EFFICACY OF *Metarhizium anisopliae* BASED-BIOPESTICIDE FOR BIOCONTROL OF *Spodoptera frugiperda* IN MAIZE PRODUCTION IN MIGORI AND HOMABAY COUNTIES, KENYA

MUNYWOKI M. JOSEPH (B.Sc. Biology)

I56/38053/2017

A THESIS SUBMITTED IN FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF MASTER OF SCIENCE (MICROBIOLOGY) IN THE SCHOOL OF PURE AND APPLIED SCIENCES OF KENYATTA UNIVERSITY

JUNE, 2025

DECLARATION

I, Munywoki M. Joseph, affirm that this thesis is my original work and has not been submitted to any other university for the award of a degree or any other academic qualification

Munywoki Matumu Joseph

I56/38053/2017

Department of Biochemistry, Microbiology and Biotechnology

Signature…………………………. Date……………………………

SUPERVISORS

We confirm that the work presented in this thesis was conducted by the candidate under our supervision.

Prof. Ezekiel Mugendi Njeru

Signature…………………………. Date……………………………

Department of Biochemistry, Microbiology and Biotechnology,

Kenyatta University

Prof. Leonidah Omosa Kerubo

Signature…………………………. Date……………………………

Dean School of Physical Sciences,

University of Nairobi

Dr. Komivi Senyo Akutse

Signature…………………………. Date……………………………

Plant Health Theme,

International Centre of Insect Physiology and Ecology

DEDICATION

I dedicate this work to the Heavenly Father, the giver of life and to my wife Queen Munanie and my daughter Hailleyesther Mwende. Secondly, I also extend this dedication to my lovely parents, my brother Mathew Mutuku, my sister Esther Munywoki, and the extended family members for the support and motivation they gave me during my period of study.

To my late grandfather “Joseph Matumu Senior”, I offer this work as a tribute.

Furthermore, I dedicate this work and its findings to those who cherish human diversity and love each other around the entire world.

ACKNOWLEDGEMENTS

Firstly, I give heartfelt thanksgiving to the Almighty God for His blessings, beauty of good health, strength and protection during the times of this research. I wish to pass my appreciation to Kenyatta University for the opportunity to further my studies.

My sincere gratitude goes to the Dissertation Research Internship Programme (DRIP) of *icipe* for financing my research studies.

Thank you Prof. Ezekiel Mugendi Njeru for your dedication and for taking me through all the steps essential for the success of this project as well as your leadership skills and steadfast supervision throughout the entire period of this work. I am particularly grateful to Prof. Leonindah Omosa Kerubo for sparing your limited time to follow up my project progress and for the opportunity to undertake part of this research at the University of Nairobi research laboratory under your guidance. Much appreciation to Dr. Komivi Senyo Akutse for believing in me and granting me this project and as well as for his supervision. You kept the door to your office always open for me whenever I had any concern about the research work. You tirelessly and constantly coxswained me in the correct path whenever you felt it necessary.

I am also grateful to the lecturers of Kenyatta University for their support in class and during various presentations. My sincere appreciation also goes to Dr. John Maingi for his guidance in class work and for encouraging me to pursue this study.

Special thanks to the whole International Centre of Insect Physiology and Ecology (*icipe*) fraternity for their candid support all through. Many thanks to the staff of the Arthropod Pathology Unit for their constant guidance and backing in all my laboratory and field experiments throughout my research period. Special thanks to Mr. David Owour and the *icipe* Mbita-Thomas campus fraternity for providing land to conduct my field experiments and for always helping me in the field. May our Heavenly Lord bless you all abundantly.

Special appreciation also goes to, Ms. Junitor Chepkemoi, Mr. Harrison Kibe Kamore, Mr. Jasper Kirui, Mr. Abdul Jalloh, Ms. Druscilla Abonyo and Mr. Ezekiel Fardolo for their encouragement and overwhelming support during my research study period. .

Lastly, my deepest thanks go to my parents Mr. David Munywoki, Mrs. Phyllis Munywoki, Mr. Mutuku Mwanza, and Mrs. Esther Munywoki for their sacrifices throughout my educational journey, their heartfelt prayers, contributions, support, and financial assistance. God Almighty bless you all.

TABLE OF CONTENTS

TITLE PAGE ... i

DECLARATION ... ii

DEDICATION ... iii

ACKNOWLEDGEMENTS ... iv

LIST OF TABLES ... xi

LIST OF FIGURES ... xii

LIST OF PLATES ... xiii

ABBREVIATIONS AND ACRONYMS ... xiv

ABSTRACT ... xvi

CHAPTER ONE ... 1

INTRODUCTION ... 1

- 1.1 Background of the study ... 1
- 1.2 Statement of the problem ... 4
- 1.3 Rationale of the study ... 5
- 1.4 Justification of the study ... 5
- 1.5 Research hypotheses ... 7
- 1.6 Objectives ... 7
  - 1.6.1 General objective ... 7
  - 1.6.2 Specific objectives ... 8
- 1.7 Significance of the study ... 8

CHAPTER TWO ... 9

LITERATURE REVIEW ... 9

- 2.1 Importance of maize production and its geo-ecological distribution in Kenya ... 9
- 2.2 Maize varieties grown in Kenya and major pests ... 9
- 2.3 Fall armyworm biology and ecology ... 10
- 2.4 Fall armyworm description and life cycle ... 11
  - 2.4.1 Egg stage ... 11
  - 2.4.2 Larval stage ... 12
  - 2.4.3 Pupal stage ... 13
  - 2.4.4 Adult stage ... 14

2.5 Taxonomy of *Spodoptera frugiperda* 16
2.6 Control of *Spodoptera frugiperda* 16
2.6.1 Cultural and physical control 16
2.6.2 Chemical control 17
2.6.3 Use of resistant varieties 18
2.6.4 Behavioral control 18
2.6.5 Biological control method 19
2.6.6 Use of biopesticides 20
2.6.7 Entomopathogenic fungi 20
2.6.8 Use of sex pheromones 23
2.6.9 Integrated pest management (IPM) 24
CHAPTER THREE 25
MATERIALS AND METHODS 25
3.1 Study site 25
3.2 Insect source and rearing conditions 25
3.3 Source and maintenance of fungal cultures 26
3.4 Conidial viability test 27
3.5 Effect of *Metarhizium anisopliae* ICIPE 41 on *Spodoptera frugiperda* 28
3.5.1 Experimental design 28
3.5.2 Experimental procedure 28
3.5.3 Pathogenicity of *Metarhizium anisopliae* on adults' moths 29
3.5.4 Mass production of fungus 31
3.6 Laboratory Experiment 33
3.6.1 Compatibility test for *Metarhizium anisopliae* ICIPE 41 with FAW sex pheromone in the laboratory 33
3.6.2 *Spodoptera frugiperda* pheromone effect on formulated conidial viability and germ tube length 34
3.7 Treatments layout 37
3.7.1 Assessment of cumulated larval mortality and lethal time (LT_{50}) 38
3.7.2 Assessment of effects of the formulations on FAW's parasitoids 38
3.8 Field Experiments 39

3.8.1 Mazao Achieve® biopesticide 39
3.8.2 Radiant pesticide 120 SC- spinetoram (XDE-175J) 40
3.8.3 Experimental design....................41
3.8.4 Treatment application 42
3.8.5 Attraction of *Spodoptera frugiperda* moths....................44
3.8.6 *Metarhizium anisopliae* ICIPE 41 conidia acquisition after field exposure .44
3.8.7 FAW mortality induced by *Metarhizium anisopliae* ICIPE 41 formulation 45
3.8.8 Assessment of conidial persistence....................46
3.8.9 *Spodoptera frugiperda* infestation and damage assessment 46
3.9 Natural enemies' assessment 46
3.10 Maize yield assessment....................47
3.11 Data analysis 47
CHAPTER FOUR 49
RESULTS....................49
4.1 Laboratory bioassays on the pathogenicity of fungal formulations....................49
4.1.1 Assessment of viability of *Metarhizium anisopliae* ICIPE 41....................49
4.2.2 Compatibility of *Spodoptera frugiperda* sex pheromone and fungus under laboratory conditions 50
4.3 Effects of *Spodoptera frugiperda* sex pheromone on formulated conidial viability and germ tube length 50
4.4 Pathogenicity of various formulations against second instar *Spodoptera frugiperda* larvae....................51
4.5 Assessment of non-target effects of the various formulations to FAW's parasitoids 53
4.6 Effect of various formulations on emergence of parasitoids 54
4.7 Effect of *S. frugiperda* sex pheromone on attraction of moths under field conditions 55
4.8: *Metarhizium anisopliae* ICIPE 41 conidia acquisition by *S. frugiperda* under field conditions....................56
4.9 FAW infestation in Migori and Mbita field sites....................57
4.10 Assessing FAW Damage 58

4. 11 Cumulated mortalities of FAW second instar larvae under field conditions.....59
4. 12 Persistence of *Metarhizium anisopliae* ICIPE 41 conidia in corn oil formulation over time in Migori and Mbita Field Sites..60
4. 13 Effect of Entomopathogenic Fungi on *Cotesia icipe* Emergence in Migori and Mbita Field Sites..61
4. 14 Maize Grain Yield in Response to Formulation Treatments in Migori and Mbita Sites..62
CHAPTER FIVE..64
DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS 64
5.1 Discussion...64
5.1.1 Viability and compatibility bioassays of *Metarhizium anisopliae* ICIPE 41 and its formulations with *Spodoptera frugiperda* sex pheromone under laboratory conditions..64
5.1.2 Pathogenicity of various formulations against second instar *Spodoptera frugiperda* larvae under laboratory conditions..66
5.1.3 Assessment of non-target effects of the various formulations to FAW's parasitoids under laboratory conditions...67
5.1.4 Effect of various formulations on emergence of parasitoids under laboratory conditions 68
5.2 Field management of *S. frugiperda* infestation using various *M. anisopliae* ICIPE 41 formulations...69
5.2.1 Effect of *S. frugiperda* sex pheromone on attraction of moths under field conditions 69
5.2.2 *Metarhizium anisopliae* ICIPE 41 conidia acquisition by *S. frugiperda* under field conditions...70
5.2.3 FAW infestation assessment in Migori and Mbita field sites as influenced by different *M. anisopliae* ICIPE 41 formulations..71
5.2.4 Cumulated mortalities of FAW second instar larvae under field conditions 72
5.2.5 Persistence of *Metarhizium anisopliae* ICIPE 41 conidia in Corn Oil Formulation over time in Migori and Mbita field sites 74

5.2.6 Effect of entomopathogenic fungi on parasitoid *Cotesia icipe e*mergence in Migori and Mbita field sites 75
5.2.7 Maize grain yield in response to formulation treatments in Migori and Mbita sites... 76
5.2 Conclusion 77
5.3 Recommendations..........78
5.3.1 Study recommendations..........78
5.3.2 Recommendations for further research 78
APPENDIX I: Research Authorization 87
APPENDIX II: Research Permit 88

LIST OF TABLES

Table 4.1: Larvae cumulated mortality caused by the fungal formulations and their corresponding lethal time 50% (LT_{50}). 52

Table 4.2: Mean percentage mortalities of FAW's parasitoids infected with different formulation of *M. anisopliae* ICIPE 41 isolate. 54

Table 4.3: Mean parasitism rate of FAW's parasitoids against different formulation of *M. anisopliae* ICIPE 41 isolate 55

Table 4.4: ANOVA table for the response *Spodoptera frugiperda* moth attraction device as affected by *Spodoptera frugiperda* sex pheromone. 56

Table 4.5: ANOVA table for *M. anisopliae* conidial acquisition. 57

Table 4.6: Average FAW density per plant eight weeks post-treatment. 58

Table 4.7: Average damage of FAW a week post-treatment examination in Migori and Mbita 58

Table 4.8: Larvae cumulated mortality caused by the fungal formulations and their corresponding lethal time 50% (LT_{50}). 59

Table 4.9: Performance FAW parasitoid *Cotesia icipe* assessed on the developmental stage in different treatments in Migori and Mbita 62

LIST OF FIGURES

Figure 4.1: Viability of the *Metarhizium anisopliae* ICIPE 41 fungal isolate conidia growing on SDA media incubated at 26 ± 2 and determined after 18 hours.... 49

Figure 4.2: Percentage germination of *Metarhizium anisopliae* ICIPE 41 when exposed to *S. frugiperda* sex pheromone for 8 days. 50

Figure 4.3: Spore germination and germ tube length after exposition to FAW pheromone for different treatment. 51

Figure 4.4: Persistence of *Metarhizium anisopliae* ICIPE 41 isolate in the corn oil formulated fungus treatment over time in Migori and Mbita fields. 61

Figure 4.5: Maize grain yield in Migori and Mbita in Kg/ha^{-1} 63

LIST OF PLATES

Plate 2.1: Fall armyworm eggs 12

Plate 2.2: Fall armyworm larvae. 13

Plate 2.3: Fall armyworm pupae. 14

Plate 2.4: Fall armyworm adult moth 15

Plate 2.5: Fall armyworm life cycle. 15

Plate 3.1: Adult *Spodoptera frugiperda* 26

Plate 3.2: *Metarrhizium anisopliae* isolate sub cultured on SDA media 27

Plate 3.3: Inoculating chamber 30

Plate 3.4: Inoculating chamber spread with the spores 30

Plate 3.5: A cage containing the FAW moths 30

Plate 3.6: Starter cultures in orbital shaker incubator 32

Plate 3.7: Mass production using rice in Milner bags 32

Plate 3.8: De-hydration *M. anisopliae* 33

Plate 3.9: (a)Vacuum filtration of conidia of *M. anisopliae, (b)* Desiccation of conidia of *M. anisopliae* using FAW sex pheromone, (c)Desiccation of conidia of *M. anisopliae* without the FAW sex pheromone (control) 37

Plate 3.10: Mazao Achieve® Biopesticide from Real IPM 40

Plate 3.11: Radiant 120 SC (Spinetoram XDE-175-J) 41

Plate 3.12: Spinetoram pesticide plot at *icipe*, Mbita campus 42

Plate 3.13: Corn oil formulation plot at *icipe*, Mbita campus 43

Plate 3.14: Mazao Achieve® plot at *icipe*, Mbita campus 43

Plate 3.15: Control plot at Awendo in Migori County 44

ABBREVIATIONS AND ACRONYMS

AEZ	Agro-ecological Zones
ANOVA	Analysis of Variance
APU	Arthropod Pathology Unit
ARQU	Animal Rearing and Quarantine Unit
BFT	Bean Flower Thrips
BCA	Biological Control Agents
CABI	Centre for Agriculture and Bioscience International
CAN	Calcium Ammonium Nitrate
DAP	Diammonium Phosphate
DF	Degree of Freedom
EPF	Entomopathogenic Fungi
EU	European Union.
FAW	Fall Armyworm
G	Grams
H	Hours
Ha	Hectares
icipe	International Centre of Insect Physiology and Ecology
IPM	Integrated Pest Management
KALRO	Kenya Agricultural and Livestock Research Organization
Kg	Kilograms
ML	Milliliters
L	Liters
LAS	Leica Application Suite
LT_{50}	Lethal Time 50%
L:D	Light Darkness photoperiod proportion
MRL	Maximum Residue Level
NCPB	National Cereals and Produce Board
R H	Relative Humidity
SAS	Statistical Analysis System
SDA	Sabouraud Dextrose Agar

SDG	Sustainable Development Goals
SE	Standard Error
SNK	Student Newman Keuls
SSA	Sub-Saharan Africa
THSD	Tukeys Honest Significance Difference
UN	United Nations

ABSTRACT

Maize is an important staple food crop for majority of individuals in Sub-Saharan Africa, particularly in East Africa, where it is cultivated on over 25 million hectares of land. However, its production in Kenya faces significant challenges from both abiotic and biotic factors, resulting in more than 50% crop losses. One of the most serious biotic threats is the invasion of Fall armyworm (FAW), scientifically known as *Spodoptera frugiperda*. This pest inflicts extensive damage in all stages of maize growth, jeopardizing sustainable production and food security. Although conventional chemical insecticides are highly relied on for management of FAW, they pose serious risks and deleterious effects on the environment and biodiversity. Therefore, there is a pressing need to promote the development of innovative and sustainable approaches for managing FAW. One promising alternative is the use of microorganisms in pest management, as they offer effective control with minimal or no negative environmental impact. A virulent isolate of *Metarhizium anisopliae* ICIPE 41 (Metschnikoff) Sorokin, obtained from the fungal culture collection germplasm housed at the International Centre of Insect Physiology and Ecology (*icipe*), and its different formulations were evaluated under the laboratory and field conditions. Initially, the study assessed the sub-lethal effects of these formulations on second instar larvae of *S. frugiperda* and on some associated parasitoids (*Cotesia icipe*, *Telenomus remus* and *Trichogramma chilonis*) of the FAW. Also, the compatibility of the fungal isolate with FAW sex pheromone (Falltrack®) was evaluated. Out of the four *M. anisopliae* ICIPE 41 formulations screened for pathogenicity against *S. frugiperda*, three formulations, namely canola oil, olive oil and corn oil, induced significantly higher mortalities, ranging from 70.4% to 76.1%. Post-treatment mortality at 50% lethal time (LT_{50}) ranged from 2.0 to 8.1 days. In contrast, the *M. anisopliae* ICIPE 41 formulations exhibited significantly lower mortality rates on the FAW parasitoids, ranging from 2.5% to 21.3%. The findings also showed that *M. anisopliae* ICIPE 41 was highly compatible with the FAW sex pheromone at eight days post-exposure with viability count ranging from 97% to 100%. The most effective formulation which caused high mortality in FAW larvae while being less toxic to the parasitoids was further evaluated under field conditions. For the field trials, evaluation of pathogenicity and conidial persistence of *M. anisopliae* ICIPE 41 corn oil formulation was carried out weekly for one month after application on maize leaves. Larval mortality resulting from exposure to the formulated fungal isolate was significantly higher than that of commercial detain biopesticide at both field sites, recording 80.1% and 81.6% mortality, respectively. Conidial germination rates on days 14, 21 and 28 showed a gradual decline at both Migori and Mbita sites, with mortality rates of 87%, 74% and 61% at Migori and 88%, 72% and 61% at Mbita, respectively. There was a significant number of *Cotesia icipe* parasitoids (56%) that emerged from field-collected dead larvae. Notably, corn oil formulation of *M. anisopliae* ICIPE 41 significantly enhanced maize yield compared to Mazao Achieve® and the control, indicating its potential as a viable biocontrol option. In conclusion, this study identified the corn oil-based formulation of *Metarhizium anisopliae* ICIPE 41 to be pathogenic to second instar larvae of *S. frugiperda* while exhibiting minimal negative effects on associated parasitoids. It therefore recommends the potential integration of locally developed delta traps, baited with FAW sex pheromone, with this fungal formulation as a lure and infect mechanism for effective field-level pest management.

CHAPTER ONE

INTRODUCTION

1.1 Background of the study

Corn (*Zea mays* L.) is a staple food for the majority of people in Africa, particularly in the sub-Saharan Africa (SSA), where it is cultivated on about 25 million hectares of land (Akutse *et al*., 2019). It is grown across diverse agroecological zones (AEZs) supporting the livelihoods of over 300 million (Akutse *et al*., 2019). However, the quest to attain sustainable production of maize in the region is continuously threatened by various abiotic and biotic constraints, which are responsible for yield losses ranging between 15%–80% (Lacey *et al*., 2015). Compounding this challenge is the rapidly growing human population, which place serious pressure on maize production systems (Pérez-Escamilla, 2017).

The most critical biotic constraint is the persistent infestation of maize by the invasive Fall Armyworm (FAW) (*Spodoptera frugiperda* [J.E. Smith], Lepidoptera: Noctuidae), which attacks various parts of the maize plant, including the leaves and cobs (Munywoki *et al*., 2022). In African countries, FAW is widely spreading, having been reported in more than 35 countries since its first reported case in 2016 (Sisay *et al*., 2018). Due to its widespread, FAW has caused wide-ranging injuries to many important crops (Prasanna *et al*., 2018) and has as well spread to the other sides of Asia (Sharanabasappa *et al*., 2019). Fall armyworm causes damages to more than 350 plant species (Meagher *et al*., 2016) with a strong preference for cereal crops. Initial estimates place the economic losses due to FAW in African maize production at between US$2.4 billion and US$6.5 billion annually (Shiferaw *et al*., 2011).

To sustain productivity, researchers have developed and implemented integrated pest management (IPM) strategies targeting the damaging lepidopteran pests (Akutse *et al*., 2019). The Centre for Agriculture and Bioscience International (CABI), through its Plantwise program, recommends for small-scale farmers to lower the pest populations through cultural practices such as collecting egg masses and destroying the larvae (Tindo *et al*., 2017). Despite the widespread use of synthetic pesticides by both small- and large-scale farmers, these methods have not been effective and economical (Opisa *et al*., 2018). Either, they are not environmentally sustainable, and thus, can negatively affect both production and consumption patterns (Muriithi *et al*., 2016).

The FAW invasion poses a significant threat to food security in Africa and undermines the progress made in agricultural productivity (Akutse *et al*., 2019). It's therefore important to identify and adopt safer, affordable and environmentally friendly pest management strategies (Opisa *et al*., 2018). The FAW larvae prefers feeding on young leaves, ears and tassels of maize, even though older instars can feed on the harder base of the stem causing harm on the whole plant (Munywoki *et al*., 2022).

In Africa, two genetic strains of *Spodoptera frugiperda* have been identified (Prasanna *et al*., 2018). Their invasions and persistence in the region has been attributed in part to poor and unsafe management practices, since farmers' responses to tackle FAW are dependent on conventional chemical applications, which have generally failed to prevent widespread crop damage. As a result, ineffective management policies have contributed to the continued spread and impact of FAW, making the search for alternative, sustainable solutions more urgent than ever (Lacey *et al*., 2015).

Alternative control strategies that are less dependent on synthetic pesticide can significantly improve maize production and enhance its utilization (Lacey *et al*., 2015). Managing FAW effectively necessitates a well-planned, comprehensive approach that complements the already existing pest control strategies used by farmers (Akutse *et al*., 2019). Fortunately, most of the potential cheaper control options in place are the ones that are built on local knowledge and ecological ideologies. These approaches are particularly relevant given that many small-scale farmers lack the financial capacity to afford synthetic pesticides or to purchase improved seed varieties (Kumela *et al*., 2019).

Some agroecological methods, which are compatible with both biocontrol strategies and plant breeding, can as well be integrated with certain synthetic pesticides (Lopes *et al*., 2011). However, majority of the synthetic chemicals currently in use are broad-spectrum in nature and tend to harm non-target organisms, including beneficial natural enemies of pests. Their continuous use, therefore, risks undermining the effectiveness of agroecological and biological control methods (Meagher *et al*., 2016). Thus, effective FAW management requires a thorough understanding of the pest control methods already employed by smallholder farmers and must be integrated into broader pest management frameworks that consider economic viability, environmental sustainability, and social acceptability (Tindo *et al*., 2017).

Biological control offers one of the most environmentally friendly and effective solutions for FAW management (Chandler *et al*., 2011; Opisa *et al*., 2018). In recent years, International Centre of Insect Physiology and Ecology (*icipe*), in collaboration with private associates, has developed and commercialized some entomopathogenic bioinsecticides aimed at managing destructive pests (Opisa *et al*., 2018; Akutse *et al*., 2020). Consequently, building on these efforts, this study is designed to evaluate the effectiveness of *M. anisopliae* isolate ICIPE 41 against the immature developmental stages of *S. frugiperda*. The results will inform the potential integration of this biological agent into sustainable pest management programs for smallholder maize farmers.

1.2 Statement of the problem

The detection of fall armyworm (FAW), a pest of the genus *Spodoptera,* singly considered as the most invasive pest of cereal crops and is grouped in quarantine list, signifies that it is a great threat to agricultural activities (Sisay *et al*., 2018). FAW inflicts substantial damage to crops, feeding on over 80 different crops, including maize (Munywoki *et al*., 2022). It feeds on all developmental stages of maize. The larvae cause damage to the leaves, tassels as well as the ears of corn (Prasanna *et al*., 2018). The injuries typically appear as cliff-edged holes on leaves, tassels and/or ears.

The threat posed by FAW is further exacerbated by the widespread application of chemical pesticides, which are often not effective and may lead to adverse environmental impacts. Additionally, the strict implementation of quarantine measures, particularly concerning maximum residue levels (MRLs), jeopardizes the worthwhile exportation of high-quality maize. Control of FAW in Africa is hampered by its capability to consume different types of plants as well as its ability to pupate and stay for long without feeding (Akutse *et al*., 2019).

Entomopathogenic fungi (EPF) represent a promising alternative in pest management, offering a cost-effective, environmentally friendly, and sustainable option compared to synthetic insecticides (Mann and Davis, 2021). Entomopathogenic fungi safeguard biodiversity by minimizing harm to non-target organisms and reduce the negative human and environmental health risks associated the use of synthetic chemical pesticides (Lacey *et al*., 2015). This study therefore proposes the use of a fungal-based biopesticide, specifically *Metarhizium anisopliae* ICIPE 41, as a cost-effective and efficacious method for managing FAW in maize production in Kenya.

1.3 Rationale of the study

Currently, the most commonly used control measures for FAW include synthetic pesticides and early harvesting (Sisay *et al*., 2018). However, these pesticides commonly used are obstinate toxicants that pose significant human and environmental risks and often leave residues in maize seeds and tissues, rendering them unfit for its utilization (Opisa *et al*., 2018). Early harvesting, a practice common in small-holder settings, has also proven ineffective, as FAW larvae can pupate within maize tissues, with adult moths emerging even after harvest and continuing the infestation cycle in storage. This study demonstrates the potential of EPF as an effective FAW management tool, offering a green alternative that could contribute to enhancing quality and quantity of maize production that meets the requirements for both local and commercial exportation.

1.4 Justification of the study

In Kenya, maize is grown in almost every homestead for either domestic or commercial use Maize production in Kenya has been experiencing a noticeable decline over the years, largely due to a combination of biotic and abiotic stresses (Kinyumu *et al*., 2018). Biotic factors such as pests, most notably the fall armyworm (FAW), have significantly compromised maize yields across major growing regions despite the improving farmers' technical capabilities, knowledge, and experiences. Consequently, despite farmers' relentless efforts, maize yields have been insufficient to meet both household and national food demands. As a staple food and primary source of carbohydrates for the majority of Kenyan households, this decline in maize production has directly contributed to rising cases of malnutrition, particularly, carbohydrate deficiency among all dependent populations. In addition, FAW invasions have significantly undermined maize productivity, with reported losses of up to 80%, contributing to higher maize prices (Kinyumu *et al*., 2018; Sisay *et al*., 2019).

To combat FAW, maize growers rely heavily on conventional insecticides, and continued application of these insecticides have led to accumulation of harmful residues in maize leaves, tassels and other edible parts. This not only poses health risks to consumers and the environment, but also threatens Kenya's trade opportunities globally due to possibility of rejection of contaminated maize (Munywoki *et al*., 2022). Hence, the utilization of microbial pest control methods, particularly using EPF, in conjunction with integrated pest management (IPM) practices, has the potential to substantially reduce the risks associated with the dependence on synthetic pesticides and safeguard food security (Murdia *et al*., 2016). Previous studies have shown the pathogenicity of various EPF isolates against FAW developmental stages (Akutse *et al*., 2019), with different *Metarhizium anisopliae* ICIPE strains being particularly effective against the immature stages of FAW (Akutse *et al*., 2019). The results of this study will contribute to the sustainable management of FAW, potentially decreasing the excessive reliance on conventional insecticides.

1.5 Research hypotheses

i. Oil and aqueous formulations of *M. anisopliae* ICIPE 41 are not effective in managing larvae of FAW in maize crop.

ii. There is no compatibility between *M. anisopliae* ICIPE 41 and FAW sex pheromone in viability, pathogenicity and persistence under laboratory and field conditions.

iii Various *M. anisopliae* ICIPE 41 formulations do not affect FAW natural enemies' fitness in laboratory and field, as well as evaluate the maize crop yield.

1.6 Objectives

1.6.1 General objective

To effectively manage *S. frugiperda* by integrating *M. anisopliae* ICIPE 41 based-biopesticide in maize production system.

1.6.2 Specific objectives

i. To evaluate the efficacy of oil and aqueous formulations of *M. anisopliae* ICIPE 41 against FAW under both laboratory and field conditions.

ii. To evaluate the compatibility of *M. anisopliae* ICIPE 41 with FAW sex pheromone under laboratory conditions in viability, pathogenicity and persistence in laboratory and field conditions.

iii. To assess the non-target effect of the oil and aqueous formulations on FAW natural enemies in laboratory and field, as well as evaluate the maize crop yield.

1.7 Significance of the study

This research anticipates that its findings will help in improving maize production by effectively managing FAW populations. By offering a sustainable and cost-effective alternative to synthetic pesticides, the study's outcomes are expected to reduce the dependence of synthetic pesticides and reduce production costs, which could, in turn, help stabilize or lower maize prices. In addition, it is expected that the formulated biopesticide product will significantly enhance food security and support income generation among smallholder farmers. These outcomes support the United Nation's (UN) sustainable development goals (SDGs), particularly SDG 1 (No Poverty) and SDG 2 (Zero Hunger) (United Nations, 2021). The scientific community will also benefit from the insights on efficacy of fungal-based biopesticides against FAW through peer-reviewed publications. Furthermore, the development of this biopesticide product could provide business opportunities for private sector through public and private partnerships.

CHAPTER TWO

LITERATURE REVIEW

2.1 Importance of maize production and its geo-ecological distribution in Kenya

Maize (*Zea mays* L.) is an annual cereal crop of family Gramineae and Genus *Zea* (Okweche *et al*., 2013). It performs best in well drained and aerated loam soils with a pH range of 5.5 – 7. Maize do grow in a varied range of agroecological zones (AEZs) of 0-2200m above sea level depending on the variety. The optimal temperature range is between 18 ºC to 27 ºC during the day and around 14 °C during the night, with rainfall ranging between 600 – 900 mm (Mumo *et al*., 2018). According to the National Cereals and Produce Board (NCPB) statistics, south rift regions of Kenya (Bomet and Kericho), western regions (Kisii and Nyamira) and some central regions are the major growers (Shiferaw *et al*., 2011; Mumo *et al*., 2018). It is a significant staple food for majority of the population in the world as it is a source of carbohydrates to the human body. It can be utilized for various purposes, including serving as livestock feed, silage, and crop residue. Additionally, the grain has industrial applications for starch and oil extraction, and it is a source of carbohydrates, vitamin B, and non-food products (Ansari *et al*., 2011).

2.2 Maize varieties grown in Kenya and major pests

In Kenya, there are over 300 different maize varieties that have been tested since 1965 as viable grade among which 100 have been successfully proved while others are still under investigation (Shiferaw *et al*., 2011). The mostly used resistant varieties in Kenya include Katumani, Pannar, Twiga, Pioneer, Duma 43, KDV1, 2, 3, 4, and 5, and Kakamega synthetic 1 and 2 (Schroeder *et al*., 2013). Despite the availability of many varieties, including the resistant ones, pest infestation in maize crops have always remained high causing significant yield losses (Sisay *et al*., 2018). The most destructive pest of maize in Kenya is the fall armyworm (FAW), although other pests such as the oriental corn borer, wireworm, pink borer, black cutworm, seed corn maggot and parasitic weeds also contribute to crop damage and reduced productivity (Sisay *et al*., 2018). Among these pests, FAW is the most invasive and destructive pest causing losses of more than 80% (Akutse *et al*., 2019). Therefore, FAW management is very important to minimize the losses posed by the pest (Prasanna *et al*., 2018). Resistant maize varieties can also serve as a strategy for pest and disease control (Bawa, 2016).

2.3 Fall armyworm biology and ecology

The fall armyworm undergoes a complete metamorphosis, consisting of distinct stages including eggs, six larval instars, puparia, and adult stages, as described by Deshmukh *et al*. (2021). Under favorable environmental conditions, typically temperature of 27 ºC, relative humidity and a 12:12 light-dark cycle, the eggs, which can be either white or greenish in color, hatch into hairy larvae. These larvae then undergo growth and molt through six instars over a period of approximately 14 days (Kalyebi *et al*., 2006). Upon reaching the sixth instar, the larvae exit the host plant, move and burrow into the soil to a depth of approximately 4-9 cm. Here, they undergo pupation, forming a puparium (Sisay *et al*., 2018). The pupae lifetime differ and ranges from 6-25 days depending on whether there is enough food and optimum favorable environmental conditions. When pupation is over, a moth emerges and then crawls to the soil surface before it flies and attaches itself to nearby plant materials (Opisa *et al*., 2018). These moths are initially sexually immature and feed for a period of 2-6 days before they begin copulating to initiate the egg-laying process, as observed in the study by Opisa *et al*. (2019). Females lay eggs in batches of about 300 to 1500 throughout their lifetime. These moths have the capacity to undergo 5-14 reproductive cycles per year (Opisa *et al*., 2018).

2.4 Fall armyworm description and life cycle

Under favorable environmental conditions, the FAW life cycle generally spans about 30 days from egg to adult emergence (Plate 2.1). However, during the summer season, this cycle can extend and take between 60 to 90 days (Tindo *et al*., 2017). Notably, FAW does not possess the ability to enter diapause, a state of dormancy or hibernation commonly seen in some insects, which allows it to remain active and invasive throughout the year. In environmentally friendly or suitable areas, migratory populations may initially appear in relatively small numbers, but they can potentially establish themselves rapidly and become persistent or difficult to eradicate (Deshmukh *et al*., 2021).

2.4.1 Egg stage

The egg is the initial stage of fall armyworm (FAW) development, as described by Sisay *et al*. (2018), at this stage, the eggs are characterized by their dome-shaped structure, featuring a flattened base that curves upwards to form a broadly rounded point at the top. These eggs have dimensions of approximately 0.5 mm in diameter length while the height is about 0.4 mm (Plate a). Egg mass size can vary, but it is most commonly observed to contain between 100 to 300 eggs. On average, each female FAW lays around 1,500 eggs, with some individuals capable of laying as many as over 2,200 eggs (Akutse *et al*., 2019). The egg masses are typically dropped in layers and are blowout over a single layer, often attached to the undersides of foliage.

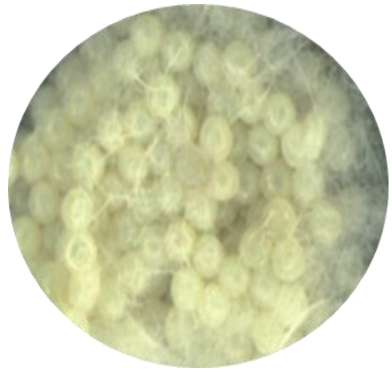


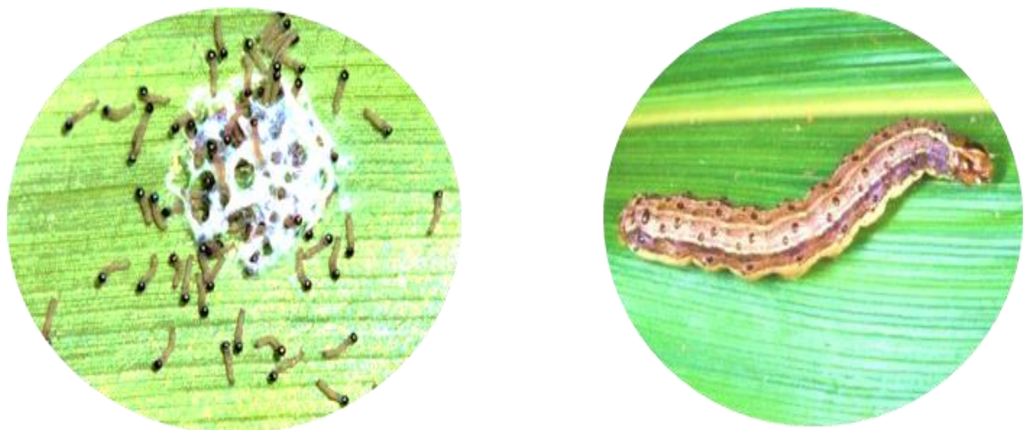
Plate 2.1: Fall armyworm eggs (Deshmukh *et al*., 2021).

2.4.2 Larval stage

This stage typically has six instars (Opisa *et al*., 2018). The youngest instar is light-green with a black head but then it turns orange in color as it molts to second instar (Opisa *et al*., 2018). The head capsule width of the FAW varies as the larvae progress through instars 1 to 6, ranging from approximately 0.5 mm to 2.6 mm. When fully grown, in instar 1, it measures about 1 mm, and in instar 6, it reaches lengths of around 45 mm (Plate 2.2 a). During the second and third in stars, the upper surface of the body turns brownish in color with the formation of lateral white lines (Plate 2.2 b) according to Deshmukh *et al*. (2021).

As the larvae continues to develop, the head turns red brown with a mottled appearance of white. The body takes on a brownish color with raised spots, typically dark in color and bearing spines. The complete developed larva may have a white inverted "Y" marking on its face, and when closely examined, its epidermis appears rough or granular in texture. However, to the touch, it doesn't feel rough because it lacks microspines. In some cases, a brownish form larva may appear mostly green dorsally, with pale elevated spots rather than dark ones.

One notable characteristic is the four prominent spots on the dorsal surface of its body. During the bright and clear parts of the day, larvae usually hide themselves. The life time is approximately 14 days during warm summer months and extends to around 30 days during cooler weather, as described by Deshmukh *et al*. (2021).



1st instar larvae (a) 4th instar larvae (b)

Plate 2.2: Fall armyworm larvae (Deshmukh *et al*., 2021).

2.4.3 Pupal stage

The larvae usually burrow into the soil at depths ranging from 2 to 8 centimeters, where they create a cocoon by binding soil particles together with silk (Munywoki *et al*., 2022). The cocoon has an oval shape and measures approximately 20 to 30 millimeters in length (Plate 2.3). If the soil is too compacted, larvae may construct a cocoon on the soil surface by weaving together leaf debris and other available materials, as described by Deshmukh *et al*. (2021).

The pupa is red brown in color, with dimensions of 15 to 19 mm in length and a width of approximately 5 mm. The life time of the pupa varies, taking approximately from 8 to 9 days in the summer seasons, though it may extend to 20 to 30 days during winter. This stage of the FAW is not able to withstand prolonged harsh colder seasons (Deshmukh *et al*., 2021).

Plate 2.3: Fall armyworm pupae (Deshmukh *et al*., 2021).

2.4.4 Adult stage

Fall armyworm adult moth's wings are typically within the range of 30 to 40 millimeters, as illustrated in Plate 2.4, according to Tindo *et al*. (2017). In male moths, the forewings are characterized by a shading of gray brown in color, with triangular white spots located at the wing's tip and near its center. Female moths, on the other hand, have less distinctive marks on their forewings, which may range from greyish brown to a fine mottling brown, as described by Deshmukh *et al*. (2021).

Adult FAW are vigorously active during warm times, particularly at night. Following a pre-oviposition period of 3 to 4 days, female moths typically deposit the majority of their eggs during the first 3 to 6 days of their adult life, although some egg-laying can continue for up to 3 weeks, according to Tindo *et al*. (2017). Adult fall armyworm moths typically live around 10 days on average, with individual lifespans varying from about 7 to 21 days (Deshmukh *et al*., 2021).

Plate 2.4: Fall armyworm adult moth (Deshmukh *et al*., 2021).

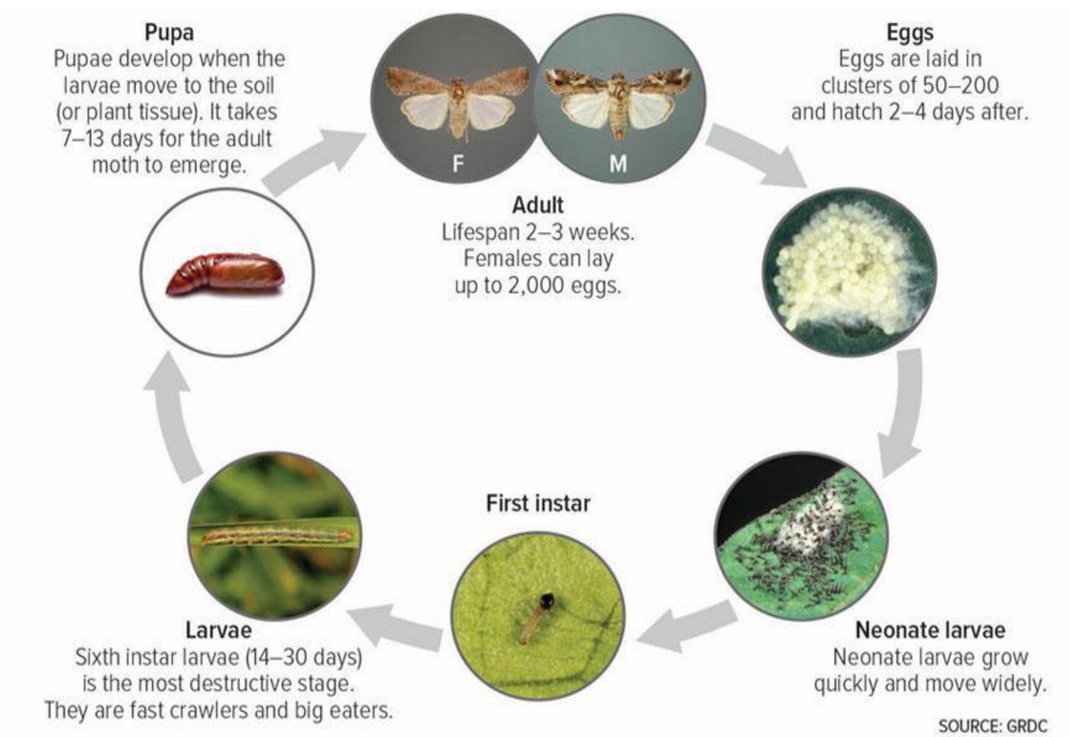


Plate 2.5: Fall armyworm life cycle (Deshmukh *et al*., 2021).

2.5 Taxonomy of *Spodoptera frugiperda*

Spodoptera frugiperda belong to the order Lepidoptera that has four main suborders; Zeugloptera, Aglossara, Heterobathmiina and Glossata which have 126 families with over 200 genera and over 160,000 species (Prasanna *et al*., 2018). The Fall Armyworm exhibits two sub-populations that are biologically similar though they differ in their plant preferences and certain physical characteristics. The first sub-population is known as the rice-strain (R-strain), which primarily favors millet and grass species as its host plants (Munywoki *et al*., 2022). The second sub-population is called the corn-strain (C-strain), and it favors maize and sorghum as its host plants. These two strains of FAW share a similar appearance but have distinct host plant preferences and ecological adaptations (Lacey *et al*., 2015).

2.6 Control of *Spodoptera frugiperda*

Fall armyworm display distinguishing varied characteristics that create prospects for control method (Day et al., 2017a). Their management is important in maintaining maize quality and quantity. Documented methods for control of maize FAW pests include cultural, biological, IPM and chemical (Lacey *et al*., 2015) as well as usage of naturally occurring products (Chandler *et al*., 2011).

2.6.1 Cultural and physical control

Cultural and physical control methods involve implementing continuous sanitation practices, which include collection of infested maize organs and burying and/or burning them to destroy soil-borne eggs. These measures are intended to decrease the population of FAW in the area (Awata *et al*., 2019). Crop rotation with solanaceous is highly recommended to manage the pest (Bawa, 2016). Crop rotation limitation is that many farmers have small field sizes, which makes it difficult for them to afford practicing long-term crop rotation (Bawa, 2016). Research conducted in Massachusetts and Oklahoma indicates that staking has been found to reduce disease incidence (Kinyumu *et al*., 2018). The incorporation of decomposed organic matter is a widely recommended practice in organic farming systems and serves as a promising control strategy. Soil amendments improve host nutrition and create heat during decomposition, which helps suppress soil-borne pathogens (Bawa, 2016).

Therefore, good farm management can highly help to control pests of maize crop (Muriithi *et al*., 2016; Prasanna *et al*., 2018). In regions with low labor costs, physical control methods, such as squashing egg masses and manually removing FAW larvae, are utilized. While such methods may be practical for small-scale farmers, they are generally unsustainable for large-scale production due to their high labor demands (Shiferaw *et al*., 2011). Consequently, ecological and integrated approaches are advocated for long-term pest control (Munywoki *et al*., 2022).

2.6.2 Chemical control

The use of synthetic insecticides remains a common practice for managing pests and diseases in maize (Kumela *et al*., 2019). Nevertheless, this method is increasingly being discouraged due to concerns over risks of human toxicity, environmental pollution, development of pest resistance, and the negative impact on beneficial organisms, including natural enemies of the FAW (Opisa *et al*., 2018).

Chemical pest control often relies on the widespread use of broad-spectrum organophosphorus insecticides, such as dichlorvos. These insecticides are formulated to target a wide range of pests, making them effective against various types of insects. However, it's important to note that while broad-spectrum insecticides can be effective in pest control, they can also have negative environmental impacts and may harm non-target species, including beneficial insects, wildlife, and even humans if not used carefully and responsibly (Munywoki *et al*., 2022). Hence, integrated pest management (IPM) approaches, which integrate biological control and cultural practices, are increasingly advocated to reduce dependency on chemical pesticides and mitigate their environmental impacts (Lacey *et al*., 2015).

2.6.3 Use of resistant varieties

The most cost-effective and environmentally sustainable approach for maize pest control is by utilizing resistant cultivars whenever they are available (Bawa, 2016; Lacey *et al*., 2015). However, breeding for resistance can pose significant challenges, particularly in the absence of a dominant resistance gene. Additionally, there is a possibility of new cases of pests overcoming host resistance, as reported by Badu-Apraku and Fakorede. (2017). Despite these challenges, the advantage of this method lies in its cost-effectiveness when compared to chemical pest control. Furthermore, it helps enhance the cultivation of previously infested fields.

2.6.4 Behavioral control

This control strategy involves manipulating insect behavior by using synthetic lure pheromones or stimulatory food attractants derived from proteins or fruit volatiles

(Chiwamba and Nkunika, 2019). These attractants are often deployed in bait traps, which are enhanced with visual cues such as colors (yellow, green, red) to improve pest detection, as outlined in the study by Lacey *et al*., (2015). In other cases, male insect lure traps are commonly designed and contaminated with pathogenic spores (Opisa *et al*., 2019). Once the male moths leave the trap, they spread the fungal spores to the FAW population, thereby extending the biological control impact (Opisa *et al*., 2019).

2.6.5 Biological control method

This approach centers on utilizing naturally occurring organisms, including parasitoids, predators, microorganisms, and pathogens to manage pests (Day *et al*., 2017b). Predators such as crickets play a crucial role in the biological control of FAW. Predatory insects and mites actively consume FAW larvae, serving as natural enemies. On the other hand, parasitoids such as *Cotesia icipe*, *Telenomus remus* and *Trichogramma* spp. lay their eggs inside or attach them to a single FAW host leading to the eventual death of the host as the parasitoid larvae develop. Both predators and parasitoids are important components of FAW's natural enemies, contributing to its biological control (Sisay *et al*., 2019). During their growth, the resulting larvae nourish themselves by consuming the tissues of the host until they reach full maturity and undergo pupation (Lacey *et al*., 2015). The larvae of parasitoids invariably result in the death of their host during their development, while parasites and microbial pathogens induce lethal infections (Sisay *et al*., 2019). Biological control is considered a fundamental component of Integrated Pest Management (IPM) and a crucial element of a harmonious and effective approach to suppress pests. By adopting such methods, higher profits can be achieved, while maintaining the environment (Chandler *et al*., 2011; Sisay *et al*., 2018).

2.6.6 Use of biopesticides

Biopesticides are naturally occurring products derived from living microorganisms or their byproducts, developed and marketed specifically for pest control purposes (Chandler *et al*., 2011). Microbial based biopesticides are also referred to as biocontrol agents (BCAs). Biopesticides provide a greener and more sustainable alternative to synthetic pesticides, making them a focal point for pest management innovation (Chandler *et al*., 2011). Unlike synthetic pesticides, which often work via neurotoxicity, biopesticides control pest in a myriad of actions including disrupting mating processes, induce anti-feedant activities, suffocate and predispose the insects to desiccation. They are highly target-specific, not harmful to the environment and users, and do not leave residues. In addition, they do not disrupt ecological balance between the pests and beneficial organisms and have short re-entry intervals after application (Lacey *et* al., 2015; Mweke *et al*., 2018). The environmentally friendly characteristics of biopesticides have intensified the search for alternative pest control methods, further fueled by rising consumer demand for reduced pesticide use and growing interest in organic products.

2.6.7 Entomopathogenic fungi

Bacteria, entomophagous nematodes, viruses, entomopathogenic fungi, and protozoa are the major entomopathogens of arthropods and have been tested for biocontrol for many years (Lacey *et al*., 2015). Entomopathogenic fungi (EPF) that are parasites of arthropods are valuable biocontrol agents and are compatible with integrated pest management strategies (Lopes *et al*., 2011). Entomopathogenic fungi represent a diverse group of nearly 1000 species that have been described from many taxonomical divisions of the fungal kingdom. Majority of the fungi used for biocontrol are Entomophthorales and Hyphomycetes of classes Zygomycota and Deuteromycota, respectively (Naranjo-Ortiz *et al*., 2019).

Unlike other biocontrol agents, EPF do not need to be ingested but instead invade the host directly through the exoskeleton. Species such as *Metarhizium anisopliae*, have the capability to infect not only the feeding stages of pests but also non-feeding stages like eggs and pupae, as described by Chandler *et al*. (2011). They offer the advantage of being amenable to mass production when required in large quantities. Furthermore, they exhibit host specificity, targeting specific pest species, and can thrive in diverse ecological conditions. This makes them valuable tools for environmentally friendly and sustainable pest management strategies (Lacey *et al*., 2015).

Metarhizium anisopliae belongs to the Hyphomycetes class of fungi. These fungi are known for being broad spectrum and are among the most commonly utilized EPF for management of pathogenic pests. Their ability to infect a variety of insect species makes them valuable assets in IPM strategies. Numerous myco-pesticides based on EPF have been created, prepared, and introduced to the market in various countries to manage economically significant pests and insects. In Kenya, *Metarhizium anisopliae* isolates have been used by the ICIPE in collaboration with Real IPM Company (Kenya) Ltd to develop biopesticides for *Spodoptera frugiperda* (fall armyworm, FAW) management. These biopesticides, formulated by *icipe*, have been successfully commercialized through public-private partnerships (Opisa *et al*., 2018). Certain isolates of EPF from the *icipe* Arthropod

Germplasm have demonstrated pathogenicity against a wide range of insect pests (Bayissa *et al*., 2017).

2.6.7.1 Mode of action of EPF

Entomopathogenic fungi exhibit an exceptional mode of action when they come into contact with the insect's hemocoel by penetrating through the cuticle, as described by Al *et al*. (2019). Infection and dissemination are initiated by asexual spore production, which allows the fungi to infect and spread within the host insect population, as noted by Al *et al*. (2019). The infection process commences when spores come contact with the host's cuticle. Subsequently, the conidia adhere to the cuticle by secreting adhesive mucus, facilitating their attachment to the host's surface (Lacey *et al*., 2015). Successful adhesion is critical for fungal virulence. Once the conidium has successfully adhered on the pest’s cuticle, it germinates into a tube-like an appressorium which then penetrates through the cuticle through either via infection pegs produced by appressoria or by direct entry (Lacey *et al*., 2015). The infection process involves a blend of mechanical and enzymatic actions. The fungus secretes enzymes such as lipases, proteases, and chitinases, which serve to hydrolyze the insect's cuticle, as detailed by Xiao *et al*. (2012). After penetrating into the insect's hemocoel, the germ tube encounters the host's cellular defense mechanisms, such as the activation of a phenol-oxidase system that generates phenols (melanin), and the existence of protease inhibitors within the cuticle. These defenses may limit the activity of fungal enzymes, as observed by Awata *et al*. (2019). The insect also forms nodules using hemocytes to trap fungal fragments, as described by Ment e*t al*. (2010). Despite these defenses, EPF often overcome the host immune system by producing mycotoxins, such as destruxins and desmethyl-destruxins, which exhibit strong insecticidal activity (Opisa *et al*., 2018; Jayshree *et al*., 2020).

Once inside the host insect, the fungus undergoes a transformation to a yeast-like hyphal form, which spreads and grow in the hemolymph and reproduce through budding, as described by Jayshree *et al*. (2020). After a certain period, the fungus reverts to a filamentous stage and begins to consume internal tissues and organs, and hence host mortality. Eventually, the fungus mycelia grow to cover the entire host body and, under suitable environmental conditions, form infectious spores (Jayshree *et al*., 2020). The fungal hyphae produce conidiospores that mature and are released into the environment and they then dispersed by any means of dispersion available (Akutse *et al*., 2019).

2.6.8 Use of sex pheromones

A pheromone is a specific semiochemical that is naturally emitted by an organism to trigger a behavioral response such as defense, mating or alarm in other organisms (Mkiga *et al*., 2021). The sex pheromone of *S. frugiperda* has a primary component (Z)-9-tetradecenyl acetate (Z9-14:Ac), alongside other secondary components such as (Z)-7-dodecenyl acetate (Z9-12:Ac), (Z)-11-hexadecenyl acetate (Z11 16:Ac), and (Z)-9-dodecenyl acetate (Z9-12:Ac) (David *et al*., 2016) These components are essentials in attracting male FAW moths in the field. Various studies have demonstrated the efficacy of sex pheromone lures in monitoring FAW populations (Mkiga *et al*., 2021). For instance, a study by Mkiga *et al*. (2021) reported that water-pan traps baited with pheromone lures captured the highest number of FAW moths, confirming their potential for use in surveillance and early warning systems.

2.6.9 Integrated pest management (IPM)

In developing countries, IPM is recognized as an effective practical approach to enhance agricultural productivity and address environmental degradation (Lopes *et al*., 2011). Its goal is to enhance the productivity of commercially valuable agricultural crops (Pérez-Escamilla, 2017). Key practices in IPM include crop rotation, incorporation of organic matter, and the use of high-residue tillage to promote healthy soils (Pérez-Escamilla, 2017). Extensive research has been undertaken worldwide to investigate how pests can be controlled by using compost products. It has been demonstrated that composts can provide effective natural biological control against pests that reside in the soil and that affects the plant collars, roots, and foliage (Sisay *et al*., 2019). Integrating green manures and cover crops into crop rotations is a highly beneficial approach to enhance soil fertility, control weeds, and disrupt pest cycles. Having different crop species in the rotation and the application of manure and/or compost guarantees the availability of a wide variety of organic matter sources. Such diversity ensures a consistent supply of organic matter, fostering balanced soil mineral content and sustained nutrient release. This reduces leaching and the potential toxicity associated with synthetic fertilizers (Mkiga *et al*., 2021). IPM involves a thorough evaluation of all available pest control methods, followed by their strategic integration to slow pest proliferation. Its goal is to minimize the use of synthetic pesticides to economically viable levels while also reducing environmental and health risks (Chandler *et al*., 2011; Muriithi *et al*., 2016). Furthermore, it places emphasis on fostering the growth of robust crops and maintaining ecosystem integrity (Pérez-Escamilla, 2017).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

This study was conducted both in the laboratory settings at *icipe*, Nairobi, Kenya. (1.22° S, 36.89° E, 1600 meters above sea level) and under field conditions at two locations: Mbita Thomas Odhiambo Campus, Homabay County (0° 26′0 06.21″ S, 32° 88′ 60.23″ E, 1142 m) and Awendo-Migori County, Kenya (0.86° S, 34.57° E, 1411 m above sea level). The two field locations experience a bimodal rainfall pattern with an average annual precipitation of approximately 1000 mm and temperatures ranging from 15°C to 24°C.

3.2 Insect source and rearing conditions

Spodoptera frugiperda were sourced from breeding stock maintained at *icipe*. The adult moths (Plate 3.1) were provided with a 10% honey solution, which had been immersed in balls of cotton wool for their nourishment, following Akutse *et al*. (2019) procedures. The moths were exposed to maize leaves and butter paper for them to lay eggs that hatched to larvae. The resulting eggs and larvae were collected and then reared on an artificial diet as described by Prasanna *et al*. (2018). The insects were maintained under controlled environmental conditions: temperature at 25 ± 2°C, relative humidity at 45%, and a photoperiod of 12 hours light and 12 hours dark (L12:D12).

Plate 3.1: Adult *Spodoptera frugiperda* (Deshmukh *et al*., 2021)

3.3 Source and maintenance of fungal cultures

The *Metarhizium anisopliae* ICIPE 41 isolate (Plate 3.2) used in this study was obtained from the entomopathogen repository maintained at the Arthropod Pathology Unit (APU), *ICIPE*. Details regarding the isolate are provided in Table 3.1. The isolate was cultured on Sabouraud Dextrose Agar (SDA) in 90 mm petri dishes and incubated at controlled ambient temperatures of approximately 25 ± 2 °C for 2 to 3 weeks (Figure 3.2). Conidiospores were harvested by scraping the sporulated fungus surface with a sterile spatula.

For autodissemination experiments, a large quantity of conidia was produced following the method described by Opisa *et al*. (2019). Rice substrate was autoclaved for 1 hour at 121°C and inoculated with a three-day-old spore culture. The inoculated substrate bags were then placed in an incubator for 21 days at a temperature of 25 ± 2°C and a relative humidity (RH) ranging from 40% to 70%. Afterward, the fungal spores were allowed to desiccate for 5 days at ambient room temperature. Conidia were collected by sieving the substrate through a 295 µm mesh sieve and stored in plastic bags inside a refrigerator at 4 ± 2°C for a maximum period of three weeks.

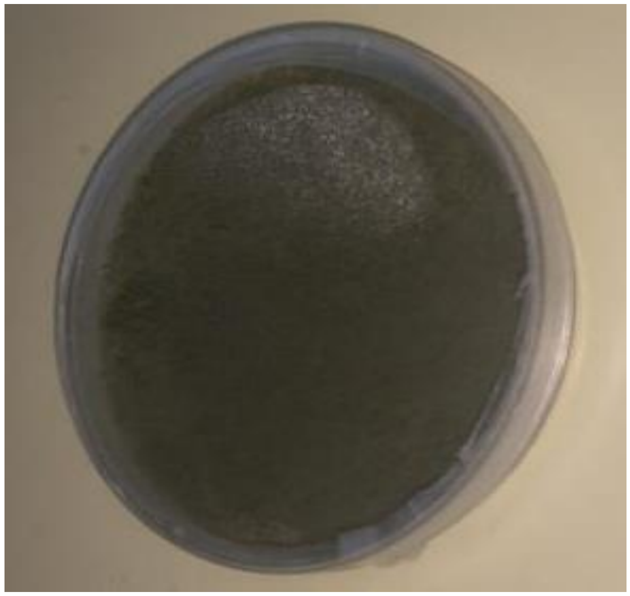


Plate 3.2: *Metarrhizium anisopliae* isolate subcultured on SDA media

Table 3.1: Fungal isolate identification and its germination rate on SDA at 26 ± 2 °C.

Fungal spp.	**Isolate**	**Source**	**Locality/Country**	**Year**	**Germination ± SE (%)**
Metarhizium anisopliae	ICIPE 41	Soil	Lemba (DRC)	1990	98.2 ± 2.2

Means ± SE by Tukey's test at $p < 0.05$

3.4 Conidial viability test

To determine the viability, a three-week-old culture was scraped, and then inoculum was put in 10 mL of sterile in a universal bottle containing glass beads with a diameter of 3 mm with 0.01% Triton water in a universal bottle, creating a concentrated solution which was vortexed for a duration of 4 minutes to achieve a homogeneous distribution of conidia. To achieve a final concentration of 3 x 10^6 spores per ml, the suspension was diluted from the stock solution and quantified using a hemocytometer.

A 100 µl aliquot from the conidial suspension was evenly spread on clean SDA plates and then incubated at 25 ± 2ºC for 16 hours, after which fungal growth was halted by adding lacto-phenol cotton blue. To each plate, four sterilized cover slips were placed on each plate, and observations were noted for each one using a light microscope at 400× magnification. Viability was assessed by counting a total of 100 conidiospores, which included both germinated and non-germinated spores. Observations were made on the length of the germ tube and when it had reached twice the size conidia's diameter, the fungal were considered viable.

3.5 Effect of *Metarhizium anisopliae* ICIPE 41 on *Spodoptera frugiperda*

3.5.1 Experimental design

The experimental design consisted of ten (10) FAW moths per replicate, which were selected randomly, with four replicates. These moths were subsequently infected with the *M. anisopliae* ICIPE 41 isolate and placed in cages. To compare means and assess the factor of virulence, analysis of variance (ANOVA) was employed.

3.5.2 Experimental procedure

Spodoptera frugiperda moths were inoculated with 1 gram of conidiospores of the isolate using a velvet material. This velvet material covered an inoculating chamber created from a tube-shaped plastic measuring 9 x 13 cm. One end of the plastic tube was fitted with a nylon mesh (5 mm diameter) to allow light and fresh air for the moths, while the other end was sealed with a plastic lid (Figure 3.2).

From a cohort of 40 FAW moths aged between 2 and 5 days, 10 adult moths were randomly selected and placed into the contaminating chamber (Figures 3.3 and 3.4) for 3 minutes. Subsequently, the contaminated moths were relocated into cages (15 x 15 x 20 cm) and provided with honey-soaked cotton wool for feeding. For the control experiment, moths were handled similarly but exposed to a clean, non-contaminated chamber.

The FAW moths were maintained at ambient temperatures, and their mortality data were recorded over a period of seven days. To determine mycosis of the cadavers, they were initially washed, surface sterilized in 1-2% sodium hypochlorite for 3 minutes, and then immersed in alcohol for another 1-2 minutes, and finally rinsed in sterile water. The cadavers were then placed in 9 cm petri dishes containing dampened filter paper. The presence of fungal growth and/or sporulation on the surface of the cadavers confirmed successful mycosis.

3.5.3 Pathogenicity of *Metarhizium anisopliae* on adults' moths

To assess the pathogenicity of *Metarhizium anisopliae* ICIPE 41 on adult *S. frugiperda*, moths aged 4–10 days were exposed to the fungal spores. Moths' mortality was recorded as described previously (section 3.3.2). For the control, ten moths were introduced into a sterilized velvet-lined chamber without fungal spores for 3 minutes. After the exposure, the moths were removed from the chamber and moved to a 20 × 20 × 20 cm cage containing honey-soaked cotton wool (Plates 3.3 – 3.5). Mortality effects of the fungal isolate on the moths was recorded after every 24 hours (Akutse *et al*., 2019).

Plate 3.3: Inoculating chamber

Plate 3.4: Inoculating chamber spread with the spores

Plate 3.5: A cage containing the FAW moths

3.5.4 Mass production of fungus

Fully mature conidiospores of the fungal isolate were mass-produced using a diphasic batch system, as outlined by Lin *et al*. (2017). Initially, a loopful of conidiospores from a plate culture was inoculated into a sterilized 50 ml liquid broth consisting of 30 g of yeast extract, 30 g of glucose, 15 g of peptone, and 1000 ml of distilled water, all contained in a shake flask. They were then incubated at room temperature in a shaker incubator (Figure 3.5) (Akutse *et al*., 2019).

The resultant liquid biomass was transferred into pre-soaked, sterilized rice (1 kg) in Milner plastic bags. The rice had been sterilized at 121 ºC and 15 psi, following the procedure described by Kiriga *et al*. (2018). The bags containing the substrate were then incubated at 26 ± 2 ºC for 10 days (refer to Plate 3.7). After incubation, the rice substrate was transferred to fresh, clean plastic basins for air drying at room temperature for one week, using a dehumidifier (Plate 3.7). The dried spores were separated by passing the substrate through a 295 µm mesh sieve and then dried in a desiccator containing anhydrous silica gel for two days until they reached a relative humidity of 5% to 13%. Finally, the dried conidia (Plate 3.8) were stored in a refrigerator at temperatures between 0 and 4 ºC.

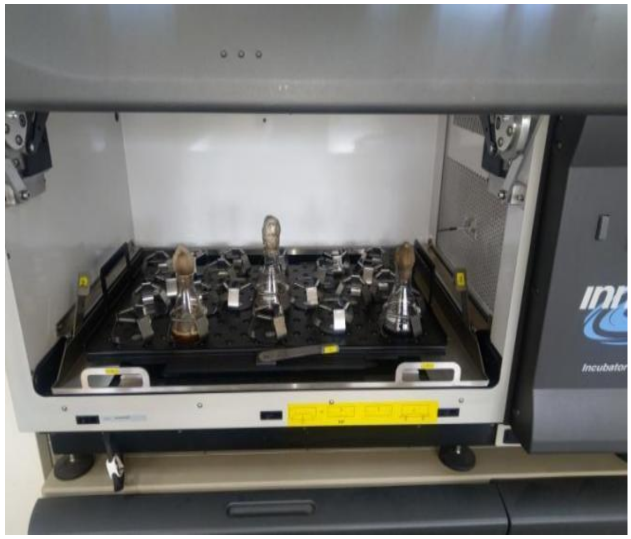


Plate 3.6: Starter cultures in orbital shaker incubator

Plate 3.7: Mass production using rice in Milner bags

Plate 3.8: De-hydration *Metarhizium anisopliae* ICIPE 41

3.6 Laboratory Experiment

3.6.1 Compatibility test for *Metarhizium anisopliae* ICIPE 41 with FAW sex pheromone in the laboratory

Fall armyworm sex pheromone along-side delta traps were procured from Agroduka suppliers, Nairobi. In the laboratory, a mating response in male FAW was triggered to facilitate the isolation and identification of the sex pheromone, (Z)-9-tetradecenyl acetate (Z9-14: Ac), from female moths. To assess the antifungal properties of the pheromone and the survival of conidiospores in the compatibility study, the protocol detailed by David *et al*. (2016) was employed.

Mature dry conidia of *M. anisopliae* ICIPE 41 from clean plates were taken using a spatula and dissolved using a vortex for 5 minutes in sterile Triton water. From the mixture, 0.1ml was aliquoted and diluted in another 9 mL of sterile 0.01% Triton in a universal bottle. The diluted mixture was further vortexed for 5 minutes to form homogenous mixture. Quantification was done under the light microscope (×400) and the quantity of spores from both chambers was recorded to come up with stock concentration and volume. The stock volume was aliquoted from stock solution and diluted into ten universal bottles containing sterile 0.01% Triton water. The mixture was vortex for about 5 minutes and then filtered through 0.45 µm membrane filters using a pressure station (Plate 3.9a) that only allowed water molecules to pass through, and fungal spores to remain as residue. The filter papers with spores were then kept in sterile containers for 30 minutes for them air-dry. Out of the ten membrane filters, five were placed in a desiccator containing the pheromone (treatment 1) (Plate 3.9-b) and the other five were placed in a desiccator with no pheromone (control) (Plate 3.9-c). The experiment ran for eight (8) days with germination test conducted on 1st, 2nd, 3rd, 5th and 8th day. Membrane filters were dissolved on Triton water and 0.1ml was pipetted and then spread plated on SDA media. A total of four plates of the isolate were cultured and a germination test was done. The cultured spores were incubated at 26 ± 2 ^{o}C for 18 - 24 hours, and then lacto-phenol cotton blue was put on the plates to terminate fungal growth. Four sterilized cover slips were placed on each plate, and spore germination was observed under a microscope at 400× magnification. Germination was ascertained by assessing the percentage of conidia that had sprouted compared to those that had not on each slide. Propagules were deemed germinated when germ-tube had reached approximately twice the diameter of the conidia

3.6.2 *Spodoptera frugiperda* pheromone effect on formulated conidial viability and germ tube length

To evaluate the antifungal properties of the FALLTRACK lure and the viability of the conidia in the formulations, a compatibility test was conducted following the method outlined by David *et al*. (2016). Mature dry conidiospores from clean plates were taken using a spatula and dissolved using a vortex for 5 minutes in a sterile universal bottle containing 0.01% Triton. From the mixture, 0.1ml was aliquoted and diluted in another 9 mL of sterilized sterile Triton water in a universal bottle. The diluted mixture was vortexed for 5 minutes to form a uniform suspension.

Quantification was done under the light microscope (×400) and the number of spores from both chambers was recorded to come up with stock concentration and volume. The stock volume was aliquoted from stock solution and diluted into ten universal bottles containing triton water and 1 mL of the specific oil. Oil formulations were prepared by mixing oil at concentration of 25% with conidial suspension while aqueous formulations had only distilled water and sterile 0.01% Triton. As previously outlined, a conidial suspension was prepared and adjusted to a concentration of 1×10^8 conidia ml^{-1}.

To capture spores from the formulation, 10 ml of the mixture was filtered using a nitrocellulose filter membrane (Plate 3.9a). This was achieved by pouring the formulation through a filter holder unit (MFS) while applying an aspirator vacuum. The nitrocellulose filter membranes were air-dried for 30 minutes in a laminar flow cabinet and subsequently transferred to glass desiccators (2.5 L) for exposure to the attractants. Nitrocellulose membranes with formulated fungus were exposed to FALLTRACK lure (Plate 3.9b) and the assessments for germination were carried out at various intervals of 1, 2, 3, 6 and 8 days. An untreated control without the FALLTRACK lure was included in the experiment, (Plate 3.9c). The treatments were randomized, and the experiment was replicated three times.

For conidial germination determination, the filter membranes with spores from desiccators were transferred into 10 ml sterile distilled water solution with 0.01% Triton X-100 and vortexed for 3 minutes to detach conidia. A 0.1 ml of the suspension was titrated to 3 ×

10^6 conidia ml^{-1} and spread onto SDA plates. The fungal plates were then placed in an incubator at 26 ± 2°C and observed after 18 - 24 hours to assess conidial germination. Subsequently, the length of germ tubes was measured. o halt growth, lactophenol cotton blue was added to any plates not assessed the same day. Germination was determined by observing 100 spores per plate and measuring germ tube lengths for five randomly selected conidia per cover slip (22 × 22 mm). Measurements were replicated three times per treatment to ensure accuracy and reliability.

a)

b)

c)

Plate 3. 9: (a)Vacuum filtration of conidia of *M. anisopliae*, (b) Desiccation of conidia of *M. anisopliae* ICIPE 41 using FAW sex pheromone Falltrack, (c) Desiccation of conidia of *M. anisopliae* ICIPE 41 without the FAW sex pheromone (control)

3.7 Treatments layout

The experiment was arranged in the laboratory and consisted of five different treatments, four of the five treatments were formulations while the fifth was a control. The treatments included: (T1) 25% Canola oil formulation, (T2) 25% Corn oil formulation, (T3) 25% Olive oil formulation, (T4) Aqueous formulation, and (T5) the Control experiment. All treatments used *M. anisopliae* at 1×10^8 conidia/ml. For the aqueous formulations, spores were suspended in water 0.1% nutrient agar (Mweke *et al*., 2019), whereas for the oil formulations, spores were suspended into 25% of canola oil, corn oil and olive oil with similar proportions of nutrient agar as described above in aqueous formulation. Controls were sprayed with water containing 0.1% nutrient agar without fungal conidia. Out of the four formulations, the most effective treatment was tested in the field with Spinetoram based pesticide alongside the commercial Mazao Achieve® biopesticide as a positive control, and a control (water only).

3.7.1 Assessment of cumulated larval mortality and lethal time (LT_{50})

The concentration of 1×10^8 conidia was formulated in an aqueous solution containing Triton X-100 and distilled water at a specific ratio of 1.5:98.5, respectively, and the emulsifiable formulations (Triton X-100 + oil + water at the ratio of 1.5:2.5:6.0). Groups of 10 second-instar larvae *S. frugiperda* were used for each treatment with each having four replicates. A hand sprayer discharging fine droplets of about 40 µm diameter was used to spray both small sized and young in age leaves and larvae with each formulation. A single cut maize leaf already sprayed was transferred to petri dishes and labelled according to the treatment applied to the leaves and larvae. The larvae were then then introduced to feed on already fungal contaminated leaves and their mortalities were recorded every day for seven consecutive days. Maize leaves were replaced daily to provide soft food to the larvae. Lethal time (LT_{50}) of the various formulations was evaluated on their effectiveness.

3.7.2 Assessment of effects of the formulations on FAW's parasitoids

Fall armyworm aggressive parasitoids *Cotesia icipe* (larval-parasitoid) and *Telenomous remus* and *Trichogramma* spp. (egg-parasitoids) were used to test the effects of the various treatments. The experiment was set to have each treatment replicated four times with twenty parasitoids and sixty hosts (1:3 ratio). For the larval-parasitoids, maize leaves were cut into small sizes and larvae were artificially sprayed with a treatment and then introduced to lunch boxes to feed and stabilize before the introduction of the parasitoids.

Both the parasitoids and host were allowed to interact, and cases of parasitoid mortality were recorded daily. For the egg-parasitoids, FAW eggs were sprayed with the formulations and placed in vials containing the parasitoids and small droplets of 10% honey as source of food for parasitoids.

3.8 Field Experiments

3.8.1 Mazao Achieve® biopesticide

Mazao Achieve® (Plate 3.10), is a fungal-based biopesticide formulated with *M. anisopliae* ICIPE 78. When fungus and insect come into contact, the conidia germinate to hyphae within two hours, penetrate the cuticle, colonize the hemolymph of the insect and eventually killing the insect within 3 to 5 days. This product was primarily registered and marketed by Real IPM-Biobest for red spider mite management. The biopesticide demonstrates high effectiveness against FAW eggs and neonate larvae, effectively preventing the build-up of the pest population (Akutse *et al*., 2019). Field application involved mixing 40 ml of the product with 20 L of clean water in a knapsack sprayer.

Plate 3.10: Mazao Achieve® biopesticide from Real IPM-Biobest

3.8.2 Radiant pesticide 120 SC- spinetoram (XDE-175J)

Spinetoram (XDE-175) (Plate 3.11) is a multicomponent tetracyclic macrolide pesticide designed for the management of lepidopteran pests. It comprises two closely related active ingredients, XDE-175-J. The formulation was prepared by diluting 10 mL of the pesticide in 20 L of clean water using a knapsack sprayer. The mixture was vigorously shaken to ensure a homogeneous blend before application.

Plate 3.11: Radiant 120 SC (Spinetoram XDE-175-J**)**

3.8.3 Experimental design

The field experiments were conducted in two distinct agroecological zones, Homabay county (Mbita) and Migori county (Awendo). Within each of these zones, the experimental trials included four distinct treatments: (i) Corn oil-based formulation, (ii**)** Mazao Achieve® biopesticide, (iii) Spinetoram (synthetic pesticide), and (iv) Control. To prepare the corn oil formulation, one gram of dried fungal conidia was mixed with 2% (v/v) corn oil in sterile water containing 1×10^8 conidia/mL. Mazao Achieve® and Spinetoram were prepared at 40 mL and 10 mL per 20 L of water, respectively. The control treatment involved distilled water without any active ingredients. Treatments were first applied one week after germination, specifically targeting the leaf collar. Subsequent applications were conducted at two-week intervals. Before each application, the level of FAW infestation was assessed daily for seven days using pheromone traps that captured adult moths. All field treatments were arranged in a randomized complete block design (RCBD) and replicated four times per treatment.

3.8.4 Treatment application

Each experimental plot was applied with respective treatment using handheld sprayer and left for 24 hours. Afterwards, 20 larvae were collected from each plot and placed in petri dishes and transferred to the laboratory. Fresh and young maize leaves cut to small sizes (5 cm) from respective plots were placed in each petri dish (Plates 3.12 – 3.15) as source of food to larvae. The leaves were replaced daily to ensure a continuous food supply. Each treatment had four replicates, each with 20 larvae.

Plate 3.12: Spinetoram pesticide plot at *icipe*, Mbita campus

Plate 3.13: Corn oil formulation plot at *icipe*, Mbita campus

Plate 3. 14: Mazao Achieve® plot at *icipe*, Mbita campus

Plate 3.15: Control plot at Awendo, Migori County

3.8.5 Attraction of *Spodoptera frugiperda* moths

A blue sticky patch measuring 5 cm × 10 cm was positioned alongside a delta trap device, either with or without *S. frugiperda* pheromone bait, to monitor the number of moths attracted to the device. For insects that stuck in the stick cards, kerosene was employed to dissolve them off and others were removed using forceps. Subsequently, the moths were cleared, mounted on slides, and their numbers were recorded

.3.8.6 *Metarhizium anisopliae* ICIPE 41 conidia acquisition after field exposure

A delta trap device was prepared and hung in the field to assess the efficiency of *S. frugiperda* sex pheromone in enhancing conidial acquisition. One delta trap device was used per experimental plot, positioned at the center and approximately 1m above the ground using a support stand. These devices were divided into two treatment groups: (i) Fungus + Sex Pheromone Group: Delta trap devices contaminated with both the fungus and *S. frugiperda* sex pheromone were placed in this group and (ii) Fungus only group: Non-fungal delta trap devices were placed in this group and served as the control.

The traps were left in place to capture moths. Captured moths were individually transferred into 2-mL cryogenic tubes containing 1 mL of sterile 0.05% Triton X-100 solution. Tubes were vortexed for 2–3 minutes to dislodge conidia, which were then quantified using a hemocytometer.

For cadavers that were found dead and infected with the fungus, they were surface sterilized using 70% alcohol and rinsed three times with distilled water. These cadavers were then individually placed in Petri dishes lined with sterile, moistened filter paper and securely sealed with parafilm. The number of cadavers displaying mycosis (fungal infection) was counted and recorded after a 7-day incubation period.

3.8.7 FAW mortality induced by *Metarhizium anisopliae* ICIPE 41 formulation

Larvae collected from the field 24 hours after application were reared at the laboratory where mortality incidence was recorded daily for 7 days (Mweke *et al*., 2019). Mycosis test was conducted for the dead cadavers. Dead cadavers were sterilized on their body surface, then placed in Petri dishes with sterile filter paper moistened with water, and left at room temperature to evaluate mycosis (Mweke *et al*., 2019). Mortality was determined by examining the presence of hyphae and sporulation on the deceased cadavers using a dissecting microscope, and slides were prepared to confirm the presence of the applied fungus (Mweke *et al*., 2018).

3.8.8 Assessment of conidial persistence

Maize leaves sprayed with fungal formulation were cut into small pieces (about 5 cm long) weekly and placed in 10 mL sterile 0.01% Triton X-100 (v/v) in universal bottles containing glass beads and then vortexed for 5 minutes for the conidia on the leaves surface to dissolve. A volume of 100 µL was pipetted and spread plated onto clean SDA plates. Four SDA plates representing the four plots treated with fungal formulation were cultured and was tested for persistence. Two replicates were obtained from the individual plots. The viability test was done on day 0, 7, 14, 21 and 28 (from February 8^{th} to March 4^{th}, 2021). Pathogenicity was assessed by monitoring the mortality rate of FAW by taking records after every 24 hours.

3.8.9 *Spodoptera frugiperda* infestation and damage assessment

Natural field infestation by FAW was assessed before treatment by inspecting 10 maize crops in each plot to quantify the number of infested maize plants. The maize was then sprayed to the specific treatment. The assessments were conducted weekly for eight weeks. Pest incidence, damage level and yields were recorded 24 hours after each treatment.

3.9 Natural enemies' assessment

Fall armyworm natural enemies were mainly parasitoids. Incidence of parasitoids were counted and recorded (Sisay *et al*., 2018; Mweke *et al*., 2019). Egg-parasitoids (*Trichogramma* spp. and *Telenomus remus*) and larval-parasitoid (*Cotesia icipe*) were evaluated by collecting egg clusters and larvae from plots treated with the oil formulation. These were transferred into perforated Petri dishes and maintained under laboratory conditions. The emergence of parasitoid species was recorded during the observation period.

3.10 Maize yield assessment

Maize yields data was collected at crop maturity by cutting down the maize, harvestable ears and kernels as well as the multiplying rate of the ears per from each plot. Maize were sun dried, threshed and its quantity from each plot noted using electronic balance. Yields were calculated and expressed in kilograms per hectare (kg/ha).

3.11 Data analysis

Laboratory data on larval and parasitoid mortality were adjusted using Abbott's formula (Abbott, 1925), and their normality was assessed using the Shapiro-Wilk test (Shapiro and Wilk, 1965) before carrying out one-way analyses of variance (ANOVA). Conidial germination of fungal isolate, FAW infestation levels, counts of natural enemies, and maize grain yield were subjected to logistic linear mixed models using the *'glmer'* function from the *lme4* package in R. These models were used to assess the impact of attractant, exposure time, and their interaction. Pairwise comparisons of least squares mean were employed for mean separation, utilizing the *'lsmeans'* package. The Spearman's rank correlation was used to assess the relationship between conidial acquisition and germination. Data on interactions between *S. frugiperda* moths and traps were analyzed using a logistic linear mixed model with an intercept slope, employing the *'glmer'* function from the *lme4* package.This analysis aimed to evaluate the interaction between treatments and sampling dates. Parasitoid mortality and parasitism data were adjusted using Abbott's formula (Abbott, 1925), and their normality was assessed using the Shapiro-Wilk test (Shapiro and

Wilk, 1965) before undergoing one-way ANOVA. Reductions in moth captures relative to the control for each treatment were computed using Abbott's formula (Abbott, 1925). All data analyses were conducted using R (version 3.2.5) statistical software, utilizing packages provided by the R Development Core Team, (2014).

CHAPTER FOUR

RESULTS

4.1 Laboratory bioassays on the pathogenicity of fungal formulations

4.1.1 Assessment of viability of *Metarhizium anisopliae* ICIPE 41

The conidia of *M. anisopliae* ICIPE 41 exhibited 100% germination after 18 hours of incubation at 26 ± 2 °C, indicating full viability (Figure 4.1).

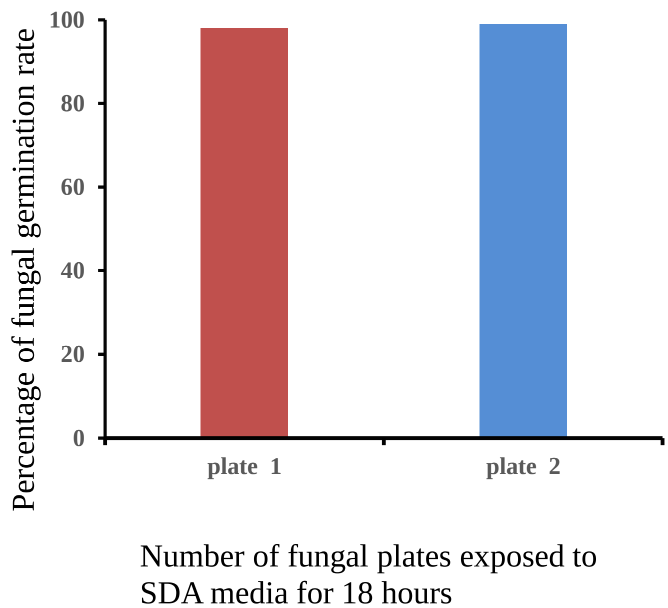


Figure 4.1: Viability of the *Metarhizium anisopliae* ICIPE 41 fungal isolate conidia growing on SDA media incubated at 26 ± 2 and determined after 18 hours

4.2.2 Compatibility of *Spodoptera frugiperda* sex pheromone and fungus under laboratory conditions

The *S. frugiperda* (FAW) sex pheromone did not significantly ($p = 0.518$) affect the spore viability in the laboratory. Nevertheless, spore germination of *M. anisopliae* ICIPE 41 did slightly decrease from 100% in day one to 95% on day 8 post treatment (Figure 4.2).

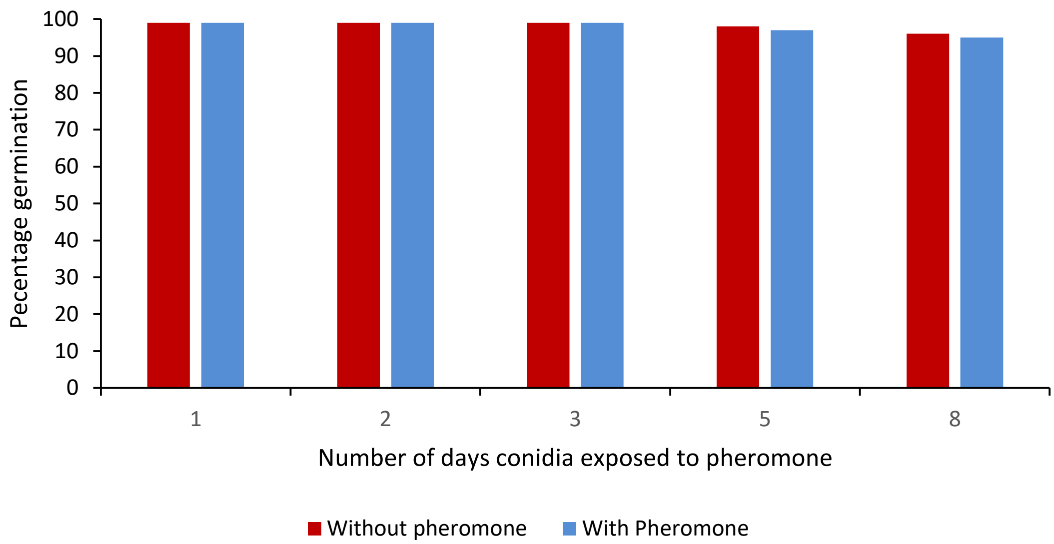


Figure 4. 2: Percentage germination of *Metarhizium* anisopliae ICIPE 41 when exposed to *S. frugiperda* sex pheromone for 8 days. The values are expressed as mean ± SE. Bar marked with same letters are not significantly different from each other at $p < 0.05$

4.3 Effects of *Spodoptera frugiperda* sex pheromone on formulated conidial viability and germ tube length

Significant variation ($p < 0.0001$) was observed among the tested formulations of *M. anisopliae* ICIPE 41 in both conidial germination and germ tube length (Figure 4.3). This distinct statistical variation shows differential compatibility. The aqueous and corn oil formulations showed germination and germ tube development comparable to the control.

In contrast, olive and canola oil formulations significantly reduced both parameters, with canola oil showing the lowest values (Figure 4.3).

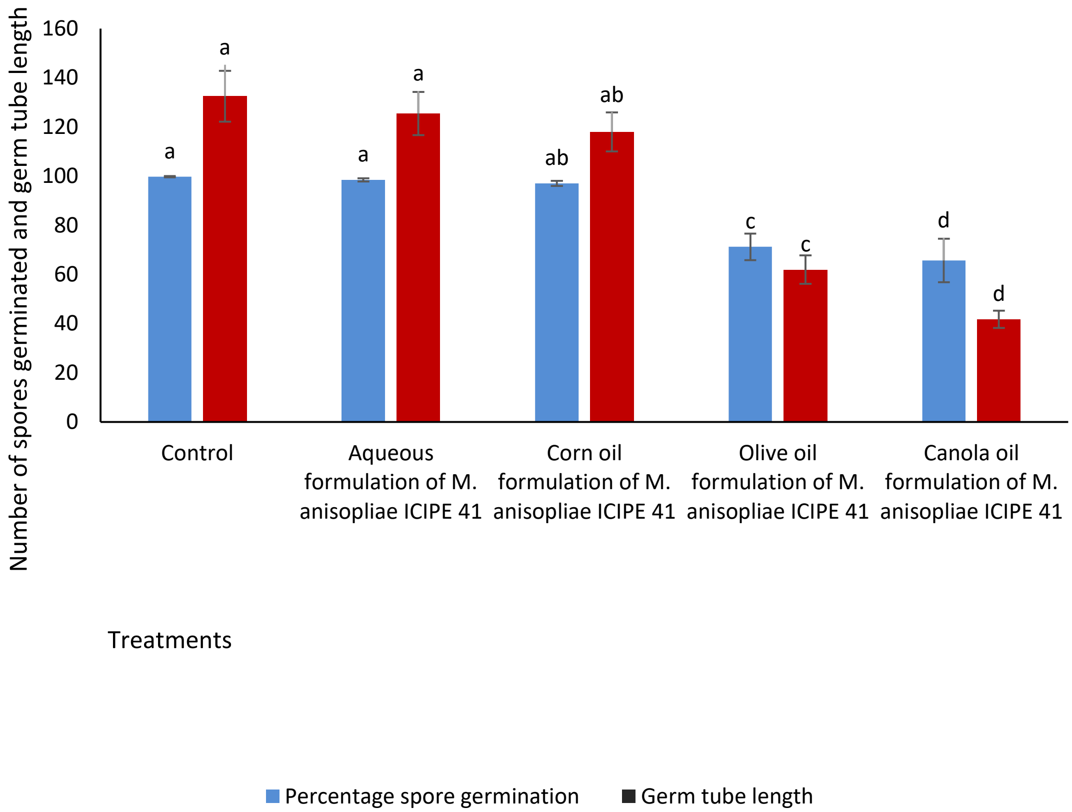


Figure 4.3: *Metarhizium anisopliae* ICIPE 41 spore germination and germ tube length after exposition to FAW pheromone for different treatment. The values are expressed as mean ± SE. Bar marked with the same letters are not significantly different from each other at $p < 0.05$

4.4 Pathogenicity of various formulations against second instar *Spodoptera frugiperda* larvae

The viability test results indicated that the germination of conidia in each fungal formulation used in this section surpassed 98% after 18 hours of incubation at 26 ± 2 °C. The fungal formulations were found to affect the 2nd instar larvae. There was a significant ($p < 0.05$) difference in larval mortality and the corresponding lethal time 50% (LT_{50})

between oil-based and aqueous formulations (Table 1). The canola oil formulation of *M. anisopliae* ICIPE 41 induced the highest larval cumulative mortality (76.0 ± 6.4%), followed by the corn oil (72.5 ± 5.5%) and olive oil (70.4 ± 6.6%) formulations, all of which were significantly more effective than the aqueous formulation (15.2 ± 2.8%) ($p < 0.01$). No significant differences were observed among the three oil-based formulations. The corresponding LT_{50} values were shortest for the canola oil formulation [2.0 days (95% CI: 1.9–2.2)], followed by corn oil [2.2 days (2.0–2.5)] and olive oil [2.5 days (2.0–2.9)], whereas the aqueous formulation had a significantly longer LT_{50} of 8.1 days (6.5–9.6). Additionally, mycosis was only observed in 58.25 ± 8.9% of the dead cadavers.

Table 4. 1: Larvae cumulated mortality caused by the fungal formulations and their corresponding lethal time 50% (LT_{50}). The values are expressed as mean ± SE.

Fungal isolate	**Fungal formulation**	**Larval cumulated mortality (%)**	**Lethal time 50% (LT_{50}) (days)**
Metarhizium anisopliae ICIPE 41	Canola oil formulation	76.0 ± 6.4b	2.0 (1.9-2.2) b
	Corn oil formulation	72.5 ± 5.5b	2.2 (2.0-2.5) b
	Olive oil formulation	70.4 ± 6.6b	2.5 (2.0-2.9) b
	Aqueous formulation	15.2 ± 2.8a	8.1 (6.5-0.6) a
F		50.3	50.3
Df		3, 12	3, 12
p		< 0.01	< 0.01

Means sharing identical letters in their mean values ± SE are not statistically different Tukey's test at p < 0.05

4.5 Assessment of non-target effects of the various formulations to FAW's parasitoids

The mean mortality of *S. frugiperda* parasitoids differed significantly among all the *M. anisopliae* ICIPE 41 formulations ($p = 0.001$ for all species; Table 4.2). For *Cotesia icipe*, the canola oil formulation induced the highest mortality (82.5 ± 4.33%) and was significantly different from all other treatments ($p < 0.05$). Olive oil and aqueous formulations showed intermediate effects (52.5 ± 9.68%). The corn oil formulation (12.5 ± 3.23%) caused significantly lower mortality than the other oil-based and aqueous formulations, but significantly higher than the control (2.5 ± 1.44%), which recorded the lowest mortality overall. while the corn oil formulation had lower mortalities than the control (2.5 ± 1.44%) which had the lowest mortality. For *Telenomus remus*, the canola oil formulation caused the highest mortality (21.3 ± 3.14%), significantly greater than olive oil (15.0 ± 2.04%), and aqueous formulation (11.3 ± 1.25%). The corn oil formulation and the control (2.5 ± 1.44%) recorded the lowest mortality among the fungal treatments. For *Trichogramma* spp., the canola oil formulation led to the highest mortality (26.3 ± 3.75%), followed by olive oil (23.8 ± 2.39%) and aqueous (23.8 ± 2.39%) formulations, which were statistically similar. The corn oil formulation (8.8 ± 1.25%) resulted in lower mortality than the other fungal treatments, but higher than the control (5.0 ± 2.04%), which again recorded the lowest mortality. Overall, *Cotesia icipe* was the most susceptible to all fungal formulations, whereas *Telenomus remus* exhibited the lowest mortality rates across treatments (Table 4.2).

Table 4. 2: Mean percentage mortalities of FAW's parasitoids infected with different formulations of *M. anisopliae* ICIPE 41 isolate

Treatments	**Parasitoids**		
	Cotesia icipe	***Telenomous remus***	***Trichogramma* spp.**
Canola oil formulation	82.5 ± 4.33d	21.3 ± 3.14d	26.3 ± 3.75d
Olive oil formulation	52.5 ± 9.68c	15.0 ± 2.04c	23.8 ± 2.39c
Corn oil formulation	12.5 ± 3.23b	2.5 ± 1.44a	8.8 ± 1.25b
Aqueous formulation	52.5 ± 9.68c	11.3 ± 1.25b	23.8 ± 2.39c
Control	2.5 ± 1.44a	2.5 ± 1.44a	5.0 ± 2.04a
F value	24.46	28.13	21.72
Df	4, 20	4, 20	4, 20
P value	0.001	0.001	0.001

Means sharing identical letters in their mean values ± SE are not statistically different Tukey's test at $p < 0.05$.

4.6 Effect of various formulations on emergence of parasitoids

The mean parasitism rates of *Cotesia icipe*, *Telenomus remus*, and *Trichogramma spp.* against *Spodoptera frugiperda* were significantly influenced by the formulation of *Metarhizium anisopliae* ICIPE 41 isolate used ($p = 0.001$) (Table. 4.3). Across all parasitoids, the corn oil formulation and the control recorded the highest parasitism rates, ranging from 79.3 ± 2.94 to 87.3 ± 2.50, with no significant difference between them. In contrast, the canola oil formulation resulted in the lowest parasitism rates (30.2 ± 2.65 to 33.5 ± 2.85), significantly lower than all the other treatments. Olive oil and aqueous formulations showed intermediate effects, with parasitism rates ranging from 38.8 ± 8.43 to 54.3 ± 4.12 (Table. 4.3).

Table 4. 3: Mean parasitism rate of FAW's parasitoids against different formulation of *M. anisopliae* ICIPE 41 isolate. The results are expressed as mean ± SE.

Treatments	**Parasitoids**		
	Cotesia icipe	***Telenomous remus***	***Trichogramma* spp.**
Canola oil formulation	31.5 ± 2.85a	30.2 ± 2.65a	33.5 ± 2.85a
Olive oil formulation	44.1 ± 7.23ab	38.8 ± 8.43ab	40.0 ± 5.21ab
Corn oil formulation	79.3 ± 2.94c	87.3 ± 2.50c	82.3 ± 2.34c
Aqueous formulation	54.3 ± 4.12b	49.1 ± 4.42b	47.1 ± 7.23b
Control	84.3 ± 3.74c	87.3 ± 2.50c	85.2 ±3.98c
F value	1.415	1.635	1.495
Df	4	4	4
P value	0.001	0.001	0.001

Means sharing identical letters are not statistically different based on Tukey's test at $p < 0.05$.

4.7 Effect of *S. frugiperda* sex pheromone on attraction of moths under field conditions

The attraction of *Spodoptera frugiperda* moths to different devices was significantly influenced by the treatment applied in both Migori and Mbita sites ($t = 15.1$, df = 3,15, $p < 0.001$) (Table 4.4). In both locations, the corn oil formulation recorded the highest adult moth attraction (79.25 ± 11.85 in Migori and 92.76 ± 18.83 in Mbita), significantly higher than other treatments. Mazao Achieve® attracted fewer moths (32.10 ± 8.49 in Migori and 37.50 ± 9.15 in Mbita), while the control recorded the lowest attraction (18.00 ± 3.21 and 15.55 ± 2.45, respectively). (Table 4.4).

Table 4.4: Analysis of *Spodoptera frugiperda* moth attraction in response to different devices incorporating different treatments in Mbita and Migori field sites. The results are expressed as mean ± SE.

	Percentage of *Spodoptera frugiperda* moth	
Treatments	**Migori**	**Mbita**
Spinetoram pesticide	-	-
Corn oil formulation	79.25 ± 11.85b	92.76 ± 18.83b
Mazao Achieve®	32.10 ± 8.49c	37.50 ± 9.15c
Control	18.00 ± 3.21a	15.55 ± 2.45a
t- value	12.1	15.1
Df	3,15	3,15
P value	< 0.001	< 0.001

Means sharing identical letters in their mean values ± SE are not statistically different Tukey's test at p < 0.05.

4.8: *Metarhizium anisopliae* ICIPE 41 conidia acquisition by *S. frugiperda* under field conditions

A significant difference in conidia acquisition was observed between the devices with the attractant (Corn oil formulation) and those without (control) ($p < 0.001$) (Table 4.5). The acquisition of *M. anisopliae* conidiospores by *S. frugiperda* was significantly higher in the corn oil formulation (79.25 ± 11.85% in Migori and 92.76 ± 18.83% in Mbita) compared to the control in both Migori and Mbita ($t = 151.25$ and 190.12; $df = 1$; $p < 0.001$). In absence of sex lure, spores were hardly acquired by moths despite the devices being fungal treated (18.00 ± 3.21% in Migori and 15.55 ± 2.45% in Mbita) (Table 4.5).

Table 4.5: Analysis on *M. anisopliae* conidial acquisition. The results are expressed as mean ± SE.

	Percentage of conidiospore acquired	
Treatments	**Migori**	**Mbita**
Corn oil formulation	79.25 ± 11.85b	92.76 ± 18.83b
Control	18.00 ± 3.21a	15.55 ± 2.45a
t- value	151.25	190.12
Df	1	1
P value	< 0.001	< 0.001

Means sharing identical letters in their mean values ± SE are not statistically different Tukey's test at $p < 0.05$.

4.9 FAW infestation in Migori and Mbita field sites

Spodoptera frugiperda (FAW) density per plant varied significantly among treatments in both Migori and Mbita (Table 4.6). The lowest infestation levels were recorded in plots treated with the spinetoram-based pesticide (3.2 ± 0.7 in Migori and 3.4 ± 0.6 in Mbita), followed by the corn oil formulation (5.3 ± 1.0 and 5.0 ± 0.8) and Mazao Achieve® (6.1 ± 1.5 and 5.1 ± 1.0), with no significant difference between the latter two. The highest infestation was recorded in the control treatments for both zones (25.7 ± 1.3 in Migori and 21.7 ± 1.3 in Mbita).

Table 4.6: Average FAW density per plant eight weeks post-treatment. The results are expressed as mean ± SE.

Treatments	FAW density per plant	
	Migori	Mbita
Spinetoram based pesticide	3.2 ± 0.7a	3.4 ± 0.6a
Corn oil formulation	5.3 ± 1.0b	5.0 ± 0.8b
Mazao Achieve®	6.1 ± 1.5b	5.1 ± 1.0b
Control	25.7 ± 1.3c	21.7 ± 1.3c

Means sharing identical letters in their mean values ± SE are not statistically different Tukey's test at $p < 0.05$.

4.10 Assessing FAW damage

A significant difference between the rate of FAW infestation among the treatments in both Migori and Mbita was reported ($P<0.001$) and ($P<0.001$), respectively, per plant after the 8 weeks of treatment evaluation (Table 4.7). Spinetoram pesticide recorded the least rate of FAW infestation. The study reported no significant difference in FAW infestation between plots treated with oil formulation and detain biopesticide. Control plots recorded the highest rate of infestation with > 20 pests per sampled plant.

Table 4.7: Average damage of FAW a week post-treatment examination in Migori and Mbita

Treatments	FAW density per plot	
	Migori	Mbita
Spinetoram based pesticide	2.2 ± 0.7a	2.4 ± 0.6a
Corn oil formulation	13.3 ± 2.0b	12.0 ± 2.8b
Mazao Achieve®	36.1 ± 5.5c	35.1 ± 5.0c
Control	75.7 ± 8.3d	71.7 ± 7.3d

Means sharing identical letters in their mean values ± SE are not statistically different Tukey's test at $p < 0.05$.

4. 11 Cumulated mortalities of FAW second instar larvae under field conditions

Larval mortality differences and their corresponding lethal time 50% (LT_{50}) varied significantly ($p < 0.0001$) among treatments in both Migori and Mbita field sites (Table 4.8). In both Migori and Mbita, the spinetoram-based pesticide caused the highest FAW larval mortality (96.1–98.7%) with the shortest lethal time (LT_{50} = 3.8 days) but did not induce mycosis. The corn oil fungal formulation showed higher mortality (81.3–83.6%) and significantly greater mycosis (66.3–70%) than *Mazao Achieve*® (73.0–74.3% mortality; 25.0–30.0% mycosis), with slightly faster LT_{50} values (4.6–5.1 vs. 4.7–5.2 days). No mortality or mycosis was observed in controls (Table 4.8).

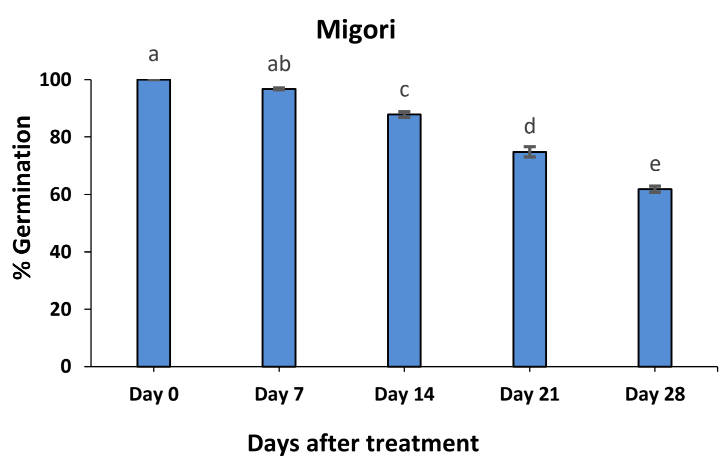
Table 4.8: Larvae cumulated mortality caused by the fungal formulations and their corresponding lethal time 50% (LT_{50}).

Treatments	**% FAW larvae mortality**	**% Mycosis**	**Lethal time 50%**
Migori			
Spinetoram-based pesticide	96.1 ± 2.5c	0.0 ± 0.0a	3.8 (3.80-3.90) a
Mazao Achieve®	73.0 ± 1.3a	30.0 ± 0.7b	5.2 (5.22-5.26) b
Corn oil formulation	81.3 ± 2.6b	70.0 ± 0.5c	5.1(5.12-5. 16) b
Control	-	0.0 ± 0.0a	-
Mbita			
Spinetoram-based pesticide	98.7 ± 1.3c	0.0 ± 0.0a	3.8 (3.80-3.88) a
Mazao Achieve®	74.3 ± 2.5a	25.0 ± 0.4b	4.7 (4.70-4.82) b
Corn oil formulation	83.6 ± 1.5b	66.3 ± 0.7c	4.6 (4.56-4.68) b
Control	-	0.0 ± 0.0a	-

Means sharing identical letters in their mean values ± SE are not statistically different Tukey's test at $p < 0.05$.

4. 12 Persistence of *Metarhizium anisopliae* ICIPE 41 conidia in corn oil formulation over time in Migori and Mbita Field Sites

A significant variation in conidial persistence over time in both Migori and Mbita field sites was observed ($p < 0.0001$) (Figure 4.4). On Day 0, germination rates were above 95%, but a gradual and statistically significant reduction was observed over time ($p < 0.05$). By Day 14, viability dropped to 87% in both locations, followed by sharper declines at Day 21 (74%) and Day 28 (61%). The same trend was recorded on both sites (Figure 4.4).



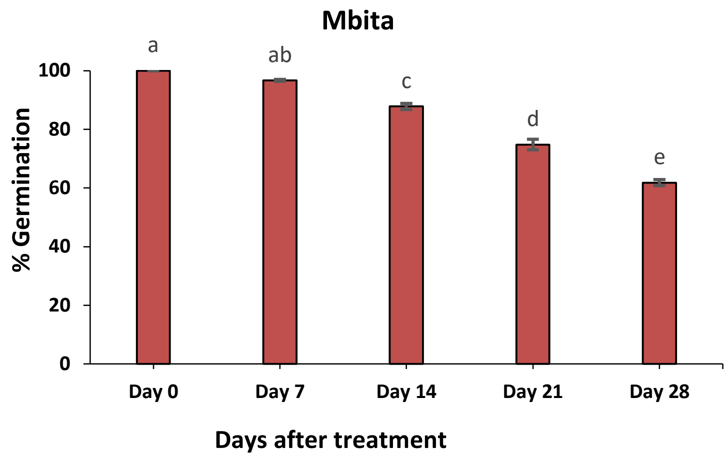


Figure 4.4: Persistence of *Metarhizium anisopliae* ICIPE 41 isolate in the corn oil formulated fungus treatment over time in Migori and Mbita fields. Bars denote means ± standard errors . Means with same letters are not statistically different based on the GLM Tukey's HSD test, $p < 0.05$.

4. 13 Effect of entomopathogenic fungi on *Cotesia icipe* emergence in Migori and Mbita field sites

Significant differences were observed in the population of parasitoids among the treatments at both Migori and Mbita sites ($p < 0.0001$ for both locations). It is worth noting that from all the incubated eggs and larvae collected across the treatments, only the larval parasitoid *Cotesia icipe* emerged from the deceased larvae. Significant differences were observed in the population of parasitoids among the treatments at both Migori and Mbita sites ((χ^2 = 1.71–3.13; $p < 0.0001$, for both locations). In both locations, the highest parasitoid emergence was recorded in the untreated control (10.00 ± 0.71 in Migori; 14.75 ± 1.25 in Mbita), followed by the corn oil fungal formulation (9.25 ± 0.85 and 11.00 ± 1.83, respectively), with significantly fewer emerging from larvae treated with *Mazao Achieve*® (2.00 ± 0.49 and 2.50 ± 0.15). No emergence was observed in the spinetoram-based pesticide treatment, indicating complete suppression of parasitoids.

Table 4.9: Performance FAW parasitoid *Cotesia icipe* assessed on the developmental stage in different treatments in Migori and Mbita

	Population of *Cotesia icipe* parasitoids that successfully emerged	
Treatments	**Migori**	**Mbita**
Spinetoram pesticide	-	-
Corn oil formulation	9.25 ± 0.85b	11.00 ± 1.83b
Mazao Achive®	2.00 ± 0.49a	2.50 ± 0.15a
Control	10.00 ± 0.71c	14.75 ± 1.25c
χ2	1.71	3.13
Df	3	3
P value	< 0.0001	< 0.0001

Means sharing identical letters in their mean values ± SE are not statistically different Tukey's test at $p < 0.05$.

4. 14 Maize grain yield in response to formulation treatments in Migori and Mbita sites

Maize grain yield varied significantly across fungal formulation treatments in both Migori and Mbita ($p = 0.001$), with the spinetoram-based pesticide producing the highest yields (902 ± 78 kg/ha in Migori; 1100± 96 kg/ha in Mbita) (Figure 4.5). The corn oil fungal formulation also resulted in significantly higher yields (708± 62 kg/ha and 842 ± 78 kg/ha, respectively) compared to Mazao Achieve® and the control. The lowest yields were observed in untreated plots (121± 10 kg/ha in Migori; 300± 48 kg/ha in Mbita) (Figure 4.5).

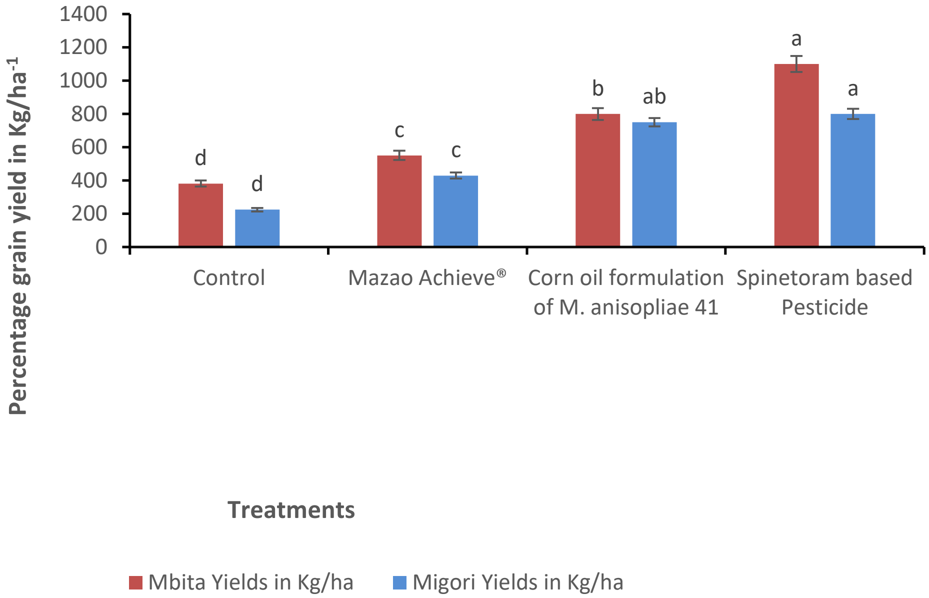


Figure 4. 5: Maize grain yield in Migori and Mbita in Kg/ha^{-1}

CHAPTER FIVE

DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

5.1 Discussion

This research work intended to formulate new environmentally friendly and effective biopesticide aimed at managing the destructive *Spodoptera frugiperda* (FAW) in maize production systems, while taking care of other friendly or non- friendly biodiversity. In summary, our study has shown that developing an effective biopesticide against FAW larvae using fungal-based formulation provides a suitable step in sustainable FAW management, while also considering the preservation of natural enemies.

5.1.1 Viability and compatibility bioassays of *Metarhizium anisopliae* ICIPE 41 and its formulations with *Spodoptera frugiperda* sex pheromone under laboratory conditions

Prior to the experiment, viability test was done and showed that 98% of the spores germinated to long mycelia when sub-cultured on SDA media and incubated at 26 ± 2 ºC for 18 hrs. Such results on this fungal isolate had been reported earlier (Akutse *et al*., 2019). The conidial germination of fungal isolate in response to exposure to *Spodoptera frugiperda* sex pheromone within a desiccator validated the fungus's compatibility with the sex pheromone. Notably, the spores of the fungus remained viable throughout the entire duration of the laboratory exposure trials, which spanned for 8 days. Although a slight decline in spore germination was observed, from 100% on day 1 to 95% on day 8 post-treatment, this reduction was minimal and within acceptable biological limits (Mann and Davis, 2021). These findings suggest that combining sex pheromone traps with fungal biocontrol agents is feasible and may support integrated pest management (IPM) strategies without compromising fungal effectiveness. The maintained viability over time also underscores the potential for co-application in field settings, where synchronized use could enhance control of FAW by both reducing adult moth populations and infecting larvae.

The findings on viability of this study were consistent with earlier studies on conidial germination, where a positive correlation with increased exposure time was observed, as previously reported by Mweke *et al*, (2018). Additionally, David *et al*. (2016) documented a decline in the germination rate of *Metarhizium* isolate in traps, both with and without an attractant, over an 8-day period.

The results of this study demonstrated that the compatibility of *Metarhizium anisopliae* ICIPE 41 with different formulation bases varied significantly, as evidenced by conidial germination and germ tube length ($p < 0.0001$). Aqueous and corn oil formulations maintained spore viability and germ tube development comparable to the untreated control, indicating that these carriers are suitable for preserving fungal infectivity. In contrast, olive and canola oil formulations significantly suppressed both germination and germ tube elongation, with canola oil exhibiting the most detrimental effects. This could be attributed to high viscous state of aqueous and corn oil which caused little or no clogging effect to the spores unlike the canola and olive oils hence high germination rates. The decreased germination rate in canola and olive oil formulation can be associated with the physical viscous state of the two oils that caused clogging effect and hence reduced germination rate. The presence of unsaturated fatty acids such as linoleic and oleic acids in these oils might have reduced the sprouting and growth of the conidiosphores (Mweke *et al*., 2019).

Such results were in concurrence with earlier report on the effects of the fatty acids on growth of *Crinipellis perniciosa* (Mweke *et al*., 2019).

These findings highlight the importance of formulation choice in fungal biocontrol applications, as incompatible carriers can impair spore performance and reduce overall efficacy. The results also suggest that corn oil, being both compatible and effective, is a promising medium for delivering *M. anisopliae* in integrated pest management strategies targeting *S. frugiperda*. Therefore, these findings reject hypothesis (ii), demonstrating compatibility between *M. anisopliae* ICIPE 41 and the FAW sex pheromone, and also reject hypothesis (i) by confirming the effectiveness of oil and aqueous formulations in maintaining fungal viability under laboratory conditions.

5.1.2 Pathogenicity of various formulations against second instar *Spodoptera frugiperda* larvae under laboratory conditions

The initial assessment of different formulations on second instar (FAW) larvae revealed a distinguishable difference between the oil-based and aqueous formulations in FAW larvae mortality. All the fungal formulations based on oil demonstrated pathogenicity against the second instar larvae, leading to mortality rates exceeding 70% in comparison to the aqueous formulation. These findings suggest that the carrier medium substantially influences fungal pathogenicity, likely due to improved spore adhesion, dispersion, and protection in oil-based formulations. In view of the chemical perspective, this difference can be attributed to the presence of chemical components such as the unsaturated and saturated fatty acids, in these vegetable oils (Lee *et al*., 2020). These can further be explained by the fact that, oils might have been hurtful to the larvae, causing suffocation due to obstruction of the spiracles (Lopes *et al*., 2011). Additionally, the non-polar nature of these oils may enhance the fungal isolate's potency, thereby increasing its infectivity rate and overall performance.

Research conducted by Opisa *et al*. (2018) elucidated that the incorporation of bentonite into fungal formulations of *B. bassiana* resulted to enhanced effectiveness against *Helicoverpa armigera* (Hübner; Lepidoptera: Noctuidae). The current study found that the oil formulations had the shortest lethal time (LT_{50}) ranging from 2.06 – 2.52 days compared to the aqueous formulations whose lethal time (LT_{50}) averaged 8.1 days. Our study aligned with the effectiveness of *M. anisopliae* in causing significant mortality and rapid lethality (LT50), as previously observed in other important arthropod pest study (Akutse *et al*., 2019).

These outcomes clearly reject hypothesis (i), which proposed that oil and aqueous formulations of *M. anisopliae* ICIPE 41 are not effective in managing FAW larvae, as all tested formulations, especially the oil-based ones, showed strong larvicidal activity under laboratory conditions. This highlights the potential of oil-based fungal formulations as viable biocontrol tools in integrated FAW management strategies.

5.1.3 Assessment of non-target effects of the various formulations to FAW's parasitoids under laboratory conditions

The non-target assessment revealed significant ($p < 0.001$) differences in susceptibility among FAW parasitoids when exposed to various *Metarhizium anisopliae* ICIPE 41 formulations. It was observed that canola and corn oil formulation caused the highest and least mortalities, respectively, in all the parasitoids (*Cotesia icipe*, *Telenomus remus* and *Trichogramma* spp.) tested. The observed differences in non-target effects among *M.*

anisopliae formulations can largely be attributed to the physiochemical properties of the carrier oils, which influence spore dispersion, persistence, and interaction with insect cuticles. The sticky and viscous characteristic of oils can cause suffocation of the parasitoids due to their small body size (Oreste *et al*., 2016). Our study results were in agreement similar research conducted at field on natural enemies of aphids on cowpea using corn oil formulation of *M. anisopliae* (Mweke *et al*., 2019).

From the results of this study, it is clear that formulation properties critically shape the selectivity of biocontrol agents, emphasizing the need to optimize for both efficacy and ecological safety. These findings support hypothesis (iii), which proposed that various *M. anisopliae* ICIPE 41 formulations affect FAW natural enemies' fitness and reproduction potentials, as significant mortality was observed in all parasitoid species, particularly with canola and olive oil formulations.

5.1.4 Effect of various formulations on emergence of parasitoids under laboratory conditions

This study reported a high parasitism rate in both the corn oil formulation and the control treatments compared to other treatments under laboratory conditions. This is could be due to low pathogenicity of the corn oil formulation to the parasitoids that resulted to high parasitizing action of the FAW larvae and eggs (Meagher *et al*., 2016). It also suggests that there is a minimal negative interaction between corn oil formulation and parasitoid activity. The significant reduction in parasitism rates of *Cotesia icipe*, *Telenomus remus*, and *Trichogramma spp.* observed in canola oil treatments may be due to its inhibitory effects on parasitoid development or survival, likely stemming from high viscosity and unfavorable fatty acid content, which may disrupt parasitoid behavior or physiology. The result of this study supports hypothesis (iii), which posited that various *M. anisopliae* ICIPE 41 formulations affect FAW natural enemies' fitness and reproduction potentials, confirming that formulation choice significantly impacts parasitoid emergence and effectiveness. These findings also highlight the importance of formulation selection to ensure compatibility with beneficial natural enemies in integrated pest management programs.

5.2 Field management of *S. frugiperda* infestation using various *M. anisopliae* ICIPE 41 formulations

5.2.1 Effect of *S. frugiperda* sex pheromone on attraction of moths under field conditions

Under field conditions, the present study has shown that adult moths positively responded to *S. frugiperda* sex pheromone, whose major active ingredients are 9-tetredecenyl acetate. The field results from both Migori and Mbita sites showed that corn oil formulation significantly enhanced the attraction of *S. frugiperda* moths compared to other treatments, including Mazao Achieve® and the control. This increased attraction could be due to the corn oil's physical and chemical properties, which may have stabilized or dispersed the sex pheromone more effectively, thereby enhancing its volatility and range of detection by the moths. This also demonstrates that corn oil can enhance pheromone-based attraction without compromising fungal viability, thus supporting its use in integrated pest management (Mkiga *et al.*, 2021). This is in line with objective (i) and (ii) of this study. The findings of this study also validate the effectiveness of the auto-inoculation device. This, in turn, indicates the device's potential utility in integrated pest control programs for managing the Fall Armyworm on maize and other crops susceptible to its infestations (Mkiga *et al*., 2021).

5.2.2 *Metarhizium anisopliae* ICIPE 41 conidia acquisition by *S. frugiperda* under field conditions

Notably, the percentage of spores' acquisition was consistent across Mbita and Migori field sites. The findings revealed that *Spodoptera frugiperda* moths acquired significantly more *Metarhizium anisopliae* ICIPE 41 conidia from devices treated with the corn oil formulation combined with the sex pheromone than from those without the attractant. This indicates that the pheromone played a key role in guiding moths to the fungal-treated surfaces, thereby enhancing conidial transfer. The markedly low acquisition rates in the absence of pheromone suggest that passive contact alone is insufficient for effective horizontal transmission of the entomopathogen under field conditions.

The considerable quantity of spores acquired by the moths in the delta trap device may be quantified to the short exposure time, which could result in more loosely attached spores on the substrate compared to those that have been exposed for an extended period in the field environment. This discovery holds significant importance, as it suggests that moths have the potential to acquire and subsequently transmit an ample inoculum within the other members of the population. It's worth noting that the impact of spore's acquisition on moth mortality was not examined in the current study. Nevertheless, Niassy *et al*. (2012) observed that an average mortality and number of conidia acquired per individual thrips were notably higher in field cages equipped with lure device at the 7-day mark after inoculation. Migiro, (2010), documented a positive relationship between the acquisition of conidia and the mortality of the leaf miner fly *Liriomyza huidobrensis.*

Nonetheless, the results of this study reject hypothesis (ii), which proposed no compatibility between *M. anisopliae* ICIPE 41 and FAW sex pheromone, by showing that the pheromone not only remains compatible but also significantly boosts the field acquisition of fungal conidia by moths, enhancing biocontrol efficacy.

5.2.3 FAW infestation assessment in Migori and Mbita field sites as influenced by different *M. anisopliae* ICIPE 41 formulations

The observed variation in FAW infestation across the different *M. anisopliae* ICIPE 41 formulations and control treatments highlights the differential efficacy of pest management strategies in both Migori and Mbita sites. The application of a Spinetoram-based pesticide led to a swift reduction in the larval density within the designated plots in both zones, as anticipated with many synthetic pesticides (Mweke *et al*., 2019), chlorantraniliprole-based (Wang and Wu, 2012), cyantraniliprole-based (Caballero *et al*., 2013), flubendiamide-based (Mohamed *et al*., 2021) synthetic pesticides. The reduction in larval numbers may be linked to the documented lack of resistance in FAW to the spinetoram pesticide (Munywoki *et al*., 2022). Synthetic pesticides, characterized by their broad-spectrum nature, can potentially harm both the pests and other beneficial natural enemies, rendering them unsuitable for pesticide use. Similar observation was recorded with Lambda-cyhalothrin based synthetic pesticide found to be toxic to aphid’s natural enemies (spiders and parasitoids) (Mweke *et al*., 2019).

Interestingly, the corn oil formulation and Mazao Achieve® treatments showed moderate and statistically similar reductions in FAW infestation and were nearly 4 times better than the untreated control, suggesting that these biopesticides could potentially contribute to FAW pest control. The notably high infestation in control plots confirms the significant pressure exerted by FAW in these regions and underscores the necessity for intervention. These findings partially reject the hypothesis (i) and (ii) as it is evident that *M. anisopliae* ICIPE 41 formulations can reduce FAW infestation and are compatible with other applications even at field level.

5.2.4 Cumulated mortalities of FAW second instar larvae under field conditions

The significant variation in cumulative FAW larval mortality and LT_{50} values across treatments in both Migori and Mbita demonstrates distinct modes and speeds of pest control by the tested formulations. High mortality rates were recorded in spinetoram-based pesticide, which also had the shortest lethal time 50% (LT_{50}). This highlights its rapid and potent insecticidal action, though it did not cause any fungal infection (mycosis), which is expected given its chemical nature. Conversely, the corn oil fungal formulation achieved substantial mortality with notably higher mycosis levels compared to Mazao Achieve®, indicating more effective fungal colonization and infection of the larvae, which likely contributed to its slightly quicker lethal time. As anticipated, no mortality was observed in the control group in either site.

Comparable outcomes were reported from other field studies conducted against native fall armyworm infestations in grain sorghum, where spinetoram application substantially resulted in decrease in the number of both infested corn whorls and the extent of damage

(Hardke *et al*., 2011). Although the pesticide outperformed other treatments, it is not recommended not only because of its adverse effects but its cost (Curl *et al*., 2020).

Interestingly, plots treated with the oil formulation caused a significantly higher mortality rate than the *M. anisopliae* ICIPE 78 commercial biopesticide. The effectiveness of this biopesticide against Fall armyworm (FAW) is being reported here for the first time, even though its efficacy has been previously documented against thrips and various other significant arthropods. Moreover, the superior performance observed in the fungal oil formulation compared to commercial biopesticide may be attributed to differences in spore viability, which can decrease over time, especially in the oil substrate used for *M. anisopliae* ICIPE 78 biopesticide. Likewise, similar findings regarding the viability of several *Metarhizium*-based when exposed to open environmental conditions were noted (Mweke *et al*., 2018). These findings support the hypothesis that *M. anisopliae* ICIPE 41 formulations can induce significant mortality through fungal infection, though chemical pesticides may deliver faster pest suppression.

This study also reported that in Mbita and Migori, respectively, 66 and 70% of the cadavers sprayed with the oil formulation had developed hyphae. This can be attributed to the fact that the conidial spores in the formulated oils came to contact with larvae and hence develop a germ tube into pest body and finally colonized the whole cadaver. Similar results on dead neonate larvae were reported on the surface of the incubated cadaver where conidia were observed on the inoculated fungus (Akutse *et al*., 2019). Additionally, the current study did report some very few hyphae on cadavers collected from plots treated with *M. anisopliae* ICIPE 78 biopesticide and spinetoram-based pesticide from each zone. This can be attributed to the fact that the *M. anisopliae* ICIPE 78 biopesticide is also made from

Metarhizium anisopliae isolate and their application on the larvae could result in the observed mycosis in dead cadavers. In the pesticide treated plots, we hypothesize that mycosis was as result of inter-plot spreading of the larvae after treatment application.

5.2.5 Persistence of *Metarhizium anisopliae* ICIPE 41 conidia in corn oil formulation over time in Migori and Mbita field sites

The persistence of *Metarhizium anisopliae* ICIPE 41 conidia in the corn oil formulation exhibited a clear temporal decline in viability across both Migori and Mbita field sites, reflecting the natural degradation of fungal propagules under field conditions. While initial germination rates were high (>95%) on Day 0, the statistically significant reduction over time—with viability declining to 87% by Day 14 and further to 61% by Day 28—highlights the limited longevity of viable conidia when exposed to environmental stressors such as UV radiation, temperature fluctuations, and humidity. The consistent trend across both locations suggests that environmental conditions in the study sites similarly influenced conidial persistence.

Oil-based formulations may increase the resistance of conidia to UV radiation, promoting enhanced germination in comparison to fungal suspended to only water-based formulations. Similar research work done on *Metarhizium acridum* IMI 330189 displayed a decline in germination percentage from 67.4%, 39.4%, 45.4% to 28.6% after 4, 8, 16 and 24-hours exposure. It is evident that formulations can be effective in the short term but may require repeated applications or improved formulation strategies to maintain efficacy over a longer period of time.

5.2.6 Effect of entomopathogenic ungi on parasitoid *Cotesia icipe* emergence in Migori and Mbita field sites

Under field conditions, application of spinetoram pesticide resulted in a rapid decline in parasitoids density on respective plots in both Migori and Mbita zones. This might be attributed by fact that the pesticide is highly toxic and has broad spectrum hence killed all pests it came in contact with. Similar results have been reported on the effect of synthetic pesticide on different naturally occurring enemies like Duduthrin® (Mweke *et al*., 2019), chlorantraniliprole-based (Wang and Wu, 2012), cyantraniliprole-based (Caballero *et al*., 2013), and flubendiamide-based (Mohamed *et al*., 2021)

The emergence patterns of *Cotesia icipe* across treatments in both Migori and Mbita reveal important ecological interactions between entomopathogenic fungi and natural enemies of FAW. The significantly higher parasitoid emergence in untreated controls suggests that natural parasitism plays a vital role in FAW population regulation when no interventions are applied. The moderate emergence from the corn oil fungal formulation treatment indicates a relatively compatible interaction between *M. anisopliae* ICIPE 41 and *Cotesia icipe*, likely because fungal infection does not immediately kill the host, allowing parasitoid development. In contrast, the markedly lower emergence of *Cotesia icipe* in Mazao Achieve® plots suggest that this intervention disrupt parasitoid survival, either by killing larvae before parasitism can occur or by creating inhospitable internal environment.

The results partially support the hypothesis, showing that while *M. anisopliae* ICIPE 41 formulations can suppress FAW populations, their compatibility with parasitoids—especially in the corn oil formulation offers an added advantage for integrated pest management strategies focused on long-term ecological balance.

5.2.7 Maize grain yield in response to formulation treatments in Migori and Mbita sites

The significant variation in maize grain yield across treatments in both Migori and Mbita reflects the direct impact of FAW management effectiveness on crop productivity. Remarkably, Mbita zone yielded significantly higher amounts compared to Migori zone. These results could be attributed to the relatively lower presence of Fall armyworm (FAW) observed at the Mbita site, resulting in lower infestation rates. The highest yields recorded in plots treated with the spinetoram-based pesticide align with its superior pest suppression and rapid action, which likely minimized plant stress and leaf damage, thereby enhancing photosynthetic efficiency and grain filling. Similar findings on maize yields had been noted in previous studies by Ogendo *et al*. (2013).

Additionally, it was observed that the corn oil formulation resulted in higher yields in both farm sites compared to Mazao Achieve® and the control, suggesting that its relatively greater efficacy in reducing FAW infestation and larval mortality contributed to improved crop performance. In contrast, the lowest yields in untreated plots underscore the severe yield losses associated with unchecked FAW pressure. Similar results on lack of treatment application on control plots of cowpea had been reported (Mweke *et al*., 2019). These results support the hypothesis that *M. anisopliae* ICIPE 41 formulations, particularly in corn oil, can positively influence maize yield by mitigating pest impact.

5.2 Conclusion

i. Corn oil formulation of *M. anisopliae* ICIPE 41 has the potential to control second instar larvae *S. frugiperda* under both laboratory and field conditions, which is the most destructive FAW developmental stage, when incorporated in integrated pest management. It was also demonstrated that the presence of corn oil in the formulation of *M. anisopliae* ICIPE 41 improved performance of the formulation EPF based biopesticide under field conditions with no negative effects on the associated natural enemies (parasitoids) of FAW. Therefore, biopesticides offer promising alternatives to synthetic chemical pesticides. The research findings have also demonstrated the efficacy of an integrated strategy for management of FAW through the application of entomopathogenic fungi in a maize cropping system.

ii. The fungal isolate was found to be compatible to FAW sex pheromone. Consequently, the combination of the sex pheromone (Falltrack) with this fungal isolate *M. anisopliae* ICIPE 41 could offer a suitable strategy of "*attract and kill*", managing the adult stage of FAW with prolonged viability, infectivity and persistence of the fungal isolate in the field.

iii. Results of this study showed the emergence of FAW associated parasitoids such as *Cotesia icipe* . Corn oil formulation of *M. anisopliae* ICIPE 41 maintained a relatively high level of compatibility with the parasitoid. In contrast, chemical control with spinetoram completely suppressed parasitoid emergence, highlighting a potential trade-off between rapid pest suppression and conservation of natural enemies. from treatments of corn oil in the formulation of *M. anisopliae* ICIPE 41. This confirms that Corn oil formulation is target specific and much effective as it takes care of other biodiversity.

iv. The findings on grain yield demonstrate that effective FAW management directly translates to improved maize productivity, with the spinetoram-based pesticide yielding the highest returns due to its rapid and efficient pest control. However, the corn oil formulation of *M. anisopliae* ICIPE 41 also significantly enhanced yield compared to Mazao Achieve® and the control, indicating its potential as a viable biocontrol option. These results underscore the role of entomopathogenic fungi in contributing to yield improvement while offering a more environmentally sustainable alternative to chemical pesticides.

5.3 Recommendations

5.3.1 Study recommendations

The promotion and utilization of EPF-based biopesticides should be prioritized among farmers to ensure sustainable maize production through the effective management of *Spodoptera frugiperda*.

5.3.2 Recommendations for further research

i. This study recommends that further evaluation of the effect of this formulation on other natural enemies of FAW, which were not evaluated, should be undertaken. It further recommends that more investigation on how to enhance formulations of the EPF based biopesticides to improve their persistence after application and reduce undesirable effects of environmental conditions/factors, while enhancing their performance under different weather conditions. Studies should be undertaken on different additives, adjuvants and protectants that improve EPF based formulations to improve and enhance their performance under different environmental conditions.

ii. More research work needs to be developed to enhance formulation carriers that eliminate expensive procedures involved in production, storage and application methods of entomopathogens while at the same time maintaining their efficacy.

REFERENCES

Abbott, W. A. (1925). Method of computing the effectiveness of an insecticide. *Journal of Entomology*, **18**, 265–267. https://doi.org/10.3109/13880209.2012.674950

Akutse, K. S., Kimemia, J. W., Ekesi, S., Khamis, F. M., Ombura, O. L. and Subramanian, S. (2019). Ovicidal effects of entomopathogenic fungal isolates on the invasive Fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Journal of Applied Entomology*, **143**(6), 626–634. https://doi.org/10.1111/jen.12634

Akutse, K. S., Subramanian, S., Maniania, K. N., Dubois, T. and Ekesi, S. (2020). Biopesticide research and product development in Africa for sustainable agriculture and food security – Experiences from the International Centre of Insect Physiology and Ecology (*icipe*). *Frontiers in Sustainable Food Systems* 4:563016. https://doi.org/10.3389/fsufs.2020.563016

Al., C. (2019). Is the insect cuticle the only entry gate for fungal infection? Insights into alternative modes of action of entomopathogenic fungi. *Journal of Fungi*, **5**(2). https://doi.org/10.3390/jof5020033

Ansari, M. A., Pope, E. C., Carpenter, S., Scholte, E. J. and Butt, T. M. (2011). Entomopathogenic fungus as a biological control for an important vector of livestock disease: The culicoides biting midge. *PLoS ONE*, **6**(1), 1–8. https://doi.org/10.1371/journal.pone.0016108

Awata .L., Chelang'at, S.,and Levi. M. S. (2019). Maize lethal necrosis and the molecular basis of variability in concentrations of the causal viruses in co-infected maize plant. *Journal of General and Molecular Virology*, **9**(1), 1–19. https://doi.org/10.5897/jgmv2019.0073

Badu-Apraku, B. and Fakorede, M. A. B. (2017). Advances in genetic enhancement of early and extra-early maize for sub-Saharan Africa. *Advances in Genetic Enhancement of Early and Extra-Early Maize for Sub-Saharan Africa*, 1–604. https://doi.org/10.1007/978-3-319-64852-1

Bawa, I. (2016). Management strategies of fusarium wilt disease of tomato incited by *Fusarium oxysporum* f. sp. lycopersici (Sacc.): A review. *International Journal of Advanced Academic Research | Sciences Technology & Engineering*, **2**(5), 2488–9849.

Bayissa, W., Ekesi, S., Mohamed, S. A., Kaaya, G. P., Wagacha, J. M., Hanna, R. and

Maniania, N. K. (2017). Selection of fungal isolates for virulence against three aphid pest species of crucifers and okra. *Journal of Pest Science*, **90**(1), 355–368. https://doi.org/10.1007/s10340-016-0781-4

Caballero, R., Cyman, S., Schuster, D. J., Portillo, H. E. and Slater, R. (2013). Baseline susceptibility of *Bemisia tabaci* (Genn.) biotype B in southern Florida to cyantraniliprole. *Crop Protection*, **44**, 104–108. https://doi.org/10.1016/j.cropro.2012.10.013

Chandler, D., Bailey, A. S., Mark Tatchell, G., Davidson, G., Greaves, J. and Grant, W. P. (2011). The development, regulation and use of biopesticides for integrated pest management. *Philosophical Transactions of the Royal Society B: Biological Sciences*, **366**(1573), 1987–1998. https://doi.org/10.1098/rstb.2010.0390

Chiwamba, S. H. and Nkunika, P. O. Y. (2019). An Application of machine learning algorithms in automated identification and capturing of fall armyworm (faw) moths in the field. *an application of machine learning algorithms in Automated Identification and Capturing of Fall Armyworm (FAW) Moths in the Field*, *3*(March), 1–4.

Curl, C. L., Spivak, M., Phinney, R. and Montrose, L. (2020). Synthetic Pesticides and Health in Vulnerable Populations: Agricultural Workers. *Current Environmental Health Reports*, **7**(1), 13–29. https://doi.org/10.1007/s40572-020-00266-5

David, K. M., Sevgan, S., Saliou, N., Daisy, S., Hannalene, du P., Sunday, E. and Nguya, K. M. (2016). Screening for attractants compatible with entomopathogenic fungus *Metarhizium anisopliae* for use in thrips management. *African Journal of Biotechnology*, **15**(17), 714–721. https://doi.org/10.5897/ajb2015.15149

Day, R., Abrahams, P., Bateman, M., Beale, T., Clottey, V., Cock, M., Colmenarez, Y., Corniani, N., Early, R., Godwin, J., Gomez, J., Moreno, P. G., Murphy, S. T., Oppong-Mensah, B., Phiri, N., Pratt, C., Silvestri, S. and Witt, A. (2017a). Fall armyworm: Impacts and implications for Africa. *Outlooks on Pest Management*. https://doi.org/10.1564/v28_oct_02

Day, R., Abrahams, P., Bateman, M., Beale, T., Clottey, V., Cock, M., Colmenarez, Y., Corniani, N., Early, R., Godwin, J., Gomez, J., Moreno, P. G., Murphy, S. T., Oppong-Mensah, B., Phiri, N., Pratt, C., Silvestri, S. and Witt, A. (2017b). Fall armyworm: Impacts and implications for Africa. *Outlooks on Pest Management*, **28**(5), 196–201. https://doi.org/10.1564/v28_oct_02

Deshmukh, S. S., Prasanna, B. M., Kalleshwaraswamy, C. M., Jaba, J. and

Choudhary, B. (2021). Fall Armyworm (*Spodoptera frugiperda*). *Polyphagous Pests of Crops*, 349–372. https://doi.org/10.1007/978-981-15-8075-8_8

Hardke, J. T., Temple, J. H., Leonard, B. R. and Jackson, R. E. (2011). Laboratory toxicity and field efficacy of selected insecticides against fall armyworm (Lepidoptera: Noctuidae). *Florida Entomologist*, **94**(2), 272–278. https://doi.org/10.1653/024.094.0221

Jayshree. A (2020). Role of bio-pesticides in recent trends of insect pest management: A review. *~ 2237 ~ Journal of Pharmacognosy and Phytochemistry*, **9**(1), 2237–2240. www.phytojournal.com

Kalyebi, A., Overholt, W. A., Schulthess, F., Mueke, J. M. and Sithanantham, S. (2006). The effect of temperature and humidity on the bionomics of six African egg parasitoids (Hymenoptera: Trichogrammatidae). *Bulletin of Entomological Research*, **96**(3), 305–314. https://doi.org/10.1079/ber2006429

Kinyumu, E. N., Odhiambo, N., Bii, H. and Kirimi, L. (2018). Food situation assessment for 2018/2019. *Policy Brief - Tegemeo Institute of Agricultural Policy and Development, No.31*, 4 pp.

Kumela, T., Simiyu, J., Sisay, B., Likhayo, P., Mendesil, E., Gohole, L. and Tefera, T. (2019). Farmers' knowledge, perceptions, and management practices of the new invasive pest, fall armyworm (*Spodoptera frugiperda*) in Ethiopia and Kenya. *International Journal of Pest Management*, **65**(1), 1–9. https://doi.org/10.1080/09670874.2017.1423129

Lacey, L. A., Grzywacz, D., Shapiro-Ilan, D. I., Frutos, R., Brownbridge, M. and Goettel, M. S. (2015). Insect pathogens as biological control agents: Back to the future. *Journal of Invertebrate Pathology*, **132**, 1–41. https://doi.org/10.1016/j.jip.2015.07.009

Lin, Y., Qasim, M., Hussain, M., Akutse, K. S., Avery, P. B., Dash, C. K. and Wang, L. (2017). The Herbivore-Induced Plant Volatiles Methyl Salicylate and Menthol Positively affect Growth and Pathogenicity of Entomopathogenic Fungi. *Nature Publishing Group, September 2016*, 1–11. https://doi.org/10.1038/srep40494

Lopes, R. B., Pauli, G., Mascarin, G. M. and Faria, M. (2011). Protection of entomopathogenic conidia against chemical fungicides afforded by an oil-based formulation. *Biocontrol Science and Technology*, **21**(2), 125–137. https://doi.org/10.1080/09583157.2010.534548

Mann, A. J. and Davis, T. S. (2021). Entomopathogenic fungi to control bark beetles: a review of ecological recommendations. *Pest Management Science*, **77**(9), 3841–3846. https://doi.org/10.1002/ps.6364

Meagher, R. L., Nuessly, G. S., Nagoshi, R. N. and Hay-Roe, M. M. (2016). Parasitoids attacking fall armyworm (Lepidoptera: Noctuidae) in sweet corn habitats. *Biological Control*, **95**, 66–72. https://doi.org/10.1016/j.biocontrol.2016.01.006

M**ent, D., Gindin, G., Rot, A., Soroker, V., Glazer, I., Barel, S. and Samish, M. (2010).** Novel technique for quantifying adhesion of *Metarhizium anisopliae* conidia, to the tick cuticle. *Applied and Environmental Microbiology*, **76**(11), 3521–3528. **https://doi.org/10.1128/AEM.02596-09**

Migiro, L. N. (2010). *Entomopathogenic fungi: a component of leafminer management*. *May*.

Mkiga, A. M., Abuelgasim, S., Hannalene, M. and Mbarak, F. (2021). Compatibility and efficacy of *Metarhizium anisopliae* and sex pheromone for controlling *Thaumatotibia leucotreta. Journal of Pest Science*, **94**(2), 393–407. https://doi.org/10.1007/s10340-020-01281-z

Mohamed, S. A., Wamalwa, M., Obala, F., Tonnang, H. E. Z., Tefera, T., Calatayud, P. A., Subramanian, S., and Ekesi, S. (2021). A deadly encounter: Alien invasive *Spodoptera frugiperda* in Africa and indigenous natural enemy, *Cotesia icipe* (Hymenoptera, Braconidae). *PLoS ONE*, **16**(7 July), 1–19. https://doi.org/10.1371/journal.pone.0253122

Morley-Davies, J., Moore, D. and Prior, C. (1996). Screening of *Metarhizium* and *Beauveria* spp. conidia with exposure to simulated sunlight and a range of temperatures. *Mycological Research*, **100**(1), 31–38. https://doi.org/10.1016/S0953-7562(96)80097-9

Mumo, L., Yu, J. and Fang, K. (2018). Assessing Impacts of Seasonal Climate Variability on Maize Yield in Kenya. *International Journal of Plant Production*, **12**(4), 297–307. https://doi.org/10.1007/s42106-018-0027-x

Munywoki, J.; Omosa, L.K.; Subramanian, S.; Mfuti, D.K.; Njeru, E.M.; Nchiozem-Ngnitedem, V.-A. and Akutse, K. S. (2022). Performance of *Metarhizium anisopliae* Isolate ICIPE 41 in the Laboratory and Field in Comparison to Another Fungal Biopesticide and a Chemical Product to Sustainably Control the Invasive Fall Armyworm *Spodoptera frugiperda.*

Murdia .L. K (2016). Maize Utilization in India: An Overview. *American Journal of Food and Nutrition*, **4**(6), 169–176. https://doi.org/10.12691/ajfn-4-6-5

Muriithi, B. W., Affognon, H. D., Diiro, G. M., Kingori, S. W., Tanga, C. M., Nderitu, P. W., Mohamed, S. A. and Ekesi, S. (2016). Impact assessment of Integrated Pest Management (IPM) strategy for suppression of mango-infesting fruit flies in Kenya. *Crop Protection*, **81**, 20–29. https://doi.org/10.1016/j.cropro.2015.11.014

Mweke, A., Akutse, K. S., Ulrichs, C., Fiaboe, K. K. M., Maniania, N. K. and Ekesi, S. (2019). Efficacy of aqueous and oil formulations of a specific M*etarhizium anisopliae* isolate against *Aphis craccivora* Koch, 1854 (Hemiptera: Aphididae) under field conditions. *Journal of Applied Entomology*, **143**(10), 1182–1192. https://doi.org/10.1111/jen.12705

Mweke, A., Ulrichs, C., Nana, P., Akutse, K. S., Fiaboe, K. K. M., Maniania, N. K. and Ekesi, S. (2018). Evaluation of the entomopathogenic fungi *Metarhizium anisopliae*, *Beauveria bassiana* and *Isaria* sp. for the management of aphis craccivora (hemiptera: Aphididdae). *Journal of Economic Entomology*, **111**(4), 1587–1594. https://doi.org/10.1093/jee/toy135

Naranjo-Ortiz (2019). Fungal evolution: diversity, taxonomy and phylogeny of the Fungi. *Biological Reviews*, **94**(6), 2101–2137. https://doi.org/10.1111/brv.12550

Niassy, S., Maniania, N. K., Subramanian, S., Gitonga, M. L., Maranga, R., Obonyo, A. B. and Ekesi, S. (2012). Compatibility of *Metarhizium anisopliae* isolate ICIPE 69 with agrochemicals used in french bean production. *International Journal of Pest Management*, **58**(2), 131–137. https://doi.org/10.1080/09670874.2012.669078

Ogendo.J (2013). *Management of stem borers using selected botanical pesticides in a maize-bean cropping system*. **13**(2073), 21–38.

Okweche, S. I., Ogunwolu, E. O. and Adeyemo, M. O. (2013). Parameters, Interrelationships with Yield and use of Carbofuran to Control Stem Borers in Maize *(Zea mays* l.) at Makurdi in the Nigerian Southern Guinea Savanna. *Greener Journal of Agricultural Sciences*, **3**(10), 702–708. https://doi.org/10.15580/gjas.2013.3.170913845

Opisa, S., du Plessis, H., Akutse, K. S., Fiaboe, K. K. M. and Ekesi, S. (2018). Effects of Entomopathogenic fungi and *Bacillus thuringiensis*-based biopesticides on *Spoladea recurvalis* (Lepidoptera: Crambidae). *Journal of Applied Entomology*, **142**(6), 617–626. https://doi.org/10.1111/jen.12512

Opisa, S., du Plessis, H., Akutse, K. S., Fiaboe, K. K. M. and Ekesi, S. (2019). Horizontal transmission of *Metarhizium anisopliae* between *Spoladea recurvalis* (Lepidoptera: Crambidae) adults and compatibility of the fungus with the attractant phenylacetaldehyde. *Microbial Pathogenesis*, **131**(February), 197–204. https://doi.org/10.1016/j.micpath.2019.04.010

Oreste, M., Bubici, G., Poliseno, M. and Tarasco, E. (2016). Effects of entomopathogenic fungi on *Encarsia formosa* Gahan. (Hymenoptera: Aphelinidae) activity and behavior. *Biological Control*, **100**, 46–53. https://doi.org/10.1016/j.biocontrol.2016.05.011

Pérez-Escamilla, R. (2017). Food security and the 2015-2030 sustainable development goals: From human to planetary health. *Current Developments in Nutrition*, **1**(7), 1–8. https://doi.org/10.3945/cdn.117.000513

Prasanna, B., Huesing, J. E., Eddy, R. and Peschke, V. M. (2018). Fall Armyworm in Africa: a Guide for Integrated Pest Management. *Mexico, CDMX: CIMMYT., First Edit*, 45–62.

R Development Core Team, (2014). On best practice optimization methods in R. *Journal of Statistical Software*, **60**(2), 1–14. https://doi.org/10.18637/jss.v060.i02

Schroeder . (2013). Volume 13 No. 2 April 2013. *African Journal of Food, Agriculture, Nutrition and Development*, **13**(2), 7357–7371.

Shapiro, S. S. and Wilk, M. B. (1965). An Analysis of Variance Test for Normality (Complete Samples). *Biometrika*, **52**(3/4), 591–611. https://doi.org/10.2307/2333709

Sharanabasappa, C. M., K., Poorani, J., Maruthi, M. S., Pavithra, H. B. and Diraviam, J. (2019). Scientific Notes Natural Enemies of *Spodoptera frugiperda* (J . E . Smith), a Recent Invasive Pest on Maize in South India. *Florida Entomologist*, **102**(3), 619–623.

Shiferaw, B., Prasanna, B. M., Hellin, J. and Bänziger, M. (2011). Crops that feed the world 6. Past successes and future challenges to the role played by maize in global food security. *Food Security*, **3**(3), 307–327. https://doi.org/10.1007/s12571-011-0140-5

Sisay, B., Simiyu, J., Malusi, P., Likhayo, P., Mendesil, E., Elibariki, N., Wakgari, M., Ayalew, G. and Tefera, T. (2018). First report of the fall armyworm, *Spodoptera frugiperda* (Lepidoptera: Noctuidae), natural enemies from Africa. *Journal of Applied Entomology*, **142**(8), 800–804. https://doi.org/10.1111/jen.12534

Sisay, B., Simiyu, J., Mendesil, E., Likhayo, P., Ayalew, G., Mohamed, S., Subramanian, S. and Tefera, T. (2019). Fall armyworm, *Spodoptera frugiperda* infestations in East Africa: Assessment of damage and parasitism. *Insects*, **10**(7), 1–10. https://doi.org/10.3390/insects10070195

Tindo, M., Tagne, A., Tigui, A., Kengni, F., Atanga, J., Bila, S., Doumtsop, A. and Abega, R. (2017). First report of the fall army worm, *Spodoptera frugiperda* (Lepidoptera, Noctuidae) in Cameroon. *Journal of Biological and Biochemical Sciences*, **25**(March), 30–32.

United Nations. (2021). *Sustainable Development Goals | United Nations in Kenya*. https://kenya.un.org/en/sdgs

V**ega, F. E., Goettel, M. S., Blackwell, M., Chandler, D., Jackson, M. A., Keller, S., Koike, M., Maniania, N. K., Monzón, A., Ownley, B. H., Pell, J. K., Rangel, D. E. N., and Roy, H. E. (2009).** Fungal entomopathogens: new insights on their ecology. *Fungal Ecology*, **2**(4), 149–159. https://doi.org/10.1016/j.funeco.2009.05.001

Wang, X. and Wu, Y. (2012). High levels of resistance to chlorantraniliprole evolved in field populations of *Plutella xylostella. Journal of Economic Entomology*, **105**(3), 1019–1023. https://doi.org/10.1603/EC12059

Xiao, W., Yin, W., Xia, S. and Ma, P. (2012). The study of factors affecting the enzymatic hydrolysis of cellulose after ionic liquid pretreatment. *Carbohydrate Polymers*, **87**(3), 2019–2023. https://doi.org/10.1016/j.carbpol.2011.10.012

Zimmermann, G. (2007). Review on safety of the entomopathogenic fungus *Metarhizium anisopliae. Biocontrol Science and Technology*, **17**(9), 879–920. https://doi.org/10.1080/09583150701593963

APPENDICESAPPENDIX I: Research Authorization

KENYATTA UNIVERSITY
OFFICE OF THE EXECUTIVE DEAN GRADUATE SCHOOL

E-mail: dean-graduate@ku.ac.ke
Website: www.ku.ac.ke

P.O. Box 43844, 00100
NAIROBI, KENYA
Tel. 020-8704150

Internal Memo

FROM: Executive Dean, Graduate School **DATE:** 30th October 2023

TO: Mr. Munywoki Joseph Matumu **REF:** I56/38053/2017
C/O Department of Biochemistry, Microbiology and Biotechnology0

SUBJECT: APPROVAL OF RESEARCH PROPOSAL

===

We acknowledge receipt of your Research Proposal after fulfilling recommendations raised by the Graduate School Board of 11th October 2023.

You may now proceed with your Data collection, subject to clearance with the Director General, National Commission for Science, Technology & Innovation.

As you embark on your data collection, please note that you will be required to submit to Graduate School completed Supervision Tracking and Progress Report Forms per semester. The forms are available at the University's Website under Graduate School webpage downloads.

Also, please ensure that you publish article(s) from your thesis before submitting it to Graduate School for examination as per the Commission for University Education and Kenyatta University guidelines.

Thank you.

KENYATTA UNIVERSITY
OFFICE OF THE EXECUTIVE DEAN
01 NOV 2023
GRADUATE SCHOOL
NAIROBI
Box 43844-00100

JULIA GITU
FOR: EXECUTIVE DEAN, GRADUATE SCHOOL

cc. Chairman, Department of Biochemistry, Microbiology and Biotechnology
Supervisors:

1. Dr. Ezekiel Mugendi Njeru
C/O Department of Biochemistry, Microbiology and Biotechnology
Kenyatta University

2. Prof. Leonidah Kerubo Omosa
Chemistry Department, University of Nairobi
C/O Biochemistry, Microbiology and Biotechnology
Kenyatta University

3. Dr. Komivi Senyo Akutse
International Centre of Insect Physiology and Ecology
C/O Biochemistry, Microbiology and Biotechnology
Kenyatta University

APPENDIX II: Research Permit

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: 571957

Date of Issue: 08/March/2021

RESEARCH LICENSE

This is to Certify that Mr.. Joseph Matumu Munywoki of Kenyatta University, has been licensed to conduct research in Homabay on the topic: Potential use of Metarhizium anisopliae based biopesticides for control of Spodoptera frugiperda in maize production system in Kenya for the period ending : 08/March/2022.

License No: NACOSTI/P/21/9251

571957

Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.