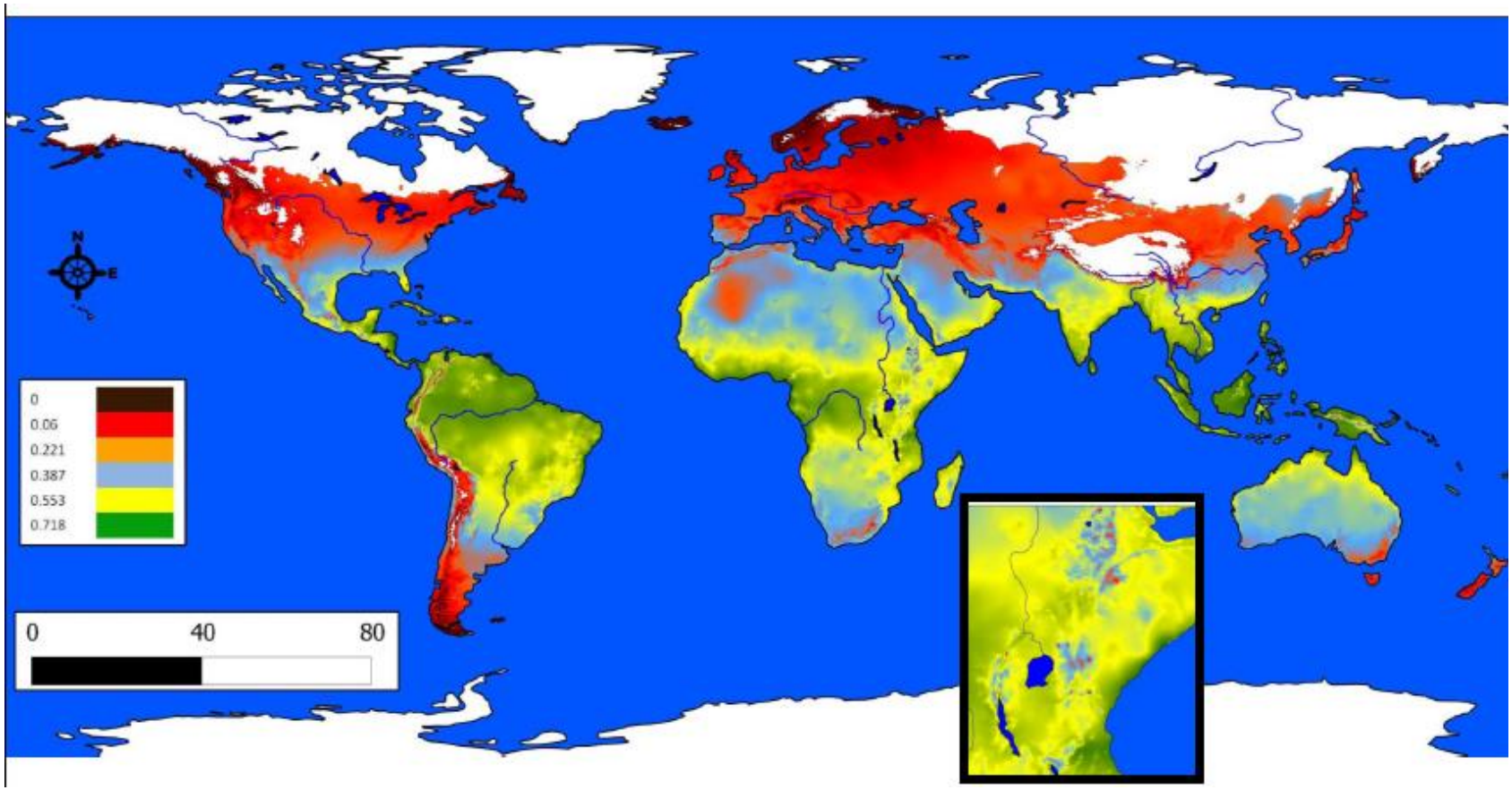

Figure 3: Global map predicting the efficacy of entomopathogenic fungus ICIPE69 against adult Western Flower thrips using the geospatial temperature data layer and the best fitting Cubic Model. (**Inset**) Predictions for efficacy of ICIPE69 in East Africa.

Legend: 0 – 0% mortality, 0.06- 6% mortality, 0.221- 22.1% mortality, 0.387- 38.7% mortality, 0.553- 55.3% mortality, 0.718- 71.8% mortality

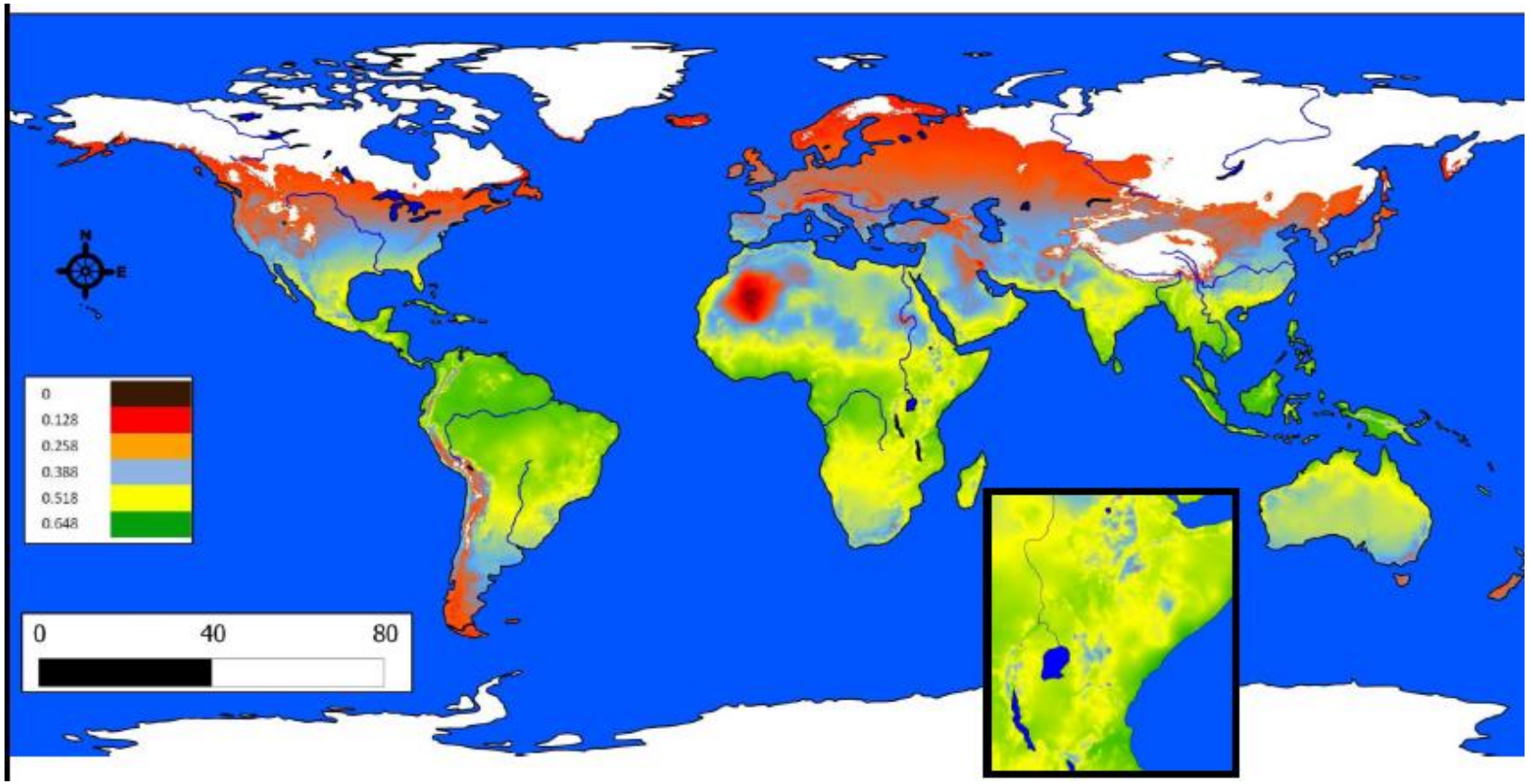

Figure 4: Global map predicting the efficacy of entomopathogenic fungus ICIPE69 against adult Western Flower thrips using the geospatial temperature data layer and the best fitting Cubic Model. (Inset) Predictions for efficacy of ICIPE69 in East Africa. Legend: 0 – 0% mortality, 0.06- 6% mortality, 0.221- 22.1% mortality, 0.387- 38.7% mortality, 0.553- 55.3% mortality, 0.718- 71.8% mortality

4.5 Effect of *Metarhizium anisopliae* isolate ICIPE69 on western flower thrips on French beans in different agro – ecological zones (Kibwezi, Mwea and Naivasha)

4.5.1 Density of western flower thrips

4.5.1.1 Kibwezi

In both seasons and the cultivars, there was a significant difference in the population of thrips among the treatments, with the highest dose (10^{13}) of fungus as effective as Imidacloprid followed by the second dose of the fungus. A high significant difference in the thrips population between the two seasons ($_{F\ 1,\ 192}$ = 125.85; P < 0.001) was observed with season one recording more thrips population than season two. Compared to seasons the thrips population also varied over weeks ($_{F\ 3,\ 192}$ = 53.00; P < 0.001) during the crop growth (Table 7). The highest dose of the fungus and Imidacloprid were the best treatments ($_{F\ 3,\ 192}$ = 3.63; P < 0.05) and the variety Serengeti as the better variety ($_{F\ 1,\ 192}$ = 6.51; P < 0.05) with least density of WFT (Table 7). The interaction between season and time for the thrips population was also observed to be highly significant ($_{F\ 3,\ 192}$ = 38.96; P < 0.001). Confidor was recorded to have the lowest mean density of thrips of (5.1 and 6.4) for the first and second seasons respectively while, *M. anisopliae* 10^{12} was recorded to have the highest mean density of thrips of (9.5 and 14.4) for trhe first and second seasons respectively.

4.5.1.2 Mwea

There was significantly higher thrips population during the first crop season (February - July 2012) than the second crop season (May - August 2012) ($_{F\ 1,\ 192}$ = 984.75; P < 0.001) (Table 8). As observed at Kibwezi, there was a significant difference in the thrips population among the treatments with the highest dose (10^{13}) of the fungus as effective as Imidacloprid in both

season and with the two varieties ($_{F\ 3,\ 192}$ = 8.22; P < 0.001). The thrips population also varied significantly over weeks ($_{F\ 3,\ 192}$ = 31.31; P < 0.001) (Table 8). Highly significant interaction for the thrips population was observed between season and weeks after treatment ($_{F\ 3,\ 192}$ =60.34; P< 0.001) and season, weeks after treatment and the treatments ($_{F\ 9,\ 192}$ = 4.8424; P < 0.001) (Table 8). Confidor was recoreded to have the lowest mean density of thrips of (19.3) for the first season, while *M. anisopliae* 10^{13} had the lowest mean density of thrips (27.4) during the second season. *M. anisopliae* 10^{12} was recorded to have the highest mean density of thrips (43 and 32.7) for the first and second seasons respectively.

4.5.1.3 Naivasha

As observed at Kibwezi and Mwea, in both the season and with the two varieties, a significant difference in thrips population was observed among the treatments with the highest dose of the fungus treatment as effective as Imidacloprid ($_{F\ 3,\ 192}$ = 282.93; P< 0.001). As observed at Mwea, there was a higher thrips population during the first crop season (February - July 2012) than the second crop season (May - August 2012) ($_{F\ 1,\ 192}$ = 21.12; P < 0.001) (Table 9). The thrips population also varied significantly over weeks ($_{F\ 3,\ 192}$ = 137.92; P < 0.001). The interaction between season x week x treatment for the thrips population was highly significant ($_{F\ 9,\ 192}$ = 13.27; P < 0.001) (Table 9). *M. anisopliae* 10^{13} was recorded to have the lowest mean density of thrips of (6.1) for the first season while, Confidor had the lowest mean density of thrips (7.2) during the second season. *M. anisopliae* 10^{12} was recorded to have the highest mean density of thrips (13.6 and 9.3) for the first and second seasons respectively

Combined analysis of all the data for the three locations, indicated that the thrips population varied significantly over location ($_{F\ 2,\ 552}$ = 551.86; P < 0.001), season ($_{F\ 1,\ 552}$ = 1117.41; P <

0.001), week ($F_{3, 552}$ = 81.87; P < 0.001) and treatment ($F_{3, 552}$ = 54.63; P < 0.001). However the two varieties; Tana and Serengeti did not differ significantly with thrips population ($F_{1, 552}$ = 3.63; P > 0.05).

The efficacy of the treatments was found to vary with location ($F_{6, 552}$ = 9.97; P < 0.001), weeks of treatment ($F_{9, 552}$ = 6.68; P < 0.001), but not with the season ($F_{3, 552}$ = 0.17; P = 0.92) and the variety ($F_{3, 552}$ = 0.33; P = 0.81). However, the efficacy of the treatments significantly differed with location and season ($F_{6, 552}$ = 9.5412; P <0.001). During the first cropping season (February - July 2012), Kibwezi had the lowest WFT population (Table 7) while Naivasha had the lowest population during the second cropping season (Table 9). Mwea had the highest thrips population for the two crop seasons (Table 8).

4.5.1.4 Percent reduction of thrips population in treatments over control

There was a high significant difference in percentage reduction of thrips population during the first crop season than during the second ($F_{1, 162}$ = 237.69; P < 0.001). High significant differences were noted in the reduction of thrips population across the three locations ($F_{2, 162}$ = 36.04; P < 0.001) (Table 10). Similarly, significant differences in reduction of thrips population were also observed between the three treatments ($F_{2, 162}$ = 5.18; P < 0.006), over time ($F_{2, 162}$ = 4.85; P < 0.009) between; location and treatment ($F_{4, 162}$ = 4.5; P =0.52692) and location and season ($F_{2, 162}$ = 6.59; P < 0.01). However, there was no significant difference between; season and treatment ($F_{2, 162}$ = 30.31; P =0.74) (Table 10). The highest percentage recorded at Naivasha (91.2 and 75.1%) for the *M. anisopliae* 1 x 10^{13} treatment while, the lowest reduction recorded at Mwea (53.2 and 24.3%) for *M. anisopliae* 1 x 10^{12} treatments during the first and second seasons respectively.

Table 7: Effect of *M. anisopliae* on population of *F. occidentalis* on two French bean varieties at Kibwezi for the two crop seasons

Variety	Treatment	Season one (February - July 2012)					Season two (May -August 2012)				
		Days after treatment					Days after treatment				
		0	7	14	21	Mean	0	7	14	21	Mean
Tana	*M.a* 10^{13}	7.3±1.2a	5.8±0.3a	6.0±0.9a	5.6±0.6a	5.8±0.1a	14.1±0.4a	11.2±0.8a	8.5±0.3a	4.5±0.2a	8.1±1.9a
	M.a 10^{12}	6.8±0.6a	10.5±0.4b	9.5±1.4b	8.6±0.7b	9.5±0.5b	15.4±0.8a	21.3±0.6b	14.1±0.4b	7.9±0.1b	14.4±3.9b
	Confidor	5.0±1.1a	5.6±0.8a	5.0±1.6a	4.8±0.6a	5.1±0.2a	21.5±0.8a	11.3±0.4a	5.7±0.1a	2.3±0.1a	6.4±2.6a
	Control	8.5±0.1a	17.0±1.1c	28.0±1.3c	35.5±0.1c	26.8±5.4c	15.4±0.9a	25.8±1.1c	36.2±0.2c	48.6±0.2c	36.9±6.6c
LSD		5.4	8.9	4.2	3.1	2.4	5.4	8.9	4.2	3.1	2.4
%CV		57.7	57.9	30.4	48.2	86.6	57.7	57.9	30.4	48.2	86.6
Serengeti	*M.a* 10^{13}	7.8±0.5a	5.3±0.7a	5.5±1.1a	5.0±0.6a	5.3±0.1a	27.8±1.3a	20.4±0.8a	10.9±0.1a	9.5±0.4a	13.6±3.4a
	M.a 10^{12}	5.5±0.2a	5.8±0.4a	8.5±0.7b	6.5±1.2b	6.9±0.8b	23.4±0.8a	22.0±1.1b	16.4±0.4b	11.2±0.8a	16.5±3.1b
	Confidor	5.0±0.8a	5.3±1.1a	6.3±0.8a	4.5±0.4a	5.4±0.5a	25.4±1.3a	16.1±0.7a	5.1±0.1a	6.1±0.1a	9.1±3.5a
	Control	5.3±0.4a	18.3±0.1b	31.0±0.2c	38.2±1.2c	29.2±5.8c	25.1±1.6a	23.1±0.9c	19.3±0.2c	37.1±0.2c	26.5±5.5c
LSD		3.3	3.1	7.1	2.5	5.3	26.1	23.1	6.6	6.6	5.3
%CV		34.6	60.1	34.8	45	72.8	30.8	33.9	25.9	25.9	72.8

M. a 10^{12} and 10^{13} = spore concentration of *M. anisopliae* at 10^{12} and 10^{13} conidia/ha, respectively. Means within a column followed by same letter do not differ significantly by Student- Newman-Keul's test ($P<0.05$). Day 0 denote pre-treatment samples

Table 8: Effect of *M. anisopliae* on population of *F. occidentalis* on two French bean varieties at Mwea for the two crop seasons

Variety	Treatment	Season one (February - July 2012)					Season two (May -August 2012)				
		Days after treatment					Days after treatment				
		0	7	14	21	Mean	0	7	14	21	Mean
Tana	*M.a* 10^{13}	68.3±1.2 a	44.3±0.8 b	31.6±0.5 a	17.3±0.7 a	31.1±7.8 a	31.8±0.5 a	24.1±1.6 a	40.9±1.1 a	12.7±0.3 a	27.4±8.1a
	M.a 10^{12}	64.4±1.3 a	51.9±1.1 c	41.6±0.3 b	37.4±1.1 b	43±4.3b	30.5±0.8 a	26.0±0.9 a	47.4±0.6 a	26.8±0.2 b	32.7±7.0a
	Confidor	60.4±0.9 a	29.3±0.7 a	19.6±2.1 a	9.1±1.3a	19.3±5.8 a	35.2±2.1 a	19.4±0.9 a	38.8±2.9 a	19.9±1.2 a	28.3±6.4a
	Control	63.8±1.1 a	59.4±0.3 c	79.9±0.8 c	82.9±0.7 c	74.1±7.4 c	38.6±1.9 a	35.1±1.4 b	65.7±3.5 b	84.3±0.7 c	45.9±9.1b
LSD		4.1	9.4	3.4	3.5	16.6	29.8	35.2	61.4	22.2	16.6
%CV		50.2	52.8	14.9	23.2	51.9	14.9	23.2	26.4	23.2	51.9
Serengeti	*M.a* 10^{13}	79.3±0.9 a	51.9±1.9 a	39.8±0.1 a	18.5±1.6 a	36.7±9.7 a	32.4±1.8 a	20.4±0.3 a	37.1±1.6 a	14.6±0.5 a	24.0±6.7a
	M.a 10^{12}	69.3±1.1 a	50.4±1.8 a	41.1±1.3 b	39.9±1.4 b	43.8±3.3 b	37.4±0.9 a	24.9±1.4 a	31.8±1.3 a	28.3±0.7 b	28.3±1.9b
	Confidor	77.8±1.2 a	48.5±0.7 a	29.2±0.7 a	17.0±0.5 a	31.6±9.1 a	39.4±2.6 a	16.7±1.0 a	27.4±0.9 a	16.4±0.6 a	20.2±3.6a
	Control	72.0±1.7 a	69.8±0.4 b	71.8±1.4 c	86.3±0.4 c	75.9±5.1 c	39.9±0.4 a	31.8±2.1 b	57.4±1.3 b	72.1±2.3 c	38.6±11.7 c
LSD		9.4	9.2	10	3.8	6.3	3.9	33.2	32.6	11.5	6.3
%CV		101.2	73.1	49.1	255.3	49	22.8	23.7	17.1	15.7	49

M. a 10^{12} and 10^{13} = spore concentration of *M. anisopliae* at 10^{12} and 10^{13} conidia/ha, respectively. Means within a column followed by same letter do not differ significantly by Student- Newman-Keul's test ($P<0.05$). Day 0 denote pre-treatment samples

Table 9: Effect of *M. anisopliae* on population of *F. occidentalis* on two French bean varieties at Naivasha for the two crop seasons

Variety	Treatment	Season one (February - July 2012)	Season two (May -August 2012)

		Days after treatment					Days after treatment				
		0	7	14	21	Mean	0	7	14	21	Mean
Tana	*M.a* 10^{13}	17.0±0.9a	8.8±0.5b	5.3±1.5a	4.3±0.8a	6.1±1.4a	2.4±0.3a	7.4±0.2a	6.1±0.6a	10.5±0.1a	8±1.3a
	M.a 10^{12}	19.3±1.2a	16.5±0.6c	12.3±2.1b	12.0±0.1b	13.6±1.5b	3.1±0.2a	9.2±0.3a	7.5±0.9b	11.3±0.5b	9.3±1.1a
	Confidor	19.5±0.1a	12.5±1.3a	5.0±0.9a	5.0±0.5a	7.5±2.5a	2.7±0.2a	7.8±0.3a	3.4±0.4a	10.4±0.2a	7.2±2.0a
	Control	24.3±0.3a	40.0±0.3d	55.0±1.2c	77.0±1.4c	57.3±10.7c	3.1±0.5a	13.2±0.6b	18.8±0.9c	22.7±0.3c	18±2.8b
LSD		7.3	8.1	3.8	3.5	5.3	6.2	7.5	1.3	6.9	5.3
%CV		153.8	71.5	270	68	110.6	44.7	18.1	36	16.7	110.6
Serengeti	*M.a* 10^{13}	22.5±0.7a	11.3±1.3a	5.0±1.5a	5.0±0.7a	7.1±2.1a	3.4±0.1a	5.6±0.3a	5.8±0.5a	8.9±0.1a	6.8±1.1a
	M.a 10^{12}	20.0±1.0a	15.3±1.1a	13.3±1.4b	13.3±0.1b	13.9±0.7b	2.7±0.2a	8.6±0.3a	7.2±0.5ab	14.3±0.1b	10±2.2ab
	Confidor	21.3±1.7a	11.5±1.4a	5.5±0.6a	5.0±0.5a	7.3±2.1a	2.3±0.1a	4.7±0.2a	4.4±0.2a	12.6±0.2a	7.2±2.7a
	Control	22.3±1.2a	41.3±1.5b	62.8±0.3c	77.5±0.3c	60.5±10.5c	2.6±0.2a	11.6±0.5a	12.3±0.6b	28.6±0.2c	17±5.5c
LSD		5.9	3.8	0.8	3.8	4.5	3.9	9.1	6.2	4.4	4.5
%CV		205.7	270	400	89	107.2	37.3	27.4	14.7	10.2	107.2

M. a 10^{12} and 10^{13} = spore concentration of *M. anisopliae* at 10^{12} and 10^{13} conidia/ha, respectively. Means within a column followed by same letter do not differ significantly by Student- Newman-Keul's test ($P<0.05$). Day 0 denote pre-treatment samples

Table 10: Percentage reduction of thrips population over control for three treatments over time at three agro ecological zones

Season	Treatment	Kibwezi				Mwea				Naivasha			
		Days after treatment				Days after treatment				Days after treatment			
		7	14	21	Mean	7	14	21	Mean	7	14	21	Mean
February - July 2012	Confidor	69.5±3.5a	70.0±8.2a	55.4±4.2a	64.9±4.7a	75.7±8.9a	61.6±4.5a	81.4±11.8a	69.6±9.1a	70.6±1.7a	91.1±0.3a	91.1±0.3a	84.3±6.8a
	M. a 10^{12}	49.7±4.6b	53.5±7.1b	50.5±1.0b	51.2±1.2b	30.4±10.7a	44.8±6.1b	54.5±16.4b	53.2±1.4b	60.9±2.6b	78.4±1.4b	78.4±1.4b	72.6±5.8b
	M. a 10^{13}	63.8±5.8a	68.9±8.2a	55.8±4.2a	62.8±3.8a	69.9±2.3a	67.0±2.9a	81.0±6.2a	72.6±4.3a	74.8±3.2a	91.2±1.0a	91.2±1.0a	85.7±5.5a
	LSD	15.2	3.2	3	5.7	25.9	9.3	15	5.7	8.2	3.2	3.2	5.7
	%CV	15.5	2.3	26.7	13.4	24.9	42.5	13	13.4	7.5	2.3	2.3	13.4
May -August 2012	Confidor	52.1±3.8a	45.8±11.3a	58.9±5.2a	52.3±3.8a	43.1±6.0a	31.4±9.7a	35.8±4.4a	36.8±3.4a	48.5±4.2a	60.1±3.2a	72.3±3.8a	60.3±6.9a
	M. a 10^{12}	34.0±9.9b	22.9±8.2b	38.5±9.4b	31.8±4.6b	22.8±7.1c	25.3±8.5b	24.7±0.8b	24.3±0.8b	34.7±5.5c	29.8±3.7b	52.3±6.3b	38.9±6.8b
	M. a10^{13}	31.9±2.6b	48.8±5.4a	53.3±6.0a	44.7±6.5a	32.8±8.9b	31.6±8.2a	41.5±3.1a	35.3±3.1a	56.3±4.3b	51.3±0.5a	75.1±8.2a	60.9±7.2a
	LSD	20.3	2.8	7.6	9.6	9	2.3	8.3	5.6	12.1	20.4	9	5.6
	%CV	32.3	36.3	34.4	21.1	45.2	47.3	52	21.1	20.2	25.9	10	21.1

Means within column followed by the same letter do not differ significantly by Student- Newman- Keul's test ($P<0.05$)

4.5.2 Effect of *M. anisopliae* on total pod yield of two French beans varieties in different agro-ecological zones

Among the treatments, the highest rate of fungus treatment was as effective in reducing thrips population as Imidacloprid followed by the second rate of fungus treatment ($_{F\ 2,\ 144}$ = 102.72; P < 0.001). The total pod yield was higher during the second crop season than the first crop season ($_{F\ 1,\ 144}$ = 180.39; P < 0.001) (Table 12). Naivasha had the highest total pod yield than the other two areas; Kibwezi and Mwea ($_{F\ 2,\ 144}$ = 43.51; P < 0.001) (Table 10 and11). Among the two varieties Serengeti recorded high mean yield than Tana, however the differences were not significant ($_{F\ 1,\ 144}$ = 1.95; P = 0.16). The total pod yield was also observed to be significantly different between the treatments over the three locations ($_{F\ 6,\ 144}$ = 4.13; P < 0.001), two seasons ($_{F\ 2,\ 144}$ = 23.74; P < 0.001) but not between the two varieties ($_{F\ 2,\ 144}$ = 0.48; P = 0.697) (Table 11 and 12). Confidor gave the highest marketable yield of 999.5, 1573.7 and 1462.5 kg/ha at Kibwezi, Mwea and Naivasha respectively while *M. anisopliae* 10^{12} gave the lowest marketable yield was 277.5, 370 and 202 kg/ha at Kibwezi, Mwea and Naivasha.

Significant differences in marketable yield also was observed between locations ($_{F\ 2,\ 144}$ = 28.26; P < 0.001), seasons ($_{F\ 1,\ 144}$ = 142.51; P < 0.001) and treatments ($_{F\ 3,\ 144}$ = 145.37; P < 0.001) but not between varieties ($_{F\ 1,\ 144}$ = 1.95; P = 0.16) (Table 11 and 12). Difference in marketable yield between treatments was also significantly influenced by the location ($_{F\ 6,\ 144}$ = 5.60; P < 0.001), season ($_{F\ 2,\ 144}$ = 22.67; P < 0.001) and not by the variety ($_{F\ 2,\ 144}$ = 0.28; P = 0.84). *M. anisopliae* 1 x 10^{13} and Imidacloprid treated plots gave the highest marketable yield while, *M. anisopliae* 1 x 10^{12} gave the lowest compared to the untreated from the three

locations and two crop seasons (Table 11 and 12). Application of both Imidacloprid and *M. anisopliae*10^{13} significantly increased the marketable pod yield of French beans for both crop seasons (Table 11 and 12).

Table 11: Efficacy of *M. anisopliae* on WFT in thrips management and yield of two French bean varieties at three AEZs for season one (February to May, 2012)

		Kibwezi		Mwea		Naivasha	
Variety	Treatment	Total	Marketable	Total	Marketable	Total	Marketable
Tana	*M.a* 10^{13}	1185.2±80b	814.8±80a	1574.1±177b	1018.5±93b	1759.3±111b	1055.6±36b
	M.a 10^{12}	629.6±21c	259.3±21b	740.7±0c	370.4±0c	888.9±0c	148.1±0c
	Confidor	1500±36a	944.4±76a	1944.4±93a	1481.5±0a	2092.6±119a	1537.0±97a
	No spray	222.2±0d	0±0c	518.5±132d	0±0d	222.2±0d	0±0d
	LSD	120.3	132.8	120.3	132.8	120.3	132.8
	%CV	26.3	31.8	26.3	31.8	26.3	31.8
Serengeti	*M.a* 10^{13}	1240.7±97b	870.4±195a	1574.1±233a	1111.1±302a	1740.7±164a	1000.0±230a
	M.a 10^{12}	666.7±0c	296.3±0b	740.7±151b	370.4±0b	907.4±19b	259.3±222b
	Confidor	1518.5±77a	1055.6±221a	2129.6±177a	1666.7±478a	2129.6±130a	1388.9±175a
	No spray	240.7±19d	0±0c	463.0±93c	0±0c	240.7±19c	0±0c
	LSD	170.8	407	170.8	407	170.8	407
	%CV	28.8	39.4	28.8	39.4	28.8	39.4

M. a 10^{12} and 10^{13} = spore concentration of *M. anisopliae* at 10^{12} and 10^{13} conidia/ ha, respectively. Means within a column followed by same letter do not differ significantly by Student- Newman-Keul's test ($P<0.05$)

Table 12: Efficacy of *M. anisopliae* on WFT in thrips management and yield of two French bean varieties at three AEZs for season two (May to August, 2012)

		Kibwezi		Mwea		Naivasha	
Variety	Treatment	Total	Marketable	Total	Marketable	Total	Marketable
Tana	*M.a* 10^{13}	1388.9±233a	740.7±262ab	3769.3±319a	3055.6±382a	5370.4±685a	4166.7±411a
	M.a 10^{12}	1203.7±233b	648.1±278b	1759.3±177b	1296.3±107b	3333.3±302b	1666.7±185b
	Confidor	2314.8±316.3a	1574.1±316a	4074.1±214a	3333.3±216a	5925.9±1571a	4907.4±1347a
	No spray	555.6±107c	0±0c	740.7±151c	92.6±93b	1111.1±151c	0±0b
	LSD	1621.2	1731	1621.2	1731	1621.2	1731
	%CV	41.2	50.7	41.2	50.7	41.2	50.7
Serengeti	*M.a* 10^{13}	2314.8±278b	1759.3±278a	2777.8±355a	2129.6±438a	4351.9±487a	3240.7±278a
	M.a 10^{12}	1296.3±239c	648.1±177b	1388.9±278b	370.4±37b	2500±177b	1388.9±93b
	Confidor	3148.1±355a	2500±411a	2592.6±642a	2129.6±553a	4722.2±949a	3888.9±778a
	No spray	833±93c	0±0b	648.1±177b	0±0b	1296.3±185c	0±0c
	LSD	805.8	859.4	805.8	859.4	805.8	859.4
	%CV	36.4	46.5	36.4	46.5	36.4	46.5

M. a 10^{12} and 10^{13} = spore concentration of *M. anisopliae* at 10^{12} and 10^{13} conidia/ ha, respectively. Means within a column followed by same letter do not differ significantly by Student- Newman-Keul's test ($P<0.05$)

CHAPTER FIVE

DISCUSSION

5.1 Effect of temperature on spore germination, radial growth and sporulation of *Metarhizium anisopliae*

The optimum temperature for spore germination, radial growth and sporulation was recorded at 25 and 30ºC. Similar results were observed by Ekesi *et al*., (1999) and Dimbi *et al*., (2004). Recently, Bugeme (2008) and Niassy *et al.* (2012) reported the optimum temperature for spore germination to be 25 - 30 ºC. Beyond 30ºC and less than 25ºC there was a sharp and significant reduction in the germination of spores with no germination at 10 and 40ºC. According to Walstad *et al*., (1970) there was a decrease in germination of *Beauveria bassiana* and *M. anisopliae* spores as temperature increased beyond 30 ºC. The change in EPF spore germination as affected by temperature is attributed to the osmotic difference/adjustment between cells surroundings of the intracellular fluid. According to Chandler *et al*., (1994) osmotic potential affects the germination of EPF spores and the osmotic potential is influenced by the accumulation of polyhols and trehalose compounds in conidia. The accumulation of the compounds is temperature dependent (Brown, 1990, Lane *et al*., 1991; Harman *et al*., 1991). As the temperature increases, spore germination increases up to a 30ºC beyond which reduction in spore germination occurs; this is due to the decrease in the amount of polyhols and trehalose in the conidia (Harman *et al*., 1991).

Radial growth of the ICIPE69 was slower at 15 and 35ºC as compared to 20, 25 and 30 ºC. Optimum temperature for growth ranged between 25 and 30 ºC which is consistent with reports of Ekesi *et al* (1999) and Dimbi *et al.* (2004). Ouedrago *et al*. (1997) also reported that

the optimum temperature for radial growth of four Ethiopian isolates of *M. anisopliae* ranged between 25 and 32 ºC.

There was low spore production at 15 and 35ºC, with higher spore production at an optimum temperature range of 25 - 30ºC. Similarly, Tefera and Vidal, (2009) also observed low sporulation at 15 and 35ºC compared to 20 - 30ºC on four different strains of *Beauveria bassiana*.

5.2 Effect of temperature on the pathogenicity of *Metarhizium anisopliae* on adult and second instars of western flower thrips

As expected, infection of *F. occidentalis* adults and second instars by *M. anisopliae* increased with spore concentrations tested. The infectivity also increased with temperature upto an optimum of 25 - 30ºC, beyond which it declined. The optimum temperature for infectivity 25 - 30ºC and corresponds to the optimum temperatures for germination and radial growth as observed in the present study. The results are consistent with those of Thomas and Jenkins (1997), Ekesi *et al*. (1999), Dimbi *et al*. (2004) and Bugeme *et al* (2008). According to Ekesi *et al*. (1999) *B. bassiana*; ICIPE 53, TP-GHA and four strains of *M. anisopliae*; ICIPE 30, ICIPE 66, ICIPE69 and ICIPE 74 were most effective to adult *M. sjostedti* at 25 and 30ºC. Vestergaard, (1995) reported that adult *F. occidentalis* was susceptible to *M. anisopliae* isolate 275 at a temperature range of 18 - 26ºC, but mortality was higher at upper temperatures of 23 and 26ºC compared to 18 and 20ºC. However, Ekesi *et al.*, (1999) showed that some isolates of *B. bassiana* and *M. anisopliae* were highly virulent against adult legume flower thrips, *M. sjostedti* at 20ºC. The LT_{50} and LT_{90} values for causing mortality of the adult thrips of *F. occidentalis* decreased as the temperatures increased up to 30ºC, beyond which it

increased with the shortest LT_{50} and LT_{90} values at 25 and 30ºC. Such observations were also made by Ekesi *et al* (1999) who reported that the LT_{50} values for the virulence of *M. anisopliae* on adult *M. sjostedti* decreased as temperature increased and no significant difference was observed in the LT_{50} values at 25 and 30ºC.

In the present study, the mortality of the second instars was low compared to the adults irrespective of the doses. Similar observation were made by Ekesi *et al*., (2000) who reported that the second instars of *M. sjostedti* had the highest LT_{50} value (9.5 days), followed by the pupa (7.2 days) and the adult stage (3.1 days) at a dose of 1×10^8 at 26ºC. Maniania *et al*., (2001) reported that application of *M. anisopliae* significantly reduced WFT in chrysanthemum crop; but the control of second instars was much lower than for adults. Similar observations were made in laboratory bioassays by Vestergard, (1995) and Ugine *et al*., (2005) with *M. anisopliae* and *Beauveria bassiana* (Balsmo) Vuillemin. Niassy *et al*., (2012) reported that the LT_{50} value for second instars were 8.2 days at 26ºC as compared to 1.9 – 2.2 days for the adults as observed in the present study at a temperature range of 25 – 30ºC. Molting is known to be an important factor in insect resistance to fungal infection, particularly when the time interval between successive molting is short (Vey and Fargues, 1977). Such an interaction between the fungus and molting can be attributed to the reduced susceptibility of the second instars in the present study.

The best fitting model relating the temperature to infectivity was the cubic model which indicated that the mortality of the adult thrips increased significantly as temperature increased up to an optimum temperature of 29 ºC beyond which the mortality reduced. While the

predictions of the Lactin 1 and cubic models were similar for the upper threshold, they differed in their prediction of lower thresholds for fungus efficacy. The cubic model predicted a sharp decline in efficacy with a lower threshold close to 5ºC while, the Lactin 1 model predicted a gradual decrease in the mortality and it was asymptotic to the x - axis. The cubic model could be used as a support tool to predict the efficacy of ICIPE69 in relation to temperature. Use of non-linear regression models for prospective analysis of efficacy could be very valuable during the implementation of a biological control program given the expense of field trials and difficulty in assessing mortality against highly mobile species especially in small – scale preliminary application trials (Klass *et al*., 2007 a, b). Having established a basic susceptibility model it enables predictions of likely performance based on relatively simple measures of local climatic conditions. The Lactin model developed by Klass *et al*., (2007a) had the potential for identifying where and when the bio-pesticide could be used effectively and hence, for assisting in development of optimum use strategies against locust infestations.

The geo-spatial fungus efficacy maps show that predictions from the cubic and Lactin 1 models indicate higher efficacy of the fungus in the tropics than the temperate regions. Several studies have shown that *M. anisopliae* has considerable potential for control of locust and grasshoppers and have been tested extensively throughout Africa, Australia, parts of Europe and Latin America (Thomas *et al*., 2000; Lomer *et al*., 2001; Thomas and Kooyaman, 2004). The predictions are in line with the reports of Klass *et al*. (2007b), who predicted lower efficacy of the *Metarhizium anisopliae var. acridum* for control of locust in the Northern South Africa. In East Africa, both the models predicted lower efficacy in very high altitude zones compared to the mid- and low-altitude regions

5.3 Effect of *Metarhizium anisopliae* on infestation of western flower thrips on French beans in different agro-ecological zones

Application of fungus at spore concentration of $1x10^{13}$conidia/ha was as effective as the use of Imidacloprid for the management of WFT. Further the application of *M. anisopliae* was more effective during the first crop season (February - May) than the second one (May – August). This is because the temperature in the first season was warmer and more favorable for the fungus than during the second season at the three locations. At Kibwezi, the mean temperatures during both the crop seasons were favourable for the growth of the fungus; hence there was no variation in the efficacy of the fungus over seasons as observed from the percentage reduction of thrips population over control. However, in Naivasha and Mwea, the mean temperature in the second season was considerably lower than the first season leading to highly significant differences in the efficacy of fungus over seasons. The geospatial map indicates that the fungus is not effective in the cold regions, which is in line with the field results for this study. The high level of field efficacy of *M. anisopliae* as a promising biocontrol is in agreement with reports by Maniania *et al*. (2001), who found that *M. anisopliae* significantly reduced both the adult and larval populations of *F. occidentalis* on chrysanthemum, although the level of control of larval populations was much lower than for adults. According to Muvea, (2011) the fungus was very effective in controlling insecticide resistant populations on Western Flower thrips. From the differences observed in the three AEZs, high, mid and low, the results indicate that temperature is an important factor in determining the effectiveness of the fungus.

Gouli *et al*. (2009) found *M. anisopliae* was found to be the most effective fungus in the management of *F. occidentalis* in bean plants compared to *Lecanicillium lecanii*, and *Beauveria bassiana* on the other hand. Arthurs *et al*. (2013) found *B. bassiana* GHA, *Metarhizium brunneum* F52 and *Isaria fumosorosea* to be effective in the management of low to moderate populations of chilli thrips; *Scirtothrips dorsalis.*

Thrips population lowers the total pod yield due to reduced pod set (Muvea, 2011; Nyasani *et al*., 2012). In this study high total pod yields were observed/ achieved where thrips population were low, particularly in Mwea and Kibwezi in the second season. While during the season two, there was no significant difference between the *M. anisopliae* 10^{13} treated and the chemical plots. This compares to Maniania *et al* (2003b) studies which reported no significant difference between the *M. anisopliae* treated and the chemical treated plots in the first trial, but in the second trial the fungal treated plots resulted in higher onion yield than the chemical treated plots. Metarhizium anisopliae has also been reported to increase the total pod yield by reducing populations of *M. sjostedti* in cowpea (Ekesi *et al*., 1998).

Although temperature was observed to be a key factor, several others such as relative humidity (Fargures *et al*., 1997), rainfall (Inyang *et al*., 2000), differences in susceptibility of thrips populations at different densities in different locations (Azaizeh *et al*., 2002) are known to influence the efficacy of the fungus. These factors may have played a role in the results obtained. Hence studies taking into consideration other weather factors, insect factors and detailed understanding of the population differences could be used to refine the prediction model used in the study.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Spore germination, radial growth and sporulation of *Metarhizium anisopliae* are affected by temperature and the optimum occurs at temperature of 25 - 30ºC. Temperatures below 15ºC and above 35ºC are not effective for the fungal activity.

The median lethal time to cause 50 and 90% mortalities is shorter at 25ºC and the lethal time decreases as temperatures increased from 15 to 25ºC, beyond which the time increased until 35ºC. The adult thrips of *F. occidentalis* are more susceptible to *M. anisopliae* than the second instars at favourable temperatures between 25 - 30ºC.

Statistically the cubic model was the best as it had a smaller AIC value than the Lactin 1 model. Predictions from the geo- spatial efficacy map indicate that the fungus ICIPE69 is likely to be effective in mid and low altitude zones of East Africa including Kenya and not in the very high altitude zones.

Among the field application rates for the fungus tested, application rate of $1x10^{13}$ conidia/ha was as effective as application of Imidacloprid for the management of *F. occidentalis*. Both laboratory and field experiments showed that temperature is a key determinant of the entomopathogenic fungus efficacy.

6.2 Recommendations

- *Metarhizium anisopliae* has a high potential in thrips management in comparison to chemical insecticides and thus should be used as one of the management options in the integrated pest management program of thrips.

- Further studies taking into consideration other weather factors, insect factors and detailed understanding of the population differences should be done to refine the prediction model used in the study.
- Further research should be done on other climatic factors other than temperature that influence the efficacy of the entomopathogenic fungus

- The role of formulation technology to counter a biotic stresses to further enhance the efficacy of fungus needs to be evaluated.

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