

**EFFECTS OF ENTOMOPATHOGENIC FUNGI AND BORDER CROPPING ON BEAN
APHID POPULATION, GROWTH, YIELD AND QUALITY OF FRENCH BEAN**

ANTHONY EMARU

**A Thesis Submitted to the Graduate School in Partial Fulfilment of the Requirements for
the Master of Science Degree in Horticulture of Egerton University**

EGERTON UNIVERSITY

SEPTEMBER, 2024

DECLARATION AND RECOMMENDATION

Declaration

I declare that this thesis is my original work and has not been presented in this University or any other for the conferment of a Degree.

Signature: 

Date: 16-09-2024

Anthony Emaru

KM14/09033/20

Recommendation

This thesis has been submitted with our recommendations as University supervisors.

Signature:..... 

Date: 25/09/2024

Prof. Mwanarusi Saidi, Ph.D.

Department of Crops, Horticulture and Soils,
Egerton University

Signature:.... 

Date: 23/09/2024

Dr. Jane G. Nyaanga, Ph.D.

Department of Crops, Horticulture and Soils,
Egerton University

COPYRIGHT

©2024, Anthony Emaru.

All rights reserved. No part of this thesis may be reproduced, stored in a retrieval system or transmitted in any form or by any means, electronic, mechanical, photocopying, recording or otherwise, without the prior permission in writing from the copyright owner or Egerton University.

DEDICATION

This thesis is dedicated to my family, Dr. Andrew Waswa who was very supportive, encouraged and mentored me to work hard and God who gave me a good health to achieve this dream.

ACKNOWLEDGEMENTS

First and foremost, I am grateful to Egerton University for providing institutional support, conducive environment and the provision of field space to conduct my research study. I sincerely convey my gratitude to MasterCard Foundation in partnership with the Regional Universities Forum for Capacity Building in Agriculture (RURORUM) for financial support through the "Transforming African Agricultural Universities to Meaningfully Contribute to Africa's Growth and Development"(TagDev) scholarship. My sincere gratitude also goes to Egerton University for giving me an opportunity to pursue this postgraduate degree in the institution. My deep sense of gratitude and appreciations go to my supervisors: Prof. Mwanarusi Saidi and Dr. Jane G. Nyaanga, for their treasured and incessant guidance, fruitful remarks, steady support and passionate inspiration to this study. I thank the entire staff in the Department of Crops, Horticulture and Soils. Special gratitude goes to Dr. Caleb Otieno and Mr. George Morara Ongoro for their support in the field. Mr. Herman Oteinno and Kipngetch Chumo Shadrack, a technician at the Microbiology and Ecology Laboratory at Egerton University, for their support during the laboratory experiment. I am also grateful to Dr. Komivi Akutse, Dr. Dennis Beesigamukama, Dr. Kisaakye James, Mr. Levi Ombura. Mr. Geoffrey Kirui and technicians at the International Centre for Insect Physiology and Ecology (ICIPE)for their training and guidance on how to conduct scientific bioassay research, Mr. Geoffry Ongoya from Koppert Biological Services, Ms. Ruth Murunde and Mr. Daniel Nene from Real IPM Co. Limited and Dr. Srinivasan Ramasamy from World Vegetables Centre- Taiwan for their inspirations and technical support throughout this study. Words cannot express my heartfelt gratitude and immeasurable indebtedness to my dear friends Mr. Enock Kwizera, Mr. Uma Apollo, and Mrs. Scolastica N. Nambafu, who helped with data collection. Additionally, Mr. Ochieng Lawrence Otieno and Mr. Rwotonen Innocent Bob, Mr. Niyokuri Nshuti Aristide, Mrs. Solome Emaru, Mrs. Doreen Mbabazize Zebosi, Mr. Andrew Waaswa, Mr. Satagnon Felix, Miss. Virginia Njuguna and Mr. Sheku Nyumah Gbollie, who actively spared time in different ways whenever I called upon them. Lastly, I thank the almighty God for His presence in my life and for granting me the strength and grace to complete the thesis and publication work.

ABSTRACT

French bean (*Phaseolus vulgaris* L.) is an important crop in Kenya that accounts for 33% of total vegetable export and immensely contributes to the Gross Domestic Product (GDP). Despite the economic value of French bean, growers still experience low yield and quality due to several biotic constraints, including the black bean aphid (*Aphis fabae*). Efforts to manage aphids have resorted to frequent use of synthetic pesticides, which have negative impact on human health and the environment. Integrating biopesticides use with cropping systems is a promising alternative. This study objectives were; to determine the bioactivity of the entomopathogenic fungus *Metarhizium anisopliae* against black bean aphid and its interactive effects with border crops on aphid population and damage, growth, yield and quality of French bean. To determine the bioactivity of *M. anisopliae* against black bean aphid, a laboratory experiment arranged in a Completely Randomized Design (CRD) with five replications was conducted. Different concentrations of Mazao supreme and Metarril wettable powder (WP) formulations were tested against a commercial biopesticide (Biomagic), a synthetic pesticide (alpha-cypermethrin) and water as control. Probit analysis was done to determine the median lethal time (LT₅₀) and median lethal concentration (LC₅₀). To determine the effect of integrating *M. anisopliae* with border crops on black bean aphid population and damage, growth, yield and quality of French bean, a field experiment was conducted using a 4×3 factorial in Randomized Complete Block Design (RCBD) with three replications. Factor one comprised of four treatments: Metarril (2×10⁸ spores/g) with Biomagic (2×10⁸ spores/ml) and alpha-cypermethrin as positive control and water as negative control. The second factor had three treatments, two border crops (Wheat and sunflower) planted and control (no border crop). Data on black bean aphid population, damage severity yield and quality of French bean were recorded. Data was analyzed using SAS version 9.4 at 5% level. The results on bioactivity showed a time and dose response on the aphid mortality. Metarril (84.2%), Mazao (80.7%) and Biomagic with lowest aphid mortality of 68.4%. In the field experiment, a significant reduction was observed in aphid population ($P < 0.0005$) and damage severity ($P < 0.001$) on the plots with border crop. Metarril WP E9 and wheat border crop reduced aphid population, damage and enhanced growth (collar diameter, number of pods), yield (pod yield) and quality of French bean. plots. Border cropping caused an increase in marketable yield (2.7% to 3.1%) compared to control for both seasons, respectively while a sunflower border caused a 4% and 5.4% decrease in the marketable yield compared to no border. This study demonstrates the integrated use of *M. anisopliae* and wheat border crop as a viable alternative to synthetic insecticides in the control of black bean aphids for improved yield and quality of French bean.

TABLE OF CONTENTS

DECLARATION AND RECOMMENDATION	i
DECLARATION	i
RECOMMENDATION	i
COPYRIGHT	ii
ACKNOWLEDGEMENTS	iii
LIST OF PLATES	xi
LIST OF ABBREVIATIONS AND ACRONYMS	xiv
CHAPTER ONE	1
INTRODUCTION	1
1.1 Background information	1
1.2 Statement of the problem	3
1.3. Objectives	4
1.3.1 General objective	4
1.3.2 Specific objectives	4
1.4 Hypotheses of the study	4
1.5 Justification of the study	4
CHAPTER TWO	6
LITERATURE REVIEW	6
2.1 The Origin, taxonomy and botany of French bean	6
2.2 Utilization and nutritional value of French bean	7
2.3 French bean production in Kenya	8
2.4 Climatic and grading requirement for French bean	9
2.5 Production constraints.....	10
2.5.1 Biology and life cycle of black bean aphid (<i>Aphis fabae</i> Scopoli).....	11
2.6 Aphids control methods	12
2.6.1 Chemical control.....	12
2.6.2 Physical and mechanical control.....	13
2.6.2 Cultural control and companion cropping	14
2.6.4 Biological control.....	15
2.7 Mode of action of <i>Metarhizium anisopliae</i> Entomopathogenic fungi.....	16
CHAPTER THREE	18

BIOACTIVITY OF <i>METARHIZIUM ANISOPLIAE</i> AGAINST BLACK BEAN APHID (<i>APHIS FABAE</i>) IN FRENCH BEAN	18
Abstract	18
3.1 Introduction.....	18
3.2 Materials and methods	20
3.2.1 Rearing of aphid colonies	20
3.2.2 <i>Metarhizium anisopliae</i> Entomopathogenic Fungus Formulation.....	22
3.2.4 <i>Metarhizium anisopliae</i> stock solution preparation and dilution.....	24
3.2.5 Experimental design and treatment application.....	25
3.3 Data Collection	26
3.4 Data analysis	27
3.5 Results.....	27
3.5.1 Mycosis test	27
3.5.2 Bioactivity of <i>Metarhizium anisopliae</i> fungi formulations against black bean aphid	28
3.6 Discussion.....	32
3.7 Conclusion	34
CHAPTER FOUR.....	35
EFFECT OF <i>Metarhizium anisopliae</i> AND COMPANION CROPPING ON APHID POPULATION AND DAMAGE IN FRENCH BEANS	35
Abstract	35
4.1 Introduction.....	36
4.2 Materials and methods	37
4.2.1 Experimental site description.....	37
4.2.3. Experimental design and treatments	39
4.2.4 Field preparation, layout and crop management.....	40
4.3 Data collection	43
4.3.1 Black bean aphid population.....	43
4.3.2 Black bean aphid damage	44
4.3.3 Ladybird beetle population at the border crop.....	45
4.3.4. Data analysis	46
4.4 Results.....	46
4.5 Discussion.....	52
4.6 Conclusion	56

CHAPTER FIVE	57
EFFECTS OF <i>Metarhizium anisopliae</i> AND BORDER CROPPING ON GROWTH, YIELD AND QUALITY OF FRENCH BEAN.....	57
Abstract.....	57
5. Introduction.....	58
5.1 Materials and method.....	59
5.1.1 Experimental site description.....	59
5.1.2 Planting materials.....	59
5.1.3. Experimental design and treatments	60
5.1.4 Field preparation, layout and crop management.....	60
5.1.5. Treatment spraying	61
5.2 Data collection	62
5.2.1 French bean growth variables	62
5.2.2. French bean yield variables	62
5.3 Data analyses	64
5.4.1 Effects of <i>Metarhizium anisopliae</i> and border crop on the growth of French bean	64
5.4.3 Effect <i>Metarhizium anisopliae</i> and border cropping quality of French bean.	80
5.6 Conclusion	87
CHAPTER SIX	88
GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS	88
6.2 Conclusions.....	91
6.3 Recommendations.....	91
6.4 Areas for further studies.....	92
REFERENCES.....	93
APPENDICES	117
Appendix A: Research permit NACOSTI	117
Appendix C: Publications	119
Appendix D: The normality test analysis sample for different variables data before being subjected for ANOVA test.....	122
Appendix E: Analysis of variance for Metarril and Mazao supreme different concntration on <i>Aphis fabae</i> mortality under laboratory bioassay.....	123
Appendix F: Analysis of variance for Metarril and Mazao supreme different concntration on <i>Aphis fabae</i> median lethal time (LT ₅₀) bioassay.....	123

Appendix G: Analysis of variance for different products on <i>Aphis fabae</i> mortality under laboratory bioassay	123
Appendix H: Analysis of variance for different products on <i>Aphis fabae</i> LT ₅₀	124
Appendix I: Mean separation effect of different products on aphid mortality and LT ₅₀	124
Appendix J: Analysis of variance for the aphid population and damage severity	124
Appendix K: Analysis of variance for the growth parameters	125
Appendix L: Analysis for number of pods, average pod weight and total pod weight yield	126
Appendix M: Analysis of variance for pod grade, quality, non-marketable and marketable yield	127

LIST OF FIGURES

Figure 2. 1: Mode of action of <i>Metarhizium anisopliae</i> (Reingold <i>et al.</i> 2021).....	17
Figure 3.1: Dose-response curves over time for (A) Mazao supreme® and (B) Metarril WP E9.	29
Figure 3.2: Mortality of black bean aphids treated with Mazao supreme and Metarril WP E9 at different concentrations	31
Figure 3.3: Percent mortality (mean \pm S.E.) of Black bean aphids treatments response over time.	32
Figure 4.1 Map showing the experimental location.....	38
Figure 4.2: Field experimental layout showing different treatment combinations and randomisation	41
Figure 4.3: Field layout showing the spacing and layout of the different crops.....	41
Figure 5.1: Percent mean pod weight per plant and quality grades.....	81

LIST OF PLATES

Plate 2. 1: French bean plant (<i>Phaseolus Vulgaris</i> . L) at podding stage.....	6
Plate 2.2: Black bean aphids (<i>Aphis fabae</i>) on the French bean	11
Plate 3.1: A set up of the black bean aphid rearing on French bean potted plants in green house cages.....	21
Plate 3.2: Microscopic mycosis test on the black bean aphid	26
Plate 3.3. Fresh French bean leaflet placed in a 90mm petri dish	32
Plate 4.1: Field layout showing border crops (A), sunflower (B) wheat crop and (C) French bean crop.....	42

LISTS OF TABLES

Table 3.1: Product description.....	23
Table 3.2: Percent Spore viability of Mazao supreme Metarril E9 and Biomagic <i>M. anisopliae</i> formulations.....	24
Table 3.3: Median Lethal time (LT ₅₀) aphid mortality for Mazao supreme and Metarril at different concentrations.....	30
Table 3.4: LC ₅₀ values for Mazao Supreme and Metarril WP with exposure time.....	30
Table 4.1: Mean monthly rainfall, relative humidity and temperature data at the site during seasons.....	38
Table 4.2: Description of the treatment combinations.....	40
Table 4.3: Aphid population score scale.....	43
Table 4.4 Damage severity score scale.....	45
Table 4.5: Mean aphid abundance and damage severity with border crops in both seasons.....	47
Table 4.6: Effect of <i>Metarhizium anisopliae</i> on bean aphid abundance and damage severity score.....	47
Table 4.7: Effects of <i>Metarhizium anisopliae</i> and border crop on aphid population in both seasons.....	49
Table 4.8: Effect of <i>Metarhizium anisopliae</i> and border crop on damage severity in both seasons	50
Table 4.9: Effects of <i>Metarhizium anisopliae</i> and border crop on ladybird beetle population in season	52
Table 5.1: Effects of <i>Metarhizium anisopliae</i> and border crop on plant height (cm) on cumulative plant height (cm) in both seasons.....	65
Table 5.2: Effects of <i>Metarhizium anisopliae</i> and border crop on plant height (cm) in both season one (April to July 2022) and season two (September to December 2022).....	67

Table 5.3: Effects of <i>Metarhizium anisopliae</i> and border crop on collar diameter (cm) in both seasons.....	69
Table 5.4: Effects of <i>Metarhizium anisopliae</i> and border crop on the average mean of collar diameter (cm) in both seasons.....	71
Table 5.5: Effects of <i>Metarhizium anisopliae</i> and border crop on the on cumulative number of branches (no. per plant) in both seasons.....	72
Table 5.6: Effects of <i>Metarhizium anisopliae</i> and border crop on number of branches of French bean in both seasons.....	74
Table 5.7: Effects of <i>Metarhizium anisopliae</i> and border crop on the number of pods (no. of pods /plant) in both seasons.....	76
Table 5.8: Effects of <i>Metarhizium anisopliae</i> and border crop on average pod weight yield (g) in both seasons.....	78
Table 5.9: Effects of <i>Metarhizium anisopliae</i> and border crop on total pod weight (tonnes/ha) in both seasons.....	79
Table 5.10: Effects of <i>Metarhizium anisopliae</i> and border crop on percent (%) marketable and non-marketable yield in both seasons.....	83

LIST OF ABBREVIATIONS AND ACRONYMS

a.i:	Active ingredient
AFA:	Agriculture and food Authority.
ANOVA:	Analysis of Variance
BBA:	Black bean aphids.
CRD:	Complete randomized design.
EPF:	Entomopathogenic Fungus
FAO:	Food and Agriculture Organization
FAO:	Food and Agriculture Organization.
FAOSTAT:	Food and Agriculture Organization Statistics
GDP:	Growth Domestic Product
HCD:	Horticultural Crop Directorate
ICIPE:	International Centre of Insect Physiology and Ecology.
ICIPE:	International Centre of Insect Physiology and Ecology
KALRO:	Kenya Agricultural Livestock Research Organization.
KNBS:	Kenya National Bureau of Statistics.
PCPB:	Pest Control Product Board.
PHI:	Pre-harvest intervals
PRiF:	Pesticide Residues in Food
RUFORUM:	Regional Universities Forum for Capacity Building in Agriculture.
SDA:	Sabouraud dextrose agar (SDA)
SNV:	Netherland Development Organization.
TAGDEV:	Transforming African Agricultural Universities to Meaningfully Contribute to Africa's
UNECE:	United Nations Economic Commission for Europe.
WHO:	World Health organization.

CHAPTER ONE

INTRODUCTION

1.1 Background information

Horticultural sector which includes production and sale of flowers, fruits and vegetables, is one of the most valued subsectors of agriculture in Kenya, contributing 29.3% to the Gross Domestic Product (GDP) and 80% of national employment (FPEAK, 2020). The domestic value of horticulture production in 2021 amounted to Kenyan Shillings (KES) 285.4 billion compared to 269.68 billion KES in 2020, equivalent to 5.8% increase (HCD, 2021). Horticultural exports earn the country a considerable income, with fresh vegetable exports fetching about 48% of the foreign exchange. Vegetable production directly offers food and nutritional security, increased incomes and employment to thousands of small holder farmers (HCD & AFA, 2020).

French bean (*Phaseolus Vulgaris* L.), also known as green beans, snap beans, kidney beans, haricot beans, or string beans (Khondoker *et al.*, 2020) is an exotic vegetable that continues to gain commercial value due to its huge demand in the export market. Kenya is the second-largest exporter of French beans in Africa after Morocco, contributing about 52% in value and 61% of the volume of total vegetable exports (Fulano *et al.*, 2021; Trade Map, 2020). French beans are grown mainly for their edible pods, which are rich sources of nutrients such as carbohydrates, dietary fibres, proteins, vitamins and other essential minerals (Didinger & Thompson, 2021; Myers *et al.*, 2019).

Despite the importance of French beans to the economy, profitable and safe crop production is still faced with several challenges. The average yield of French bean in Kenya, estimated to be 5.6- 8.8 tonnes per hectare, falls below the world average yield of 14 tonnes per hectare. China has an average yield of 26 tonnes per hectare (Mwangi *et al.*, 2019). Since 2016, Kenya's vegetable sector, inclusive of French bean has suffered a decrease in export volumes from 22% to 16% in 2020 (HCD, 2020). The decline has been attributed to abiotic factors (drought, temperature, light, soil fertility and relative humidity) and biotic stress, mainly diseases and insect pests (FAO, 2021). These factors reduce the quality and yield of French bean pods (Kalawa *et al.*, 2018; Njenga *et al.*, 2021). Black bean aphid (*Aphis fabae* Scopoli) is among the economically important insect pests limiting the realisation of maximum yields and quality of French beans in the country.

Aphis fabae is considered among the most serious insect pests worldwide, capable of causing 70-80% yield loss in various crops, particularly vegetables (Nordey *et al.*, 2017). The losses are either due to direct damages caused by sucking plant sap and wounding plant tissues (Boni *et al.*, 2020) or indirect damage through the transmission of pathogens to healthy plants. The honeydew secreted by aphids forms sooty mould on plant foliage and subsequently reduces the photosynthetic capacity of French bean leaves, reducing yield and quality (Wamonje *et al.*, 2020). French bean growers use different insect pest control strategies such as cultural, mechanical and chemicals/ pesticides to minimise such losses.

Synthetic pesticides are the most preferred in managing the aphids because they are considered easy to apply (labour saving), more effective and accessible than most other methods. However, several drawbacks are associated with frequent and indiscriminate use of insecticides. These include environmental pollution (bio-magnification), and threat to natural enemies and pollinators (Bass *et al.*, 2015; Marete *et al.*, 2021). Repeated use of synthetic insecticides often renders the management approach less effective at suppressing aphid populations. This is could be partly attributed to increase chance of resistance build-up by the insect pest (Mweke *et al.*, 2020). In addition, use of synthetic insecticides may lead to the accumulation of pesticide residues in fresh and processed products (Marete *et al.*, 2020; Sharma *et al.*, 2019).The adverse effects of synthetic pesticides create an impetus for French bean growers to seek environmentally safe and acceptable alternative control measures against this important pest.

Natural pest regulation using natural biological agents such as predators, parasitoids, or entomopathogenic fungi and companion planting as an intercrop or trap crop present safe alternatives to synthetic pesticides (Colmenarez *et al.*, 2020). Several species of entomopathogenic fungi (EPF) have been studied for use as an eco-friendly strategy for management of a wide range of agricultural pests (Kisaakye *et al.*, 2021; Nawaz *et al.*, 2022; Paradza *et al.*, 2022; Shanker *et al.*, 2023) presenting an opportunity to be used to manage insect pests such as aphids. Entomopathogenic fungi such as *Metarhizium* ssp are a beneficial group living in the soil that infects insects by penetrating insect cuticles and their bodies to eventually kill the pest (Rajula *et al.*, 2021). According to Bamisile *et al.* (2021), EPFs are pathogenic to insect pests with broad host plants and are potential pest management approaches. Farmers in other parts of the world, including some parts of Asia, North America, Europe and South Africa, have successfully used

EPF products to control a wide range of pests alone or in combination with other pest management methods.

Companion planting, on the other hand, is a strategy used to manage insect pests and support a natural enemy population through vegetative diversification (Sarkar *et al.*, 2019). Companion crops act as either repellent or attractant of pests and can reduce pest effects on the primary crop. This has proved an effective control strategy for aphids in crops like collards (Gontijo *et al.*, 2018), hot pepper (Waweru *et al.*, 2021) and sweet pepper (Ben-Issa *et al.*, 2017a), reducing on the use of high dosages of pesticides (Parker *et al.*, 2013). Furthermore, companion cropping is a traditional practice among most vegetable farmers used to diversify income sources for the farmers due to the economic value of the additional crop as well as to preserve the biodiversity of living organisms within the ecosystem (Ben-Issa *et al.*, 2017a). However, there is limited empirical research on the integrated pest management (IPM) approach to controlling insect pests like the black bean aphid in Kenya. Integrating the use of entomopathogenic fungi with companion cropping presents a potential opportunity to enhance their effectiveness in managing black bean aphids in French bean production. This will ensure the safety of human health and the environment and minimize the development of resistance by the pest to the synthetic pesticide.

1.2 Statement of the problem

Increased aphid incidences and severity accentuated by climatic variability and limited defined and safe strategies to counteract their effects remain a significant constraint to French bean production in Kenya. Black bean aphids account for 37-90% of yield loss and direct damage to legume crops annually in Kenya, including French beans. As a result, growers have engaged in continuous and unregulated pesticide application. Synthetic pesticides are preferred because of their accessibility, quick pest knock-down effect and ease of application to the target pests compared to other methods. However, the use of synthetic pesticides has raised concerns about their threats and adverse effects on humans, and other animals' health, environmental pollution, build-up of pest resistance and residual accumulation on agricultural produce. Strict guidelines on quality and maximum residue levels (MRL) in the export market have seen several tonnes of French beans continuously rejected and listed as unsafe for human consumption. The constant economic losses and damage caused by black bean aphids significantly threaten growers' income, food and nutrition security. Integrated pest management achieved through the combined use of

biopesticides containing *Metarhizium anisopliae* and companion cropping in French beans has not been fully researched.

1.3. Objectives

1.3.1 General objective

To contribute to food and nutritional security as well as improve livelihoods through the use of entomopathogenic fungus and companion cropping to control black bean aphid in French beans.

1.3.2 Specific objectives

- i. To determine the bioactivity of *Metarhizium anisopliae* entomopathogenic fungi against the black bean aphid in French beans.
- ii. To determine the effects of *Metarhizium anisopliae* entomopathogenic fungus and companion cropping on aphid population and damage on French beans
- iii. To determine the effects of *Metarhizium anisopliae* entomopathogenic fungus and companion cropping on French bean growth, yield and quality.

1.4 Hypotheses of the study

The following hypotheses were tested;

- i. *Metarhizium anisopliae* entomopathogenic fungus has no significant bioactivity against black bean aphid in French bean.
- ii. *Metarhizium anisopliae* entomopathogenic fungus and companion cropping have no significant effects on the black bean aphid population and damage on French bean.
- iii. *Metarhizium anisopliae* entomopathogenic fungus and companion cropping have no significant effects on French bean growth, yield and quality.

1.5 Justification of the study

Kenya's fresh vegetable export sector accounted for 30%, 22% and 24.6% of the total export volume in 2019, 2020, and 2021, respectively (FPEAK, 2020; HCD, 2021). French beans are among the fresh export crops that contribute significantly to Kenya's Gross Domestic Product (GDP) and are a leading export vegetable that accounts for 15.7% of foreign exchange. French bean production earns substantial income for small-holder farmers in Kenya, especially women and youth actively involved in its production. An increase in French bean production, yield and quality would therefore be an important step towards enterprise diversification and improved living standards among farmers.

French bean production relies on high usage of synthetic pesticides to manage most insect pests, including the black bean aphid. Increased dosage and repeated applications of these chemicals have negatively impacted compliance with the maximum residual level requirement in the export markets. This resulted in the European Union (E.U.) regulation amendment of 669/2009 that has seen Kenyan fresh beans (French beans and peas) subjected to 10% increased mandatory inspection for chemical residues and quality checks, leading to frequent rejection of substantial volumes of the produce. To maintain the economic role of French beans in improving livelihood amidst the increasing concerns on unacceptable pesticide use and residue levels in the export market. There is a need for alternative, safer, environmentally friendly pest management strategies that must be developed, implemented and promoted.

Biopesticide and companion planting provide multiple benefits to the growers. They are environmentally safe and have lower residues, less resistance build-up, and a lower post-harvest interval (PHI). Fungi are ubiquitous natural entomopathogens that often cause epizootics in host insects and possess many desirable traits that favour their development as Microbial control agents. The difficulties in isolation and characterization of novel strains, negative effects of geographical location, vegetation type and human disturbance on fungal entomopathogens are among the numerous setbacks that have been documented (Bamisile *et al.*, 2021; Lacey *et al.*, 2015). More testing under field conditions is required to identify effects of biotic and abiotic factors on efficacy and persistence. Lastly, greater attention must be paid to their use within integrated pest management programs to ensure compatibility and maximize efficacy. There is limited knowledge of the additive effect of entomopathogenic fungi and companion cropping used in an integrated pest management (IPM) context to control black bean aphids in French beans. The study's findings stand to contribute to safer and sustainable production of high-quality French beans amongst Kenyan producers and SDGs (2, 3, 15 & 17), Kenya's big four agenda and blueprint vision 2030. The study results also contribute to the existing information with French bean growers (small, medium and large-scale farmers), other scientists and policy makers on the combined use of EPF (*Metarhizium anisopliae*) and companion cropping as an effective integrated pest management approach against the black bean aphid in French bean production.

CHAPTER TWO

LITERATURE REVIEW

2.1 The Origin, taxonomy and botany of French bean

French bean (*Phaseolus Vulgaris* L.) is one of the most important and nutritious vegetable crops and is native to Central and South America (Zeder, 2006) and is grown worldwide for its immature tender edible pods (Herron *et al.*, 2020). French beans have two distinct gene pools: the Andean gene pool that originates from the highlands of South America and the Mesoamerica gene pool from Central America (Arunga *et al.*, 2015; Wallace *et al.*, 2018). Arunga and Oriama (2020) recorded and reported that about 85% of the French bean genotypes in East Africa are of the Andean gene pool. French bean does belongs to the Fabaceae family and the genus *Phaseolus* that consists of five cultivated species; *Phaseolus Vulgaris* (common bean/French bean), *Phaseolus lunatus* (lima bean), *Phaseolus coccineus* (runner bean), *Phaseolus polyanthus* (year bean) and *Phaseolus acutifolius* [teparty bean] (Chaurasia, 2020).



Plate 2. 1: French bean plant (*Phaseolus Vulgaris*. L) at podding stage

French bean is an annual herbaceous dicotyledonous plant with pods harvested at the immature, tender and green stage (Arunga *et al.*, 2015; Khondoker *et al.*, 2020). The crop undergoes epigeal germination with a taproot system of highly branched secondary roots with nodules. There are two common growth habits, for example: determinate and indeterminate. The determinate type grows up to 30-60 cm in height and is preferred to the indeterminate type that grows to more than 3 m high. The plant consists of slender stems and trifoliate leaves with a hermaphrodite flower forming a raceme of about 12 individual flowers. Most of the flowers

undergo self-pollination, with the chance for cross-pollination depending on the varieties. After fertilization, each flower will produce slender, cylindrical, single green pods with a diameter (0.6-2.0) cm and a length (8-20) cm depending on the variety (Infonet Biovision, 2020). Masiga *et al.* (2014) observed that a self-fertilization situation lessens the chance of parthenocarpy or apomixis, which enhances pod uniformity influenced by seed set consistency. For example, where all ovules are fertilized, the pods grow uniformly, giving the producer the first-grade product.

2.2 Utilization and nutritional value of French bean

The fresh French bean pods are eaten in different forms, such as salads and desserts, or as a fresh cut, soups, frozen, and canned. The French bean pod constitutes 1.85g of protein, 4.5g of carbohydrates, 0.1 g fats, 0.16 mg iron, 1.8 g of fibre, 1.76 mg of calcium, and 3.43 mg zinc per 100g and 0.42 mg of potassium per 100g compared to meat (Didinger & Thompson, 2021; Myers *et al.*, 2019). Myers *et al.* (2019), in their study on improving the health benefits of snap bean noted that regular consumption of French beans increased because consumers were aware of the nutritional components such as soluble fibre, low-fat and cholesterol levels. French bean pod also contains lower protein and carbohydrate levels due to high water content (90%) than dry bean. It also possesses pro-vitamins A, carotenoids B₃, C and K, folic acid, thiamine and Omega-3 fatty acids that are a vital part of human nutrition compared to other leafy vegetables (Chaurasia, 2020; Myers *et al.*, 2019).

Other nutritional benefits of French bean pods are that they contain polyphenols, anthocyanin, vitamin C, and carotenoids with polyphenol component being the highest compared to bush beans and Romano beans (Ci *et al.*, 2017). These bioactive compounds act as an anticancer and anti-diabetes, aid weight loss due to slow digestibility help reduce food appetite. It is cost-effective as it requires less energy to cook than any other vegetable. Chaurasia (2020) observed that consuming quality French bean pods does significantly lowers blood pressure and improves the digestive system. The antioxidant properties and bioactive molecules like phenols, carotenoids and flavonoids can lower the damaging effect of cancerous cells, neurological and chronic diseases. The mineral (folic acid) contained in French bean pods increases fertility among women due to its ability to promote fetus development and disorder. Despite the importance of French beans as a vegetable to Kenya's economy, there has been a significant decline in export volume from 140,000 tonnes in 2012 to 20,000 tonnes in 2018 (FAOSTAT, 2021). Marete *et al.* (2020) attributed the decline in the export volume to low-quality produce, insect pest presence, and

chemical residues above the set limits. The presence of residues above the maximum limit causes a double impact on the consumers' health and the product quality due to rejection in the market (Omwenga *et al.*, 2021). This is due to the indiscriminate and continuous application of chemicals and noncompliance to the postharvest quality requirement of the export market by most small-scale growers of French beans.

2.3 French bean production in Kenya.

Kenya is the leading producer of French beans in East Africa, with an estimated production of 40 416 tonnes annually, followed by Tanzania, Uganda, Rwanda, Burundi, and South Sudan, respectively (FAOSTAT, 2021; Mwangi *et al.*, 2019). French bean production in Kenya contributed to the 6.1% increase in fresh horticulture export to 322.6 tonnes in 2018. French beans as an export crop account for 61% of the volume of total vegetable export and 52% of the value and this translates to growth in the country's GDP (Horticultural Crop Directorate (HCD), 2020; Kenya National Bureau of Statistic (KNBS), 2019). This led to the creation of 80% of employment opportunities directly and indirectly for more than a million people in Kenya (Fulano *et al.*, 2021; Mwangi *et al.*, 2019). According to NSV (2012), the French bean production sector employs over 60,000 people, 60% of whom are women, followed by youth.

In Kenya, French bean cultivation is concentrated around the Central, Eastern, Western, and Coast provinces due to the favourable conditions for its production (Arunga *et al.*, 2015). These areas include Meru, Embu, Kirinyaga, Nyeri, Muranga, Maragua and Kiambu. Other areas include Machakos, Makueni, Naivasha, Bungoma, Vihiga, Trans Nzoia and Kericho (Matere, 2020). Approximately 80% of French bean growers in Kenya do it on a small scale and the production is highly structured on contract-based farming between small to medium-scale farmers and a few large-scale growers (Mwangi *et al.*, 2019; Rosch & Ortega, 2019). Dwarf (determinate) and climbing (indeterminate) are commonly grown French bean types. The determinate varieties include Samantha, Enclave, Loby, Gloria, Paulista, Amy, Serengeti, Teresa, Belcampo and Cupvert for fresh markets (Gozaimasu, 2016). Enclave cultivar of French beans is preferred by most growers for export compared to other cultivars because of its high yielding potential for the extra fine grade, circular and straight in shape, most pods (12-13 cm in length and 6-8mm in diameter). It can also mature in 54 days and with medium-dark, green-coloured pods. French bean is mainly considered a cash crop due to its low consumption rate and demand in the domestic or local market. This is because most farmers are organized in groups and based on a contract

agreement that restricts sales to the local markets of the agreement (Fulano *et al.*, 2021; Kimno *et al.*, 2016).

2.4 Climatic and grading requirement for French bean

French bean is a warm-season vegetable crop, sensitive to high and chilling temperatures at all stages of growth and development (Infonet Biovision, 2020). The optimum temperature for growth ranges between 20-25 °C but can accommodate temperatures in the range of 14-32°C depending on the variety (Greenlife Crop protection Africa, 2021). On the contrary, Kakon *et al.* (2018) observed that the optimum growth of French beans occurs at a temperature range between 14-25 °C, with a minimum at 10 °C and a maximum at 30 °C where there is no significant growth. Ideal soil pH range between 5.5.-6.5. The seeds germinate faster in (4-9 days) within 18-24 °C, but below 15 °C, the process is hindered. Uwiringiyimana (2020) observed that temperature ranges influence plant height, pod quality, and abscission of flower buds. French beans can grow at an altitude range of 1000-2100m above sea level and, for optimal growth, require moderate and sustainable annual rainfall between 900-1200mm (Greenlife Crop protection Africa, 2021; Infonet Biovision, 2020).

Depending on water availability, soil fertility, variety and other factors, spacing of 30 x 15cm (one seed per hole) for a single row or 60 x 30cm (two seeds per hole) for double rows is recommended (Greenlife Crop protection Africa, 2021). Diammonium Phosphate (DAP) is applied at 200 kg /ha by incorporating it into the soil before seeding to enhance the root development of French beans (Oseko & Dienya, 2015). Calcium Ammonium Nitrate (CAN) at a rate of 150-kg/ha as a top dressing in two equal split applications, first at three true leaf stages and second at the onset of flowering to enhance nutrient availability suitable for growth (Oseko & Dienya, 2015). Most varieties reach maturity and are ready for harvesting between 45-65 days after planting, depending on growing conditions, but Enclave cultivars mature about 54 days after planting, depending on the growing conditions. According to UNECE (2017), French beans are graded into three groups: extra-fine, fine, and bobby grade. Extra fine grade is the finest and the pods are straight, tender and seedless with no strings and should be less than 6 mm in diameter and 10cm less in length, respectively. Fine grades are short with soft strings, may have small seeds and are usually (6-9) mm and (12-14) cm of diameter and length, respectively. The bobby grade is bigger than the fine grade with a more than 9 mm diameter and is reasonably tender with small seeds (Gozaimasu, 2016). The extra fine grade is considered the highest grade in the export market

and fetches a higher pricing value than the fine grade. On the other hand, bobby grade is for domestic use or as feeds in the poultry industry and fetches low price per gram sold.

2.5 Production constraints

French bean production and marketing is still constrained by abiotic and biotic factors such as harsh climatic conditions, poor soil fertility, high temperatures, pests and diseases that cause a reduction in growth, yield and quality (Matere, 2020). This causes significant crop losses and acts as a major barrier to achieving global food security and poverty eradication goals (Pretty & Bharucha, 2015). Globally, insect pests account for 18-35% of average annual yield losses worth more than US\$ 470 billion, which is considerably higher in developing countries (Sharma *et al.*, 2017). The most common insect pests of French bean causing significant damage include bean aphids (*Aphis fabae Scopoli*), bean stem maggot (*Ophiomyia* spp.), western flower thrips [*Frankliniella occidentalis* (Pergande)], red spider mites (*Tetranychus* spp.), whiteflies [*Bemisiatabaci* (Gennadius)] and others (Mwanauta *et al.*, 2015). Bean aphids are among the economic insect pests causing significant loss in yield and quality of French beans grown in Kenya.

In Kenya, black bean aphids account for 37-90% of yield loss among legume crops annually (Ndakidemi *et al.*, 2021). Thus, despite the economic contribution of French beans, many growers still face low yield and quality of 5.6- 8.8 tonnes per hectare compared to the world average of 14 tonnes/hectare. Sharma *et al.* (2017) reported that insect pests are responsible for about 20% of rejection losses in French beans due to quality issues such as short pod, deformation, black sooty mould, discolouration and non-uniformity in shape. Sucking insect pests such as aphids causes injuries and excessive extraction of plant sap that induces stress, hindering photosynthesis and plant growth rate (Perring *et al.*, 2018). Rapid expansion in trade for fresh quality horticultural produce for export purposes and changes in production and marketing systems worldwide call for an urgent need to manage the aphid population to ensure quality products (Fulano *et al.*, 2021). Aphids directly extract plant sap from the phloem and inject toxic saliva that disrupts the physiology of the plant, causing stunted growth, curled or deformed leaves, curved pods, flower abortion and death of young plants (Kinyanjui *et al.*, 2016; Koppert Biological Systems, 2021). Aphids excrete honeydew on the plant's leaves, stems and petioles, which encourages the growth of black sooty moulds that hinders photosynthetic efficiency rate, aesthetic value and reduced quality. Aphids indirectly act as vectors transmitting plant viruses, for example,

cucumber mosaic virus, bean yellow mosaic virus (BYMV), bean common mosaic necrosis virus (BCMNV) and many different potyviruses (Wamonje *et al.*, 2020).

2.5.1 Biology and life cycle of black bean aphid (*Aphis fabae* Scopoli)

Aphis fabae Scopoli (Hemiptera: Aphididae) is a highly polyphagous insect pest and lives in clusters on plant tender parts like stems, petioles, flowers and pods (Koppert Biological Systems, 2021). Aphids show polyphenism behaviour (ability to have wings or not). They reproduce either by asexual or sexual reproduction, depending on the ecosystem conditions (Kumar, 2020; Mehrparvar *et al.*, 2013). The adult female can hatch 40-100 live wingless nymphs in her lifetime. Adult aphids land on a suitable host plant and deposit numerous live nymphs on tender plant tissues. The nymphs start to feed on plant sap, moults four times increase in size and become adults in 7-10 days under favourable conditions. The cycle starts when mature adults deposit eggs or nymphs on the host plant, depending on the conditions and live for about four weeks before it dies (Barbercheck, 2014). Due to aphids' faster and short reproductive rate, they can build up resistance to commonly applied synthetic pesticides with a short duration (Mehrparvar *et al.*, 2013). Winged aphids are formed in search of new hosts due to limited resources, predation, overcrowded colonies and stress alarm pheromones release. They have a more developed sensory system beneficial for locating new habitats, resistant to starvation and sensitive to environmental cues (Mehrparvar *et al.*, 2013).



Plate 2.2: Black bean aphids (*Aphis fabae*) on the French bean plant (a) reproductive stage, (b) vegetative stage

2.6 Aphids control methods

2.6.1 Chemical control

Broad-spectrum chemical pesticides have been developed over time and have contributed significantly to increased yield and income among farmers. Several systemic and contact pesticides such as alpha-cypermethrin, Imidacloprid, Dimethoate, Deltamethrin, Lambda-cyhalothrin, Beta-Cyfluthrin, Abamectin and many others registered in Kenya have shown effectiveness against the aphid population (PCPB, 2022). Omwenga *et al.* (2021) reported that generally, in Kenya, the amounts of pesticides used in vegetable crops are three times higher than in cereal crops. Most vegetable farmers prefer using synthetic pesticides to control different agricultural pests because of their quick effectiveness, affordability and accessibility to enhanced productivity to fetch more income (Bass *et al.*, 2015). However, this has encouraged indiscriminate and increased application frequency that poses potential severe threats to human health, environmental pollution, residual accumulation and development of resistance by the pests (Alfaro *et al.*, 2021; Marete *et al.*, 2021; Sharma *et al.*, 2019). A study by Omwenga *et al.* (2021) in the urban and peri-urban areas of Nairobi found that tomatoes (22%) had the highest residues of different pesticides above the Maximum Residue Limit (MRL) accepted on produce, followed by French beans (21%), Kales and Spinach respectively. This reduced the quality of vegetables produced and posed severe threats to consumer health and the environment due to the harmful effects of chemical pesticides compared to biopesticides.

Alfaro *et al.* (2021) observed that the pyrethroid lambda-cyhalothrin sub-lethal concentrations caused severe side effects on natural enemies such as the parasitic wasp *Aphidius colemani* used to control peach potato aphid *Myzus persicae*. This reduced the effectiveness of controlling cotton aphids with the combined use of chemical pesticides and biocontrol. This affects the reproduction rate, disorientation and disruption of cues interaction by the parasitoids. Sharma *et al.* (2019) reported that the continuous developments of resistance by pests are due to the repetitive application of some pesticides with the same active ingredient insecticide and have adverse effects on the environment and human health. A study around lake Naivasha a horticultural hub, shows most workers (weeders, sprayers and harvesters) had health complications associated with exposure to synthetic pesticides (Tsimbiri *et al.*, 2015). Marete *et al.* (2021) report in Kenya indicated that about 350,000 cases of pesticide poisoning occur annually. A survey study conducted in Meru County, the eastern region of Kenya, among the farming households in Buuri,

Imenti South and Imenti North indicated that 26% of the farmers experienced health effects after using synthetic pesticides (Marete *et al.*, 2021). There is an increase in health problems associated with synthetic pesticide use, among most farmer in developed countries with high pesticides use index (Kim *et al.*, 2020).

As a result, several drawbacks associated with the indiscriminative use of synthetic pesticides include excess residues in produce, pollution and harm to non-target organisms. Kumar and Omkar (2018) observed pest resurgence, resistance build-up, pollination, death of non-target organisms, an outbreak of secondary pests and human health hazards due to repeated use of synthetic pesticides. This presents an urgent need for an environmentally safe alternative approach to tackle increased pest infestation. Several findings (Boni *et al.*, 2020; Mweke *et al.*, 2018; Reingold *et al.*, 2021) recommended using biopesticides as part of an IPM approach for promoting aphids and implemented over chemical pesticides. This is because using *Metarhizium anisopliae* as biopesticide has shown no side effects to the environment and humans, reduced resistance build-up, low residues and zero pre-harvest intervals, yet still can effectively suppress the aphid pest population.

2.6.2 Physical and mechanical control

The physical management approach reduces the access of insect pests to crops by either disrupting their behaviour or causing their direct mortality (Kumar & Omkar, 2018). The use of warm water treatment, flooding, bagging of fruits, roughing of the infested plant, hand picking, flooding, light or coloured trap, use of agro-net, repelling and trap crop have been documented in the control of various insect pests (Thakur *et al.*, 2021). Nawaz *et al.*'s (2016) study on using different types of hollow nozzles with tap water to control Mustard aphid (*Lipaphis erysimi*) in *Brassica juncea*. The result found that high-pressured water application using a hollow nozzle reduced the aphid population by 90.8% compared to the control (no water applied). A study by Gogo *et al.* (2014) on the use of agronet covers found a reduction in the populations of Silverleaf whitefly (*Bemisia tabaci* Gennadius) and black bean aphids (*Aphis fabae* Scopoli) in French beans compared to the control plots. Despite the use and contribution of the physical control approach in managing insect pests, the costs involved, such as the use of agronet, time and training, are not cost-effective for smallholder farmers (Kumar & Omkar, 2018). For example, a detasseling technique to control corn borers is labour intensive, reducing the effectiveness and practicability of the technique for pest management.

2.6.2 Cultural control and companion cropping

Several cultural methods, like crop rotation, intercropping, and field sanitation, are evidenced to increase the productivity of a given crop with timely application. Hedges *et al.* (2018) observed that intercropping kale with culinary herbs such as green onion, parsley, cilantro and coriander effectively reduced the aphid population and their losses. However, intercropping alone was not adequate to manage pests during kale production and thus need for better compatible intercropping designs with other methods that could lower aphid populations. Waweru *et al.* (2021) also observed a significant reduction in the incidence of aphid-transmitted viral diseases, particularly *Cucumber mosaic virus*, *Potato virus Y* and *Pepper veinal mottle virus* in the hot pepper with border crops such as sorghum, maize and sunflower. The border crops did not reduce the number of aphids on the main crop (hot pepper) and recommended further field trials to monitor the aphid population at the border plants.

In East Africa, mixed cropping is common among small-scale farmers, particularly in Kenya (Tesemma *et al.*, 2010). This aims to diversify sources of income, replenish the soil, spread risk, and manage pests and diseases. Nyasani *et al.* (2012) observed that intercrops are not only grown for their market value but have been known for several centuries as a remedy for pest control and management, for example, in the management of thrips. His research concluded that intercropping French beans with other crops compromised its yield but reduced damage to the bean pods, thereby enhancing marketable yield. Ben-Issa *et al.* (2017a), Sarkar *et al.* (2018) and Reddy (2017) observed that intercrops, when used as traps, for example, sunflower and nasturtium are more attractive to some specific pests and lure them away from the main crop. According to Kumar (2017), using insecticides on trap crops reduces the application rate to the main crop and exposure to chemicals. This also lowers the cost and lessens the negative effect that could be inflicted on the natural enemies with the feasibility of employing IPM. As a result, this also enhances the quality of the produce like the French bean pods and their shelf life after harvesting. Mweke *et al.* (2020) observed that the application of entomopathogenic fungus on maize-cowpea intercrop successfully controlled *Aphis craccivora* Koch population without any effects on aphid-associated natural enemies. Studies have shown the successful use of the sunflower *Helianthus annuus*. L (Asteraceae) as a trap crop to manage some insect pest populations that belong to the coleopteran, lepidopteran and hemipteran orders (Sarkar *et al.*, 2018; Shelton & Badenes-Perez, 2006). Other authors (Bortolotto *et al.*, 2015; Khan *et al.*, 2017; Parker

et al., 2013; Sharma, *et al.*, 2019) have also documented the successful use of Wheat (*Triticum aestivum*. L) as a companion crop for aphid (*Rhopalosiphum padi* and *green peach*) to manage their population below the economic inquiry level. Hansen *et al.*(2008) observed that intercropping Field bean (*Vicia faba*) with spring wheat and spring barley reduced the number of black bean aphids per plant and the number of infested plants significantly. When intercropped for two years (2000 and 2002), there was an increase in profits from 42 and 70% in intercrop compared to field bean as monocrop.

The use of companion cropping approach is an important tool in pest management. It allows auto conservation, pests regulation, and stability of the ecosystem by modifying habitat to support and sustain the population of native natural enemies for insect pests like parasitoids and predators, thus reducing the use of synthetic pesticides (Amala & Shivalingaswamy, 2018; Ben-Issa *et al.*, 2017). Companion cropping provides shelter and acts as a food bank for beneficial pollinators, parasitoids and predators (Mwani *et al.*, 2021). Despite the successful use of companion crop and intercropping approaches in managing some pest populations, combining their use with other IPM strategies such as biopesticide, multiple trap crops, among others would enhance their effectiveness in managing the aphid population. When used solely, most cultural control approaches are less effective in insect pest management (Kumar & Omkar, 2018). Additionally, there are drawbacks to the use of cultures, such as the cost involved in acquiring an in-depth understanding of how to synchronize the approach with the pest infestation, which is time-consuming and labour intensive. There is a need for research on an integrated pest management approach that could enhance the effectiveness of cultural methods, with other techniques such as biopesticides.

2.6.4 Biological control

The use of biological control dates back to around 4,000 years ago by ancient Egyptians (Kwenti, 2017). After world war II, there was a significant decline in the use of biological control due to the innovation of synthetic pesticides to increase productivity(Miller &Rebek, 2018; Teresa *et al.*, 2019). Between the 1960s and 70s, pesticide resistance by pests surfaced. This led to the conceptualization of the integrated pest management (IPM) approach to tackle the drawback of synthetic pesticides to non-targeted organisms and environmental pollution documented by Rachael Carson (1962). This also aims to reduce residual accumulation, resistance, or cross-resistance of pests and pest resurgence due to synthetic pesticides (Matere, 2020). Biological

control strategies like predators, parasitoids and EPF have been used commercially to manage the aphids population (Sharma *et al.*, 2019).

Metarhizium anisopliae (Metschn) Sorokin (Hypocreales: Clavicipitaceae) is an important entomopathogenic fungus with a broad host range (Srinivasan *et al.*, 2019; Villamizar *et al.*, 2021). It is a broad pathogenic fungus to more than 200 species of insects belonging to different orders like Coleoptera, Lepidoptera, Orthoptera, Hemiptera and Thysanoptera (Akutsee *et al.*, 2020; Boni *et al.*, 2020; Iwanicki *et al.*, 2019). *Metarhizium anisopliae* is also known as green muscardine fungus. *Metarhizium anisopliae* as biopesticides accounts for increased area usage from 43,290 hectares in 2015 to more than 132,980 in 2019 (Akutse *et al.*, 2020). More than 37,600 farmers use *Metarhizium spp* products in sub-Saharan Africa, as reported by ICPE (2019). In Kenya, commercialized EPFs include *Lecanicillium lecanii*, *Metarhizium anisopliae*, *Beauveria bassiana* and *Isaria spp* (Mweke *et al.*, 2019). Different products containing *Metarhizium anisopliae*, in particular, are available in different brand names like Metarril WP (Koppert Biological System), Mazao Supreme (Real IPM) and ICIPE62 (Srinivasan *et al.*, 2019). The EPFs have a wide host range but are more specific; insect pests such as aphids have also been shown to be susceptible to infections caused by fungi and capable of under natural conditions to regulate their populations. There is a need to introduce more efficient strains of entomopathogenic fungi than those already occurring in an area. The EPFs infest pests through physical penetration or enzymatic degradation of the cuticle, then secrete toxins that kill; hence, the pest is less likely to build resistance and cause negative effects than the chemical. This presents an alternative pest management approach for bean aphids and minimizes the use of synthetic pesticides. As the EPFs are rendered safe to humans, less harmful to the environment, relatively host-specific, reduced resistance build-up, low residues and zero pre-harvest intervals requirements compared to chemical pesticides (Akutse *et al.*, 2020; J. Kumar *et al.*, 2021; Mweke *et al.*, 2018).

2.7 Mode of action of *Metarhizium anisopliae* Entomopathogenic fungi.

The pathogenic fungus can infect the host by directly penetrating the insect cuticle through physical pressure and cuticle degrading enzymes (Villamizar *et al.*, 2021). Therefore, in contact with the suitable host cuticle using hydrophobic interaction and adhesion. Fungal conidia or asexual spores germinate, conidia and develop infective peg. The infective peg penetrates the host cuticle through the combined use of mechanical pressure from the appressorium. The action of cuticle-degrading enzymes like trypsin, metalloproteases and aminopeptidases as detailed in

Figure 2.1. The fungus hyphae penetrate the host haemocoel to obtain nutrients while releasing insecticidal toxin cyclic peptides substance called destruxins that affect host immunity. This eventually kills the host and infective conidia re-emerge from the mummified host body to infect a suitable healthy host again (Boni *et al.*, 2020; Brunner-Mendoza *et al.*, 2019). Reingold *et al.*(2021) found infective green spores on host cadavers capable of infesting new healthy pest populations and causing death. This makes microbial insecticides more effective for sucking insects, particularly aphids, because of the need for only contact with the suitable target host to cause an effect in form of the disease that suppresses the host population.

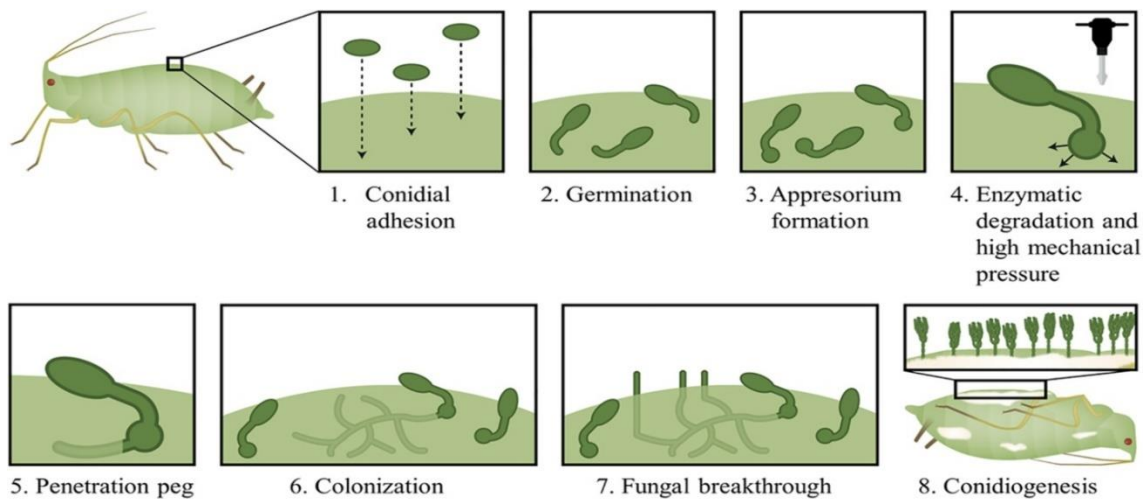


Figure 2. 1:Mode of action of *Metarhizium anisopliae*.

Mkiga *et al.* (2021) observed that combining EPF *Metarhizium anisopliae* ICIPE 69 with sex hormone effectively suppressed the false codling moth (FCM) population in an orange orchard and increased the marketability yield. Sajid *et al.* (2017) observed 83.23% effectiveness of biopesticide containing *Metarhizium anisopliae* in the invitro control of mustard aphids on kale leaves compared to *Beauveria bassiana* (78.33%) and *Bacillus thuringiensis* (73%), respectively. According to Kim *et al.* (2020) and Srinivasan *et al.* (2019), microbial pesticide use, in particular, the entomopathogenic fungi, is safe, cheaper and with a broad host range compared to synthetic pesticides with increased negative folds on the environment and humans. Fungus production cost is considered lower compared to synthetic pesticides.

CHAPTER THREE
BIOACTIVITY OF *METARHIZIUM ANISOPLIAE* AGAINST BLACK BEAN APHID
(*APHIS FABAE*) IN FRENCH BEAN

Abstract

A two-step bioassay to evaluate the efficacy of two commercial *Metarhizium anisopliae* formulations to control the black bean aphid was conducted in petri dish experiments in the laboratory at Egerton University using a Complete Randomized Design (CRD) design with five replications. In step 1; Mazao SUPREME® aqueous formulation was tested at 1×10^8 , 2×10^8 and 4×10^8 cfu/ml spore concentration and Metarril WP E9 Wettable powder at 1×10^8 , 2×10^8 and 4×10^8 cfu/g. Triton water used as a control. In step 2; Mazao SUPREME® was tested at 2×10^8 cfu/ml spore concentration and Metarril WP E9 2×10^8 cfu/g. Biomagic 1.5 Liquid Formulations (L.F), *Metarhizium anisopliae* product registered to control aphids, alpha-cypermethrin synthetic insecticide and triton water were used as checks. Data (mortality) was analysed using SAS version 9.4, (2021). The results showed concentration and exposure time-dependent increase in efficacy of both formulations on black bean aphids. Significant effects on median lethal time (LT_{50}) of about 4 days for both Metarril WP E9 and Mazao supreme formulations. The median lethal concentration (LT_{50}) was 0.4×10^8 cfu/ml for Mazao Supreme and 0.43×10^8 cfu/g for Metarril WP E9. Mazao supreme (4×10^8 spores/ml) and Metarril (4×10^8 spores/ml) caused the highest mortality (95.5%), Alpha-cypermethrin caused the highest aphid mortality (99.3%) and the lowest was Biomagic treatment (68.4%). The study recommended Metarril (2×10^8 spores/g) for further open-field evaluation.

3.1 Introduction

Insect pest management is a pre-requisite for enhanced horticultural crop productivity and subsequent impact because of their significant damage and loss to the crop (Srinivasan *et al.*, 2019; Zhao *et al.*, 2020). French bean (*Phaseolus Vulgaris* L.) is a leading among vegetable crop grown primarily for export in Kenya (Fulano *et al.*, 2021). Besides French bean's economic and nutritional importance, its productivity in Kenya is still constrained by many biotic and abiotic factors such as insect pests, diseases, drought, and soil fertility, which affect the productivity and profitability of the crop (Bansode *et al.*, 2019; Shaban, 2021; Yang *et al.*, 2023). The most common

pests of French bean are the sap-sucking insect pests, among them being aphids, thrips, leafhoppers and spider mites that cause significant losses and quality reduction in the vegetable.

Black bean aphid (*Aphis fabae* Scopoli) (Hemiptera: Aphididae), is among the most significant insect pests causing a threat to vegetable productivity (Singh & Singh, 2021). It is French beans' most destructive phytophagous insect pest, with more than 200 host species globally (Saruhan, 2018). Black bean aphid causes severe damage and crop loss to French bean, usually grown for their edible immature pods, purposely for export. The damage to the plant is to all plant parts, such as leaves, stems, pods and flowers, through sap-sucking, resulting in stunted growth and reduced yields, besides playing an essential role as a vector for viral diseases (Srinivasan *et al.*, 2019). Black bean aphid also produce honeydew, a sticky substance that can attract sooty mold and other fungi, further reducing the crop's productivity and aesthetic value of the pods. The destructive nature of aphids is associated with their rapid reproduction and parthenogenesis (Zhao *et al.*, 2020). To manage the damage caused by aphids, prevention and management approaches rely more on synthetic insecticides to suppress their population (Zaller, 2020). This results in indiscriminative and frequent use of insecticide, which negatively impacts the environment and may lead to the development of resistance by aphids. In addition, several studies (Alfaro-Tapia *et al.*, 2021; Ara & Haque, 2021; Castellanos *et al.*, 2021; D'Ávila *et al.*, 2018; Nayak *et al.*, 2020; Ochieng *et al.*, 2022; Zaller, 2020) have agreed that many synthetic insecticides used to manage aphids are not easily degradable. Besides, they persist in soil, leach into groundwater, and contaminate the environment. These synthetic chemicals have been documented to pose a serious threat to natural enemies of insect pests and pollinators such as ladybird beetles, parasitoids, lacewing and hoverflies that can significantly impact food security (Ochieng *et al.*, 2022). Therefore, alternative approaches to synthetic chemical use in managing insect pests are important to reduce environmental impact and enhance crop productivity. Biological control is considered one of the most effective direct green management approaches for many sucking insect pests that attack French beans with less or no harm to humans and the environment. This is considered the first line of protection in an integrated pest management (IPM) program (Srinivasan *et al.*, 2019).

There are many practices that can be incorporated into integrated systems directed at the management of bean aphids in French beans. Among them is the use of biopesticides and companion cropping to promote natural enemies. Similarly, other studies done so far have reported that use of biopesticides products such as entomopathogenic fungi (EPF) containing *Metarhizium*

anisopliae as comparatively safe for natural enemies and the environment with less residue and no pre- and post-harvest interval (Akutse *et al.*, 2020; Kumar *et al.*, 2021; Mweke *et al.*, 2018, 2019; Sain *et al.*, 2019; Sajid *et al.*, 2017; Srinivasan *et al.*, 2019). Fungal biopesticides are biodegradable, more host-specific, and safer for the environment than persistent synthetic insecticides (Kumar *et al.*, 2021; Warra & Prasad, 2020). These fungal biopesticides are potentially considered a safer and more sustainable alternative to synthetic chemicals. As a result, the use of fungal biopesticide to manage insect pests is in line with the International Organization for Biological and Integrated Control of Noxious Animals, Diseases and Plants (IOBC) principles and guidelines I and II that emphasize biological control (Baur *et al.*, 2019). Where their guidelines are focused on optimizing agroecology use in designing and managing sustainable production systems, supporting and enhancing biological diversity are naturally preferable and safe technologies to manage insect pests. As a result, the demand for microbial pesticides is driven primarily by the need to support high-value export horticultural crops due to the increasingly strict standards for pesticide residues on produce, especially in the export market. However, there is still need for empirical research in the laboratory and open field in different ecological zones to ascertain the effectiveness and efficacy of *Metarhizium anisopliae* products against black bean aphids. This process will also lead to more understanding of the product and its effectiveness against black bean aphids to be used by farmers. Moreover, there is limited information on the overall efficacy and sustainability of microbial biopesticides containing *Metarhizium* species as a management strategy for black bean aphid in sub-Saharan Africa. Because of this, there is a need to evaluate and test microbial biopesticides containing *Metarhizium anisopliae* as potential alternative approach that could minimize residues in produce compared to synthetic chemicals (Gwynn & Maniana, 2017). This study sought to determine the bioactivity of *Metarhizium anisopliae* against black bean aphids.

3.2 Materials and methods

3.2.1 Rearing of aphid colonies

The rearing of the black bean aphid was done on caged potted plants according to Nyaanga *et al.* (2012), with slight modifications. The three cages used in this study measured 2.5 m × 2 m × 1.5 m in size, with 2 mm thick metal frames covered with insect-proof net mesh size 0.4mm. An insect-proof net procured from AgroZ® Insect Net Nairobi was used to prevent any prior parasitism from natural enemies or prevent the entry and exit of aphids and other insect pests into

the cage (Mkenda *et al.*, 2019; Ochieng *et al.*, 2022). Pots measuring 18cm wide and 15cm deep, were filled with containing 1.5 kg peat moss media., Peat moss was used as growing media to provide optimal water absorption, retention rate, good drainage and pathogen free media compared to soil. Six pots were placed in each cage and planted with two seeds of French bean variety enclave. The cages were placed inside a greenhouse to protect them from the effect of direct rain and sunshine

Black bean aphids were collected from infested common bean plants grown within the Horticulture Research and Demonstration Field at Egerton University. They were collected by detaching aphid infested leaves from a bean plant and placing them in a plastic container (7.5 × 5.5 × 9.8 cm) with a screened lid. The aphids were transferred to the Microbiology and Ecology Laboratory Egerton University. The aphid species identity was confirmed under a light compound microscope and taxonomic aphid keys (Blackman & Eastop, 2000). Twelve (12) adult BBA guided by research study by Boni *et al.* (2020), were introduced onto French bean leaf sections inserted in moist sterile cotton wool placed in several 90mm in diameter Petri dishes. The petri dishes were placed on a laboratory bench at 25 ± 2 °C and aphids to reproduce for 21 days (Boni *et al.*, 2020) after which they were carefully transferred into the clean potted and caged French bean plants in the greenhouse using a fine brush grade 00. Aphids were allowed to multiply freely in the greenhouse cages into a large colony for use in laboratory bioassay. A fresh potted healthy French bean plants free from aphids were introduced to the aphid-rearing cages once every beginning of the week (Monday) to replace the deteriorating ones and maintain a continuous supply of aphid culture **Plate 3.1**.

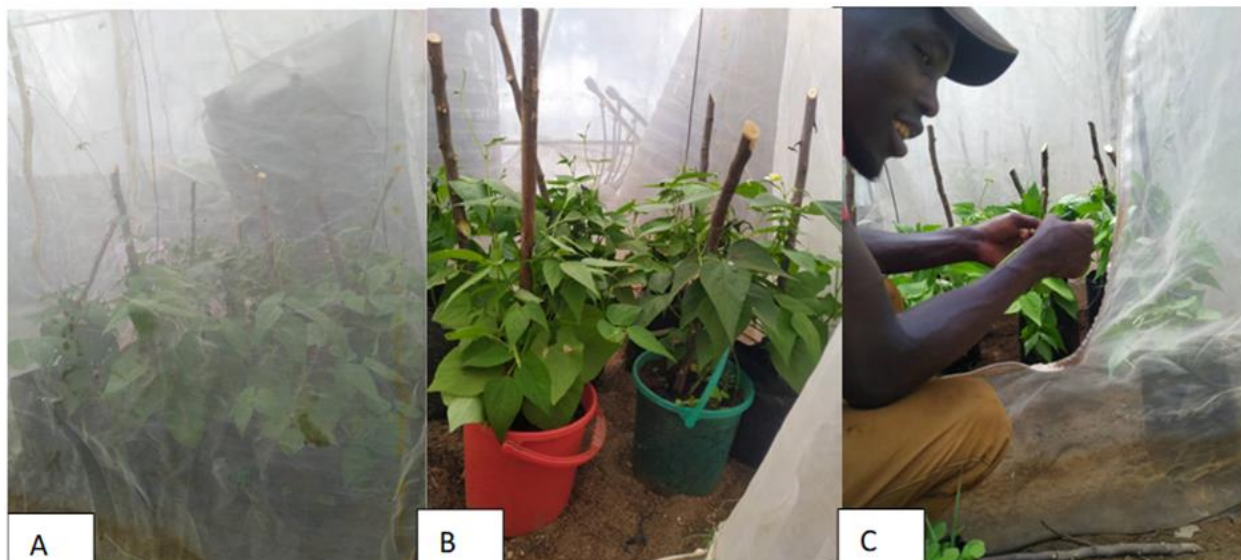


Plate 3.1: A set up of the black bean aphid rearing in green house cages (a) French bean plant free from black bean aphid, (b) French bean plants inoculated with black bean aphid (c) French bean plants with pure colonies of black bean aphid

3.2.2 *Metarhizium anisopliae* Entomopathogenic Fungus Formulation

Three commercial entomopathogenic fungus products containing *Metarhizium anisopliae*: Mazao SUPREME® packed in an aqueous formulation was procured from Real IPM Co. Thika, Kenya, and Metarril WP E9 (wetable powder) from Koppert Biological Systems (K) Ltd, Kenya. Biomagic 1.5 Liquid Formulation (LF), registered by the Pest Control Product Board (PCPB, 2022) as a bio-control agent for aphids was purchased from Osho Company Ltd. Detailed information on the different products is shown in Table 3.1.

Table 3.1: Product description

Trade name	Active Ingredient (a.i)	Formula tion	Fungal spore concentration	Manufacturer/ Suppliers in Kenya	%Viability confirmation (Mean \pm SE)
Mazao supreme [®]	<i>M. anisopliae</i>	Oil	2 \times 10 ⁸ cfu/ml	Real IPM Company (K) Ltd	95.6 \pm 0.63
Metarril WP E9	<i>M. anisopliae</i>	Powder	2 \times 10 ⁸ cfu/g	Koppert Biological Systems	96.9 \pm 1.60
Biomagic 1.5 LF	<i>M. anisopliae</i>	Liquid	2 \times 10 ⁸ cfu/ml	Osho Chemical Industries Ltd.	94.5 \pm 1.00
Alpha 10 EC	Alpha-cypermethrin	Liquid	-	Osho Chemical Industries Ltd.	-

WP = Wetable Powder, CFU = Colony Forming Unit, LF= liquid formulation, EC= Emulsifiable concentrate, ml= milliliters: Source: Products' label 2022.

3.2.3 Spore viability confirmation test

Isolates from the three EPF products were subjected to a spore viability percentage test. Fungal spores from each isolate were quantified using an improved Neubauer hemocytometer (VWR International, USA) adjusted to 10⁶ conidia per milliliter by dilution with 0.05% Triton X-100 as described by Inglis *et al.* (2012). The improved Neubauer hemocytometer was used with the help of a light microscope (Microscope X107, Marty Enterprises Ltd, Kenya) at 400 \times magnification attached to an HD screen. In a serial dilution, each isolate, one milliliter of each suspension was removed and diluted to 10⁶ conidia mL⁻¹ using 0.05% Triton X-100, then transferred to a universal bottle containing 9mL of 0.05% Triton X-100 water and vortexed. Each isolate concentration was adjusted to 10⁶ conidia/ml, and 100 μ L of the suspension was spread plated on five individual petri-dishes as a replicate containing Sabouraud Dextrose Agar (SDA) media. SDA media is the

most preferred for cultivation and isolation of pathogenic fungi attributed to its ability to provide high glucose concentration, nitrogen, carbon and vitamin source obtained from the peptone component in the media that enhances fungal growth and have bacterial growth inhibition ability compared to potato dextrose agar (PDA) (Sagar, 2022). The plated media was incubated for 16-18 hours (25 ± 1 °C). The plates were sealed with parafilm and maintained in the incubator for 16–18 hours. Spore germination was halted by spreading ~1 mL lactophenol blue solution on the SDA surface in each petri-dish. Under four microscopic observation fields, four sterile glass coverslips were placed on each petri dish's agar surface. One hundred (100) conidia were selected randomly under each coverslip mounted on a compound microscope (400×magnification) and used to determine the percentage spore viability. Germinated and non-germinated conidia were counted and recorded as germinated if their germ tubes had twice the conidium's diameter or a longer propagule diameter (Goettel *et al.*, 2000). The mean values of the replicates were determined and used to calculate the conidia viability percentage (Table 3.2). The samples (Mazao supreme, Metarril and Biomagic) were adjusted to a final concentration of 10^8 conidia mL⁻¹ to be used in the bioactivity study.

Table 3.2: Percent Spore viability of Mazao supreme Metarril E9 and Biomagic *M. anisopliae* formulations.

<i>Metarhizium anisopliae</i> products	Spore concentration	Spore viability (%)
Mazao supreme®	2×10^8 cfu/ml	95.6
Metarril E9	2×10^8 cfu/g	96.9
Biomagic	2×10^8 cfu/ml	94.5

3.2.4 *Metarhizium anisopliae* stock solution preparation and dilution

Five hundred (500) µml of triton X-100 (0.05% Tween 80®) measured using a micropipette was dissolved in 1000ml of distilled water in a clean container to make a triton water solution. The triton water solution enhanced the proper mixing and homogeneity of the fungal formulation by reducing the surface and interfacial tension of water. Using a twenty millilitre (20 ml) syringe procured from a registered pharmaceutical shop in Nakuru town, 10 ml of triton water was measured into a sterile clean universal bottle to reduce the possibility of contamination from pathogens (Plate 3.2). The stock solution was prepared by separately measuring 10 ml of Mazao and Biomagic using a micropipette and 10g of Metarril using an analytical balance LINB-A10.

The measured products were then dissolved in a litre of triton water. In addition, alpha-cypermethrin stock solution was prepared by adding 0.4ml in one litre of triton water and dissolving in a litre of triton water. For all *M. anisopliae* products, serial dilution of the stock solution (1×10^{-6} to 1×10^{-1} cfu/ml) was done and vortexed for 5 min to achieve suspension homogeneity according to Sayed *et al.* (2019). The serial dilutions were then used to determine the concentration (colony forming units, cfu) for the spore viability percentage test.

3.2.5 Experimental design and treatment application

A two-step bioassay to evaluate the efficacy of the two *Metarhizium anisopliae* formulations, Mazao supreme ® (aqueous) and Metarril WP E9 (wettable powder) against the black bean aphid was conducted in a sterile 90 mm plastic Petri dish lined with moist cotton wool in the laboratory. Step 1 was conducted following procedures described by Glazer and Lewis (2000) with modifications. A fresh French bean leaflet stalk inserted in a moist cotton wool and placed in each a petri dish. (Plate 3.2). The leaflet served as a food source for the aphids. Twelve adult aphids from the greenhouse cage stock culture were carefully transferred onto the fresh and clean French bean leaf sections in each petri dish using a moistened fine camel hairbrush. The petri dishes containing aphids on leaf sections were separately sprayed with 10 ml of three different concentrations (1×10^8 , 2×10^8 and 4×10^8 cfu/ml) of Mazao supreme® suspension and 1×10^8 , 2×10^8 and 4×10^8 spore/g of Metarril WP E9 suspensions and 10 ml of sterile 0.05% Triton X-100. Spraying was performed using Alison's hand sprayer held at 0.5 m from the Petri dishes to ensure uniform delivery of the fungal spores (Erdos *et al.*, 2020). Each treatment was replicated five times. Petri dishes were sealed with parafilm and maintained in a sterilized chamber at 26 ± 2 °C, photoperiod of 12:12h (light: dark) and relative humidity of 70 - 75% and monitored daily for aphid mortality for a period seven days. Aphids were considered dead when no movement was observed after probing with the tip of a camel hairbrush (Macuphe *et al.*, 2021). Dead aphids were counted and disinfected with 1% sodium hypochlorite and 70% ethanol, then rinsed three times in sterile distilled water before being transferred to sterile 90 mm Petri dishes lined with a moist sterile filter paper. The Petri dishes were then sealed with parafilm, incubated (25 ± 2 °C) for 7 days, and monitored for mycosis (Mweke *et al.*, 2020).

Step 2 of the bioassay was conducted by applying Metarril WP 90 at 2×10^8 cfu/g) and Mazao supreme at 2×10^8 cfu/ml spore concentration (selected based on their performance in bioassay 1 above). Biomagic 1.5 LF (2×10^8 spores/ml), a registered biopesticide for the control of

aphids (PCPB, 2022) and alpha-cypermethrin, a commercial synthetic insecticide, were used as controls. The experimental setup was designed and performed as described in step 1 above.



Plate 3.2. Fresh French bean leaflet placed in a 90mm petri dish with the stalk inserted in a moist cotton wool.

3.3 Data Collection

Aphid mortality data was collected at twenty four (24) hours post-treatment and continued daily for seven days. The period of seven days is the most preferred for insect mortality as it provides enough time for the pathogenic fungus to cause death to the host pest (Mweke *et al.*, 2018). Mortality was assessed by counting the number of dead aphids in each petri-dish and corrected % mortality for each treatment computed using Abbott's formula (Abbott, 1925):

$$\text{Corrected \% mortality} = \left(1 - \frac{\text{number of insects in T after Treatment}}{\text{number of insects in } C_0 \text{ after treatment}}\right) \times 100, \dots\dots \text{equation 1}$$

Where; T= treated and C_0 =control.

3.3.1 Mycosis test

The mycosis test assessment was done at 24 hours intervals, using microscopic inspection at $\times 40$ magnification (Microscope X107, Marty Enterprises Ltd, Kenya). The presence of green muscardine fungal outgrowth on the dead aphids, as described by Boni *et al.* (2020), confirmed that the death of the aphid was caused by *Metarhizium anisopliae* treatment. In cases where a dead aphid showed no fungal outgrowths with similar characteristics in contrast to the green muscardine, the aphid death was considered to be caused by other factors and discarded.

3.4 Data analysis

Data on percent corrected aphid mortality using was first checked for normality (using Shapiro-Wilk test) and homogeneity of variances (using Levene's test) before analysis was done. The statistical software SAS version 9.4 (SAS Institute, Cary, NC) was used for data analysis. Aphid corrected mortality data were then subjected to analysis of variance (ANOVA) using a generalized linear model assuming a binomial distribution and probit analysis treatments post application. Medial lethal time (LT₅₀) and median lethal concentration (LC₅₀) for treatments that caused > 50% mortality of adult aphids was determined. Tukey's honestly significant difference test was used to separate treatment means significant at 5%. In both experiments, Statistical model below:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \varepsilon_{ijk},$$

where: Y_{ijk} = overall observation on aphid's mortality, μ = overall mean, α_i =effect of the i^{th} different treatments and control, β_j =effect of j^{th} period post-treatment application, ε_{ij} = random error component which is normally and independently distributed about zero mean with a common variance σ^2 .

Probit (p) analysis to determine the bioactivity response of the treatment with days post-treatment application $p\left(Y = \frac{1}{X}\right) = \Phi(X^T \beta)$, where p is the probability and Φ is the cumulative distribution function of the standard normal distribution. The parameters β are typically estimated by maximum likelihood.

3.5 Results

3.5.1 Mycosis test

Green muscardine which is a confirmation for fungal growth was observed on the 96 % aphids' cadavers treated with Mazao supreme and Metarril WP E9 *Metarhizium anisopliae* formulations. Green muscardine was, however absent on the aphids sprayed with alpha-cypermethrin (Plate 3.3).

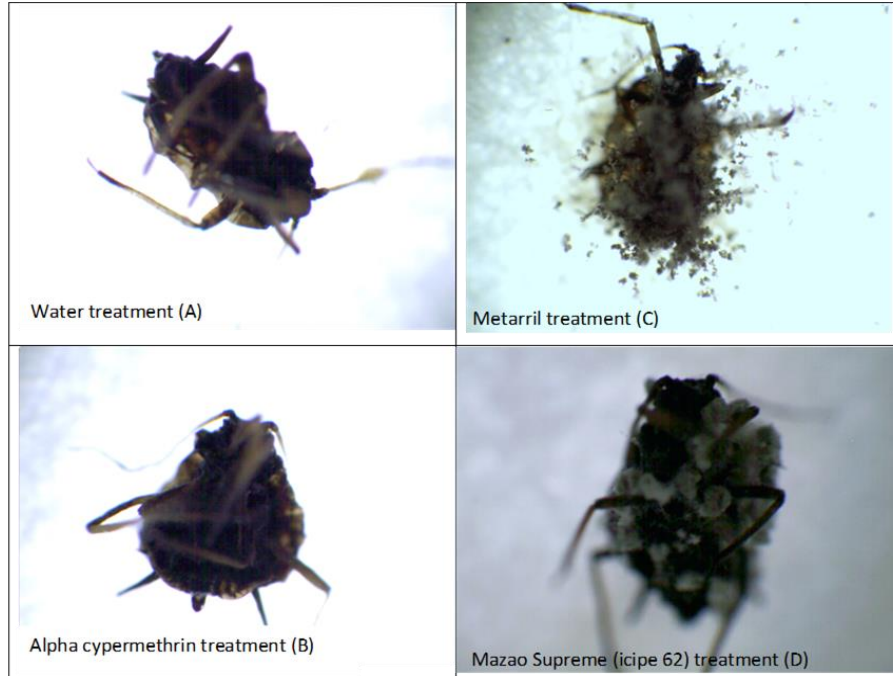


Plate 3.3: Microscopic mycosis test on the black bean aphid cadavers (a) water and (b) alpha-cypermethrin treatment without green muscardine growth (c) Metarril WP E9 and (d) Mazao supreme treatment showing green muscardine growth.

3.5.2 Bioactivity of *Metarhizium anisopliae* fungi formulations against black bean aphid

The probit analysis showed concentration and exposure time-dependent increase in the efficacy of Mazao supreme aqueous suspension and Metarril wettable powder *M. anisopliae* formulations against black bean aphids in the laboratory. The seven-day exposure time showed a higher bean aphid mortality at all tested concentrations for Mazao supreme (Fig. 3.4a) and Metarril WP E9 (Fig. 3.4b). The shorter exposure times of 1, 2, 3 and 4 days for Mazao supreme and 1, 2,3,4 and 5 days for Metarril WP were generally not significantly different at all concentrations tested.

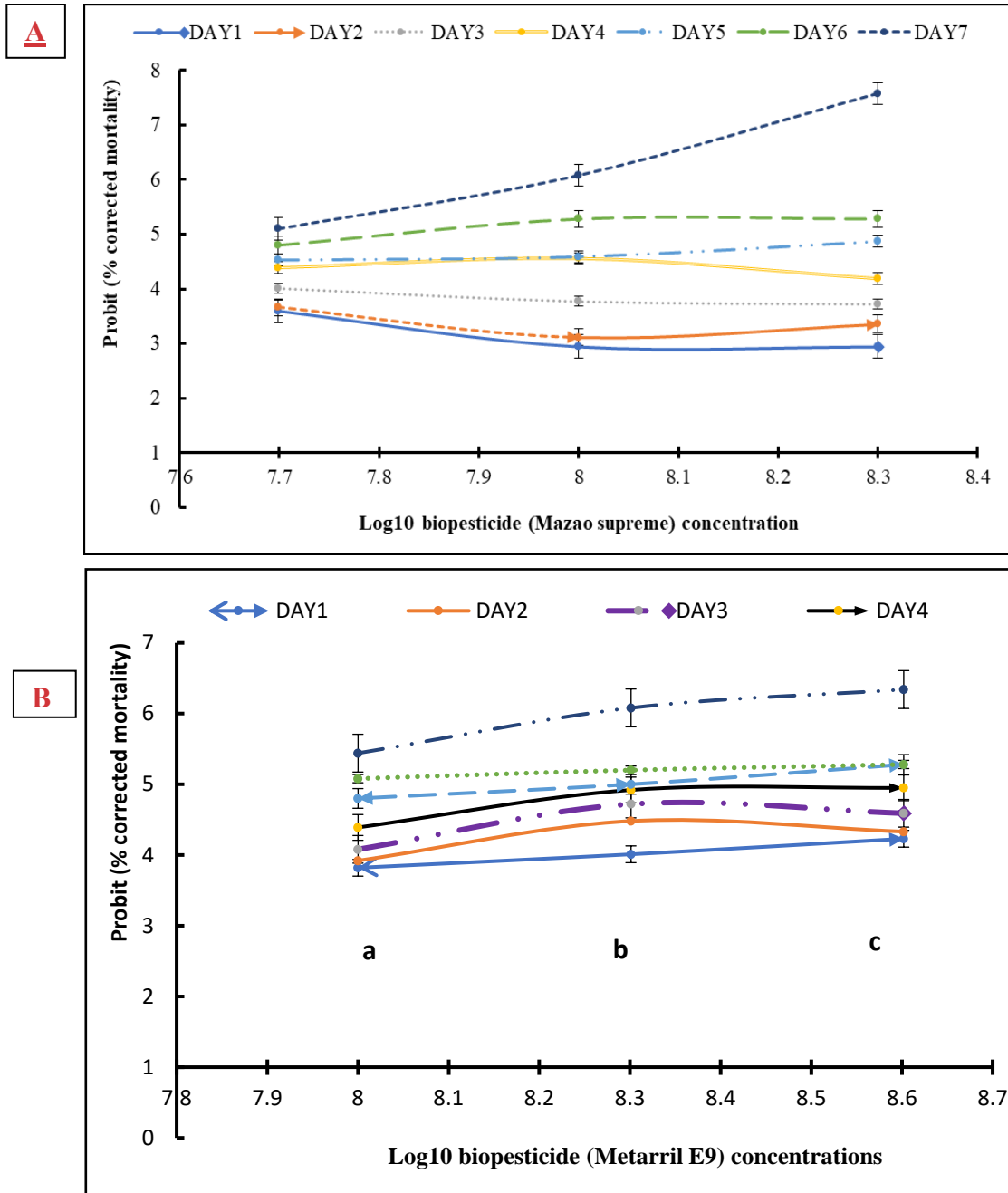


Figure 3.1: Dose-response curves over time for (A) Mazao supreme® and (B) Metarril WP E9

Results showed that the median lethal time (LT_{50}) for Mazao supreme® and Metarril WP E9 were significantly different ($p < 0.00004$) at different concentrations (Table 3.3). The Mazao supreme® (1×10^8 cfu/ml) and Metarril (1×10^8 cfu/g) took the longest time (7 days) to kill 50% of

the bean aphid population, whereas 2×10^8 cfu/ml of Mazao supreme and 4×10^8 cfu/g of Metarril concentration took the shortest time (4) days.

Table 3.3: Median Lethal time (LT₅₀) time it took to cause 50% aphid mortality for Mazao supreme and Metarril at different concentrations after treatment application

<i>Metarhizium anisopliae</i> products	Concentration	Median lethal time (LT ₅₀)
Mazao supreme	1×10^8 cfu/ml	7.0±0.9a
	2×10^8 cfu/ml	5.0±0.4b
	4×10^8 cfu/ml	4.8±0.3b
Metarril E9	1×10^8 cfu/g	7.0±0.9a
	2×10^8 cfu/g	5.0±0.4b
	4×10^8 cfu/g	4.0±0.3b

Means followed by the same letter within a column are not significantly different according to Tukey's honestly significant test ($p < 0.05$).

Results for probit regression analysis show that the LC₅₀ values (median lethal concentrations to kill 50% of the aphid population), reduced with exposure time (Table 3.4). Metarril WP E9 achieved an LC₅₀ of 0.95×10^8 on day 6 and was reduced to 0.43×10^8 cfu/mg on 7 days of exposure time. Mazao supreme did not achieve a mortality of > 50% by the 6th day of exposure. It however attained an LC₅₀ of 0.40×10^8 on day seven (7) but was not significantly different from Metarril WP E9 formulation with 0.43×10^8 cfu/g.

Table 3.4: LC₅₀ values for Mazao Supreme and Metarril WP with exposure time

Product	LC ₅₀	
	6 days	7 days
Mazao supreme	-	0.40×10^8
Metarril WP E9	0.95×10^8	0.43×10^8

Bioassay results showed that two biopesticide products of Mazao supreme and Metarril E9, at different concentrations, significantly ($p < 0.00007$) influenced the mortality of black bean aphids (Figure 3.2). There was a significant difference in black bean aphid mortality between Metarril E9 applied at 2×10^8 cfu/g and Mazao supreme at 2×10^8 cfu/ml only at 5- and 6-days exposure time,

with Metarril E9 causing more black bean aphid mortality compared to Mazao. Also, Metarril E9 applied at 2×10^8 spores /g caused significant black bean mortality compared to Mazao supreme 2×10^8 spores/ml at 3, 4- and 5-day exposure time. There was, however, no significant difference ($p > 0.05$) in black bean mortality for Metarril WP E9 applied at 4×10^8 cfu/g and Mazao supreme applied at 2×10^8 cfu/ml. Metarril E9 (4×10^8 cfu/g) caused the highest average mortality (95%) of black bean aphid and lowest mortality (54%) was obtained at 1×10^8 spores /ml of Mazao supreme.

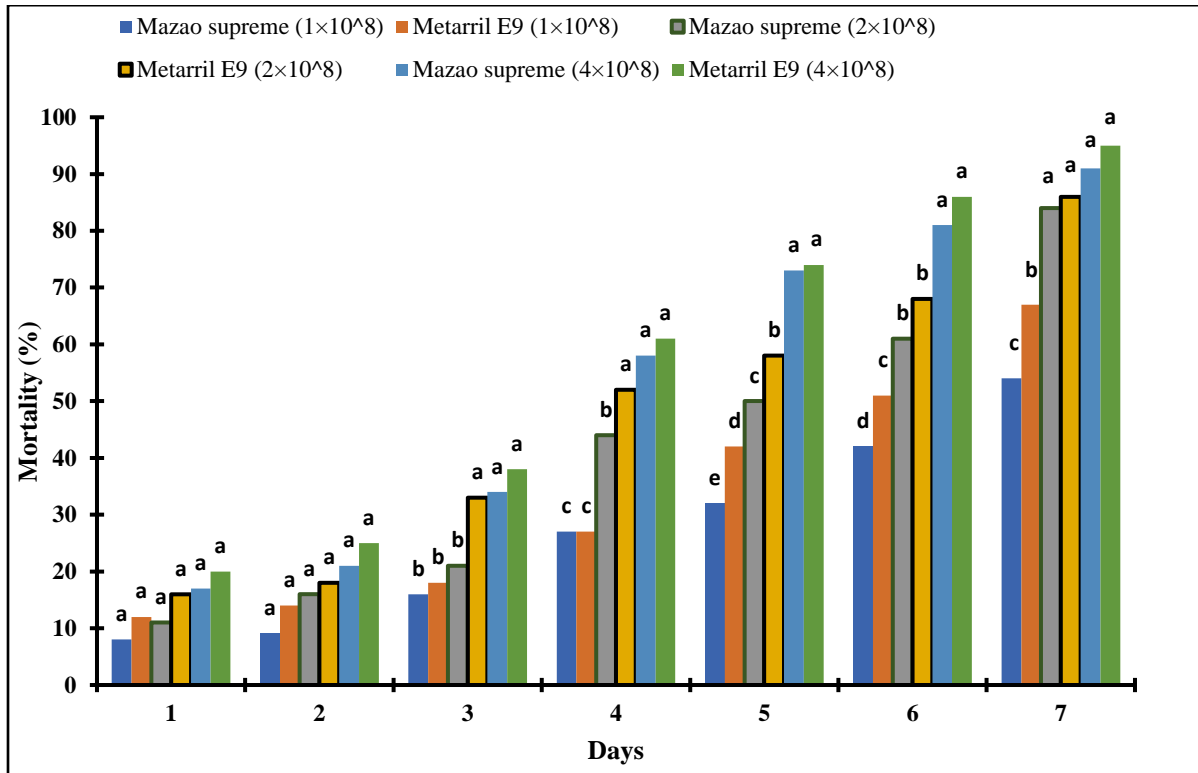


Figure 3.2: Mortality of black bean aphids treated with Mazao supreme and Metarril WP E9 at different concentrations

A comparison for the efficacy of Mazao supreme (2×10^8 cfu/ml) and Metarril E9 (2×10^8 cfu/g) formulations tested alongside Biomagic *Metarhizium anisopliae* biopesticide and alpha-cypermethrin a synthetic commercial insecticide indicated significant differences ($p < 0.0005$). The results showed that Metarril WP E9 and Mazao supreme *M. anisopliae* formulations significantly affected black bean aphid mortality by 84.2% and 80.7%, respectively, compared to the negative control (water). Metarril WP E9, Mazao supreme and Biomagic recorded the lowest (68.4b) aphid percentage mortality (Figure 3.3). Alpha-cypermethrin caused the highest corrected percentage mortality (99.3% a) of aphids, followed by. However, there were no significant

differences between Metarril E9 (84.2% ab) and Mazao supreme (80.7% ab) in percentage mortality caused to black bean aphids. In general, among the biopesticides, Metarril E9 causes the highest mortality, followed by Mazao supreme and the lowest mortality was recorded for the treatment of Biomagic. Metarril E9 caused a higher mortality percentage compared to Mazao supreme post-treatment application as shown in figure 3.3

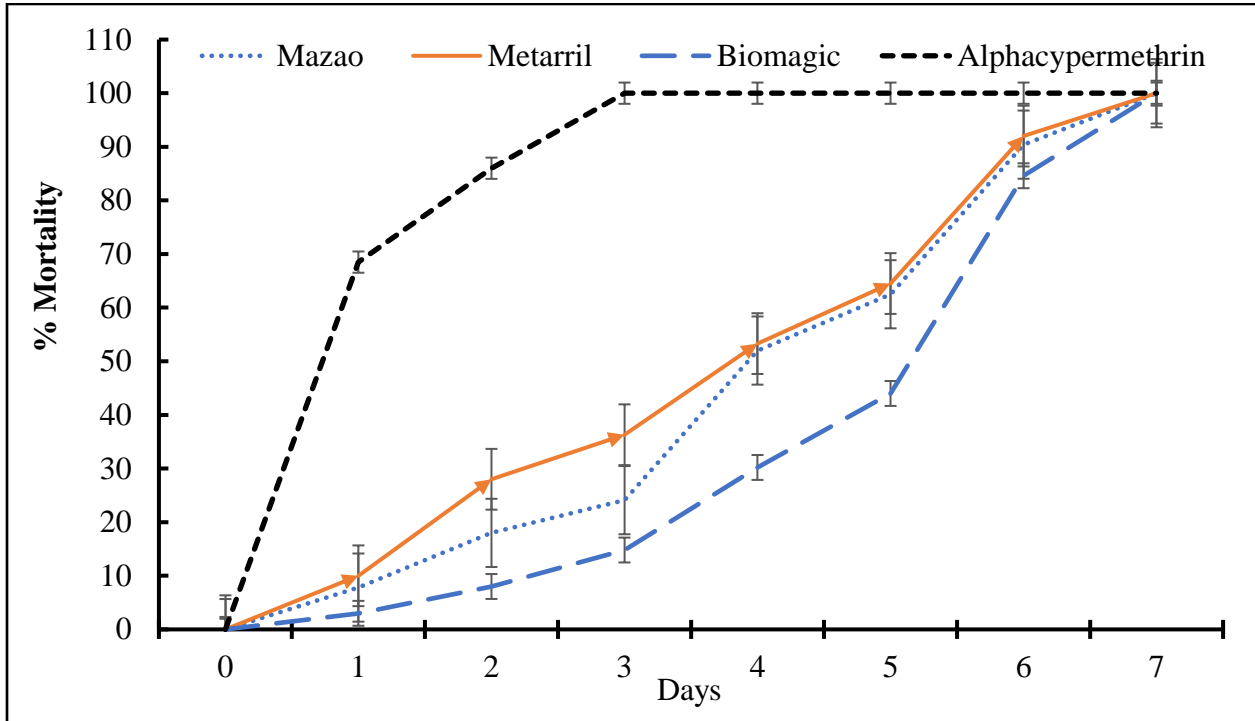


Figure 3.3: Percent mortality (mean \pm S.E.) of Black bean aphids treated with Metarril (2×10^8 spores/g), Mazao supreme (2×10^8 spores/ml), biomagic (2×10^8 spores/ml) and synthetic pesticide alpha-cypermethrin response over time.

3.6 Discussion

The study demonstrated a positive relationship between the efficacy and biopesticide concentration in the two formulations. The black bean aphid mortality was lower at 1×10^8 cfu/ml for Mazao supreme and 1×10^8 cfu/mg for Metarril WP E9 concentrations. However, it increased up to 95% at the highest spore concentration of 4×10^8 cfu/ml and 4×10^8 cfu/mg, indicating a dose dependent response for both biopesticide formulations tested with an LC_{50} value of 0.40 cfu/ml and 0.43 cfu/mg/ respectively. A study by Latiff *et al.* (2022) reported and confirmed that aphids are susceptible to Metarhizium species fungal treatment irrespective of the host plant. The strain, characteristic and concentrated levels are associated with the virulence rate of the pathogen isolate

of fungi. Conversely, the concentration of each isolate, as in the number of conidia per gram or ml, is associated with enhancing the ability of the isolate to cause mortality to the target pest (Iqbal *et al.*, 2021; Qubbaj & Samara, 2022).

Similarly, the two biopesticide formulations indicated an exposure time response in their efficacy against the black bean aphid. The aphid mortality was lower at increased to more than 90% at 7 days, indicating a time dependent response for formulations tested with an LT_{50} of 4 days. Mweke *et al.* (2018) conducted a study on different isolates of *Metarhizium anisopliae* and reported a mortality of 90% and 45% for ICIPE62 (1×10^8 spores/ml) on *Aphis craccivora* and LT_{50} (3.3 to 6.3 days) post-treatment. The findings were associated with the difference in fungal strains as some strains take longer for the pathogen to contact and cause harm to the target insect pest. *Metarhizium anisopliae* isolates ICIPE 62 were reported to produce more conidia on aphid cadavers, with a high ability and enhanced chances for secondary infection to the host. Srinivasan *et al.* (2019) reported a mortality rate (83.23%) caused by *Metarhizium anisopliae* against aphids and also attributed the effects to pathogen strain isolated and its ability to cause secondary infection to target pests or hosts within a given period

The current study further demonstrated that the two *Metarhizium anisopliae* formulations are effective against the black bean aphids. Mazao supreme and Metarril WP E9 indicated an efficacy of up to 84% exposure compared to Biomagic. A registered biopesticide for aphids used as a standard check indicating 68.4% in the laboratory bioassay. The efficacy for biopesticide products was, however significantly lower than alpha-cypermethrin with 99.3%. A similar study by Trinh *et al.* (2020) reported maximal mortality (85.3%) and lowest (60.0%) at the highest concentration of *Metarhizium anisopliae* at eight days post-treatment. Several other studies have also reported similar results (Akutse *et al.*, 2020; Boni *et al.*, 2020; Mweke *et al.*, 2018, 2019).

Metarril WP E9 (1×10^8 spores/g) formulation indicated higher aphid mortality compared to Mazao supreme at 5- and 6-days exposure time. This could be attributed to fungal strain formulation. Mweke *et al.* (2019) studied the effects of the different formulations and reported higher performance on oil than aqueous formulations. This could be attributed to increased contact and adhesion to the sprayed plant or target insect pest. In addition, Mweke *et al.* (2018) also found that *Metarhizium anisopliae* isolates ICIPE 62 produced more conidia on aphid cadavers, increasing their virulence after treatment application. The difference in efficacy could also be associated with the ability of the isolate to produce an immense extent of toxins associated with

higher mortality or focus more energy on vegetative growth, thus lowering the mortality rate on the target insect pest (Leger & Wang, 2020). In addition, *Metarhizium anisopliae*' flexibility at the genetic level, nutritional heterogeneity, host switching and virulence. A study by Erol *et al.* (2020) suggested that the difference in the fungal ability to cause mortality depends on the secondary bioactive metabolites produced by pathogens associated with the initial formulation of the isolate, affecting the isolate infection rate. Kisaakye *et al.* (2021) study reported *Beauveria bassiana* isolates, ICIPE 648, ICIPE 660 and ICIPE 273 caused over 80% mortality to adult banana weevil (*Cosmopolites sordidus*) associated to the highest spores per cadaver. but with no significant difference.

3.7 Conclusion

Based on this study, *Metarhizium anisopliae* in different concentrations caused mortality to black bean aphid and aphid nymphs. Under laboratory conditions, Metarril E9 (2×10^8 spores/g) formulation caused the highest mortality rate and with a short median lethal time. The study recommends Metarril biopesticide for further trials under field conditions to evaluate their effectiveness with border cropping as part of integrated pest management.

CHAPTER FOUR
EFFECT OF *Metarhizium anisopliae* AND COMPANION CROPPING ON APHID
POPULATION AND DAMAGE IN FRENCH BEANS

Abstract

Small-holder farmers, who are the major French bean growers in Kenya, often rely on insecticides to manage black bean aphids. There is a need for alternative pest management strategies. A field study to determine the combined effects of integrating *Metarhizium anisopliae* biopesticide and border cropping to manage aphid population and damage on French beans was conducted at the Horticulture Demonstration Field 3 of Egerton University, Njoro. The study was done between May and December 2022 using a two-factor experiment arranged in a Randomized Complete Block Design (RCBD) with three replications. The first factor comprised two border crops (sunflower and wheat) and a control (no border crop). The second factor involved spraying the French bean crop with Metarril (2×10^8 cfu/g) E9 *Metarhizium anisopliae* alongside Biomagic (2×10^8 cfu/ml), alpha-cypermethrin (8ml/20L) as positive controls and water as a negative control. Data analysis was done using SAS 9.4 version. Results showed a significant reduction in aphid population ($p < 0.0005$) and damage severity ($P < 0.0005$) on French bean grown in plots with border crops compared to those grown with no border crop. Plots with no border crop recorded the highest aphid population (3.18, 2.95) for seasons 1 and 2, respectively. A low aphid population (2.76, 2.32) was recorded in plots with sunflower as a border crop in both seasons one and two, respectively. Plots with no border crop showed the highest damage severity of 2.40 and 2.32 in season 1 and 2. Plots treated Metarril WP E9 had significantly lower aphid populations (2.96, 2.44) for seasons 1 and 2. The lowest aphid mortality of 1.56 was recorded in plots treated with alpha-cypermethrin. There were no significant differences in the damage severity between Metarril WP E9 and Biomagic in both seasons. Metarril and wheat combination reduced aphid population (1.87) compared to Biomagic (2.20) and water (2.93) at 56 days post-treatment in season two. The lowest aphid population (1.00) was recorded in the second spray for plots treated with alpha-cypermethrin. Plots planted with wheat as border crop in combination with Metarril, Biomagic and water had, more ladybird beetles (7-9) compared to those with sunflower. Alpha-cypermethrin recorded the lowest ladybird beetle population (<3) across all treatment combinations in both seasons. Therefore, biopesticide products containing *M. anisopliae* should be included in the integrated pest management of black bean aphids in Kenya.

4.1 Introduction

Crop productivity is often constrained by several biotic constraints, including insect pests leading to a decline in the quality and yields of most crops in Africa (Ogecha *et al.*, 2019). French bean (*Phaseolus Vulgaris L.*), whose production is mostly in the open fields, has faced declining productivity over time (Mwangi *et al.*, 2019). Under open field conditions, more than 37 insect pest species are associated with the crop through its production cycle (Mondal *et al.*, 2018). The most common insect pests include aphids, mites, whiteflies, leaf miners and pod borers. An increase in the incidence and severity of these pests has been associated with climatic variability that significantly affects the productivity of most crops (Mondal *et al.*, 2018; Sahoo & Giraddi, 2022).

In French bean farming, the black bean aphid (*Aphis fabae*) is considered a major biotic constraint causing significant yield losses and reduced crop quality and profitability. The preferred synthetic chemical insecticides used to control aphids have been associated with environmental pollution and develop resistance, besides being a health hazard. Therefore, there is a need to explore alternative, sustainable, integrated approaches to managing pests. Integrating border crops with biopesticides could enhance insect pest management and offer a safer option to synthetic chemicals in managing crop pests. Moreover, using border or companion crops has been reported to enhance the efficient use of available plant growth resources as well as reduce the population of insects such as aphids, white flies and many other pests (Afrin *et al.*, 2017; Hendges *et al.*, 2018; Huss *et al.*, 2022; Lithourgidis *et al.*, 2011). A field experiment by Waweru *et al.* (2021) reported that the use of maize (*Zea Mays L.*), sorghum (*Sorghum bicolor L.*) and sunflower (*Helianthus annuus*) as either a border or companion crop managed to reduce viral disease incidences spread by aphids in Hot pepper.

Furthermore, adding biopesticides to the border cropping approach to manage sap-sucking insect pests could be enhanced through more research on the interactive effects between border crops and biopesticides. Biopesticides containing entomopathogenic fungi (EPF) strains like *Beauveria*, *Metarhizium*, *Isaria*, *Hirsutella* and *Lecanicillium* have also been reported as safer for the environment and humans and can be a better addition to other methods used to manage insect pests (Sain *et al.*, 2019). However, the effectiveness of some EPFs, particularly *Metarhizium anisopliae*, has been reported as more influenced by several factors such as weather, the presence of ultra-violet light, temperatures, crops and its associated cropping system. A cropping system

that explores wheat and sunflower can offer the advantages of a push and pull strategy (Huss *et al.*, 2022; Waweru *et al.*, 2021). In addition, wheat is alternatively attacked by a different aphid species compared to French bean, particularly the Russian wheat aphid (*Diuraphis noxia*).

On the contrary, research by Varenhorst *et al.* (2021) described sunflower as a crop that is a suitable host for various aphid species, inclusive of bean aphids for bean plants, however, preference of aphids for sunflower compared to bean species has not been fully studied. Therefore, the sunflower crop could provide an alternative host for bean aphids, thus a potential option that could be used as a border crop for French bean grown plants and offer an additional purpose for integrated management of aphids by attacking them. Border crops like wheat attract generalist natural enemies of aphids such as lacewings, ladybird beetles, hoverflies, and other parasitoids that can prey on back bean aphids. This study aimed to investigate the effects of integrating border cropping systems of wheat and sunflower to enhance the efficiency of *Metarhizium anisopliae* in controlling black bean aphid infestations and subsequent damage to French bean plants.

4.2 Materials and methods

4.2.1 Experimental site description

The study was conducted at the Horticulture Research and Demonstration Field, Egerton University, Njoro-Kenya, as shown in Figure 4.1. The field lies at latitude 0.23°S and longitude 35.35 °E in the Lower Highland III Agroecological Zone (LH3) at about 2238 m above sea level. The site receives an annual average rainfall of 1180-1400mm with an annual daily temperature range of 16-22°C. The soil types are predominately Mollic Andosols, well-drained, friable, moderate in fertility containing medium levels of organic carbon, low levels of phosphorus and a pH range of 6.0 to 6.5 (Jaetzold *et al.*, 2012). The rainfall pattern and distribution during the study period are shown in Table 4.1.

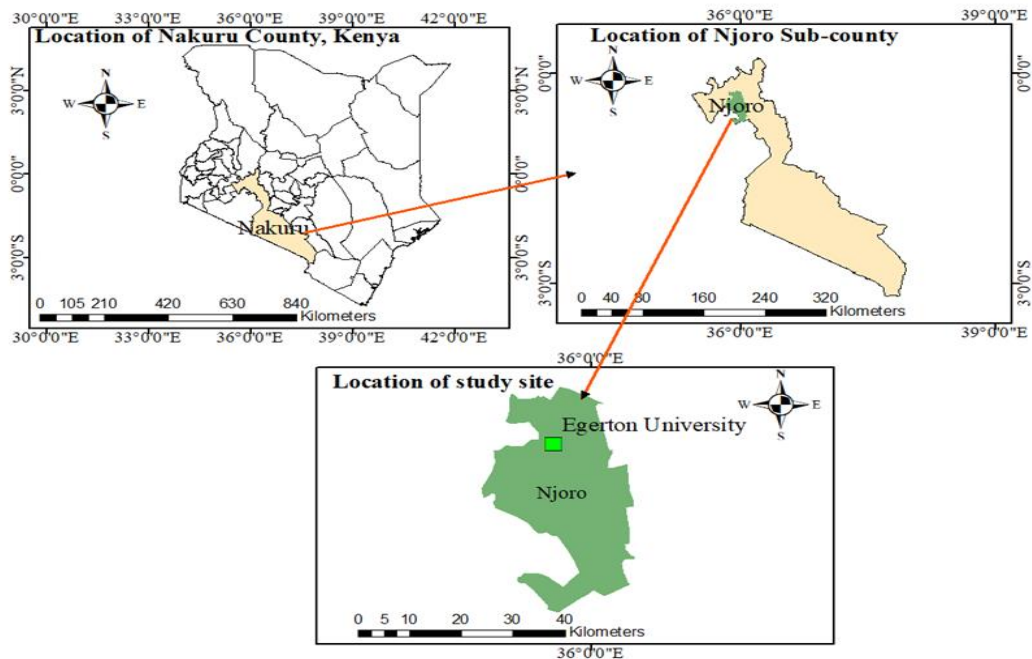


Figure 4.1 Map showing the experimental location.

Table 4.1: Mean monthly rainfall, relative humidity and temperature data at the site during season one (April to July 2022) and season two (September to December 2022).

Month	Temperature (°C)		Rainfall		Relative Humidity
	Max (°C)	Min (°C)	Month (mm)	Day (mm)	(%)
January	25.53	10.92	43.21	5.02	65.37
February	27.04	11.6	35.98	6.46	62.29
March	27.03	12.69	107.05	12.82	65.62
April	25.39	12.16	147.53	23.71	75.13
May	25.48	12.89	149.93	25.26	74.34
June	24.05	12.5	132.06	25.45	80.06
July	24.05	11.26	73.86	13.69	75.82
August	24.04	11.56	182.86	27.38	80.96
September	25.33	13.17	201.36	23.33	78.37
October	24.3	12.29	172.43	25.54	83.93
November	23.85	11.11	137.62	23.61	83.97
December	23.29	10.75	147.73	26.13	83.86

Source: Egerton University Engineering Meteorological Station.

4.2.2 Planting materials

French bean cultivar 'Enclave', a determinate cultivar that is highly susceptible to black bean aphids, was used in this study. It is among the commonly grown cultivars in many of Kenya's French bean growing regions. Enclave cultivar produces more extra fine-grade quality pods, is high yielding, and is adaptable to wide agroecological zones compared to other cultivars. French bean seeds were procured from Hygrotech East Africa Ltd, Naivasha, Kenya. Sunflower (*Helianthus annuus* L.) 'Kenya Fedha' and wheat (*Triticum aestivum* L.) 'Njoro bread wheat 2' were procured from Kenya Agricultural and Livestock Research Organization (KALRO) Njoro, Kenya. The wheat variety is highly preferred by aphids, with a dwarf growth habit that could reduce shade effects. It is the most commonly grown commercial variety and most adopted by farmers within the agroecological zones of Nakuru County. On the other hand, the sunflower cultivar used in this study is an open-pollinated variety with uniform maturity, well suited to medium and high altitudes, and the most preferred in the region where this study was conducted.

4.2.3. Experimental design and treatments

A 3x 4 factorial experiment arranged in a Randomized Completely Block Design (RCBD) with three replications was conducted in the open field. A biopesticide product; Metarril (2×10^8 cfu/g) E9, was chosen based on its superior performance during the laboratory bioassay experiment in chapter 3 above. The first factor under study was companion cropping at three levels; (i) sunflower as a border crop, (ii) wheat as border crops and (iii) no border crop as a control. The second factor was foliar spray application at four levels; (i) Metarril WP (2×10^8 cfu/g) E9, (ii) Biomagic (2×10^8 cfu/ml) 15 Liquid Formulation commercial biopesticide (iii) alpha-cypermethrin (8ml /20L) a commercialised synthetic insecticide as positive controls and (iv) water as the negative control. The treatment combinations used are as shown in Table 4.2.

Table 4.2: Description of the treatment combinations

	Border crops		
	Sunflower (S)	Wheat (W)	No border crop (N)
Metarril (M)	M×S	M×W	M×N
Biomagic (B)	B×S	B×W	B×N
Alpha-cypermethrin (A)	A×S	A×W	A×N
Water (W)	W×S	W×W	W×N

4.2.4 Field preparation, layout and crop management.

The experimental field was ploughed and harrowed using a tractor before planting to obtain a moderate tilth suitable for planting French beans and the border crops. Experimental plots measuring 4.2m by 3m each were laid out, as shown in Figure 4.1. Sunflower was planted along the border of the designated plots at a spacing of 30 cm x 75 cm with two (2) seeds placed in each hill and later thinned to one seedling per hill two weeks after germination of the seeds. At the same time, two (2) wheat seeds were planted at 30 cm x 30cm in the designated plots and were later thinned two weeks after the plant's emergency to ensure that a healthy plant per hole was allowed to continue growing for further data collection. The distance between border crops and the first row of French bean crop was 60 cm. French bean was planted three weeks (3 weeks) after sunflower and wheat border crops were planted. This allowed the border crops time to establish at least 2-3 leaves before planting the French bean, enabling the companion crops to establish well before the primary crop (French bean) was planted. French bean was planted at a spacing of 30 x by 15cm, with two (2) seeds planted per hole and later thinned to one plant per hole (Fulano, 2016). Diammonium phosphate (DAP) fertilizer was applied during planting at the rate of 200 kg/ ha (Greenlife Crop Protection Africa, 2021). Calcium Ammonium Nitrate (CAN) at a rate of 150 kg/ha was applied as a top dress in two equal split applications at three (3) true leaves and at the onset of flowering as the growth stages recommended for application of CAN fertilizer to French bean plants to enhance essential nutrient availability (Oseko & Dienya, 2015). Weeding was done manually three weeks after emergence. The experiment was rain-fed with supplemental irrigation provided using a drip irrigation system at an equal rate in all experimental units during dry spells. Details of the experiment's field layout are shown in Figures 4.2, 4.3 and plate 4.1.

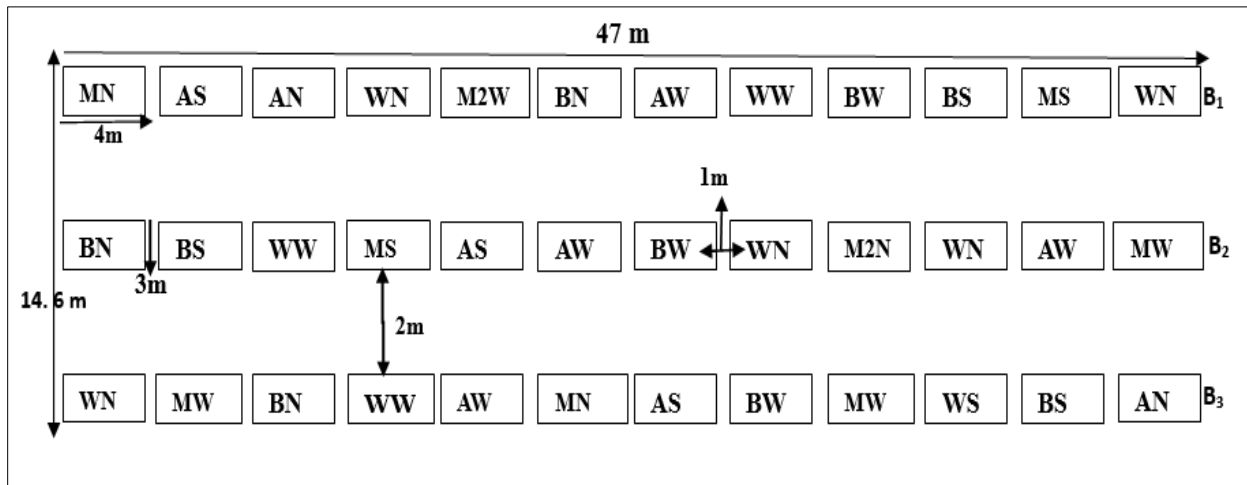


Figure 4.2: Field experimental layout showing different treatment combinations and randomisation

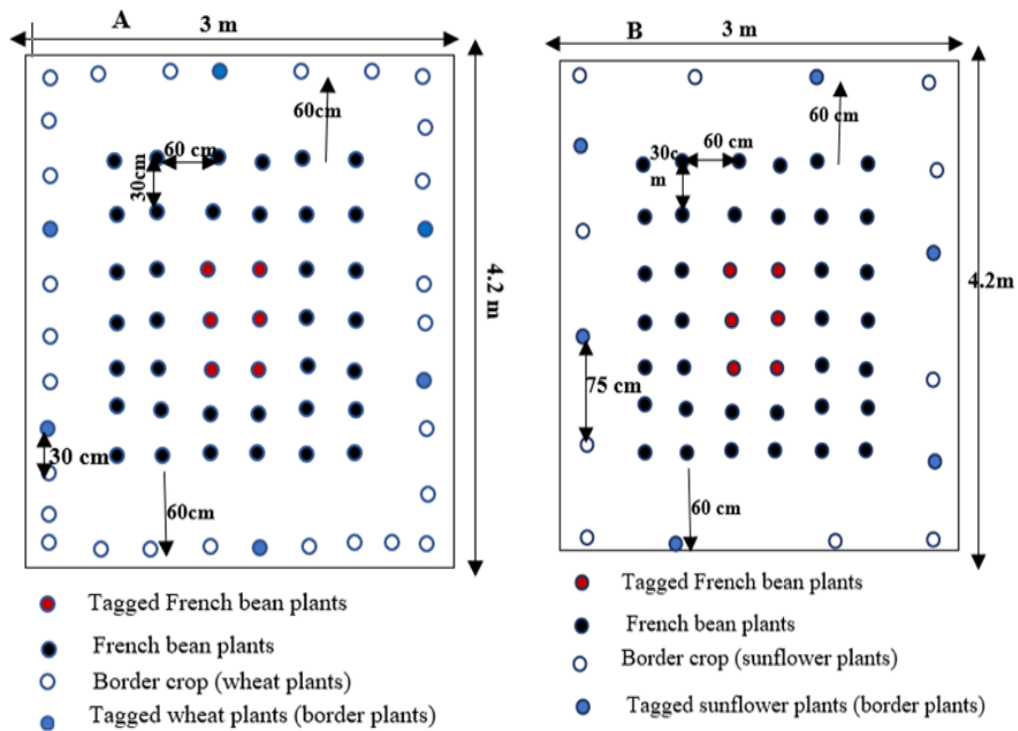


Figure 4.3: Field layout showing the spacing and layout of the different crops



Plate 4.1: Field layout showing border crops (A), sunflower (B) wheat crop and (C) French bean crop.

4.2.5 Black bean aphid introduction to the field experiment

Black bean aphids were collected from the greenhouse cultures (described in chapter three). The aphids were introduced into the field by placing six infested French bean leaves with ten (10) aphids per leaf at the center of each experimental unit to allow the aphids equal chance to spread to other parts of the plots. This was done 14 days after emergence of French bean and would allow the aphids equal chance to spread to other parts of the plots.

4.2.6 Treatment application

Treatment application started 21 days after the emergence of the French bean. Spraying was repeated four times at every 14 days interval, targeting mainly the vegetative growth stage of French bean plants when insect pests cause severe damage by attacking auxiliary buds and tender growing points as Sayed *et al.* (2019) described. The a 14-day spray interval was adopted and based on a previous study by Boni *et al.* (2020) as the most effective range for insect pest mortality in open field experiments using entomopathogenic fungi to cause death as a result of both primary and secondary infection. Metarril wettable powder E9 (2×10^8 spore/g), Biomagic 1.5 LF (2×10^8 spores /ml), and alpha-cypermethrin were thoroughly mixed in water containing 0.05% Integra (3ml per 20l) for 5 minutes prior to application to ensure a homogenous solution before being applied to each experimental unit. The 0.05% integra acts as a surfactant to reduce the surface tension of the treatment spray solution for even and uniform spreading on the plant's surface, improving the absorption and retention rate. To minimize the likelihood of contamination, four different knapsack sprayers (Kenplastic Knapsack sprayer, 20L) but with similar calibration to discharge 350L/acre and nozzle pressure of 2.88 kgf m⁻² were used. Each of the four knapsack

sprayers was used to apply only one product, that is, Metarril (2×10^8 spores/g) E9, Biomagic (2×10^8 spores/ml), alpha-cypermethrin (8ml/20L) or water. Treatment application was done early in the morning, between 06:00 and 08:00 hours East Africa Time (EAT), to minimize treatment drift caused by wind.



4.3 Data collection





After the first spray was initiated, data on aphid population, damage severity score and lady bird population (number per sampled 6 plants) of the border crop were collected at 14 days intervals thereafter up to a period of the 75th day after the emergence of the French beans

4.3.1 Black bean aphid population

Data on aphid population were collected from six tagged plants in the inner row of each experimental unit just before each spraying. The use of visual observation and scoring of aphid population using 6 points scale as described by Aken *et al.* (2013) and Mkenda *et al.* (2015) was adopted as shown in table 4.1. The 6 point scale used to assess aphid population was; 1 = no aphids; 2 = a few scattered aphids; 3 = a few small colonies; 4 = several small colonies; 5 = large isolated colonies; and 6 = large continuous colonies and data obtained were used to compute the average number of aphids per plant (no. of aphids/plant). Data collection was done in the early morning hours between 07:00- 08:00 hours when most aphids were inactive on the leaves.

Table 4.3 Aphid population score scale

Number	Aphid population	Visual expression
1	No aphid infestation and damage	
2	A few scattered aphid	






3	A few isolated colonies	
4	Several isolated aphid colonies	
5	Large isolated colonies	
6	Large continues colonies	

Sources: (Aken *et al.*, 2013; Mkenda *et al.*, 2015)

4.3.2 Black bean aphid damage

The aphid damage score was also determined by visually observing and scoring the level of aphid damage on six randomly selected plants using a 5-point scale, according to Mkenda *et al.* (2015), where: 1 = no infestation or damage, 2 = light damage and infestation (< 25%) of plant parts damaged, 3 = average damage and infestation (26 - 50%) of plant parts damaged, 4 = high infestation and damage (51 - 75%) of plant parts and 5 = severe infestation damage (>75%) of plants or a dead plant (Table 4.2). The data obtained were later used to compute the average damage caused on each French bean plant.

Table 4.4 Damage severity score scale

Scale	Description of damage	Visual expression
1.	No infestation or damage	
2.	Showing light damage or an infestation of less than 25%	
3.	Showing damage or infestation 26%-50%	
4.	Showing light infestation and damage 51 -75%	
5.	Severe infestation and damage leading to death greater than 75%	

Sources: Mkenda *et al.* (2015).

4.3.3 Ladybird beetle population at the border crop.

This study also recorded the population of ladybird beetles at the border crop. Visual count on ladybird beetles was done on the top three leaves on six randomly sampled plants as border crops (wheat and sunflower). The data obtained were later used to compute the average number of ladybird beetles per plant at the border crops.

4.3.4. Data analysis

Data were subjected to a normality test using the Shapiro-Wilk test and Levene's tests for homogeneity of variances at $p=0.05$ using the SAS 9.4M8 version (SAS Institute, 2023) before analysis. Data on aphid population and damage did not meet the assumption of normality and equal variance and were first subjected to arcsine transformation before analysis of variance (ANOVA) was done. The analysis procedure followed a statistical model:

$$Y_{ijk} = \mu + a_i + \beta_j + (a\beta)_{ij} + block_k + \varepsilon_{ijk}.$$

Where; Y_{ijk} the response from k^{th} experimental unit receiving the i^{th} type of spray and j^{th} type of border crop, μ is the overall mean, a_i effect due to the i^{th} type of spray, β_j effect due to the j^{th} type of the border crop, $(a\beta)_{ij}$ is the interaction effect of the i^{th} type of spray with the j^{th} type of border crop, $block_k$, effects due to k^{th} block, ε_{ijk} is the random error associated with the response from the k^{th} experimental unit receiving the i^{th} type of spray combined with the j^{th} level of border crop. Mean separation for significant treatments at F-test was done using Tukey's honestly significant difference (HSD) at $p \leq 0.05$. The results obtained have been presented and discussed in the subsequent sections of this chapter.

4.4 Results

4.4.2: Effects of border crop on aphid population and damage severity

The use of border crops had significant ($p < 0.0005$) effects on the population of aphids and damage severity in both seasons (Table 4.5). Plots with no border crop recorded the highest aphid population (3.18, 2.95) for season 1 and 2, respectively. A low aphid population (2.76, 2.32) was recorded in plots with sunflower as border crop in both seasons one and two, respectively. This was, however, not significantly different from plots with wheat as border crops. A similar trend was observed for damage severity with plots with no border crop the highest damage severity was 2.40 and 2.32 in seasons one and two, respectively. There were no significant differences in the damage severity between wheat and sunflower border crops in both seasons. Generally, season one had a higher aphid population and damage severity compared to season two

Table 4.5: Mean aphid abundance and damage severity with border crops in season 1 and 2

Border crop	Season one		Season two	
	Aphid population	Damage severity	Aphid population	Damage severity
No border crop	3.18a*	2.40a*	2.95a*	2.32a*
Wheat	2.80b	2.18b	2.39b	2.08b
Sunflower	2.76b	2.13b	2.32b	1.97b

*Means in a column followed by the same letter are not significantly different using Tukey's test at $p < 0.05$

4.4.3: Effects of *Metarhizium anisopliae* on aphid population on French bean plants

Results showed that plots treated with *M. anisopliae* biopesticide (Metarril WP E9) had significantly lower aphid populations (2.96, 2.44) for season 1 and 2, respectively (Table 4.6). This was, however not significantly different in plots treated with Biomagic (3.18) in season 1. The lowest aphid mortality of 1.56 was recorded in plots treated with alpha-cypermethrin A similar trend was observed for damage severity with the negative control (water) showing the highest damage severity of 2.64 and 2.40 in seasons one and two, respectively. There were no significant differences in the damage severity between Metarril WP E9 and Biomagic in both seasons. Generally, season one had a higher aphid population and damage severity compared to season two

Table 4.6: Effect of *Metarhizium anisopliae* on bean aphid abundance and damage severity score in season 1 and 2

Biopesticide	Season one		Season two	
	Aphid population	Damage severity	Aphid population	Damage severity
Water	3.54a*	2.64a*	3.20a*	2.40a*
Biomagic	3.18b	2.46ab	3.04a	2.22a
Metarril	2.96b	2.36b	2.44b	2.19a
Alpha cyper	1.56c	1.49c	1.56c	1.59b

*Means in a column followed by the same letters are not significantly different using Tukey's

4.4.4: Combined effects of *M. anisopliae* and border crops on aphid population on French bean plants

The results showed that all interaction effects between border crop and *M. anisopliae* biopesticide application on the aphid population were significant ($p < 0.05$) in season two (Table 4.7). The highest aphid population (4.76) was recorded at 14 days for plots with no border and treated with water. The lowest aphid population (1.00) was recorded in the second spray for plots with no border and treated with alpha-cypermethrin.

The interaction between *Metarril biopesticide* and wheat as a border crop significantly ($p < 0.0005$) influenced aphid population. Reduction in aphid population was observed in plots with wheat as a border crop and treated with Metarril (1.87) or alpha-cypermethrin (1.17) compared to plots treated with Biomagic (2.20) or water (2.93) at 56 days post-treatment in season two compared to sunflower. The highest aphid population (4.66) was recorded at 14 days for the first spray for plots with no border and treated with water. The lowest aphid population (1.00) was recorded in the second spray for plots treated with alpha-cypermethrin.

Table 4.7: Effects of *Metarhizium anisopliae* and border crop on aphid population in season one (April to July 2022) and season two (September to December 2022).

Treatment *	Aphid population (no./plant)				
	Days after spraying (DAS)				
	Pre-spray	14 DAS	28 DAS	42 DAS	56 DAS
Season one					
WN	3.60**	3.96**	4.13a***	4.14a***	4.07a***
BN	3.50	3.70	3.06ab	3.50ab	4.06a
MN	3.50	3.50	2.66bc	3.40ab	2.73b
AN	3.26	2.40	2.00bc	1.78cd	1.36cd
WW	3.50	3.86	2.80bc	3.40ab	3.26ab
BW	3.46	3.16	2.53bc	3.06b	3.20ab
MW	3.60	2.56	2.56bc	2.40bc	2.76b
AW	3.36	2.16	2.00bc	1.33d	1.16d
WS	3.56	3.13	2.93abc	3.60ab	3.26ab
BS	3.30	2.73	2.43bc	3.50ab	3.16ab

MS	3.63	2.70	2.23bc	2.43bc	2.10bc
AS	3.43	2.30	1.77c	1.30d	1.00d
Season two					
WN	3.17**	3.73a***	4.67a***	4.06a***	3.46a***
BN	3.10	3.60a	4.40ab	3.87a	2.76ab
MN	3.03	2.40bc	3.26b	3.20ab	2.16bc
AN	3.03	1.67cd	1.43cd	1.10d	1.00d
WW	3.13	3.33ab	3.87ab	2.87ab	2.93ab
BW	3.10	3.00ab	3.00b	2.17bc	2.20bc
MW	3.10	2.00cd	2.13c	1.60cd	1.87bc
AW	3.00	1.00d	1.43cd	1.00d	1.17cd
WS	3.16	2.33bc	2.90b	2.43bc	3.13a
BS	3.16	2.20cd	2.87b	2.20bc	2.20bc
MS	3.10	2.00cd	2.50bc	1.60cd	2.10bc
AS	3.23	1.33d	1.00d	1.00d	1.00d

*WN (water and no border), BN (Biomagic and no border), MN (Metarril WP E9 and no border), AN (alpha-cypermethrin and no border), WW (water and wheat), BW (Biomagic and wheat), MW (Metarril WP E9 and wheat), AW (alpha-cypermethrin and wheat), WS (water and sunflower), BS (Biomagic and sunflower), MS (Metarril WP E9 and Sunflower), AS (alpha-cypermethrin and sunflower). **Means not followed by a letter within a column in each season are not significantly different according to Tukey's HSD test ($p \leq 0.05$). ***Means followed by the same letter within a column are not significantly different according to Tukey's HSD test ($p \leq 0.05$).

4.4.5 Combined effects of *Metarhizium anisopliae* and border crops on aphid damage severity on French bean

Results showed that there were significant differences in damage severity amongst the different treatment combinations and dates in season one (Table 4.8). In season two, however, significant differences in damage severity were observed. Plots without border crops had higher damage severity across the treatment combinations and dates compared to plots planted with border crop. The lowest damage severity (<1.22) was recorded in plots sprayed alpha cypermethrin at all treatment combinations. Plots sprayed with Metarril and Biomagic sunflower combinations had no significant difference in damage severity except 28 days after spraying in season two. Plots

sprayed with Metarril and Biomagic significantly differed in damage severity at 14, 28 and 42 days after spraying in season two. Generally, plots with Metarril with wheat combination had a significantly lower damage severity compared to sunflower.

Table 4.8: Effect of *Metarhizium anisopliae* and border crop on damage severity in season one (April to July 2022) and in season two (September to December 2022)

Treatment*	Damage severity (no. /plant)				
	Days after spray (DAS)				
	Pre-spray	14 DAS	28 DAS	42 DAS	56 DAS
Season one					
WN	2.33**	3.33**	2.33**	3.33**	3.40**
BN	2.66	3.00	2.00	3.00	2.80
MN	2.00	2.67	2.33	2.67	2.33
AN	2.66	2.00	1.33	1.28	1.28
WW	2.00	3.33	2.33	3.00	3.00
BW	2.33	2.66	2.00	1.67	2.00
MW	2.33	3.33	2.00	1.33	2.66
AW	2.00	2.00	1.33	1.26	1.22
WS	2.00	3.00	2.00	2.33	2.00
BS	2.00	2.33	2.00	2.33	2.67
MS	2.66	2.33	1.67	2.33	2.00
AS	2.33	2.00	1.33	1.30	1.22
Season two					
WN	3.26**	3.60a***	4.76a***	4.06a***	2.76ab***
BN	3.10	2.4bc	3.20b	3.20ab	2.16bc
MN	3.03	3.73a	4.40a	3.86a	3.46a
AN	3.06	1.66de	1.43dc	1.10d	1.00d
WW	3.48	3.0ab	3.86ab	2.86ab	2.93ab
BW	3.13	2.00cd	2.13c	1.60cd	1.86bc
MW	3.10	3.3ab	3.00b	2.16bc	2.20bc
AW	3.00	1.66de	1.43cd	1.00d	1.16cd

WS	3.16	2.33bc	2.80bc	2.43b	3.13a
BS	3.16	2.10cd	2.50bc	1.93bc	2.10bc
MS	3.16	2.20bc	2.90bc	2.20bc	2.83ab
AS	3.23	1.33e	1.00d	1.00d	1.00d

*WN (water and no border), BN (Biomagic and no border), MN (Metarril WP E9 and no border), AN (alpha-cypermethrin and no border), WW (water and wheat), BW (Biomagic and wheat), MW (Metarril WP E9 and wheat), AW (alpha-cypermethrin and wheat), WS (water and sunflower), BS (Biomagic and sunflower), MS (Metarril WP E9 and Sunflower), AS (alpha-cypermethrin and sunflower). **Means not followed by a letter within a column within a season are not significantly different according to Tukey's HSD test ($p \leq 0.05$). ***Means followed by the same letter within a column in each season are not significantly different according to Tukey's HSD test ($p \leq 0.05$).

4.4.3 Effects of *Metarhizium anisopliae* and border crops on ladybird beetle population

The results of the study indicated that the different treatments and treatment combinations significantly ($p < 0.0005$) influenced ladybird beetle population on border crops (Table 4.9). In both seasons, for plots planted with wheat as border crop had, more ladybird beetles of between 7 and 9 in all the treatment combinations Metarril, Biomagic and water compared to plots planted with sunflower in both season one and two. Plots sprayed with alpha-cypermethrin recorded the lowest ladybird beetle population (<3) across all treatment combinations in both seasons. An increase in ladybird beetle population was observed up to 42 DAS followed by a decrease in at 56 DAS in all treatment combinations and both seasons.

Table 4.9: Effects of *Metarhizium anisopliae* and border crop on ladybird beetle population in season one (April to July 2 022) and season two (September to December 2022)

Ladybird beetle population (no. per plant)					
Days after spray (DAS)					
Treatments	Pre-spray	14DAS	28 DAS	42DAS	56 DAS
Season one					
WW	1.33*	8.67a**	14.67a**	14.00a**	9.00a**
BW	1.67	4.67ab	9.67ab	13.33a	7.33ab

MW	1.67	6.67ab	10.00ab	13.33a	7.00ab
AW	1.67	1.33e	1.33dc	1.67b	3.33b
WS	1.67	4.00b	9.33ab	10.67ab	7.33ab
BS	1.67	4.00b	5.67bc	7.67ab	7.33ab
MS	1.13	3.00cd	7.00bc	9.00ab	4.70ab
AS	1.33	2.33d	1.00d	1.03c	1.00c

Season two

WW	1.33**	4.67b***	10.00ab***	14.00a***	7.33ab***
BW	1.67	8.67a	9.66ab	13.33a	9.00a
MW	1.67	6.67ab	14.67a	13.33a	7.00ab
AW	1.67	1.33d	1.33d	1.67c	1.33c
WS	1.33	4.00b	9.33ab	10.67ab	7.00ab
BS	1.67	4.00b	5.67bc	7.67b	7.33ab
MS	1.13	3.00bc	7.00b	9.00ab	6.00ab
AS	1.33	2.33c	1.00d	1.33c	1.23c

*WN (water and no border), BN (Biomagic and no border), MN (Metarril WP E9 and no border), AN (alpha-cypermethrin and no border), WW (water and wheat), BW (Biomagic and wheat), MW (Metarril WP E9 and wheat), AW (alpha-cypermethrin and wheat), WS (water and sunflower), BS (Biomagic and sunflower), MS (Metarril WP E9 and Sunflower), AS (alpha-cypermethrin and sunflower). *Means not followed by a letter within a column in each season are not significantly different according to Tukey's HSD test ($p \leq 0.05$). **Means followed by the same letter within a column in each season are not significantly different according to Tukey's HSD test ($p \leq 0.05$).

4.5 Discussion

Metarril WP E9 *Metarhizium anisopliae* formulation tested in this study is a potentially efficient biopesticide for black bean aphids and would contribute significantly to the implementation of IPM of this pest French on beans. A greenhouse study by Bayissa *et al.*'s (2017) attributed *Metarhizium anisopliae* to be responsible for about 73-98% mortality cabbage aphids, cotton aphids and turnip aphids on kale and okra, seven days after inoculation. Mweke *et al.* (2018) also reported that *Metarhizium anisopliae* under laboratory test was less pathogenic against aphid predator *Cheilomenes lunata*, but had high conidial production responsible for 34.5-90% mortality rate on *Aphis craccivora*. Murerwa *et al.* (2014) observed higher virulence of

Metarhizium anisopliae compared to *Beauveria bassiana* against *Rhopalosiphum padi* and *Metopolophium dirhodum* aphids.

Based on the current study, the aphid population were significantly reduced in plots where wheat or sunflower border crop was used compared to plots with no border crops. This current study showed that border cropping reduced aphid population and damage severity but increased ladybird populations and could translate to increased yields of French bean in open field production systems, Offers extra shelter and diet for aphid predators and other natural enemies (Amala & Shivalingaswamy, 2018; Daryanto *et al.*, 2020; Mweka *et al.* 2020. The study also showed a positive interaction between the effect of biopesticides and border crops on aphid abundance and damage severity, demonstrating the potential of integrating biopesticides and border crop systems in open fields. This is consistent with other studies that have reported that the integration of biopesticides and border cropping has been shown to effectively suppress insect pest populations and the subsequent damage caused to plants (Afrin *et al.*, 2017; Farhat *et al.*, 2014; Huss *et al.*, 2022; Mwani *et al.*, 2021; Waweru *et al.*, 2021).

Increasing crop biodiversity at the border offers not only an alternate habitat (s) but also provides alternate food or intermediate hosts for predators and parasitoids population build-up, thus increasing natural enemies in the border cropping system (Mwani *et al.*, 2021; Nyaanga *et al.*, 2006; Ochieng *et al.*, 2022). Border cropping has also been shown to significantly reduce aphid population by distracting pests to other crops and attracting their natural enemies, reducing the target pest population and economic loss impact on the crop and enhancing biological control (Ndakidemi *et al.*, 2022; Sarkar *et al.*, 2018). More than 60% of angiosperm plants emit floral scent in the form of volatile compounds (limonene, (*E*)-ocimene, myrcene, linalool, α -pinene, aldehydes, alcohols, esters, terpenoids and benzaldehyde) when these plants are used as border crop or companion plants thereby attract not only pollinators but also natural enemies that will contribute significantly in pest management (Sánchez-Sánchez *et al.*, 2017; Thomas *et al.*, 2023). Thus, higher plants such as sunflower or wheat have been shown to contain some secondary plant compounds which are considered vital components of the plant defense system against phytophagous insects or released volatile organic compounds that attract natural enemies (Nalam *et al.*, 2019; Yeshe *et al.*, 2022; Zhu, 2016). Additionally, plant trichomes on plant surfaces like wheat and sunflower have been shown to provide a physical barrier to insect pest movement (Singh *et al.*, 2021). At the border crop based on this study, ladybird beetle and aphid populations

increased for treatment combination where wheat or sunflower was used as a border crop compared to where there was no border crop. In both seasons, the ladybird population under plots where wheat was used as a border crop accounted for 21.3% and 19.9% more ladybird beetle in season one and two, respectively, compared to plots where sunflower was used as a border crop. The aphid population at the border was also higher under plots where wheat was used as a border crop, thus accounting for 0.7% and 9.8% compared to plots under sunflower. Higher plants used as border crops have been shown to release secondary compounds such as (E) β -farnesene (aphid alarm pheromone), which promotes ‘dispersal behavior’ in aphids or may act through exhibiting attractancy, oviposition deterrence or attract natural enemies, thus masking effect from the mix and/or luring pests away from the main crop (Sánchez-Sánchez *et al.*, 2017; War *et al.*, 2012). Such diverse effects of border cropping on insect pests could have worked in the current study either in a synergistic or additive manner that led to lower aphid population on French bean and an increased ladybird beetle population at the border of plots with wheat or sunflower.

As a result, natural enemies at the border preyed on pests that avoided insecticide sprayed or recolonized the main crop, thus reducing pest resurgence (Serée *et al.*, 2022). Border crops also act as a physical barrier or trap or repellent for various sucking pests such as aphids and whiteflies, thereby delaying outbreaks on vegetables, population build-up and subsequent damage impact to the main crop (Ben-Issa *et al.*, 2017; Chopkar *et al.*, 2020; Mutisya *et al.*, 2016). Border crop structure and arrays of color have often been known to cause camouflage of odor and appearance, thus interrupting with insect pest search, identification and migration to suitable hosts (Thomas *et al.*, 2023).

Also, the current study observed black bean aphid species at the border crop, particularly on sunflower plants, thus enhancing border crop use in pest management as they attract aphids and obstruct them from migrating to the main crop. Also, other aphid species, such as the Russian wheat aphid (*Diuraphis noxia*) were observed on wheat at the border, which acts as a harbor for generalist natural enemies that could contribute to and enhance the effectiveness of border crop use in managing aphids (Alarcón-Segura *et al.*, 2022). Other authors have documented similar findings (Khan *et al.*, 2017; Omkar & Pervez, 2016; Srinivasan *et al.*, 2019) where the use of border crops increased the population of natural enemies such as ladybird beetles, hoverflies, parasitoids throughout the crop growth stage compared to sunflowers, which mainly obstruct and interrupt the movement of flying insect pests such as aphids. This could be partly attributed to the

continuous production of tillers by wheat, which provides habitat and releases a volatile chemical that attracts more generalist natural enemies of aphids like ladybird beetle to the border crop.

Mwani *et al.* (2021) study finding reported that the maize-dolichos intercrop system had a 24.6% lower bean aphid population compared to mono-crop with a significantly higher infestation of 51.6%. A similar finding was reported by Chopkar *et al.* (2020), where aphid population was significantly reduced in Lablab bean (*Lablab purpureus*) plots with border crops, where sweet corn as a border crop had the least mean aphid population, followed by marigold, safflower, cowpea, mustard, coriander, sunflower, and sesame, respectively. Based on the current study, border crop plant structures, such as sunflower could have provided a barrier by interfering with the movement and access of the inset pest to the intended host plant, thereby enhancing main crop performance and reducing pest effects. Mansion-Vaquié *et al.* (2020) studied an intercrop of *Triticum aestivum* L. with *Pisum sativum* L., or *Trifolium repens* L. and concluded that intercropping wheat with clover significantly reduced cereal aphid densities and thus less damage caused. The study attributed this response to chemical and physical responses that interfered with host location, slower female development, decreased fecundity and increased mortality. Most plants elicit compounds that may exhibit chemical repellency, attractancy, oviposition deterrence, insecticidal effects, or luring pests away from the main crop, leading to decreased pest pressure (Alarcón-Segura *et al.*, 2022; Huss *et al.*, 2022).

Management of sap-sucking insects like aphids relies on broad-spectrum insecticides known to cause high mortality on natural enemies (Alfaro-Tapia *et al.*, 2021; Barros *et al.*, 2018). Based on the current study, the synthetic chemical alpha-cypermethrin was the most effective insecticide in reducing aphid infestation and subsequent damage of French bean. A similar result was reported by Aljassani *et al.* (2020), where their findings documented the effectiveness of alpha-cypermethrin in managing black bean aphids and Green aphids in peas. Indiscriminative use of synthetic insecticides in French production has been considered dangerous because of the need to observe post-harvest intervals (PHI) and maximum residual limits (MRL) export market requirements for French beans that are harvested frequently (Barros *et al.*, 2018; Marete *et al.*, 2020). However, continuous and indiscriminative use of synthetic chemicals poses a higher risk of excess residue above the maximum residue limit requirement in the export market, promotes insect pest resistance development, causes health hazards to growers and harms the environment negatively, reducing biodiversity reduction. Resistance development by bean aphids and toxicity

of synthetic insecticides to natural enemies has been documented by several authors (Bass *et al.*, 2015; Liu *et al.*, 2021; Ullah *et al.*, 2023).

Biopesticides are considered safer for the environment and humans, with no residual accumulation on the produce, but they have shown variability in their efficacy rate under natural conditions. The variability by biopesticides could be partly attributed to the sensitivity of the spores to higher temperatures, Ultra-Violet light and unfavorable conditions (very dry) for the pathogen to colonize and infest the host pest (Iqbal *et al.*, 2021; Mweke *et al.*, 2020). Use of intercrop or border cropping has been documented to enhance the efficacy of entomopathogenic fungi against insect pests under open field conditions (Boni *et al.*, 2020; Mweke *et al.*, 2020; Peng *et al.*, 2021). Therefore, integrating biopesticides with a border cropping system provides a suitable alternative because they are less likely to develop resistance and are less harmful to most natural enemies; thus, they offer a better comparative advantage to alpha-cypermethrin based on its impact to the ecosystem.

4.6 Conclusion

Based on the results of this study under open field conditions, the combined use of *Metarhizium anisopliae* and border planting managed aphid population, reduced damage severity on French bean. Our results recommend integrating biopesticides with wheat as border crops in managing black bean aphids in the open field. The results also present this approach as a viable, environmentally friendly insect pest management strategy that, apart from being easy to use for small-to-medium scale farmers. A well as provide an option for reducing the indiscriminate application of synthetic insecticides that not only increase the cost of production but also harmful to humans and the environment.

CHAPTER FIVE

EFFECTS OF *Metarhizium anisopliae* AND BORDER CROPPING ON GROWTH, YIELD AND QUALITY OF FRENCH BEAN

Abstract

Despite the economic value of French bean, growers still experience low yield and quality due to several biotic and abiotic constraints. The study aimed to determine the effects of *Metarhizium anisopliae*, an entomopathogenic fungus (EPF) and border cropping on French bean growth, yield and quality in an open field production system. A factorial experiment was conducted in a Randomized Complete Block Design (RCBD) with three replications for two growing seasons at the Horticulture Demonstration Field of Egerton University- Njoro, Kenya, between May and December 2022. The first factor comprised border crops at three levels (a sunflower, wheat, and no border crop). The second factor involved spraying the French bean plants with Metarril (2×10^8 spores/g) E9 containing *Metarhizium anisopliae* as a biopesticide alongside Biomagic (2×10^8 spores/ml) and alpha-cypermethrin (8ml/20L) as positive controls and water as negative control. Data on growth (collar diameter, plant height, number of leaves and branches), pod yield (tonnes/hectare) and quality (pod grade) variables of French bean were collected. Data obtained were analyzed using the SAS 9.4M8 version (2023). Results showed that the use of *Metarhizium anisopliae* ($p < 0.0005$) and border cropping ($p < 0.0005$) significantly enhanced growth (collar, plant height and branching) as well as yield and quality compared to negative control treatment in both seasons. Among the biopesticide treatments, an increase in marketable pod yield (4-5%) and a decrease in non-marketable pod yield (4-5%) was recorded in plots sprayed with Metarril compared to those sprayed with Biomagic in both seasons. Plants under plots with a wheat border crop yielded the highest total pod yield (14.95 tonnes /ha). Sunflower border crop recorded the tallest plants and caused a decrease in the total harvested yield of French beans compared to a wheat border or no border crop. A wheat border or sunflower border caused a 7.9% and 7.1% increase in marketable yield, respectively, compared to plants in plots with no border crop. The highest total pod yield (18.96 tonnes/ha) was obtained from plots sprayed with alpha-cypermethrin and with a wheat border crop. French bean plants sprayed with Metarril with a wheat border recorded more total yield and marketable yield than Biomagic sprayed plots with a wheat border crop. Therefore, *Metarhizium anisopliae* and border cropping influenced the growth, yield and quality of French beans and thus could be considered and promoted among growers.

5. Introduction

French bean (*Phaseolus Vulgaris* L.) is an exotic and economical vegetable grown for its rich source of nutrients, essential minerals and is primarily targeted for the export market (Didinger & Thompson, 2021; Myers *et al.*, 2019). French bean production has contributed significantly to the horticultural sector of Kenya, creating about 80% of employment opportunities for more than a million people, both skilled and unskilled labour (Fulano *et al.*, 2021; Mwangi *et al.*, 2019). A constant reduction in crop productivity has, however, been recorded, thereby causing increasing emphasis and concern on how to sustain crop production without compromising yield and quality. As a result, the integration of traditional cultural practices such as intercropping in production has received attention (Zhang *et al.*, 2020) because of the added advantages that come with such systems besides the main crop yield, such as improved crop quality and the environment offered by such diversified and integrated approaches of crop production.

Intercropping is a traditional agronomic measure to improve crop yield and diversify income, which involves combining two or more crops grown simultaneously with the association of time and space. Several studies have documented the advantages of appropriate intercropping as a companion crop to include efficient resource utilization, increased crop productivity in terms of yield and quality, conservation or attraction of natural enemies, obstruction, trapping, repelling or suppressing pest population as well as increased economic benefits for the growers (Afrin *et al.*, 2017; Daryanto *et al.*, 2020; Huss *et al.*, 2022; Xu *et al.*, 2020). A study by Bao-jie *et al.* (2021) on intercropping reported a boost in the total yield, increased plant diversity with more significant and stable crop productivity, increased economic benefit and also reduced occurrence of pests and diseases as a result of altered micro-climate, ecological structure and environmental conditions to favor natural enemies growth and development in the fields (Chi *et al.*, 2021). Companion cropping strategy as an intercrop within rows or as a border crop has been documented to reduce the competition for arable land between French bean and other crops of economic importance (Singh *et al.*, 2021; Zhang *et al.*, 2021). Other studies (Ben-Issa *et al.*, 2017b; Gontijo *et al.*, 2018; Saldanha *et al.*, 2019; Waweru *et al.*, 2021) have documented the use of companion crops either as border or intercrop crops without compromising the yield within the crop production process as one of the strategies being promoted to achieve sustainable integrated crop management in horticultural crops. Thus, using companion crops and/or with biopesticides such as

entomopathogenic fungi could comply partly with safe production requirements by international export market criteria on consumer protection at the crop production level.

Studies (Abdollahdokht *et al.*, 2022; Fenibo *et al.*, 2021; Lengai & Muthomi, 2018) have also shown that biopesticides derived from entomopathogenic fungus (EPF) have the advantage of being compatible with integrated pest management (IPM) strategies although, their low efficacy under field conditions slows down their widespread use (Mweka *et al.*, 2020). EPF-based biopesticides containing *Metarhizium anisopliae* have been recommended as safe in crop production. They are cheap, environment-friendly, specific in their mode of action, sustainable, do not leave residues and are not associated with the release of greenhouse gases (Ayilara *et al.*, 2023; Kumar *et al.*, 2021; Srinivasan *et al.*, 2019). Combining the use of *Metarhizium anisopliae* with other practices, such as cropping systems using border crops, can enhance their performance. However, limited or no previous studies have been carried out with the focus on assessing the effects of applying biopesticide products containing *Metarhizium anisopliae* to plots of French beans with sunflower or wheat as border crops for their subsequent impact on growth, yield and quality of the French bean crop. Therefore, the current study aimed to determine the effects of *Metarhizium anisopliae* entomopathogenic fungus application and companion cropping using wheat or sunflower as a border crop on French bean growth, yield and quality under open field conditions.

5.1 Materials and method

5.1.1 Experimental site description

The study was conducted at the Horticulture Research and Demonstration Field, Egerton University, Njoro-Kenya. The field lies at latitude 0.23°S and longitude 35.35 °E in the Lower Highland III Agroecological Zone (LH3) at approximately 2238 m above sea level (ASL). The site receives an annual average rainfall of 1180-1400mm with an annual daily temperature range of 16-22 °C. The soil types at the experimental site are predominately Mollic Andosols, well-drained, friable, moderate in fertility, containing medium levels of organic carbon, low levels of phosphorus, and a pH range of 6.0 to 6.5 (Jaetzold *et al.*, 2012). The rainfall pattern and distribution during this study were recorded as shown in chapter four, section 4.2.1.

5.1.2 Planting materials

French bean cultivar 'Enclave', a determinate cultivar and among the commonly grown cultivars in Kenya's French bean growing regions, such as Naivasha, was used in this study. The

Enclave cultivar produces more extra fine-grade quality grade pods, is high yielding, and adaptable to wide agroecological zones compared to other cultivars. French bean seeds used in the study were procured from Hygrotech (EA) Ltd- Naivasha. Sunflower (*Helianthus annuus* L.) variety 'Kenya Fedha' and wheat (*Triticum aestivum* L.) variety 'Njoro bread wheat two' were procured from Kenya Agricultural and Livestock Research Organization (KALRO) Njoro, Kenya. The wheat variety is highly preferred by insect pests, with a dwarf growth habit that could reduce shade effects. It is the most commonly grown commercial variety and most adopted by farmers within the agroecological zones of Nakuru County. On the other hand, the sunflower used in this study is an open-pollinated variety with uniform maturity, well suited to medium and high altitudes, and the most preferred in the agricultural region where this study was conducted.

5.1.3. Experimental design and treatments

The study was a 4 x 3 factorial experiment arranged in a Randomized Completely Block Design (RCBD) with three replications. The first factor under study was foliar spray application at four levels: (i) Metarril Wettable powder (2×10^8 spores/g) (M_1), chosen based on its superior performance during the laboratory bioassay experiment, (ii) Bio-magic 15 LF as commercialised biopesticide product, (iii) alpha-cypermethrin (8ml/20L) as a commercialised synthetic insecticide as positive control and (iv) water as the negative control. The second factor was companion cropping at three levels: (i) sunflower as a border crop, (ii) wheat as border crops and (iii) no border crop as a control. Twelve (12) treatment combinations were therefore studied namely, Metarril (2×10^8 spores /g) + sunflower as border crop (MS), Metarril (2×10^8 spores /g) + wheat as border crop (MW), Metarril (2×10^8 spores /g) + no border crop (MN), Biomagic (2×10^8 spores /ml) + sunflower as a border crop (BN), Biomagic (1×10^8 spores /ml) + wheat as border crop (BW), Biomagic (2×10^8 spores /ml) + no border crop (BN), water (20L) + wheat as border crop (WW), water (20L) + sunflower as border crop (WS), water (20L) + no border crop (WN); alpha-cypermethrin (8ml/20L) +wheat as border crop (AW), alpha-cypermethrin (8ml/20L) + sunflower as border crop (AS) and alpha-cypermethrin (8ml /20L) + no border crop (AN).

5.1.4 Field preparation, layout and crop management

The experimental field was ploughed and harrowed using a tractor before planting to obtain a moderate tilth suitable for planting French beans and the border crops. Experimental plots measuring 4.2m by 3m each were laid out as shown in Figure 4.1 in chapter four. Sunflower was planted along the border of the designated plots at a spacing of 30 cm x 75 cm with two (2) seeds

per hole and later thinned to one healthy and strong seedling per hill two weeks after emergence of the seedlings. At the same time, wheat was also planted at a spacing of 30 cm x 30cm in the designated plots. The distance between the border crops and the first French bean row was 60 cm in all plots. French bean seeds were planted three weeks (3 weeks) after planting the sunflower and wheat to allow the border crops time to establish at least 2-3 leaves before planting the French bean so that the companion crops could provide a habitat for natural enemies and other invertebrates by the time the primary crop (French bean) was planted. French bean was planted at a spacing of 60 x by 30cm with two (2) seeds planted per hole. Diammonium phosphate (DAP) fertilizer was applied to French bean plots during planting at 200 kg/ ha rate (Greenlife Crop Protection Africa, 2021). Calcium Ammonium Nitrate (CAN) at a 150 kg/ha rate was applied as a top dress in two equal split applications at three (3) true leaves and at the onset of flowering as the growth stages recommended for application in French bean production to enhance essential nutrient availability (Oseko & Dinya, 2015). Weeding was done manually three weeks after germination. The experiment was rain-fed with supplemental irrigation provided using a drip irrigation system during extended periods of a dry spell.

5.1.5. Treatment spraying

Spraying of the experimental plots started 21 days after the emergence (DAE) of the French bean. Spraying was repeated every 14 days, with four sprays targeting mainly the vegetative growth stage of French bean plants when insect pests cause severe damage by attacking auxiliary buds and tender growing points, as described by Sayed *et al.* (2019). The selection of a 14-day spray interval was guided and adopted based on a previous study by Boni *et al.* (2020) as the most effective range for insect pest mortality in open field experiments using entomopathogenic fungi, as this period provides enough time for the pathogenic fungi such as *Metarhizium anisopliae* to cause death as a result of both primary and secondary infection. Metarril wettable powder E9 (2×10^8 spore /g), Biomagic 1.5 LF (2×10^8 spores /ml), and alpha-cypermethrin were thoroughly mixed in water containing 0.05% Integra (3ml per 20l) for 5 minutes prior to application to ensure a homogenous solution of each before being applied to each experimental unit. Integra product acts as a surfactant to reduce the surface tension of the treatment spray solution for even and uniform spreading on the plant's surface, improving the absorption and retention rate. To minimize the likelihood of contamination, four different knapsack sprayers (Kenplastic Knapsack sprayer, 20L) but with similar calibration to discharge 350L/acre and nozzle pressure of 2.88 kgf m⁻² were

used. Each of the four knapsack sprayers was used to apply only one product, that is, Metarril (2×10^8 spores/g) E9, Biomagic (2×10^8 spores/ml), alpha-cypermethrin (8ml/20L) or water. Treatment application was done early in the morning, between 06:00 and 08:00 hours to minimize treatment drift caused by wind.

5.2 Data collection

Six (6) plants from inner rows of each experimental unit were randomly selected at the start of data collection and tagged for data collection on French bean growth, yield and quality.

5.2.1 French bean growth variables

Data collection on growth variables started at 21 days after emergence (DAE) of French bean, when the crop had on average three (3) true leaves and thereafter continued at a two-weeks interval for ten (10) weeks. The growth variables of interest in this study were plant height, collar diameter and number of branches. These latter growth variables were chosen to provide valuable insights on combined effects of border cropping and *Metarhizium anisopliae* on plant growth and development. However, shoot dry weight, root dry weight and leaf area index were not collected due to resource limitations.

(a) Plant height and number of branches

The height of the tagged plants in each experimental unit was measured in centimeters (cm) using a meter ruler from the ground level to the highest point tip of the plant, beginning at 21 DAP and continuing to the first harvest. The number of branches on each tagged plant was counted and recorded during each data collection date as the number of branches per plant (no./plant).

(b) Collar diameter

The stem collar diameter of each tagged plant was measured in millimeters (mm) using a digital vernier caliper (Model 599-577-1/USA) at ≈ 4 cm from the ground level. Measurement of collar diameter began 21 DAP to the first harvest. Data obtained were used to compute the average stem collar diameter for the different treatments.

5.2.2. French bean yield variables

Pod numbers and fresh pod weights were used as the yield variables in this study.

(a) Pod numbers and fresh pod weight

Harvesting was started at 77 DAP and was done twice per week for four weeks by removing pods that had attained the horticultural maturity stage. The pods harvested from the six tagged plants from each experimental unit were counted separately at each harvest and the number was recorded

and later used to compute the average number of pods per plant (no./plant). The pods from each experimental unit were then weighed in grams (g) using a weighing balance (Advanced Technocracy Inc. Ambala) weights obtained were recorded in grams per experimental unit and later converted to tonnes per hectare (t/ha) of the total weight.

(b) French bean pod grading and quality

At each harvest, after the average number of pods per plant was computed, French bean pods harvested from each experimental unit were sorted, graded and eventually separated into three grades. These grades were (i) extra-fine/extra class grade for pods with a measurement of 4 - 6 mm in width as the diameter of the pod cross section and 8 -10 cm in length, (ii) fine grade/class I (6 - 9 mm width and 10 - 17 cm length) and (iii) bobby pods/ class II (more than 9 mm width and with small seeds that were not too large) and considered as marketable yield. The grading criteria was based on an internationally accepted guide used by AAA Growers Limited, Kenya, a leading grower and exporter of French beans from Kenya to the European market (Fulano *et al.*, 2021; United Nations Economic Commission for Europe, 2017). Pods that did not meet the latter grading specifications were considered as unmarketable yield. A vernier caliper and a ruler were used to measure the pod diameter and length. Thereafter, the weight of each grade was measured using a weighing balance (Advanced Technocracy Inc. Ambala) and recorded as weight in grams (gms) per grade. The weight of each grade obtained during each harvest was summed up per week and recorded as the total yield weight (gm) and later converted to tonnes per hectare. Thereafter, different grades (extra fine, fine and bobby) were re-summed up in marketable and then converted to tonnes per hectare (t/ha). Harvested pods that did not meet the marketable grading scale (overgrown pods, off-type, blemished pods due to insect pests, sooty mould or injured and those with physical or physiological defects) were classified as non-marketable. Thereafter, the weight of the extra fine, fine and bobby pods per treatment for each week was computed and expressed as a percentage exportable yield. The total grade weight (sum of extra-fine, fine and bobby) percentage per treatment combination was computed by dividing the weight per grade by the total sum of grade weight multiplied by 100 to convert to percentages as shown in equation 3;

$$\text{Percent total weight (\%)} = \left(\frac{\text{Total grade weight}}{\text{Total weight}} \right) \times 100 \dots\dots\dots \text{equation 3}$$

5.3 Data analyses

Data were subjected to normality tests based on the Shapiro-Wilk test and Levene's tests for homogeneity of variances ($P = 0.05$) using the Proc univariate procedure of SAS 9.4M8 version (January 2023, SAS Institute, Cary, NC) before Analysis of Variance (ANOVA) was done. Data on growth and yield variables did not meet the ANOVA normality and equal variance assumptions. As a result, square root transformation and arcsine transformation were used, respectively, to fix the problem for growth and yield variables data, before ANOVA was done. ANOVA was then done using the GLM procedure of SAS at $p \leq 0.05$. Means for significant treatments at the F test were separated using Tukey's Honestly Significant Difference (THSD) test at $P \leq 0.05$. The RCBD model fitted for the experiment was. $Y_{ijk} = \mu + \alpha_{(i)} + \beta_j + (\alpha\beta)_{ij} + block_k + \varepsilon_{ijk}$. Where Y_{ijk} the response from k^{th} experimental unit receiving the i^{th} type of sprayed and j^{th} type of border crop, μ is the overall mean, $\alpha_{(i)}$ effect due to the i^{th} type spray, β_j effect due to the j^{th} type of the border crop, $(\alpha\beta)_{ij}$ an interaction effect of the i^{th} type spray and the j^{th} type of border crop, $block_k$ is effect due to k^{th} block, ε_{ijk} is the random error associated with the response from the k^{th} experimental unit receiving the i^{th} type spray and the j^{th} level of border crop.

5.4 Results

5.4.1 Effects of *Metarhizium anisopliae* and border crop on the growth of French bean

The results indicated that different treatments significantly ($p < 0.0001$) influenced growth variables measured in both seasons (Table 5.3). The main effects of spray type significantly influenced plant height ($p < 0.002$), collar diameter ($p < 0.0001$) and number of branches ($p < 0.0001$) in season two while in season one, significant differences were observed in the variable plant height ($p < 0.0001$) only. The main effect of border crops was also highly significant for plant height ($p < 0.0005$), collar diameter ($p < 0.0001$) and number of branches ($p < 0.0001$) in season two with significant differences amongst the different border crops recorded only for the variable plant height ($p < 0.001$) in season one. Similarly, the interaction effect between the type of spray applied and border crop grown at the different data collection dates was significant for plant height ($p < 0.0001$), collar diameter ($p < 0.0001$) and number of branches ($p < 0.0001$) in season two and only for plant height ($p < 0.0001$) in season one.

(a) Plant height

Analysis of data during the different data collection dates showed insignificant differences in plant height amongst the different treatments during the early data collection days at 14, 28 and

42 DAP, but the difference in plant height was significant amongst the different treatment combinations at 56 and 70 DAP in both seasons (Table 5.1). Over the two seasons, plants were generally tallest under the sunflower border treatment, followed by plants in plots with a wheat border and shortest in plots with no border crop during most data collection dates. Amongst all the treatment combinations, Alpha-cypermethrin sprayed plots with sunflower or wheat as a border crop had the tallest plants, followed by plants in Metarril sprayed plots with sunflower or wheat as a border crop, then plants in Biomagic sprayed plots with sunflower or wheat as a border crop, then plants in plants in water sprayed plots with sunflower or wheat as a border crop compared to plants in grown under similar spray treatments but with no border crop during most data collection dates in both seasons. Amongst *Metarhizium anisopliae* treatments, plants sprayed with Metarril and Biomagic were not significantly different in plant heights during most data collection days except at 56 and 70 DAS in both seasons.

Table 5.1: Effects of *Metarhizium anisopliae* and border crop on plant height (cm) in both season one (April to July 2022) and season two (September to December 2022).

Treatment*	14 DAP	28 DAP	42 DAP	56 DAP	70 DAP
Season one					
WN	10.73**	14.00**	16.43**	16.39b***	14.16b***
BN	11.91	14.55	15.26	21.50ab	18.53b
MN	13.03	16.12	18.81	21.61ab	20.27b
AN	14.84	17.68	22.36	30.38ab	33.33ab
WW	12.59	16.53	21.59	17.23ab	16.72b
BW	12.53	18.22	24.11	23.83ab	26.78ab
MW	14.26	17.66	19.90	27.44ab	26.78ab
AW	11.64	16.11	18.83	27.55ab	31.69ab
WS	13.59	15.19	18.75	20.05b	20.17b
BS	12.47	18.08	22.93	25.83ab	32.67ab
MS	13.83	19.19	23.50	26.20ab	30.30ab
AS	15.24	19.08	26.45	39.61a	42.83a
Season two					
WN	18.23	23.37	40.36	49.10b	50.17b

BN	17.30	29.53	41.83	56.07ab	58.70ab
MN	16.93	28.93	43.33	57.83ab	64.07a
AN	15.30	27.56	44.60	62.37a	58.40ab
WW	14.50	20.70	43.33	52.20ab	54.43ab
BW	16.86	27.13	43.83	53.57ab	58.73ab
MW	16.93	28.93	44.13	56.73ab	59.23ab
AW	17.30	25.90	42.33	62.13a	63.90a
WS	18.90	29.23	44.60	55.06ab	55.13ab
BS	18.30	31.63	47.27	55.83ab	57.83ab
MS	17.06	28.47	48.88	60.67ab	64.73a
AS	18.63	31.13	53.43	63.26a	65.93a

*WN (water and no border), BN (Biomagic and no border), MN (Metarril E9 and no border), AN (alpha-cypermethrin and no border), WW (water and wheat), BW (Biomagic and wheat), MW (Metarril and wheat), AW (alpha-cypermethrin and wheat), WS (water and sunflower), BS (Biomagic and sunflower), MS (Metarril and Sunflower), AS (alpha-cypermethrin and sunflower)

Means not followed by a letter within a column are not significantly different according to Tukey's HSD test ($P \leq 0.05$). *Means followed by the same letter within a column are not significantly different according to Tukey's HSD test ($P \leq 0.05$). Days after planting (DAP).

Averaged over the growing season, the main effect of spraying French bean with *Metarhizium anisopliae* on the height of French bean plants from the different treatments was significant in both seasons (Table 5.2). In season one, significant differences in plant height were observed in plants amongst all plots that received the different treatments with plants in plots sprayed with alpha-cypermethrin recording the highest plant height (24.52 cm), followed by plants in plots sprayed with Metarril E9 (20.04 cm) and Biomagic (19.80 cm) compared to plots sprayed with water (16.88 cm). Generally, plants were shortest in plots sprayed with water in both seasons. Plants from plots sprayed with Metarril E9 and Biomagic were not statistically different in plant height. Similar trend was observed in season two, although the difference in height of plants recorded for the different treatments was not statistically significant during all data collection dates.

During both seasons, French bean plants grown in plots with border crops (wheat or sunflower) were significantly taller than grown in plots which had no border crop (Table 5.2). In season one, French bean plants were tallest in plots with a sunflower border crop compared to those grown in

plots with a wheat border crop or with no border crop. Although French bean plants grown with a wheat border generally tended to be taller than those grown with no border crop, the difference in the height of plants from the two treatments was not significant in both seasons. During both growing seasons, therefore, the tallest plants were obtained in plots with a sunflower border crop while the shortest plants were obtained in plots that had no border crop. French bean plants in plots with sunflower or wheat as a border crop and applied with the different spray types were generally taller than plants in plots sprayed with the respective spray types but with no border crop in both seasons. Plants in plots sprayed with Metarril E9 and Biomagic were not significantly different in plant height, although plants in plots sprayed with Metarril recorded higher plant height than those in plots sprayed with Biomagic in both seasons. On overall, the highest plant height was recorded for French bean plants grown in plots sprayed with alpha-cypermethrin and had a sunflower border crop (46.24 cm) while the shortest plant height was recorded for plants grown in plots sprayed with water and had no border crop (14.54 cm) during the study as shown in table 5.2.

Table 5.2: Effects of *Metarhizium anisopliae* and border crop on plant height (cm) in both season one (April to July 2022) and season two (September to December 2022)

Spay type	No border	Wheat	Sunflower	Spray Means
Season one				
Water	14.54e**	18.52bcde**	17.55cde**	16.88c**
Biomagic	15.26de	21.18bc	22.33bc	19.80b
Metarril	17.23cde	20.48bcd	22.40bc	20.04b
Alpha-cypermethrin	21.16bc	23.72a	28.62a	24.51a
Border crop means	18.68b	19.51b	22.73a	
Season two				
Water	38.24d**	40.28bcd**	43.93ab**	40.40*
Biomagic	38.43dec	40.98bcd	39.55bcd	41.50
Metarril	42.55abc	41.01bcd	42.17abc	41.03
Alpha-cypermethrin	39.83bcd	43.51ab	46.24a	41.95
Border crop means	40.16b	40.54b	42.97a	

*Means not followed by the same letter within a column and within a season are not significantly different according to Tukey's HSD test ($p \leq 0.05$). **Means followed by the same letter within a column

and within a season are not significantly different according to Tukey's HSD test ($P \leq 0.05$). Data were subjected to square root transformation before analysis, but the values presented are original means.

(b) Collar diameter

Analysis of data during the different data collection dates showed no significant differences in collar diameter among the different treatments during the early data collection days at 14 DAP but the difference in plant collar diameter was significant amongst the different treatment combinations from 28 DAP all the way to 70 DAP in season one. Although a similar trend on treatment effect could be picked in season two, the difference in collar diameter amongst the different treatment combinations was not significant throughout the entire season (Table 5.3). During the two seasons, plants that generally had larger collar diameters were grown with a wheat border crop, followed by plants grown in plots with a sunflower border crop, which were thinnest in plots with no border crop during most data collection dates. Amongst all treatment combinations, alpha-cypermethrin sprayed plots with a sunflower or wheat border crop had the largest collar diameter, followed by plants in Metarril sprayed plots with sunflower or wheat as a border crop, plants in Biomagic with sunflower or wheat as a border crop, then plants in plots in water sprayed plots with sunflower or wheat as a border crop compared to plants grown under similar spray treatments but with no border crop during most data collection dates in both seasons. Amongst *Metarhizium anisopliae* treatments, plants sprayed with Metarril and Biomagic significantly differed in collar diameter size during most data collection days in season one except at 14 DAP, with Metarril sprayed plants being thicker than Biomagic sprayed plants. During season two, plants sprayed with the different treatment combinations did not differ significantly in collar diameter in all data collection dates (Table 5.3).

Table 5.3: Effects of *Metarhizium anisopliae* and border crop on collar diameter (cm) in both season one (April to July 2022) and season two (September to December 2022).

Treatment	14 DAP	28 DAP	42 DAP	56 DAP	70 DAP
Season one					
WN	0.31**	0.35b***	0.39b***	0.43d***	0.48c***
BN	0.32	0.37b	0.45ab	0.51dc	0.56bc
MN	0.33	0.40ab	0.45ab	0.52bcd	0.72ab
AN	0.34	0.42ab	0.53ab	0.53bcd	0.76a
WW	0.33	0.38ab	0.46ab	0.63abc	0.68ab
BW	0.31	0.42ab	0.53a	0.59abc	0.72ab
MW	0.35	0.42ab	0.56a	0.65abc	0.76a
AW	0.32	0.47a	0.54a	0.69a	0.82a
WS	0.32	0.36b	0.45ab	0.51dc	0.57bc
BS	0.32	0.38ab	0.49ab	0.58abc	0.69ab
MS	0.35	0.43ab	0.55a	0.63abc	0.70ab
AS	0.34	0.43ab	0.53a	0.66ab	0.78a
Season two					
WN	0.28**	0.44**	0.67**	0.63**	1.16**
BN	0.29	0.48	0.67	0.79	1.43
MN	0.28	0.48	0.69	0.80	1.38
AN	0.30	0.51	0.78	0.70	1.40
WW	0.25	0.40	0.54	0.78	1.02
BW	0.20	0.48	0.66	0.91	1.16
MW	0.20	0.45	0.62	1.02	1.46
AW	0.29	0.44	0.74	1.14	1.13
WS	0.27	0.47	0.65	0.75	1.16
BS	0.32	0.49	0.65	0.75	1.13
MS	0.30	0.48	0.74	0.86	1.30
AS	0.29	0.53	0.75	0.78	1.30

* **WN** (water and no border), **BN** (Biomagic and no border), **MN** (Metarril E9 and no border), **AN** (alpha-cypermethrin and no border), **WW** (water and wheat), **BW** (Biomagic and wheat), **MW** (Metarril and wheat), **AW** (alpha-cypermethrin and wheat), **WS** (water and sunflower), **BS** (Biomagic and sunflower), **MS** (Metarril and Sunflower), **AS** (alpha-cypermethrin and sunflower)

Means not followed by a letter within a column are not significantly different according to Tukey's HSD test ($P \leq 0.05$). *Means followed by the same letter within a column are not significantly different according to Tukey's HSD test ($P \leq 0.05$).

Averaged over a growing season, the main effect of spraying French bean with *Metarhizium anisopliae* resulted in significant differences in collar diameter amongst the different spray types in season one only (Table 5.4). During season two, no significant differences in plant collar diameter were observed amongst plants that received the different spray types. Generally, French bean plants in plots sprayed with alpha-cypermethrin recorded the largest collar diameter, followed by plants in plots sprayed with Metarril E9, then those sprayed with Biomagic compared to plants in plots sprayed with water in both seasons. Although plants in alpha-cypermethrin treated plots tended to be thicker than those in Metarril treated plots in season one, the difference in collar diameter of plants from these treatments was not statistically significant. Plants in plots sprayed with Biomagic and water were not statistically different in collar diameter in both seasons. Overall, French bean plants were thinnest in plots sprayed with water and thickest in plots sprayed with alpha-cypermethrin. Amongst *Metarhizium anisopliae* sprayed plots, plants in plots sprayed with Metarril E9 tended to be thicker than plants sprayed with Biomagic, but this difference was significant in season one not in season two.

During season one, French bean plants grown in plots with a border crop of wheat or sunflower had significantly higher collar diameters compared to plants grown in plots with no border crop (Table 5.4). A similar trend was established in season two although the difference in collar diameter of plants from a wheat, sunflower and no border crop treatments were not significantly different. The collar diameter of plants grown in plots with a wheat border crop significantly differed from that of plants grown in plots with a sunflower border crop in both seasons. Overall, the largest mean collar diameter (0.72 cm) was obtained in plots with wheat border crop while the thinnest collar diameter (0.39 cm) was obtained in plots with no border crop. A significant difference was observed in the collar diameter for plants in plots sprayed with different treatments and had a sunflower or wheat border or no border combination. Amongst all the treatment

combinations, the largest collar diameter of 0.57 cm and 0.76 cm and were recorded under plots sprayed with alpha-cypermethrin and a wheat border and the thinnest collar diameter of 0.39 cm and 0.47 cm were recorded for plants in plots sprayed with water and no border crop in season one and two, respectively. Amongst *Metarhizium anisopliae* treatments, plants sprayed with Metarril E9 had significantly higher collar diameter than plants sprayed with Biomagic with either of the border type crops in season one compared to plants in plots with similar treatment with no border crop.

Table 5.4: Effects of *Metarhizium anisopliae* and border crop on the average mean of collar diameter (cm) in both season one (April to July 2022) and season two (September to December 2022).

Spay type*	No border	Wheat	Sunflower	Spray Means
Season one				
Water	0.39e**	050b**	0.44c**	0.46c**
Biomagic	0.44c	0.51ab	0.49b	0.47c
Metarril	0.48b	0.54ab	0.54ab	0.51ab
Alpha-cypermethrin	0.53ab	0.57a	0.55a	0.55a
Border crop means	0.47b	0.53ab	0.51ab	
Season two				
Water	0.66*	0.72*	0.67*	0.67*
Biomagic	0.68	0.73	0.68	0.70
Metarril	0.69	0.75	0.70	0.71
Alpha-cypermethrin	0.71	0.76	0.71	0.72
Border crop means	0.70	0.71	0.69	

*Means not followed by a letter within a column and a season are not significantly different according to Tukey's HSD test ($p \leq 0.05$). **Means followed by the same letter within a column and within a season are not significantly different according to Tukey's HSD test ($P \leq 0.05$). Data were subjected to square root transformation before analysis, but the values presented are original means.

(c) Number of branches

Analysis of data during the different data collection dates showed significant differences in the number of branches among the different treatment combinations during the data collection days at 28, 42, 56 and 77 DAP in season two only (Table 5.5). Although the trend in treatment effects on number of branches in season one was similar to that depicted in season two, the differences in treatment effects were not significant during this season. Over the two seasons, the highest number of branches were generally observed in plots with wheat as a border crop or no border crop compared to plots with sunflower as a border crop. Amongst all the treatment combinations, water sprayed plots with a no border crop or wheat border had the highest number of branches per plant, followed by plants in Biomagic sprayed plots with no border crop or a wheat border crop.

Table 5.5: Effects of *Metarhizium anisopliae* and border crop on the number of branches (no. per plant) in both season one (April to July 2022) and season two (September to December 2022).

Table 5.5: Effects of *Metarhizium anisopliae* and border crop on number of branches of French bean in both season one (April to July 2022) and season two (September to December 2022).

Treatments*	14 DAP	28 DAP	42 DAP	56 DAP	70 DAP
Season one					
WN	1.00**	3.33**	6.00**	7.00**	8.33**
BN	1.00	3.66	4.67	6.33	8.33
MN	2.00	3.66	5.33	6.67	8.33
AN	1.20	3.33	4.67	6.67	7.66
WW	1.30	3.00	4.67	6.33	7.33
BW	1.02	3.66	4.67	6.33	7.66
MW	1.52	3.67	4.67	6.67	7.66
AW	1.33	3.67	4.67	6.67	8.33
WS	2.03	3.33	4.67	6.67	7.66
BS	1.55	3.66	5.00	6.00	8.33
MS	2.02	3.33	5.00	7.33	8.33
AS	1.43	4.00	5.33	6.67	8.33
Season two					

WN	1.00**	4.33a***	7.00a***	9.67a***	11.00a***
BN	1.00	4.33a	6.00a	8.33ab	9.00ab
MN	1.00	4.00ab	5.00bc	7.00bc	7.67b
AN	1.67	3.00abc	4.67bc	6.33bc	7.33b
WW	1.33	2.67bc	4.33c	6.00c	7.67b
BW	1.33	3.67ab	4.67bc	6.33bc	7.67b
MW	1.33	3.33abc	4.00c	7.33bc	8.00b
AW	2.00	2.67bc	4.33c	6.67bc	7.67b
WS	1.33	3.00abc	4.33c	6.33bc	7.33b
BS	1.33	3.33abc	4.00c	6.00c	7.67b
MS	1.22	2.67bc	4.00c	6.67bc	7.00b
AS	1.33	2.00c	3.67c	6.00c	7.67b

***WN** (water and no border), **BN** (Biomagic and no border), **MN** (Metarril E9 and no border), **AN** (alpha-cypermethrin and no border), **WW** (water and wheat), **BW** (Biomagic and wheat), **MW** (Metarril and wheat), **AW** (alpha-cypermethrin and wheat), **WS** (water and sunflower), **BS** (Biomagic and sunflower), **MS** (Metarril and Sunflower), **AS** (alpha-cypermethrin and sunflower)

Means not followed by a letter within a column and within a season are not significantly different according to Tukey's HSD test ($P \leq 0.05$). *Means followed by the same letter within a column and within a season are not significantly different according to Tukey's HSD test ($P \leq 0.05$). **DAS**-days after planting.

Averaged over the growing season, the main effect of spraying had significant effects on the number of branches on French bean plants in season two (Table 5.6). Although a similar trend in spray type effects on the number of French bean plants was depicted in season one, the difference in the number of branches amongst the different spray types was not significantly different during all data collection dates of this season. In season two, significant differences in the number of branches were observed in plants amongst all plots that received the different treatments, with plants in plots sprayed with water recording the highest number of branches, followed by plants in plots sprayed with Biomagic and Metarril E9 compared to plots sprayed with alpha-cypermethrin. Plants from plots with border crops (wheat or sunflower) recorded lower number of branches from plots with no border crop for season two only (Table 5.6). A similar trend was observed in season

one, although the difference in the number of branches per plant recorded for the different treatments was not statistically significant during all data collection dates.

During season one, French bean plants grown in plots with different border types were not statistically different in the number of branches per plant. During season two, however, French bean plants grown in plots with wheat or sunflower border crops had significantly lower number of branches per plant compared the plants grown in plots with no border crop (Table 5.6). In both seasons, plants from plots with no border crop had the highest number of branches per plant, followed by plants in plots with a wheat border crop with the lowest number of branches per plant recorded in plots with a sunflower border crop. French bean plants in plots that received the different spray treatments with no border crop had the highest number of branches per plant, followed by those with a wheat border crop compared to those that had a sunflower border crop in both seasons. Over the two seasons, the highest number of branches was recorded under plots sprayed with water and no border crop and the lowest number of branches per plant was recorded in plots sprayed with alpha-cypermethrin and had a sunflower border crop. Although a similar trend in spray type effects on the number of French bean plants was depicted in season one that had no border crop recorded a higher number of branches per plant compared to plants in plots that received Metarril spray and had a wheat or sunflower border crop in both seasons.

Table 5.6: Effects of *Metarhizium anisopliae* and border crop on number of branches of French bean in both season one (April to July 2022) and season two (September to December 2022).

Spray type	No border	Wheat	Sunflower	Spray Means
Season one				
Water	4.73*	4.80*	4.53*	4.91*
Biomagic	5.00	4.80	4.60	4.91
Metarril	5.00	4.80	4.66	4.73
Alpha-cypermethrin	5.13	4.86	4.63	4.71
Border crop means	4.88	4.88	4.68	
Season two				
Water	6.80a**	4.60dc**	4.47dc**	5.28a**
Biomagic	5.80b	4.73dc	4.40dc	4.97ab
Metarril	4.93c	4.80dc	4.20dc	4.64bc
Alpha-cypermethrin	4.47dc	4.33dc	4.13d	4.31c

Border crop means

5.50a

4.62b

4.30c

*Means not followed by a letter within a column and within a season are not significantly different according to Tukey's HSD test ($P \leq 0.05$). **Means followed by the same letter within a column and within a season are not significantly different according to Tukey's HSD test ($P \leq 0.05$). Data were subjected to square root transformation before analysis, but the values presented are original means.

5.4.2 Effects of *Metarhizium anisopliae* and border cropping on the yield of French bean

The results showed that different treatments significantly influenced the number of pods per plant ($p < 0.0319$), ($p < 0.0001$), average pod weight ($p < 0.0057$), ($p > 0.0405$) and total pod weight ($p < 0.0086$), ($p < 0.0012$) of French bean in season one and two, respectively (Appendix B). Application of *Metarhizium anisopliae* significantly influenced number of pods ($p < 0.0001$) ($p < 0.0021$), average pod weight ($p < 0.0004$) ($p > 0.2713$), total pod weight ($p < 0.0003$) ($p < 0.0004$) in season one and two respectively, except for average pod weight only in season two. Similarly, use of border crops influenced the number of pods ($p < 0.0390$) and total pod yield ($p > 0.3295$) significantly for season two only. The interaction effect between border crops and *Metarhizium anisopliae* application was insignificant for the number of pods and average pod weight except for the total yield of French bean plants in both seasons.

(d) Number of pods

Averaged over the growing season, the main effect of French bean plants with *Metarhizium anisopliae* resulted in a significant difference in the number of pods per plant of French bean amongst the different treatment combinations in both seasons (Table 5.7). However, a similar trend in spray type effects on the number of French bean pods was depicted in season one, with plants in plots sprayed with alpha-cypermethrin recording the highest number of pods per plant (437.78), followed by plants in plots sprayed with Metarril E9 (280.44) and Biomagic (239.78) compared to plots sprayed with water (192.55). Plants from plots sprayed with Metarril E9 and Biomagic were statistically different in the number of pods per plant in both seasons. A similar trend was observed in season two, although more pods were recorded for plants in plots sprayed with Metarril E9 compared to the Biomagic sprayed plot. However, the number of branches for French bean plants grown in plots sprayed with Biomagic and water treatments was not statistically different in season one. During season two, French bean plants grown in plots with different border crop types had

significant differences in the number of pods compared to plants in plots that had no border crop (Table 5.7). The number of pods per plant of plants grown in plots with a wheat border crop significantly differed from plants grown in plots with a sunflower border crop. During both seasons, the highest number of pods per plant was recorded for plants grown in plots with a wheat border compared to plots with a sunflower border, which recorded the lowest number of pods in all border crop types. Over the two seasons, the highest number of pods (648.33) were recorded for plants in plots sprayed with alpha-cypermethrin and had a wheat border crop and the lowest number of pods per plant (117.00) was recorded in plots sprayed with water and had a no border crop treatment combination (Table 5.7). Amongst biopesticide treatments, plants in plots sprayed with Metarril E9 and had a wheat border crop recorded the highest number of pods per plant (557.33b) while the lowest number of pods (158.66) was recorded for plants in plots sprayed with Biomagic and had a no border crop.

Table 5.7: Effects of *Metarhizium anisopliae* and border crop on the number of pods (no. of pods /plant) in both season one (April to July 2022) and season two (September to December 2022).

Treatment	Border crop			Treatment Means
	No border	Wheat	Sunflower	
Season one				
Water	117.00*	288.33*	172.33*	192.55b**
Biomagic	158.66	345.67	225.67	239.78b
Metarril	197.33	345.00	298.33	280.44ab
Alpha-cypermethrin	475.33	447.00	361.00	437.78a
Border crop Means	289.67	293.33	279.91	
Season two				
Water	313.00c**	349.00c**	338.67c**	333.56c**
Biomagic	406.33bc	433.33bc	351.33c	451.00b
Metarril	444.33bc	557.33b	448.00bc	429.33bc
Alpha-cypermethrin	602.33a	648.33a	515.67b	558.78b
Border crop Means	447.91bc	497.08b	407.00bc	

*Means not followed by a letter within a column and a season are not significantly different according to Tukey's HSD test ($P \leq 0.05$). **Means followed by the same letter within a column and a season are not significantly different according to Tukey's HSD test ($P \leq 0.05$). Data were subjected to square root transformation before analysis, but the values presented are original means.

(d) Average pod weight

Averaged over the growing season, the main effect of French bean plants with *Metarhizium anisopliae* resulted in a significant difference in the average pod weight of French bean amongst the different treatment combinations in both seasons (Table 5.8). During both seasons, significant differences in average pod weight were observed amongst all plots in season one with plants in plots sprayed with alpha-cypermethrin recording the highest average pod weight (2.87g), followed by plants in plots sprayed with Biomagic (2.30g) and Metarril E9 (2.08g) compared to plants in plots sprayed with water (1.82g). The average pod weights for plants in plots sprayed with Biomagic and Metarril E9 were not statistically different in both seasons, although the highest average pod weights were recorded for plants in plots sprayed with Biomagic compared to the average pod weight of plants in plots sprayed with Metarril E9 in both seasons. During both seasons, French bean plants grown in plots with different types of border crops did not differ statistically on the average pod weight (Table 5.8). Furthermore, plants in plots with a wheat border crop recorded the highest average pod weight, followed by plants in plots with a no border crop compared to plants in plots with a sunflower border crop that recorded the lowest average pod weight. The highest average pod weights were recorded for plants in plots sprayed with alpha-cypermethrin and had a wheat border crop. The lowest average pod weights were recorded in plots sprayed with water and had a no border crop for both seasons (Table 5.8). Amongst biopesticide treatments, plants in plots sprayed with Biomagic and had a wheat border crop recorded the highest average pod weight per plant (3.92g). The lowest average pod weight (1.81g) was recorded for plants in plots sprayed with Metarril E9 and had a no border crop in season two. During season one, plants in plots sprayed with Biomagic and had a wheat border crop recorded the highest average pod weight per plant (2.63g) while the lowest average pod weight (2.06g) was recorded for plants in plots sprayed with Biomagic and had a no border crop or a sunflower border.

Table 5.8: Effects of *Metarhizium anisopliae* and border crop on average pod weight yield (g) in both season one (April to July 2022) and season two (September to December 2022).

Treatment	Border crop			Treatment Means
	No border	Wheat	Sunflower	
Season one				
Water	1.40*	2.00*	2.06*	1.82b**
Biomagic	2.06	2.63	2.06	2.30b
Metarril	2.10	2.13	2.10	2.08b
Alpha-cypermethrin	2.86	3.13	2.73	2.87a
Border crop means	2.25	2.34	2.25	
Season two				
Water	1.74*	2.40*	1.89*	2.15*
Biomagic	2.64	3.92	3.68	3.10
Metarril	1.81	2.97	2.31	2.56
Alpha-cypermethrin	3.35	3.99	2.99	3.42
Border crop means	2.60	3.30	2.53	

*Means not followed by a letter within a column and within a season are not significantly different according to Tukey's HSD test ($P \leq 0.05$). **Means followed by the same letter within a column and within a season are not significantly different according to Tukey's HSD test ($P \leq 0.05$). Data were subjected to square root transformation before analysis, but the values presented are original means.

(e) Total pod weight.

Averaged over the growing season, the main effect of French bean plants with *Metarhizium anisopliae* resulted in significant difference in the total pod weight of French beans amongst the different treatment combinations in both seasons (Table 5.9). In season one, significant differences in total pod yield were observed amongst all plots that received the different treatments with plants in plots sprayed with alpha-cypermethrin recording the highest total pod weight (11.82 t/ha), followed by plants in plots sprayed with Metarril E9 (6.20 t/ha), Biomagic (5.26 t/ha) compared

to the total pod yield of plants in plots sprayed with water (4.63 t/ha). Generally, the highest total pod weight was recorded for plants sprayed with alpha-cypermethrin, and the lowest pod weight was recorded for plants in plots sprayed with water during both seasons. Plants from plots sprayed with Metarril E9 (5.26 t/ha) and Biomagic (6.20 t/ha) were not statistically different in the total pod weight in season one. A similar trend was observed in season two, although among the biopesticides, the difference in total pod weight of plants recorded was not statistically different. French bean plants grown in plots under different border crop (wheat or sunflower) treatments had significant differences in total pod weight compared to plants grown in plots that had no border crop for season two only (Table 5.9).

Significant differences in total pod weights were observed for plants grown in plots with a sunflower border crop compared to the total pod weight of plants grown in plots with a wheat border crop or with no border crop in season two. During both seasons, the highest total pod weight was obtained in plots with a wheat border crop while the lowest was obtained in plots that had no border crop. In season two, plots with a wheat border crop had the highest total pod weight (14.95 t/ha), followed by the total pod weight (13.43 t/ha) of plants in plots with no border compared to plots with a sunflower border crop which recorded the lowest total pod weight (11.49 t/ha). Over the two seasons, the highest total pod weight (18.43 t/ha) was recorded under plots sprayed with alpha-cypermethrin and had a wheat border crop while the lowest total weight (2.93 t/ha) of French bean plants in plots sprayed with water and had a no border crop treatment combination. Among the biopesticides, French bean plants in plots sprayed with Metarril and had a wheat border crop recorded the highest total weight (17.48 t/ha) while the lowest total weight (12.69 t/ha) of French bean plants was recorded in plots sprayed with Biomagic and had no border crop in season two and the similar trend was observed in season one (Table 5.9).

Table 5.9: Effects of *Metarhizium anisopliae* and border crop on total pod weight (tonnes/ha) in both season one (April to July 2022) and season two (September to December 2022).

Treatment	Border crop			Spray means
	No border	Wheat	Sunflower	
Season one				
Water	2.93c*	7.86abc*	3.10c*	4.63b*
Biomagic	3.33bc	7.43abc	5.00abc	5.26b

Metarril	3.87bc	7.03abc	7.70abc	6.20b
Alpha-cypermethrin	12.70ab	13.63a	9.13abc	11.82a
Border crop means	6.94a	7.35a	6.63a	
Season two				
Water	8.17b	10.26ab	11.32ab	9.92b
Biomagic	12.69ab	13.60ab	10.92ab	12.07b
Metarril	10.72ab	17.48ab	11.89ab	13.70ab
Alpha-cypermethrin	18.43a	18.96a	14.97ab	17.45a
Border crop means	13.43ab	14.95a	11.49b	

*Means followed by the same letter within a column are not significantly different according to Tukey's Honestly Significant Difference at ($p \leq 0.05$). Data were subjected to square root transformation before analysis values presented are original means.

5.4.3 Effect *Metarhizium anisopliae* and border cropping quality of French bean.

The use of *Metarhizium anisopliae* significantly influenced pod grade and marketable yield except for non-marketable French bean pods in both seasons. On the other hand, the use of border crops did not significantly influence pod grade and marketable yield except for non-marketable French bean in both seasons. Comparing the interaction effect between border crops and *Metarhizium anisopliae* sprayed significantly affected pod grade in both seasons except for the marketable yield of French bean, which differed significantly only in season one.

(a) Pod grade

Pod grades, (extra-fine, fine and bobby grades), were used in this study. The study results showed that growing French bean plants under different spray treatments and border crop types had some effects on the pod maturation rate, as judged by the pod shape, diameter, and length under the different pod grades in both seasons (Figure 5.1). In both seasons, more extra fine than fine grade pods were obtained for plants under plots sprayed with the different treatments and had a wheat or sunflower border crop compared to plants in plots sprayed with the different treatments and had no border crop in the total yield of the different grades. Among different treatment combinations, the highest amount of extra-fine grade pod was obtained under the alpha-cypermethrin and had a wheat border crop while plants in plots sprayed with water and had no border crop registered the lowest quantity of extra-fine grade pods in both seasons of the total

harvest of pod grade. A similar trend was recorded for fine grade yield in both seasons. On the other hand, more bobby grade pods were obtained under plots sprayed with water and had no border crop in both seasons. Among the biopesticides, more extra fine than fine grade pods were obtained for plants under plots sprayed with Metarril and had a wheat or sunflower border crop, followed by plants in plots sprayed with Biomagic and had wheat or sunflower border crop.

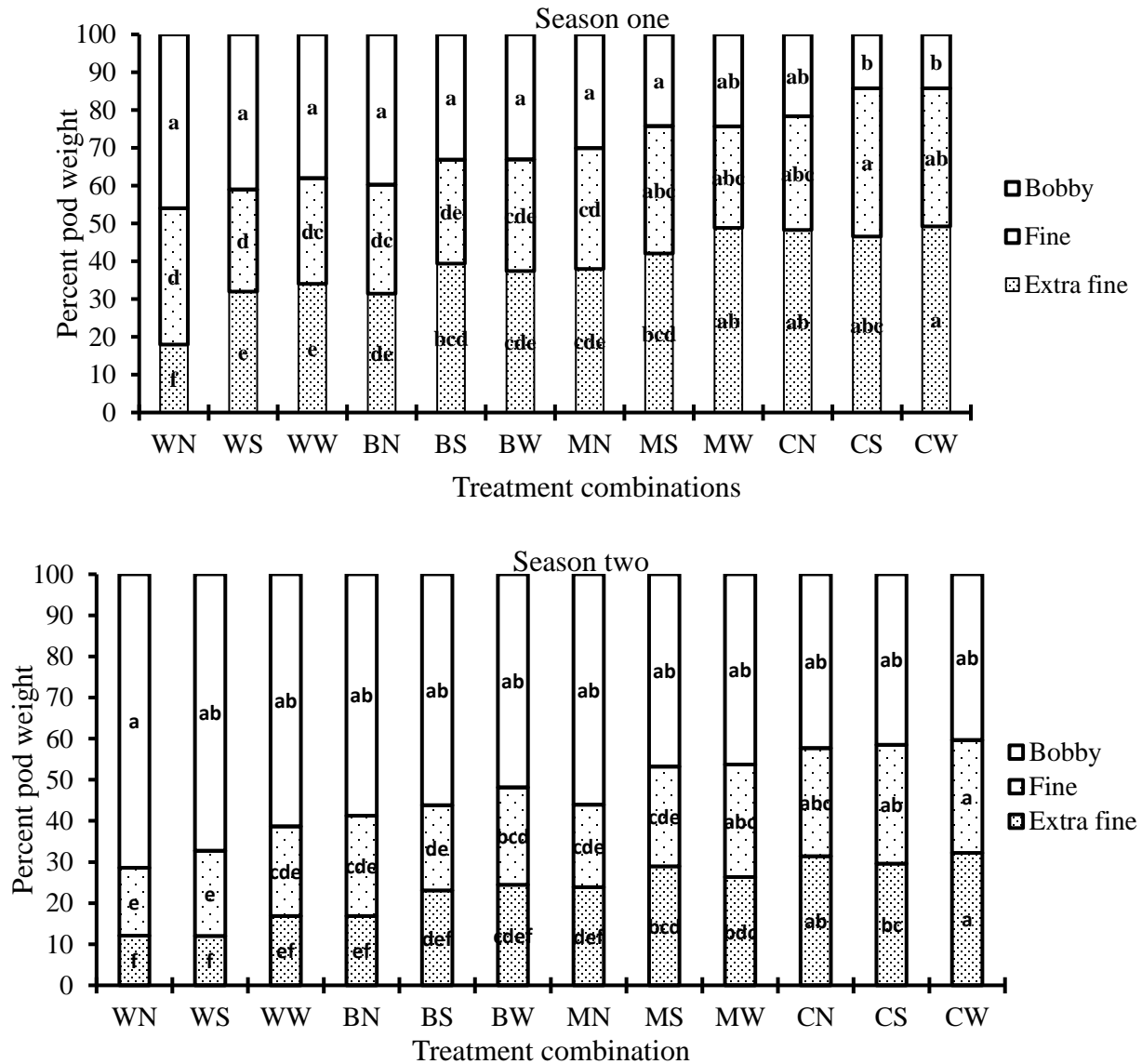


Figure 5.2: Percent mean pod weight per plant and quality grades.

Key: WN (water and no border), BN (Biomagic and no border), MN (Metarril E9 and no border), AN (alpha-cypermethrin and no border), WW (water and wheat), BW (Biomagic and wheat), MW (Metarril and wheat), AW (alpha-cypermethrin and wheat), WS (water and sunflower), BS (Biomagic and sunflower), MS (Metarril and Sunflower), AS (alpha-cypermethrin and sunflower).

Growing French bean plants under the different treatments sprayed and border crop type covers improved the pod quality (extra fine, fine and bobby grades) compared to no border crop in both seasons (Table 5.10). During both seasons, French bean plants grown under plots sprayed with alpha-cypermethrin recorded the highest marketable pod weights, followed by plants in plots sprayed with Metarril, Biomagic compared to the marketable pod weight of plants in plots sprayed with water. Similarly, French bean plants grown under plots with a wheat border also produced higher marketable pod weights, followed by plants in plots with a sunflower border compared to plants grown in plots with no border. Among *Metarhizium anisopliae* treated plots in both seasons, more marketable pod yield was obtained from plants sprayed with Metarril than plants sprayed with Biomagic. Furthermore, marketable French bean yield recorded a substantial increase for plants in plots with a wheat border (7.9% increase) or a sunflower border (7.1% increase) compared to plants in plots with no border. A similar trend was observed in season one; however, there was no statistical difference, although plants in plots with a wheat border recorded slightly higher marketable yield, followed by plants with a sunflower border compared to marketable yield for plants that had no border crop. The study found that French bean plants grown under alpha-cypermethrin and a wheat border had the highest marketable yield, while those sprayed with water and no border crop had the lowest yield. On the other hand, plants in plots sprayed with alpha-cypermethrin recorded the lowest percentage of non-marketable yield, followed by plants in plots sprayed with Metarril E9, Biomagic while the highest marketable yield percentage was recorded for plants in plots sprayed with water (Table 5.10). French bean plants in plots sprayed with Metarril E9 and Biomagic, Biomagic and water treatment combinations were statistically different in both seasons. However, French bean plants in plots sprayed with water recorded the highest non-marketable yield. Different border crop types had no statistical difference, although French bean plants in plots with no border crop recorded slightly higher non-marketable yield percent compared to yield from plants in plots with either a sunflower or wheat border (Table 5.10). During the two seasons, the highest percentage of non-marketable yield was recorded for French bean plant plots sprayed with water and no border crop. In both seasons, the lowest non-marketable yield percentage was recorded for French bean plants grown under plots sprayed with alpha-cypermethrin with a wheat border crop.

Table 5.9: percent (%) marketable and non-marketable yield in both season one (April to July 2022) and season two (September to December 2022).

Treatment (Trt)	Non-marketable yield			Border crop			Marketable yield		
	No border	Wheat	Sunflower	Spray	No border	Wheat	Sunflower	Spray	
				Means				Means	
Season one									
Water	59.31a**	41.97ab**	37.47abcd**	46.25a**	67.41*	71.42*	68.19*	69.01c**	
Biomagic	40.31abc	27.88abcd	26.20abcd	31.46ab	79.97	71.21	69.83	73.67bc	
Metarril	30.65abcd	26.92abcd	25.12bcd	27.56b	76.99	77.62	81.32	78.64ab	
Cypermethrin	16.55bcd	12.74d	14.49dc	14.59c	85.55	86.42	82.41	84.79a	
Border means	35.77a	25.81a	28.31a		75.44	77.48	76.67		
Season two									
Water	32.58a*	28.58ab*	31.80a*	30.99a	40.68d**	58.02c**	62.53bc**	53.74c**	
Biomagic	20.02bc	28.79ab	30.17a	26.33ab	59.69bc	69.35bc	73.79b	68.53bc	
Metarril	23.01ab	22.38bc	18.67bc	21.35bc	73.08b	72.12b	74.88abc	72.43b	
Cypermethrin	18.67bc	14.59c	17.59bc	15.21c	83.45a	87.25a	85.51a	85.40a	
Border means	24.55a	22.52a	23.33a		70.32b	78.19a	77.43a		

*Means not followed by a letter within a column within a season are not significantly different according to Tukey's Honestly Significant Difference at ($p \leq 0.05$). **Percent means followed by the same letter within a column within a season are not significantly different according to Tukey's Honestly Significant Difference at ($p \leq 0.05$). Data were subjected to square root transformation before analysis, but values presented are original means.

5.5 Discussion

This study showed that growing French bean plants with border crops and application of biopesticides significantly influenced growth, yield and quality variables. Plots of French beans bordered with wheat or sunflower had significantly higher plant height, collar diameter, number of branches, number of pods, export grade pod yield, quality and marketable pod yield compared to the control plots with no border crop. The result of this study could be associated with findings reported by Nelson *et al.* (2021), where use of border cropping enhanced plant growth (plant height, collar diameter and branching) and was also able to increase yield (number of pods and marketable pod yield) by 11.4% and 34.2% for Faba bean intercropped with wheat compared to sole cropping. Other authors (Wang *et al.*, 2017; Wei *et al.*, 2022; Xu *et al.*, 2019; Youn *et al.*, 2021) also reported enhanced growth and productivity on the primary crop as a result of intercropping approach, findings that partly support for the high yield and enhanced growth obtained in the current study for French bean grown with a border crop. The current study results showed that French bean plants sprayed with biopesticides and with a sunflower border crop recorded taller plants, thinner collar diameter, fewer branches and low pod yield compared to French bean plants grown with either wheat as a border crop or no border crop. The use of taller border crops as reported by Wei *et al.* (2022), causes shading effects that lead to rapid stem elongation, thereby increasing the plant height of the primary crop. Similar observations were made in the current study, where taller French bean plants were obtained in plots with a sunflower border as compared to those with either a wheat border or no border crop. Similar results were reported by Kabir *et al.* (2020), where shading resulted in taller plants with thinner leaves, thus decreasing photosynthetically active radiation (PAR), which is a spectral range of sunlight that plants use to undergo photosynthesis ($\mu\text{mol m}^{-2} \text{s}^{-1}$), resulting in low bell pepper (*Capsicum annuum*) yield. This is because thinner leaves have less ability to capture and utilize PAR for photosynthesis narrower capacity for storing water compared to thicker leaves.

Although companion crops, such as intercrop or border crops, can potentially improve net yields, they can also negatively influence plant growth, thus causing yield reduction, specifically when taller border crops are used. These effects are associated with a reduction in the red and far-red light ratio due to the shading effect as a result of shade avoidance syndrome activation, thus promoting internode elongation, reduced branching and decreased yield per plant as reported by Dhale *et al.* (2022) and Green-Tracewicz *et al.* (2011). Similar research by Kalaitzoglou *et al.*

(2019) and Demotes-Mainard *et al.* (2016) reported that low-red to far-red light was associated with shade effects due to whole plant morphology promoting stem length, internodes, petiole, leaf length, reduced chlorophyll content, apical dominance, and reduced basal branching with increased assimilate partitioning toward the stem. Border crops with taller plant morphology can also increase the competition for resources required by the plant, light interception, and cause shade effect responsible for the etiolation process in plants due to variations in hormonal activities (Demotes-Mainard *et al.*, 2016). Shade avoidance syndrome causes the plants to adapt to shade or enhance apical growth. Low branching is primarily a shade effect response (Rameau *et al.*, 2015). Also, excessive shade increases plant height and lodging rate, affecting leaf orientation, hindering the transportation of nutrients, water, and photosynthetic products and ultimately causing considerable losses in yield through interspecific competition (Feng *et al.*, 2019; Gao *et al.*, 2014). The current study showed the combined use of a wheat border and spraying the French bean plants with *Metarhizium anisopliae* positively affected growth and yield of French bean plants and this could be attributed to diverse effects that lead to either additive, synergistic, or antagonistic responses (Huss *et al.*, 2022) such as increased relative humidity and minimized ultra-violet light penetration into the lower canopy crop that can outcompete or enhance pathogenic fungi (Mweke *et al.*, 2020). In French bean plants grown with a companion border crop could have enhanced the efficacy and performance of the biopesticide used in the current study. Raai *et al.* (2020) demonstrated that shading regimes (heavy (60%), moderate (30%) and control (0% shade)), negatively interferes with morphological features (plant height, reduced branching ability and thinner stems), photosynthetic, gas exchange and growth characteristics of the plant of wing beans. Similar to these observations, the current study recorded higher branching ability on French bean plants in plots with a wheat border crop or no border crop compared to those grown with a sunflower border, which is attributable to the higher shading effect observed in plots grown with sunflower border than with a wheat border crop. A study by Wang *et al.* (2017) reported that wheat or maize used as a border crop positively influences the plant plasticity and structure of the main crop. Higher branching ability recorded in French beans grown with a wheat border or no border crop in the current study could be attributed to the optimal light intensity absorbed by French bean in these treatments compared to the amount absorbed by French bean plants grown with a sunflower border crop with a higher shade effect (Babec *et al.*, 2020). The current study's findings

support results from previous studies in *Arabidopsis* where shade effects promoted low-red or far-red light, which reduced branching ability of the plant (Rameau *et al.*, 2015; Youn *et al.*, 2021).

Based on this study, sunflower as a border crop caused a reduction in yield compared to plots with wheat as a border which was not significantly different from the control in terms of yields. According to Damicone *et al.* (2017), a yield reduction of up to 50% was recorded in soybean with sorghum as a border crop which compromised on light inception lowered the photosynthetic rate while primarily prioritizing the partition and distribution of assimilates for vegetative growth over reproductive activities. Also, the current study results showed that treatment combination with wheat as a border crop increased marketable yield by 2-8%, export grade yield by 2-5% and lowered non-marketable yield by 2-10% in both seasons compared to control (no border). In addition, also research by Kabaale *et al.* (2022) recorded higher marketable yield gain exceeding 22% and a cost-benefit ratio greater than 2.8 (BCR~3) when plots were sprayed with biopesticide products containing *Metarhizium anisopliae*. Based on the current study, farmers who want to grow French bean crop as sole and use environmentally safer management practices could integrate biopesticide and wheat border crop as the best and most productive system. A study by Mollaei *et al.* (2021) on intercropping of canola and faba beans, field peas, or garlic reported improved canola crop yield due to reduced insect pest density. The latter results were attributed to better resource use efficiency, such as physical barrier, increased biodiversity (Mohd *et al.*, 2022), repelling pests and attracting natural enemies (Lian *et al.*, 2019), and impeding weed growth (Elsalahy *et al.*, 2019). Nawar *et al.* (2020) attributed the increase in yield quality of soybean and sunflower intercrop with less shade effect to reduced canopy layers, increased light interception efficiency and reduced interplant competition between sunflower and soybean. Based on this study, plots sprayed with Metarril and had a wheat border crop recorded more extra fine and fine grade pods. Similar to our results, cypermethrin sprayed plots and wheat border recorded an increase in yield of French beans; however, continuous and indiscriminate use of alpha-cypermethrin as non-selective could cause to non-target organisms and with a great possibility of resistance development of insect pest (Fenibo *et al.*, 2021; Srinivasan *et al.*, 2019; Zekeya *et al.*, 2022). As a result, use of biopesticide products containing *Metarhizium anisopliae* compared to synthetic insecticides offers a comparative added advantage such as less residues on produce since synthetic products have been documented as toxic to insect pests, humans and the environment (Sammama *et al.*, 2021; Zhu *et al.*, 2015). Putnoky-Csicsó *et al.* (2020) compared alpha-

cypermethrin to *Metarhizium. anisopliae* and their results showed a significantly lower number of survived *Melolontha melolontha* larvae for α -cypermethrin treatment than *Metarhizium anisopliae* treatment and control. Research by Zekeya *et al.* (2022) to evaluate the use of biopesticide *Metarhizium anisopliae*, pheromone traps and chemical pesticides to manage *Phthorimaea absoluta* in field conditions reported a significant increase in marketable yield of Faba bean. *Metarhizium anisopliae* could have benefited from favorable moisture conditions facilitating its growth in the rhizosphere and uptake by plant roots, thus increasing the plant performance, growth and productivity in support the recorded increase in yield. The quality of French bean plant pods in the current study was also enhanced by the combined use of border crops and biopesticides containing *Metarhizium anisopliae* compared to the control in the open field. These results could provide support to studies (Kumar *et al.*, 2021; Munywoki *et al.*, 2019; Wan *et al.*, 2018) that reported enhanced quality through integrated crop management through a significant reduction in insect pest population. Besides the general advantages associated with use of border cropping and biopesticides approach rely on combined ability to conserve or attract of natural enemies, obstruct, trap, repel or suppress insect pest populations and in turn enhance the quality of the crop produce for the growers (Afrin *et al.*, 2017; Daryanto *et al.*, 2020; Huss *et al.*, 2022; Xu *et al.*, 2020).

5.6 Conclusion

Based on the findings of this study,

- i. Use of border crops and *Metarhizium anisopliae* influences growth, yield and quality of French bean with plots sprayed with Metarril and a wheat border crop recording the highest collar diameter, total pod weight and weight per hectare of extra-fine and fine grade yield

CHAPTER SIX

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

6.1 General discussion

In sub-Saharan Africa, farming is mostly by conventional agriculture laden with high-input, synthetic chemical fertilizers, fungicides, insecticides, and herbicides (Sheahan *et al.*, 2017; Sheahan & Barrett, 2017). Most farmers therefore, prefer synthetic insecticides because of their significant role in managing major insect pests with less consideration of their impact on beneficial arthropods, such as parasitoids and predators (Ochieng *et al.*, 2022). However, indiscriminate use of synthetic chemicals has irreversible consequences on the environment and people (Alarcón-Segura *et al.*, 2022; Sánchez-Bayo, 2021). Alternative pest management approaches that employ Integrated pest management (IPM) programs have been considered safer as they involve using different control methods to manage insect pest populations and reduce the impact on beneficial arthropods, farmers and the environment (Baker *et al.*, 2020). Adopting such approaches, for example the use of border cropping, offers an optimal ecosystem services with diverse beneficial organisms (predator and parasitoids), therefore promoting synergies and compatibilities to natural enemies (Amala & Shivalingaswamy, 2018; Daryanto *et al.*, 2020). Conservation biological control strategies are also considered the pillar of IPM; thus, to enhance the effectiveness and success of the integrated conversation pest control strategy, repeated and indiscriminate use of synthetic pesticides must be minimised and reduced. Studies have reported that the entomopathogenic fungus *Metarhizium ansiopliae* has the ability to produce a wide range of secondary metabolites that are toxic to insect pests, such as destruxins, which disrupt insect cell membranes and cause cell death to the target pest (Sbaraini *et al.*, 2016). In addition, natural enemies' populations have shown more sensitivity to synthetic insecticides, thus making conservation biological control impractical with the integration of prohibited synthetic insecticides (Ndakidemi *et al.*, 2016; Sánchez-Bayo, 2021).

In this study, although Metarril E9 and Mazao supreme did not differ statistically, at the same concentration, Metarril caused a slightly higher percentage mortality of a 3.5% of black bean aphids under in vitro conditions. Some research has associated the different bioactivity of *Metarhizium anisopliae* with the ability of some isolates to be highly specific, cause significant reduction in the egg-laying capacity of female insects, cause secondary infection to offspring and

others infect a wide host range as reported by Domingues *et al.* (2020). Therefore, this latter finding by Domingues *et al.* (2020) could possibly offers some degree of support for the success of Metarril compared to Mazao Supreme in the current study. Similarly, the Latiff *et al.* (2022), highlighted that the difference in pathogenicity and virulence of five *Metarhizium anisopliae* against *Aphis gossypii* reared on *Capsicum annum* (chilli) and *Solanum melongena* (brinjal) were also attributed to isolate initial concentration the insect received, fungal strain and ability of the isolate to produce an immense extent of toxins or focus more energy on vegetative growth than the target pest. In addition, a study report by Erol *et al.* (2020) highlighted the difference in fungal pathogenicity associated with secondary bioactive metabolites, including Destruxins produced by the pathogen that could be responsible for the difference in the mortality against aphids.

The current study recorded a significant reduction in the aphid population and the damage severity caused on French bean plants when wheat or sunflower were used as border crop compared to where no border crop was used. These findings are similar to those of a study by Khanal *et al.* (2023), which reported a reduction in the number of Mustard aphids (*Lipaphis erysimi*) in plots sprayed with *Metarhizium anisopliae* treatments. The latter treatment was found to be efficient in reducing the subsequent damage severity due to high aphid infestation. Other studies also confirm the mortality of wheat aphids (*Diuraphis noxia*) (Hafiz *et al.*, (2020), cowpeas aphids (*Aphis craccivora*) (Mweke *et al.*, 2018), and whitefly (*Bemisia tabaci*) (Singh & Kaur, 2020) as a result of *Metarhizium anisopliae* treatment. Such findings offer support to the findings of the current study. In addition, controlled and uncontrolled condition studies have demonstrated the potential pathogenicity of biopesticides containing *Metarhizium anisopliae* against aphids. Conversely, border crop diversity correlates to harboring more diverse generalist natural enemies (Tavares *et al.*, 2019) hence able to reduce the population of insect pest and their effects on the primary crop

The use of border crops significantly reduced aphid population and their damage severity and enhanced the growth, yield, and quality of French bean plants compared to the control plot in the current study. A wheat border performed better, particularly in the growth, yield and quality of French beans than sunflower. Use of wheat as a border crop accounted for 4.3-7.4% while sunflower resulted in to a 4.8%- 8.2% reduction in aphid population and 3.3%-4.9% reduction in damage severity in compared to the no border control treatment Waweru *et al.* (2021) reported the use of higher plants such as sorghum, maize, and wheat as border crops to reduce aphid

infestation in the primary crop. In addition, a similar study by Mansion-Vaquié *et al.* (2019) highlighted that aphids show clear preferences or are attracted to some wheat varieties with specific semio-chemicals released as well as some predators, specifically ladybird beetle. The results of the interactive effects, either additive or syngenetic, could be attributed partly to the high population of aphids recorded at the border crop of the current study. Emery *et al.* (2021) and Mweka *et al.*'s (2020) reported a reduction in insect pest population with intercropping and biopesticide treatment combination. Non-host pest plants used as a border crop (wheat and sunflower) in the current study lowered aphid population and damage effects on French bean plants probably because of their ability to obstruct, act as host, or repellent of insect pests lure or repel natural enemies and pest.

The combined use of *Metarhizium anisopliae* and border crops leads to either additive, synergistic, or antagonistic relationships. A study by Behie and Bidochka (2014) reported a symbiotic relationship by which plants and *Beauveria bassiana* but not *Lecanicillium lecanii* showed the ability to reacquire lost nitrogen through the transfer of nitrogen to plants, an important role in the ecological cycling of nutrients that lead to increased overall plant productivity. On the other hand, some species of border crops when used, is reported to offer antagonistic or synergistic effect for better resource use efficiency, such as physical barrier, increased biodiversity (Mohd *et al.*, 2022), repelling pests and attracting natural enemies (Lian *et al.*, 2019), and impeding weed growth (Elsalahy *et al.*, 2019). Mutual relation associated with improved acquisition of nutrients, accelerated growth, resilience against pathogens, activation of signals that trigger immune system response and improved resistance against abiotic stress have been reported in plant and pathogens relation (Rodriguez *et al.*, 2019) which could support the better performance of realized with combined use of *Metarhizium anisopliae* and border cropping compared to the control treatment.

Although intercrops or border crops have the potential to improving net yields, they can also negatively influence plant growth, thus resulting in yield reduction, specifically when taller border crops are used. Research showed that using taller plants as border crops, such as sunflowers, as used in the current study, could lead to slightly higher shade effects than where shorter border crops such as wheat or no border crop is used, thus affecting final productivity. Similar results have been reported by Kabir *et al.* (2020), where shading effects resulted in taller plants with larger but thinner leaves thus decreased photosynthetically active radiation (PAR, $\mu\text{mol m}^2\text{s}^{-1}$) which is a spectral range of sunlight that plants use to undergo photosynthesis, resulting in low bell pepper (*Capsicum annuum*) yield. Reduction in the red and far-red light ratio as a result of shading effect

activates shade avoidance syndrome situation in plants, thus promoting internode elongation, reduced branching and decreased yield per plant as reported by Dhale *et al.* (2022) and Green-Tracewicz *et al.* (2011). Similarly research by Kalaitzoglou *et al.* (2019) and Demotes-Mainard *et al.* (2016) highlighted that low-red to far-red light due to shading effects caused by the whole border crop structure, mainly taller and shady plant promotes stem length elongation, internodes length, petiole, and leaf length, reduced chlorophyll content, apical dominance, and reduced basal branching with increased assimilate partitioning toward the stem. Use of taller border crop with plant structure that causes about 50% shading effects or more interferes with light intensity and interception and leads to etiolation of the shaded crop which could also offer an explanation for better growth, yield, and quality.

For the current study, combined use of border crops and biopesticides in the open field led to significant increase in marketable yield, quality and lowered aphid population on French bean compared to the control treatment where there was no border crop. A study by Mansion-Vaquié *et al.* (2020) attributed such effects of non-host plants to the reduced ability of insect pests to locate the host and thus deter colonisation process of the host plants by the pest through chemical and physical interference, reducing damage impact. The presence of a border crop could also increase predator abundance, thus decreasing the abundance of pests and over dependence on insecticides resulting in increased grain yield and economic profits (Wan *et al.*, 2018). In addition, border crop use in IPM approach has enhanced the performance of entomopathogenic fungi containing *Metarhizium anisopliae* and compatibility with natural enemies such as predators and parasitoids (Li *et al.*, 2021).

6.2 Conclusions

Based on the current study,

- i. *Metarhizium anisopliae*, entomopathogenic fungi, caused mortality to black bean aphid in the laboratory.
- ii. Under open field condition, combined use of *Metarhizium anisopliae* and border cropping significantly reduced aphid population and damage severity.
- iii. The integrated use of *Metarhizium anisopliae* spray and border cropping influenced French beans' growth, yield and quality.

6.3 Recommendations

Based on the results of this study, the following recommendations were made.

- i. The bioactivity of *Metarhizium anisopliae* based on the current study resulted in mortality of black bean aphids particularly Metarril E9 at a concentration of 2×10^8 spores/g, should be recommended and included in the integrated management of black bean aphids in French bean production.
- ii. *Metarhizium anisopliae*, an entomopathogenic fungus and border cropping, could be used to manage and reduce aphid population and damage on field grown French beans.
- iii. The study recommends using wheat as a border crop integrated with *Metarhizium anisopliae* for the field grown French beans to enhance growth, yield and quality without compromising pod quality in regions with relatively similar conditions as the study area. The approach is considered safer products with less or no environmental harm compared to synthetic chemicals; thus, this current study recommends their incorporation in French bean production.

6.4 Areas for further studies

The following areas for further research are highlighted in the current study:

- i. Isolation of new *M. anisopliae* native to Kenya as they would be more adapted to natural conditions and evaluation of their efficacy against black bean aphid to identify more pathogenic isolate.
- ii. There is need for multi-location research to evaluate different entomopathogenic fungi isolate other than the one considered in this study against black bean aphids.
- iii. Further research on the long-term sustainability and cost-effectiveness of *Metarhizium anisopliae* and different border crop types could be done in the open field for its efficacy against black bean aphids

REFERENCES

- Abbott, W. S. (1925). A Method of Computing the Effectiveness of an Insecticide. *Journal of Economic Entomology*, 18(2), 265–267. <https://doi.org/10.1093/jee/18.2.265a>
- Abdollahdokht, D., Gao, Y., Faramarz, S., Poustforoosh, A., Abbasi, M., Asadikaram, G., & Nematollahi, M. H. (2022). Conventional agrochemicals towards nano-biopesticides: An overview on recent advances. *Chemical and Biological Technologies in Agriculture*, 9(1), 13. <https://doi.org/10.1186/s40538-021-00281-0>
- Afrin, S., Latif, A., Banu, N. M. A., Kabir, M. M. M., Haque, S. S., Ahmed, M. M. E., Tonu, N. N., & Ali, M. P. (2017). Intercropping Empower Reduces Insect Pests and Increases Biodiversity in Agro-Ecosystem. *Agricultural Sciences*, 8(10), Article 10. <https://doi.org/10.4236/as.2017.810082>
- Akutse, K., Subramanian, S., Maniania, N., Dubois, T., & 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, 1–14. <https://doi.org/10.3389/fsufs.2020.563016>
- Alarcón-Segura, V., Grass, I., Breustedt, G., Rohlf, M., & Tscharrntke, T. (2022). Strip intercropping of wheat and oilseed rape enhances biodiversity and biological pest control in a conventionally managed farm scenario. *Journal of Applied Ecology*, 59(6), 1513–1523. <https://doi.org/10.1111/1365-2664.14161>
- Alfaro-Tapia, A., Alvarez-Baca, J. K., Fuentes-Contreras, E., & Figueroa, C. C. (2021). Biological Control May Fail on Pests Applied with High Doses of Insecticides: Effects of Sub-Lethal Concentrations of a Pyrethroid on the Host-Searching Behavior of the Aphid Parasitoid *Aphidius colemani* (Hymenoptera, Braconidae) on Aphid Pests. *Agriculture*, 11(539), 1–14. <https://doi.org/10.3390/agriculture11060539>
- Aljassani, A., Al-Hussainawy, K., Jassim, Z., & Abdulaali, R. (2020). Efficacy of some insecticides and plant extract against black bean aphid, *Aphid fabae* and Green aphid, *Myzus persicae* (Aphididae:Hemiptera). *Indian Journal of Ecology*, 47(12), 284–286.
- Amala, U., & Shivalingaswamy, T. M. (2018). Effect of intercrops and border crops on the diversity of parasitoids and predators in agroecosystem. *Egyptian Journal of Biological Pest Control*, 28(11), 1–4. <https://doi.org/10.1186/s41938-017-0015-y>

- Ara, Z. G., & Haque, A. R. (2021). A Comprehensive Review on Synthetic Insecticides: Toxicity to Pollinators, Associated Risk to Food Security, and Management Approaches. *Journal of Biosystems Engineering*, 46(3), 254–272. <https://doi.org/10.1007/s42853-021-00104-y>
- Arunga, E. E., Miriam, K., Julius, O., James, O., & Emy, C. (2015). Genetic diversity of determinate French beans grown in Kenya based on morpho-agronomic and simple sequence repeat variation. *Journal of Plant Breeding and Crop Science*, 7(8), 240–250. <https://doi.org/10.5897/JPBCS2015.0503>
- Arunga, E. E., & Oriama, S. O. (2020). Characterization of Kenyan French Bean genotypes into gene pool affiliations using allele specific markers. *African Journal of Biotechnology*, 19(9), 653–660. <https://doi.org/10.5897/AJB2020.17149>
- Ayilara, M. S., Adeleke, B. S., Akinola, S. A., Fayose, C. A., Adeyemi, U. T., Gbadegesin, L. A., Omole, R. K., Johnson, R. M., Uthman, Q. O., & Babalola, O. O. (2023). Biopesticides as a promising alternative to synthetic pesticides: A case for microbial pesticides, phytopesticides, and nanobiopesticides. *Frontiers in Microbiology*, 14(1), 1040–0901. <https://doi.org/10.3389/fmicb.2023.1040901>
- Babec, B., Šeremešić, S., Hladni, N., Terzić, S., Vojnov, B., Čuk, N., & Gvozdenac, S. (2020). Effect of intercropping sunflower with legumes on some sunflower morphological traits. *Ratarstvo i Povrtarstvo*, 57(2), 1–61.
- Baker, B. P., Green, T. A., & Loker, A. J. (2020). Biological control and integrated pest management in organic and conventional systems. *Biological Control*, 140, 1–19. <https://doi.org/10.1016/j.biocontrol.2019.104095>
- Bamisile, B. S., Akutse, K. S., Siddiqui, J. A., & Xu, Y. (2021). Model Application of Entomopathogenic Fungi as Alternatives to Chemical Pesticides: Prospects, Challenges, and Insights for Next-Generation Sustainable Agriculture. *Frontiers in Plant Science*, 12, 1–28. <https://doi.org/doi:10.3389/fpls.2021.741804>
- Bamisile, B. S., Siddiqui, J. A., Akutse, K. S., Ramos Aguila, L. C., & Xu, Y. (2021). General Limitations to Endophytic Entomopathogenic Fungi Use as Plant Growth Promoters, Pests and Pathogens Biocontrol Agents. *Plants*, 10(10), 2119. <https://doi.org/10.3390/plants10102119>

- Bansode, M. D., Vp, S., & Ka, C. (2019). Production constraints analysis of kharif French bean (*Phaseolus vulgaris* L.) in vertisols. *Journal of Pharmacognosy and Phytochemistry*, 8(4), 252–254.
- Barbercheck, E. M. (2014). *Biology and Management of Aphids in Organic Cucurbit Production Systems*. Penn State University. <https://eorganic.org/node/5304>
- Barros, E. M., da Silva-Torres, C. S. A., Torres, J. B., & Rolim, G. G. (2018). Short-term toxicity of insecticides residues to key predators and parasitoids for pest management in cotton. *Phytoparasitica*, 46(3), 391–404. <https://doi.org/10.1007/s12600-018-0672-8>
- Bass, C., Denholm, I., Williamson, M. S., & Nauen, R. (2015). The global status of insect resistance to neonicotinoid insecticides. *Pesticide Biochemistry and Physiology*, 121, 78–87. <https://doi.org/10.1016/j.pestbp.2015.04.004>
- Baur, R., Wijnands, F. G., Malavolta, C., Emilia-Romagna, R., & Agricoltura, D. (2019). *IOBC guidelines on Integrated Production and tools for the implementation on farm level* (pp. 1–1). IOBC.
- Bayissa, W., Ekesi, S., Mohamed, S. A., Kaaya, G. P., Wagacha, J. M., Hanna, R., & 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>
- Behie, S. W., & Bidochka, M. J. (2014). Ubiquity of Insect-Derived Nitrogen Transfer to Plants by Endophytic Insect-Pathogenic Fungi: An Additional Branch of the Soil Nitrogen Cycle. *Applied and Environmental Microbiology*, 80(5), 1553. <https://doi.org/10.1128/AEM.03338-13>
- Ben-Issa, R., Gomez, L., & Gautier, H. (2017a). Companion Plants for Aphid Pest Management. *Insects*, 8(4), 112. <https://doi.org/10.3390/insects8040112>
- Ben-Issa, R., Gomez, L., & Gautier, H. (2017b). Companion Plants for Aphid Pest Management. *Insects Science*, 8(4), 1–12. <https://doi.org/10.3390/insects8040112>
- Blackman, R., & Eastop, V. F. (2000). Aphids on The World's Trees; An Identification and Information Guide. *CAB International*, 1(2), 1–58.
- Boni, S. B., Mwashimaha, R. A., Mlowe, N., Sotelo-Cardona, P., & Nordey, T. (2020). Efficacy of indigenous entomopathogenic fungi against the black aphid, *Aphis fabae* Scopoli under

- controlled conditions in Tanzania. *International Journal of Tropical Insect Science*, *14*, 1643–1651. <https://doi.org/10.1007/s42690-020-00365-8>
- Castellanos, N. L., Ferreira-Filho, N. A., Rodrigues, H. S., Martínez, L. C., Serrão, J. E., & Oliveira, E. E. (2021). Imidacloprid-mediated alterations on the salivary glands of the Neotropical brown stink bug, *Euschistus heros*. *Ecotoxicology*, *30*(4), 678–688. <https://doi.org/10.1007/s10646-021-02388-4>
- Ceolin Bortolotto, O., de Oliveira Menezes Júnior, A., & Thibes Hoshino, A. (2015). Aphidophagous Parasitoids can Forage Wheat Crops Before Aphid Infestation, Parana State, Brazil. *Journal of Insect Science*, *15*(1). <https://doi.org/10.1093/jisesa/iev027>
- Chaurasia, S. (2020). Chapter 17—Green beans. In A. K. Jaiswal (Ed.), *Nutritional Composition and Antioxidant Properties of Fruits and Vegetables* (pp. 289–300). Academic Press. <https://doi.org/10.1016/B978-0-12-812780-3.00017-9>
- Chi, B., Zhang, D., & Dong, H. (2021). Control of cotton pests and diseases by intercropping: A review. *Journal of Integrative Agriculture*, *20*(12), 3089–3100. [https://doi.org/10.1016/S2095-3119\(20\)63318-4](https://doi.org/10.1016/S2095-3119(20)63318-4)
- Chopkar, P., Desai, V., Samrit, R., Uparkar, A., Choudhari, R., & Shelke, S. (2020). Effect of border crops on pest population in Lablab bean (*Lablab purpureus* L.). *Entomology and zoology studies*, *8*, 1407–1412.
- Ci, Z., Jiang, C., Tsukamoto, C., & Kojima, M. (2017). DPPH Radical Scavenging Activity and Polyphenols in the Pods of 3 Common Beans. *Journal of Food and Nutrition Research*, *5*(12), Article 12. <https://doi.org/10.12691/jfnr-5-12-4>
- Colmenarez, C. Y., Corniani, N., Mundstock Jahnke, S., Sampaio, M. V., & Vásquez, C. (2020). Use of Parasitoids as a Biocontrol Agent in the Neotropical Region: Challenges and Potential. In H. Kossi Baimey, N. Hamamouch, & Y. Adjiguita Kolombia (Eds.), *Horticultural Crops* (2nd ed., pp. 1–23). Intech Open. <https://doi.org/10.5772/intechopen.80720>
- Daryanto, S., Fu, B., Zhao, W., Wang, S., Jacinthe, P.-A., & Wang, L. (2020). Ecosystem service provision of grain legume and cereal intercropping in Africa. *Agricultural Systems*, *178*, 102761. <https://doi.org/10.1016/j.agsy.2019.102761>
- D'Ávila, V. A., Barbosa, W. F., Guedes, R. N. C., & Cutler, G. C. (2018). Effects of Spinosad, Imidacloprid, and Lambda-cyhalothrin on Survival, Parasitism, and Reproduction of the

- Aphid Parasitoid *Aphidius colemani*. *Journal of Economic Entomology*, *111*(3), 1096–1103. <https://doi.org/10.1093/jee/toy055>
- Demotes-Mainard, S., Péron, T., Corot, A., Bertheloot, J., Le Gourrierec, J., Pelleschi-Travier, S., Crespel, L., Morel, P., Huché-Thélier, L., Boumaza, R., Vian, A., Guérin, V., Leduc, N., & Sakr, S. (2016). Plant responses to red and far-red lights, applications in horticulture. *Environmental and Experimental Botany*, *121*, 4–21. <https://doi.org/10.1016/j.envexpbot.2015.05.010>
- Dhale, S., Bv, I., Head, A., Wasim, S., Parbhani, Maharashtra, I., Asewar, B., Chand, S., Mirza, I., & Narkhede, W. (2022). Effect of soybean: Pigeonpea strip cropping on growth parameters, yield attributes and yield under mechanization. *Pharma Innovation*, *11*, 684–687.
- Didinger, C., & Thompson, H. J. (2021). Defining Nutritional and Functional Niches of Legumes: A Call for Clarity to Distinguish a Future Role for Pulses in the Dietary Guidelines for Americans. *Nutrients*, *13*(4), Article 4. <https://doi.org/10.3390/nu13041100>
- Domingues, M. M., Becchi, L. K., Velozo, S. G. M., de Souza, A. R., Barbosa, L. R., Soares, M. A., Serrão, J. E., Zanuncio, J. C., & Wilcken, C. F. (2020). Selectivity of mycoinsecticides and a pyrethroid to the egg parasitoid *Cleruchoides noackae* (Hymenoptera: Mymaridae). *Scientific Reports*, *10*, 145–617. <https://doi.org/10.1038/s41598-020-71151-2>
- Emery, S. E., Anderson, P., Carlsson, G., Friberg, H., Larsson, M. C., Wallenhammar, A.-C., & Lundin, O. (2021). The Potential of Intercropping for Multifunctional Crop Protection in Oilseed Rape (*Brassica napus* L.). *Frontiers in Agronomy*, *3*, 1–8. <https://doi.org/doi:10.3389/fagro.2021.782686>
- Erdos, Z., Halswell, P., Matthews, A., & Raymond, B. (2020). Laboratory sprayer for testing of microbial biocontrol agents: Design and calibration. *University of Exeter, United Kingdom*, 1–8. <https://doi.org/10.1101/2020.04.22.054551>.
- Erol, Alime. B., Abdelaziz, O., Birgücü, A. K., Senoussi, M. M., Oufroukh, A., & Karaca, İ. (2020). Effects of some entomopathogenic fungi on the aphid species, *Aphis gossypii* Glover (Hemiptera: Aphididae). *Egyptian Journal of Biological Pest Control*, *30*(1), 108. <https://doi.org/10.1186/s41938-020-00311-3>
- FAO, F. and A. O. (2021). *Climate change fans spread of pests and threatens plants and crops* (pp. 1–38). Rome. <http://www.fao.org/news/story/en/item/1402920/icode/>

- FAOSTAT. (2021, September 15). *FAOSTAT Global snap bean production*.
<http://www.fao.org/faostat/en/#compare>
- Farhat, T., Ali, R., Rahman, N. F., Kabir, M. M. M., & Kochi, M. N. (2014). Effect of Border Crops on the Incidence of Insect Pests in Chickpea. *International Journal of Innovative Science, Engineering & Technology*, *1*(10), 453–456.
- Feng, L., Raza, M. A., Li, Z., Chen, Y., Khalid, M. H. B., Du, J., Liu, W., Wu, X., Song, C., Yu, L., Zhang, Z., Yuan, S., Yang, W., & Yang, F. (2019). The Influence of Light Intensity and Leaf Movement on Photosynthesis Characteristics and Carbon Balance of Soybean. *Frontiers in Plant Science*, *9*.
<https://www.frontiersin.org/articles/10.3389/fpls.2018.01952>
- Fenibo, E. O., Ijoma, G. N., & Matambo, T. (2021). Biopesticides in Sustainable Agriculture: A Critical Sustainable Development Driver Governed by Green Chemistry Principles. *Frontiers in Sustainable Food Systems*, *5*(0), 1–12.
<https://doi.org/10.3389/fsufs.2021.619058>
- FPEAK, F. P. E. A. of K. (2020). *Update on The State of The Horticulture Industry in Kenya 2020* –. <https://fpeak.org/update-on-the-state-of-the-horticulture-industry-in-kenya-2021/>
- Fulano, A. M., Lengai, G. M. W., & Muthomi, J. W. (2021). Phytosanitary and Technical Quality Challenges in Export Fresh Vegetables and Strategies to Compliance with Market Requirements: Case of Smallholder Snap Beans in Kenya. *Sustainability*, *13*(3), 1–21.
<https://doi.org/10.3390/su13031546>
- Gao, Y., Wu, P., Zhao, X., & Wang, Z. (2014). Growth, yield, and nitrogen use in the wheat/maize intercropping system in an arid region of northwestern China. *Field Crops Research*, *167*, 19–30. <https://doi.org/10.1016/j.fcr.2014.07.003>
- Goettel, M. S., Inglis, G. D., & Wraight, S. P. (2000). Fungi. In L. A. Lacey & H. K. Kaya (Eds.), *Field Manual of Techniques in Invertebrate Pathology: Application and evaluation of pathogens for control of insects and other invertebrate pests* (2nd ed., pp. 255–282). Springer Netherlands. https://doi.org/10.1007/978-94-017-1547-8_10
- Gogo, E. O., Saidi, M., Ochieng, J. M., Martin, T., Baird, V., & Ngouajio, M. (2014). Microclimate Modification and Insect Pest Exclusion Using Agronet Improve Pod Yield and Quality of French Bean. *HortScience*, *49*(10), 1298–1304.
<https://doi.org/10.21273/HORTSCI.49.10.1298>

- Gontijo, L. M., Saldanha, A. V., Souza, D. R., Viana, R. S., Bordin, B. C., & Antonio, A. C. (2018). Intercropping hampers the nocturnal biological control of aphids: Companion plants hinder predators at night. *Annals of Applied Biology*, 172(2), 148–159. <https://doi.org/10.1111/aab.12407>
- Gozaimasu, D. A. (2016). *Changing Farmers' Mindset from "Grow and Sell" to "Grow to Sell" in french bean production*. (pp. 1–69). Japan International Cooperation Agency, Horticultural Crops Directorate Presented to the County & AFA (HCD), Agriculture and Food Authority (AFA). <https://www.jica.go.jp>
- Greenlife Crop protection Africa. (2021). *French Beans* (pp. 1–15). Nairobi, Kenya. <https://www.greenlife.co.ke/french-beans/>
- Green-Tracewicz, E., Page, E. R., & Swanton, C. J. (2011). Shade Avoidance in Soybean Reduces Branching and Increases Plant-to-Plant Variability in Biomass and Yield Per Plant. *Weed Science*, 59(1), 43–49. <https://doi.org/10.1614/WS-D-10-00081.1>
- Gwynn, L. R., & Maniania, J. N. K. (2017). Africa with special reference to Kenya. In *The use and regulation of microbial pesticides in representative jurisdictions worldwide*, IOBC Global, (pp. 1–11). <https://doi.org/10.4060/cb3571en>
- Hafiz, S. S., Asif, J., Jalal Arif, M., & Dildar Goggi, M. (2020). Tritrophic Effect of *Metarhizium anisopliae* on Wheat Aphids and its Parasitoids. *Acta Scientific Agriculture*, 4(3), 01–05. <https://doi.org/10.31080/ASAG.2020.04.0813>
- Hansen, L. M., Lorentsen, L., & Boelt, B. (2008). How to reduce the incidence of black bean aphids (*Aphis fabae* Scop.) attacking organic growing field beans (*Vicia faba* L.) by growing partially resistant bean varieties and by intercropping field beans with cereals. *Acta Agriculturae Scandinavica, Section B — Soil & Plant Science*, 58(4), 359–364. <https://doi.org/10.1080/09064710701788844>
- HCD, H. C. D. (2020). *Overview of Horticultural Crops Directorate* [Nairobi Horticultural Centre, Kenya.]. Horticultural Crops. <https://horticulture.agricultureauthority.go.ke/index.php/sectors/overview#>
- HCD, H. C. D. (2021). *Statistics; Fresh export statistics for january—July, 2021* [Horticultural Crop Directorate]. <https://horticulture.agricultureauthority.go.ke/index.php/statistics/statistics>

- HCD, H. C. D., & AFA, A. and F. A. (2020). *Validated Horticulture Annual Report 2019- 2020*. Horticultural Crop Directorate. <https://horticulture.agricultureauthority.go.ke/index.php/statistics/statistics>
- Hendges, A. R. A. de A., Melo, J. W. da S., Guimaraes, M. de A., & Rabelo, J. da S. (2018). Intercropping Kale with Culinary Herbs Alters Arthropod Diversity and Hinders Population Growth in Aphids. *HortScience*, 53(1), 44–48. <https://doi.org/10.21273/HORTSCI12010-17>
- Herron, S. A., Rubin, M. J., Ciotir, C., Crews, T. E., Van Tassel, D. L., & Miller, A. J. (2020). Comparative Analysis of Early Life Stage Traits in Annual and Perennial Phaseolus Crops and Their Wild Relatives. *Frontiers in Plant Science*, 11. <https://www.frontiersin.org/articles/10.3389/fpls.2020.00034>
- Huss, C. P., Holmes, K. D., & Blubaugh, C. K. (2022). Benefits and Risks of Intercropping for Crop Resilience and Pest Management. *Journal of Economic Entomology*, 115(5), 1350–1362. <https://doi.org/10.1093/jee/toac045>
- icipe (International Centre of Insect Physiology and Ecology). (2019). *Biopesticide- Metarhizium anisopliae* (pp. 1–6). icipe. <http://www.icipe.org/research/plant-health/biopesticide>
- Infonet Biovision. (2020). *French Beans*. <https://infonet-biovision.org/PlantHealth/Crops/Beans>
- Inglis, G. D., Enkerli, J., & Goettel, M. S. (2012). Laboratory techniques used for entomopathogenic fungi. *Invertebrate Pathology* (pp. 189–253). Elsevier. <https://doi.org/10.1016/B978-0-12-386899-2.00007-5>
- Iqbal, M., Gogi, M. D., Atta, B., Nisar, M. J., Arif, M. J., & Javed, N. (2021). Assessment of pathogenicity of Beauveria bassiana, Metarhizium anisopliae, Verticillium lecanii and Bacillus thuringiensis var. kurstaki against Bactrocera cucurbitae Coquillett (Diptera: Tephritidae) via diet-bioassay technique under controlled conditions. *International Journal of Tropical Insect Science*, 41(2), 1129–1145. <https://doi.org/10.1007/s42690-020-00298-2>
- Iwanicki, N. S., Pereira, A. A., Botelho, A. B. R. Z., Rezende, J. M., Moral, R. de A., Zucchi, M. I., & Delalibera Júnior, I. (2019). Monitoring of the field application of Metarhizium anisopliae in Brazil revealed high molecular diversity of Metarhizium spp in insects, soil and sugarcane roots. *Scientific Reports*, 9(1), 1–12. <https://doi.org/10.1038/s41598-019-38594-8>

- Kabaale, F. P., Tumuhaise, V., Tinzaara, W., Turyasingura, G., Subramanian, S., Khamis, F. M., & Akutse, K. S. (2022). First Report of Field Efficacy and Economic Viability of *Metarhizium anisopliae*-ICIPE 20 for *Tuta absoluta* (Lepidoptera: Gelechiidae) Management on Tomato. *Sustainability*, 14(22), Article 22. <https://doi.org/10.3390/su142214846>
- Kakon, S. S., Mian, M. a. K., Saha, R. R., Nasreen, S. S., Naher, Q., & Hossain, M. A. (2018). Phenology, Growth and Pod Yield of French Bean (*Phaseolus vulgaris* L.) as Influenced by Temperature Variations Under Different Sowing Dates. *Bangladesh Agronomy Journal*, 21(2), Article 2. <https://doi.org/10.3329/baj.v21i2.44489>
- Kalaitzoglou, P., van Ieperen, W., Harbinson, J., van der Meer, M., Martinakos, S., Weerheim, K., Nicole, C. C. S., & Marcelis, L. F. M. (2019). Effects of Continuous or End-of-Day Far-Red Light on Tomato Plant Growth, Morphology, Light Absorption, and Fruit Production. *Frontiers in Plant Science*, 1-10. <https://www.frontiersin.org/articles/10.3389/fpls.2019.00322>
- Kalawa, I. L., Wafula, W. N., Korir, N. K., & Gweyi-Onyango, J. (2018). *Interaction Effect of Growth Regulators and Irrigation Schedules on Growth and Yield of French Beans in Kiambu County, Kenya* [Kenyatta University]. <https://ir-library.ku.ac.ke/handle/123456789/18403>
- Kenya National Bureau of Statistic (KNBS). (2019). *Kenya Economic Survey* (pp. 1–335). Kenya National Bureau of Statistics. <http://www.knbs.or.ke>
- Khan, A. A., Khan, A. M., & Afzal, M. (2017). Olfactory Response of Ladybird Beetle, *Coccinella septempunctata* L. (Coccinellidae: Coleoptera) towards Aphids and their Host Plants. *Pakistan Journal of Zoology*, 49(4), 1539–1541. <https://doi.org/10.17582/journal.pjz/2017.49.4.sc11>
- Khanal, D., Upadhyaya, N., Poudel, K., Adhikari, S., Maharjan, S., Pandey, P., & Joseph, M. N. (2023). Efficacy of entomo-pathogenic fungus and botanical pesticides against mustard aphid (*Lipaphis erysimi* Kalt.) at field condition Rupandehi Nepal. *Journal of King Saud University - Science*, 35(8), 102849. <https://doi.org/10.1016/j.jksus.2023.102849>
- Khondoker, N. A., Uddin, F. M. J., & Sarker, Md. A. R. (2020). Influence of nitrogen and phosphorus level for the performance of french bean (*Phaseolus vulgaris* L.). *Acta Scientifica Malaysia*, 4(1), 34–38. <https://doi.org/10.26480/asm.01.2020.34.38>

- Kim, S., Kim, J. C., Lee, S. J., Lee, M. R., Park, S. E., Li, D., Baek, S., Shin, T. Y., Gasmi, L., & Kim, J. S. (2020). Soil Application of *Metarhizium anisopliae* JEF-314 Granules to Control, Flower Chafer Beetle, *Protaetia brevitarsis seulensis*. *Mycobiology*, *48*(2), 139–147. <https://doi.org/10.1080/12298093.2020.1735765>
- Kimno, S., Kiplagat, O., Arunga, E., & Chepkoech, E. (2016). Evaluation of Selected French Bean (*Phaseolus vulgaris* L.) Genotypes for Resistance to Angular Leaf Spot (*Pseudocercospora griseola*) in Western Kenya. *American Journal of Experimental Agriculture*, *13*(4), 1–6. <https://doi.org/10.9734/AJEA/2016/26874>
- Kinyanjui, G., Khamis, F. M., Mohamed, S., Ombura, L. O., Warigia, M., & Ekesi, S. (2016). Identification of aphid (Hemiptera: Aphididae) species of economic importance in Kenya using DNA barcodes and PCR-RFLP-based approach. *Bulletin of Entomological Research*, *106*(1), 63–72. <https://doi.org/10.1017/S0007485315000796>
- Kisaakye, J., Fourie, H., Coyne, D., Cortada, L., Masinde, S., Subramanian, S., & Haukeland, S. (2021). Evaluation of the Entomopathogenic Potential of *Beauveria bassiana*, *Metarhizium anisopliae* and *Isaria fumosorosea* for Management of *Cosmopolites sordidus* Germar (Coleoptera: Curculionidae). *MDPI*, *11*(12), Article 12. <https://doi.org/10.3390/agriculture11121290>
- Koppert Biological Systems. (2021). *Black bean aphid (Aphis fabae)* (pp. 1–8). Koppert Biological Services. <https://www.koppert.com/challenges/aphids/black-bean-aphid/>
- Kumar and Omkar. (2018). Insect Pest Management. In Omkar (Ed.), *Pests and Their Management* (1st ed., Vol. 2, pp. 1015–1078). Springer Singapore. https://doi.org/10.1007/978-981-10-8687-8_27
- Kumar, J., Ramlal, A., Mallick, D., & Mishra, V. (2021a). An Overview of Some Biopesticides and Their Importance in Plant Protection for Commercial Acceptance. *Plants Science*, *10*(6), 1–28. <https://doi.org/10.3390/plants10061185>
- Kumar, J., Ramlal, A., Mallick, D., & Mishra, V. (2021b). An Overview of Some Biopesticides and Their Importance in Plant Protection for Commercial Acceptance. *Plants*, *10*(6), Article 6. <https://doi.org/10.3390/plants10061185>
- Kumar, S. (2017). Potential of Ethiopian mustard, *Brassica carinata* as a trap crop for large white butterfly, *Pieris brassicae* infesting Indian mustard, *Brassica juncea*. *Journal of Pest Science*, *90*(1), 129–137. <https://doi.org/10.1007/s10340-016-0771-6>

- Kumar, S. (2020). Aphid Plant Interactions: Implications for Pest Management. In M. T. Oliveira, F. Candan, & A. Fernandes-Silva (Eds.), *Plant Communities and Their Environment* (1st ed., pp. 1–19). IntechOpen. <https://doi.org/10.5772/intechopen.84302>
- Kwenti, T. E. (2017). Biological Control of Parasites. In H. Khater, M. Govindarajan, & G. Benelli (Eds.), *Natural Remedies in the Fight Against Parasites* (2nd ed., Vol. 4, pp. 23–46). InTech. <https://doi.org/10.5772/68012>
- Lacey, L. A., Grzywacz, D., Shapiro-Ilan, D. I., Frutos, R., Brownbridge, M., & 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>
- Leger, R. J., & Wang, J. B. (2020). Metarhizium: Jack of all trades, master of many. *Open Biology*, *10*(12), 200307. <https://doi.org/10.1098/rsob.200307>
- Lengai, G. M. W., & Muthomi, J. W. (2018). Biopesticides and Their Role in Sustainable Agricultural Production. *Journal of Biosciences and Medicines*, *6*(6), Article 6. <https://doi.org/10.4236/jbm.2018.66002>
- Lithourgidis, A. S., Dordas, C. A., Damalas, C. A., & Vlachostergios, D. N. (2011). Annual intercrops: An alternative pathway for sustainable agriculture. *Australian Journal of Crop Science*, *5*(4), 396–410.
- Liu, B., Kang, B., Tao, F., & Hu, G. (2021). Modelling the Effects of Pest Control with Development of Pesticide Resistance. *Acta Mathematicae Applicatae Sinica, English Series*, *37*(1), 109–125. <https://doi.org/10.1007/s10255-021-0988-x>
- Macuphe, N., Oguntibeju, O. O., & Nchu, F. (2021). Evaluating the Endophytic Activities of *Beauveria bassiana* on the Physiology, Growth, and Antioxidant Activities of Extracts of Lettuce (*Lactuca sativa* L.). *Plants*, *10*(6), Article 6. <https://doi.org/10.3390/plants10061178>
- Mansion-Vaquié, A., Ferrer, A., Ramon-Portugal, F., Wezel, A., & Magro, A. (2020). Intercropping impacts the host location behaviour and population growth of aphids. *Entomologia Experimentalis et Applicata*, *168*(1), 41–52. <https://doi.org/10.1111/eea.12848>
- Mansion-Vaquié, A., Wezel, A., & Ferrer, A. (2019). Wheat genotypic diversity and intercropping to control cereal aphids. *Agriculture, Ecosystems & Environment*, *285*, 106–604. <https://doi.org/10.1016/j.agee.2019.106604>

- Marete, G. M., Lalah, J. O., Mputhia, J., & Wekesa, V. W. (2021). Pesticide usage practices as sources of occupational exposure and health impacts on horticultural farmers in Meru County, Kenya. *Heliyon*, 7(2), 1–13. <https://doi.org/10.1016/j.heliyon.2021.e06118>
- Marete, G. M., Shikuku, V. O., Lalah, J. O., Mputhia, J., & Wekesa, V. W. (2020). Occurrence of pesticides residues in French beans, tomatoes, and kale in Kenya, and their human health risk indicators. *Environmental Monitoring and Assessment*, 192(692), 1–13. <https://doi.org/10.1007/s10661-020-08662-y>
- Masiga, R., Kasina, M., Mbugi, J., Odhiambo, C., Kinuthia, W., Gemmill-Herren, B., & Vaissiere, B. (2014). Do french beans (*Phaseolus vulgaris* L.) grown in proximity to Mt Kenya forest-Kenya-experience pollination deficit? *Journal of Pollination Ecology*, 14, 255–260. [https://doi.org/10.26786/1920-7603\(2013\)25](https://doi.org/10.26786/1920-7603(2013)25)
- Matere, N. J. (2020). *Developing ecofriendly approach for management of pests and diseases of French beans in Murang'a and Kirinyaga Counties, Central Kenya* [Kenyatta University]. [https://ir-](https://ir-library.ku.ac.ke/bitstream/handle/123456789/21734/Developing%20ecofriendly%20)
library.ku.ac.ke/bitstream/handle/123456789/21734/Developing%20ecofriendly%20
- Mehrpavar, M., Zytynska, S. E., & Weisser, W. W. (2013). Multiple Cues for Winged Morph Production in an Aphid Metacommunity. *PLoS ONE*, 8(3), 1–10. <https://doi.org/10.1371/journal.pone.0058323>
- Miller, P. . T. L., & Rebek, E. J. (2018). Banker Plants for Aphid Biological Control in Greenhouses. *Journal of Integrated Pest Management*, 9(9), 1–8. <https://doi.org/10.1093/jipm/pmy002>
- Mkenda, Ndakidemi, Stevenson, Arnold, Belmain, Chidege, Gurr, & Woolley. (2019). Characterization of Hymenopteran Parasitoids of *Aphis fabae* in an African Smallholder Bean Farming System through Sequencing of COI ‘Mini-Barcodes’. *Insects*, 10(10), 331. <https://doi.org/10.3390/insects10100331>
- Mkenda, P., Mwanauta, R., Stevenson, P. C., Ndakidemi, P., Mtei, K., & Belmain, S. R. (2015). Extracts from Field Margin Weeds Provide Economically Viable and Environmentally Benign Pest Control Compared to Synthetic Pesticides. *PLOS ONE*, 10(11), 1–14. <https://doi.org/10.1371/journal.pone.0143530>
- Mkiga, A. M., Mohamed, S. A., du Plessis, H., Khamis, F. M., Akutse, K. S., Nderitu, P. W., Niassy, S., Muriithi, B. W., & Ekesi, S. (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>
- Mohd, S., Mir, Saxena, A., Kanth, R., Raja, W., Dar, K., Bhat, A., Naikoo, N., Nazir, D., Amin, Z., Mansoor, T., Myint, M., Khan, M., Mohammad, I., & Mir, S. (2022). Role of Intercropping in Sustainable Insect-Pest Management: A Review. *International Journal of Environment and Climate Change*, 12(11), 3390–3404. <https://doi.org/10.9734/IJECC/2022/v12i111390>
- Mollaie, M., Fathi, S. A. A., Nouri-Ganbalani, G., Hassanpour, M., & Golizadeh, A. (2021). Effects of strip intercropping of canola with faba bean, field pea, garlic, or wheat on control of cabbage aphid and crop yield. *Plant Protection Science*, 57(1), 59–65. <https://doi.org/10.17221/132/2019-PPS>
- Mondal, A., Shankar, U., Abrol, D. P., Kumar, A., & Singh, A. K. (2018). Incidence of major insect pests on French bean and relation to environmental variables. *Indian Journal of Entomology*, 80(1), 51–55.
- Munywoki, N., Otieno, O. J., & Mwanarusi, S. (2019). Improving water Use efficiency and insect pest exclusion on french bean (*Phaseolus vulgaris* L.) using different coloured agronet covers. *Journal of Agricultural Science*, 11(3), 1–159. <https://doi.org/10.5539/jas.v11n3p159>
- Mutisya, S., Saidi, M., Opiyo, A., Ngouajio, M., & Martin, T. (2016). Synergistic Effects of Agronet Covers and Companion Cropping on Reducing Whitefly Infestation and Improving Yield of Open Field-Grown Tomatoes. *Agronomy*, 6(3), Article 3. <https://doi.org/10.3390/agronomy6030042>
- Mwanauta, R. W., Mtei, K. M., & Ndakidemi, P. A. (2015). Potential of controlling common bean Insect pests [Bean stem maggot (*Ophiomyia phaseoli*), *Ootheca* (*Ootheca bennigseni*) and Aphids (*Aphis fabae*)] using agronomic, biological and botanical practices in field. *Agricultural Sciences*, 06(05), 489–497. <https://doi.org/10.4236/as.2015.65048>
- Mwangi, W. P., Otieno, A., & Anapapa, A. (2019). Assessment of French Beans Production at Kariua in Kandara, Murang'a County- Kenya. *Asian Journal of Probability and Statistics*, 5, 1–16. <https://doi.org/10.9734/ajpas/2019/v5i430141>
- Mwani, N. C., Cheruiyot, K. E., Nyaanga, J., Ogendo, Joshua. O., Bett, K. P., Mulwa, R., Stevenson, C. P., Arnold, E. J. S., & Belmain, R. S. (2021). Intercropping and diverse field

- margin vegetation suppress bean aphid infestation in dolichos. *Journal of Plant Protection Research*, 61(3), 290–301. <https://doi.org/10.24425/jppr.2021137953>
- Mweke, A., Akutse, K. S., Ulrichs, C., Fiaboe, K. K. M., Maniania, N. K., & Ekesi, S. (2019). Efficacy of aqueous and oil formulations of a specific *Metarhizium 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., Akutse, K. S., Ulrichs, C., Fiaboe, K. K. M., Maniania, N. K., & Ekesi, S. (2020). Integrated Management of *Aphis craccivora* in Cowpea Using Intercropping and Entomopathogenic Fungi under Field Conditions. *Journal of Fungi (Basel, Switzerland)*, 6(60), 1–16. <https://doi.org/10.3390/jof6020060>
- Mweke, A., Ulrichs, C., Nana, P., Akutse, K. S., Fiaboe, K. K. M., Maniania, N. K., & Ekesi, S. (2018). Evaluation of the Entomopathogenic Fungi *Metarhizium anisopliae*, *Beauveria bassiana* and *Isaria* sp. for the Management of *Aphis craccivora* (Hemiptera: Aphididae). *Journal of Economic Entomology*, 111(4), 1587–1594. <https://doi.org/10.1093/jee/toy135>
- Myers, J. R., Wallace, L. T., Mafi Moghaddam, S., Kleintop, A. E., Echeverria, D., Thompson, H. J., Brick, M. A., Lee, R., & McClean, P. E. (2019). Improving the Health Benefits of Snap Bean: Genome-Wide Association Studies of Total Phenolic Content. *Nutrients*, 11(10), Article 10. <https://doi.org/10.3390/nu11102509>
- Nalam, V., Louis, J., & Shah, J. (2019). Plant defense against aphids, the pest extraordinaire. *Plant Science*, 279, 96–107. <https://doi.org/10.1016/j.plantsci.2018.04.027>
- Nawar, A. I., Salama, H. S. A., Khalil, H. E., Nawar, A. I., Salama, H. S. A., & Khalil, H. E. (2020). Additive intercropping of sunflower and soybean to improve yield and land use efficiency: Effect of thinning interval and nitrogen fertilization. *Chilean Journal of Agricultural Research*, 80(2), 142–152. <https://doi.org/10.4067/S0718-58392020000200142>
- Nawaz, A., Razzaq, F., Razzaq, A., Gogi, M. D., Fernández-Grandon, G. M., Tayib, M., Ayub, M. A., Sufyan, M., Shahid, M. R., Qayyum, M. A., Naveed, M., Ijaz, A., & Arif, M. J. (2022). Compatibility and synergistic interactions of fungi, *Metarhizium anisopliae*, and insecticide combinations against the cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae). *Scientific Reports*, 12(1), Article 1. <https://doi.org/10.1038/s41598-022-08841-6>

- Nawaz, M., Ali, S., & Abbas, Q. (2016). High Pressure Water Spray Technique for Controlling Mustard Aphid (*Lipaphis Erysimi*) on Brassica Crop. *American-Eurasian Journal of Agriculture & Environmental Science*, 16(2), 224–228. <https://doi.org/10.5829/idosi.aejaes.2016.16.2.12754>
- Nayak, S. B., Sahoo, A. K., K., E., & Rao, K. S. (2020). Role of pesticide application in environmental degradation and its remediation strategies. In *Environmental Degradation: Causes and Remediation Strategies* (1st ed., Vol. 1, pp. 36–46). Agro Environ Media - Agriculture and Environmental Science Academy, Haridwar, India. <https://doi.org/10.26832/aesa-2020-edcrs-03>
- Ndakidemi, B. J., Mbega, E. R., Ndakidemi, P. A., Belmain, S. R., Arnold, S. E. J., Woolley, V. C., & Stevenson, P. C. (2022). Plant-Rich Field Margins Influence Natural Predators of Aphids More Than Intercropping in Common Bean. *Insects*, 13(7), 569. <https://doi.org/10.3390/insects13070569>
- Ndakidemi, B. J., Mbega, E. R., Ndakidemi, P. A., Stevenson, P. C., Belmain, S. R., Arnold, S. E. J., & Woolley, V. C. (2021). Natural Pest Regulation and Its Compatibility with Other Crop Protection Practices in Smallholder Bean Farming Systems. *Biology*, 10(8), Article 8. <https://doi.org/10.3390/biology10080805>
- Ndakidemi, B., Mtei, K., & Ndakidemi, P. A. (2016). Impacts of Synthetic and Botanical Pesticides on Beneficial Insects. *Agricultural Sciences*, 7(6), Article 6. <https://doi.org/10.4236/as.2016.76038>
- Nelson, W. C. D., Siebrecht-Schöll, D. J., Hoffmann, M. P., Rötter, R. P., Whitbread, A. M., & Link, W. (2021). What determines a productive winter bean-wheat genotype combination for intercropping in central Germany? *European Journal of Agronomy*, 128, 126–294. <https://doi.org/10.1016/j.eja.2021.126294>
- Netherland Development Organization (SNV). (2012). *The bean value chain in Kenya*. (pp. 1–59). Netherland Development Organization. <https://s3-eu-west-1.amazonaws.com/s3.sourceafrica.net/documents/119074/Kenya-National-Bureau-of-Statistics-Economic>.
- Njenga, J. K., Gathungu, G. K., & Mbaka, J. N. (2021). Efficacy of neem, tithonia and tephrosia leaf extracts in the management of root-knot nematodes in French beans (*Phaseolus vulgaris* L.). *African Journal of Horticultural Science*, 15, 1–85.

- Nordey, T., Basset-Mens, C., De Bon, H., Martin, T., Déletré, E., Simon, S., Parrot, L., Desprez, H., Huat, J., Biard, Y., Dubois, T., & Malézieux, E. (2017). Protected cultivation of vegetable crops in sub-Saharan Africa: Limits and prospects for smallholders. A review. *Agronomy for Sustainable Development*, 37(6), 53. <https://doi.org/10.1007/s13593-017-0460-8>
- Nyaanga, J. G., Kamau, A. W., Pathak, R. S., & Tuey, R. K. (2012). The effect of different cereal aphid species on the performance of two coccinellid predators. *Journal of Entomology*, 9(1), 41–49.
- Nyaanga, J. G., Kamau, A. W., & Wanjama, J. K. (2006). Niche and Temporal Differences in Colonization of Wheat Varieties by Three Species of Cereal Aphids. *Asian Journal of Plant Sciences*. <http://docsdrive.com/pdfs/ansinet/ajps/2006/713-716.pdf>
- Nyasani, J. O., Meyhöfer, R., Subramanian, S., & Poehling, H.-M. (2012). Effect of intercrops on thrips species composition and population abundance on French beans in Kenya. *Entomologia Experimentalis et Applicata*, 142(3), 236–246. <https://doi.org/10.1111/j.1570-7458.2011.01217.x>
- Ochieng, L. O., Ogendo, J. O., Bett, P. K., Nyaanga, J. G., Cheruiyot, E. K., Mulwa, R. M. S., Arnold, S. E. J., Belmain, S. R., & Stevenson, P. C. (2022). Field margins and botanical insecticides enhance *Lablab purpureus* yield by reducing aphid pests and supporting natural enemies. *Journal of Applied Entomology*, 146(7), 838–849. <https://doi.org/10.1111/jen.13023>
- Ogecha, J., Arinaitwe, W., Muthomi, J., Aritua, V., & Obanyi, J. (2019). Incidence and Severity of Common Bean (*Phaseolus vulgaris* L.) Pests in Agro-Ecological Zones and Farming Systems of Western Kenya. *East African Agricultural and Forestry Journal*, 83(3), 191–205. <https://doi.org/10.1080/00128325.2019.1599151>
- Omkar, O., & Pervez, A. (2016). Ladybird Beetles. In Omkar (Ed.), *Ecofriendly Pest Management for Food Security* (pp. 281–310). Academic Press Elsevier, India. <https://doi.org/10.1016/B978-0-12-803265-7.00009-9>
- Omwenga, I., Kanja, L., Zomer, P., Louisse, J., Rietjens, I. M. C. M., & Mol, H. (2021). Organophosphate and carbamate pesticide residues and accompanying risks in commonly consumed vegetables in Kenya. *Food Additives & Contaminants: Part B*, 14(1), 48–58. <https://doi.org/10.1080/19393210.2020.1861661>

- Oseko, E., & Dienya, T. (2015). *Fertilizer consumption and fertilizer use by crop (FUBC) in Kenya*. Africafertilizer.Org. <https://africafertilizer.org/wp-content>
- Paradza, V. M., Khamis, F. M., Yusuf, A. A., Subramanian, S., & Akutse, K. S. (2022). Efficacy of *Metarhizium anisopliae* and (E)-2-hexenal combination using autodissemination technology for the management of the adult greenhouse whitefly, *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae). *Frontiers in Insect Science*, 2, 1–15. <https://doi.org/10.3389/finsc.2022.991336>
- Parker, Joyce. E., E., W., C., G., & RodriguezSao, C. (2013). Companion Planting and Insect Pest Control. In S. Soloneski (Ed.), *Weed and Pest Control—Conventional and New Challenges* (pp. 1–30). InTech. <https://doi.org/10.5772/55044>
- PCPB. (2022). *Pest control products registered for use in Kenya* (pp. 1–8). Pest Control Product Board. <https://www.pcpb.go.ke/crops/>
- Peng, G., Xie, J., Guo, R., Keyhani, N. O., Zeng, D., Yang, P., & Xia, Y. (2021). Long-term field evaluation and large-scale application of a *Metarhizium anisopliae* strain for controlling major rice pests. *Journal of Pest Science*, 94(3), 969–980. <https://doi.org/10.1007/s10340-020-01313-8>
- Perring, T. M., Battaglia, D., Walling, L. L., Toma, I., & Fanti, P. (2018). Chapter 2 - Aphids: Biology, Ecology, and Management. In W. Wakil, G. E. Brust, & T. M. Perring (Eds.), *Sustainable Management of Arthropod Pests of Tomato* (pp. 15–48). Academic Press. <https://doi.org/10.1016/B978-0-12-802441-6.00002-4>
- Putnoky-Csicsó, B., Tonk, S., Szabó, A., Márton, Z., Tóthné Bogdányi, F., Tóth, F., Abod, É., Bálint, J., & Balog, A. (2020). Effectiveness of the Entomopathogenic Fungal Species *Metarhizium anisopliae* Strain NCAIM 362 Treatments against Soil Inhabiting *Melolontha melolontha* Larvae in Sweet Potato (*Ipomoea batatas* L.). *Journal of Fungi*, 6(3), 116. <https://doi.org/10.3390/jof6030116>
- Raai, M. N., Zain, N. A. M., Osman, N., Rejab, N. A., Sahruzaini, N. A., & Cheng, A. (2020). Effects of shading on the growth, development and yield of winged bean (*Psophocarpus tetragonolobus*). *Ciência Rural*, 50(2), e20190570. <https://doi.org/10.1590/0103-8478cr20190570>
- Rameau, C., Bertheloot, J., Leduc, N., Andrieu, B., Foucher, F., & Sakr, S. (2015). Multiple pathways regulate shoot branching. *Frontiers in Plant Science*, 5(0), 1–23.

- Reddy, P. P. (2017). Companion Planting. In P. P. Reddy (Ed.), *Agro-ecological Approaches to Pest Management for Sustainable Agriculture* (pp. 149–164). Springer. https://doi.org/10.1007/978-981-10-4325-3_10
- Reingold, V., Kottakota, C., Birnbaum, N., Goldenberg, M., Lebedev, G., Ghanim, M., & Ment, D. (2021). Intraspecies variation of *Metarhizium brunneum* against the green peach aphid, *Myzus persicae*, provides insight into the complexity of disease progression. *Pest Management Science*, 77(5), 2557–2567. <https://doi.org/10.1002/ps.6294>
- Rodriguez, P. A., Rothballer, M., Chowdhury, S. P., Nussbaumer, T., Gutjahr, C., & Falter-Braun, P. (2019). Systems Biology of Plant-Microbiome Interactions. *Molecular Plant*, 12(6), 804–821. <https://doi.org/10.1016/j.molp.2019.05.006>
- Rosch, S. D., & Ortega, D. L. (2019). Willingness to contract versus opportunity to contract: A case study in Kenya’s French bean export market. *Agricultural Economics*, 50(1), 27–37. <https://doi.org/10.1111/agec.12463>
- Sahoo, S., & Giraddi, R. S. (2022). Insect pest spectrum of french bean *Phaseolus vulgaris* (pole type) grown under nethouse. *Indian Journal of Entomology*, 1–3. <https://doi.org/10.55446/IJE.2021.389>
- Sain, S. K., Monga, D., Kumar, R., Nagrale, D. T., Kranthi, S., & Kranthi, K. R. (2019). Comparative effectiveness of bioassay methods in identifying the most virulent entomopathogenic fungal strains to control Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae). *Egyptian Journal of Biological Pest Control*, 29(1), Article 1. <https://doi.org/10.1186/s41938-019-0130-z>
- Sajid, M., Bashir, N. H., Batool, Q., Munir, I., Bilal, M., Jamal, M. A., & Munir, S. (2017). In-vitro evaluation of biopesticides (*Beauveria bassiana*, *Metarhizium anisopliae*, *Bacillus thuringiensis*) against mustard aphid Lipaphis erysimi kalt. (Hemiptera: Aphididae). *Journal of Entomology and Zoology Studies*, 5(6), 331–335.
- Saldanha, A. V., Gontijo, L. M., Carvalho, R. M. R., Vasconcelos, C. J., Corrêa, A. S., & Gandra, R. L. R. (2019). Companion planting enhances pest suppression despite reducing parasitoid emergence. *Basic and Applied Ecology*, 41, 45–55. <https://doi.org/10.1016/j.baae.2019.10.002>
- Sammama, H., Kaoua, M., Hsissou, D., Latique, S., Selmaoui, K., & Mohamed Najib, A. (2021). The impact of wheat and faba bean intercrop on the competitive interactions, grain yield,

- biochemical parameters and mineral content of leaves. *Zemdirbyste*, 108, 233–240.
<https://doi.org/10.13080/z-a.2021.108.030>
- Sánchez-Bayo, F. (2021). Indirect Effect of Pesticides on Insects and Other Arthropods. *Toxics*, 9(8), 1–77. <https://doi.org/10.3390/toxics9080177>
- Sánchez-Sánchez, H., Morquecho-Contreras, A., Sánchez-Sánchez, H., & Morquecho-Contreras, A. (2017). Chemical Plant Defense Against Herbivores. *Herbivores* (pp. 1–34). IntechOpen. <https://doi.org/10.5772/67346>
- Sarkar, S. C., Wang, E., Wu, S., & Lei, Z. (2018). Application of trap cropping as companion plants for the management of agricultural pests: A review. *Insects*, 9(4), 1–128. <https://doi.org/10.3390/insects9040128>
- Saruhan, I. (2018). Efficacy of some entomopathogenic fungi against *Aphis fabae* Scopoli (Hemiptera: Aphididae). *Egyptian Journal of Biological Pest Control*, 28(1), 1-89. <https://doi.org/10.1186/s41938-018-0096-2>
- Sayed, S. M., Ali, E. F., & Al-Otaibi, S. S. (2019). Efficacy of indigenous entomopathogenic fungus, *Beauveria bassiana* (Balsamo) Vuillemin, isolates against the rose aphid, *Macrosiphum rosae* L. (Hemiptera: Aphididae) in rose production. *Egyptian Journal of Biological Pest Control*, 1–29(1), 19. <https://doi.org/10.1186/s41938-019-0123-y>
- Sbaraini, N., Guedes, R. L. M., Andreis, F. C., Junges, Â., de Moraes, G. L., Vainstein, M. H., de Vasconcelos, A. T. R., & Schrank, A. (2016). Secondary metabolite gene clusters in the entomopathogen fungus *Metarhizium anisopliae*: Genome identification and patterns of expression in a cuticle infection model. *BMC Genomics*, 17(8), 654–736. <https://doi.org/10.1186/s12864-016-3067-6>
- Shaban, N. (2021). Correlation and path analysis of interaction between snap beans yield and its components with crop management. *Trakia Journal of Sciences*, 19(1), 86–96. <https://doi.org/10.15547/tjs.2021.01.014>
- Shanker, R., Prajapati, M. R., Singh, R. P., Singh, R., Singh, J., & Kumar, P. (2023). Isolation, molecular characterization of indigenous *Metarhizium anisopliae* (Metchnikoff) isolate, using ITS-5.8s rDNA region, and its efficacy against the *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae). *Egyptian Journal of Biological Pest Control*, 33(1), 23. <https://doi.org/10.1186/s41938-023-00670-7>

- Sharma, A., Kumar, V., Shahzad, B., Tanveer, M., Sidhu, G. P. S., Handa, N., Kohli, S. K., Yadav, P., Bali, A. S., Parihar, R. D., Dar, O. I., Singh, K., Jasrotia, S., Bakshi, P., Ramakrishnan, M., Kumar, S., Bhardwaj, R., & Thukral, A. K. (2019). Worldwide pesticide usage and its impacts on ecosystem. *SN Applied Sciences*, *1*(11), 585–1446. <https://doi.org/10.1007/s42452-019-1485-1>
- Sharma, A., Sandhi, R. K., & Reddy, G. V. P. (2019). A Review of Interactions between Insect Biological Control Agents and Semiochemicals. *Insects*, *10*(12), 280–439. <https://doi.org/10.3390/insects10120439>
- Sharma, A., Shrestha, G., & Reddy, G. V. P. (2019). Trap Crops: How Far We Are From Using Them in Cereal Crops? *Annals of the Entomological Society of America*, *112*(4), 330–339. <https://doi.org/10.1093/aesa/say047>
- Sharma, Kooner, R., & Arora, R. (2017). Insect Pests and Crop Losses. In R. Arora & S. Sandhu (Eds.), *Breeding Insect Resistant Crops for Sustainable Agriculture* (pp. 45–66). Springer. https://doi.org/10.1007/978-981-10-6056-4_2
- Sharma, S., Kooner, R., & Arora, R. (2017). Insect Pests and Crop Losses. In R. Arora & S. Sandhu (Eds.), *Breeding Insect Resistant Crops for Sustainable Agriculture* (pp. 45–66). Springer. https://doi.org/10.1007/978-981-10-6056-4_2
- Sheahan, M., & Barrett, C. B. (2017). Ten striking facts about agricultural input use in Sub-Saharan Africa. *Food Policy*, *67*, 12–25. <https://doi.org/10.1016/j.foodpol.2016.09.010>
- Sheahan, M., Barrett, C. B., & Goldvale, C. (2017). The unintended consequences of agricultural input intensification: Human health implications of pesticide use in Sub-Saharan Africa. *African Development Bank (AfDB)*, *8*(4), 1–8.
- Shelton, A. M., & Badenes-Perez, F. R. (2006). Concepts and applications of trap cropping in pest management. *Annual Review of Entomology*, *51*(1), 285–308. <https://doi.org/10.1146/annurev.ento.51.110104.150959>
- Singh, A., Dilkes, B., Sela, H., & Tzin, V. (2021). The Effectiveness of Physical and Chemical Defense Responses of Wild Emmer Wheat Against Aphids Depends on Leaf Position and Genotype. *Frontiers in Plant Science*, *12*. <https://www.frontiersin.org/articles/10.3389/fpls.2021.667820>

- Singh, H., & Kaur, T. (2020). Pathogenicity of entomopathogenic fungi against the aphid and the whitefly species on crops grown under greenhouse conditions in India. *Egyptian Journal of Biological Pest Control*, 30(1), 84. <https://doi.org/10.1186/s41938-020-00287-0>
- Singh, R., & Singh, G. (2021). Aphids. Omkar (Ed.), *Polyphagous Pests of Crops* (pp. 105–182). Springer. https://doi.org/10.1007/978-981-15-8075-8_3
- Srinivasan, R., Sevgan, S., Ekesi, S., & Tamò, M. (2019). Biopesticide based sustainable pest management for safer production of vegetable legumes and brassicas in Asia and Africa. *Pest Management Science*, 75(9), 2446–2454. <https://doi.org/10.1002/ps.5480>
- Tavares, P. D., Uzêda, M. C., Pires, A. dos S., Tavares, P. D., Uzêda, M. C., & Pires, A. dos S. (2019). Biodiversity Conservation in Agricultural Landscapes: The Importance of the Matrix. *Floresta e Ambiente*, 26(4). <https://doi.org/10.1590/2179-8087.066417>
- Teresa, Mogoi. K., Nyaora Moturi, W., Nyaanga, J., Kinyoro Macharia, J., & Jerop Birech, R. (2019). Determination of pesticide residues in organic and conventional exotic vegetables. *International Journal of Agriculture Environment and Bioresarch*, 04(04), 06–11. <https://doi.org/10.35410/IJAEB.2019.4402>
- Tesemma, Z. K., Mohamed, Y. A., & Steenhuis, T. S. (2010). Trends in rainfall and runoff in the Blue Nile Basin: 1964-2003. *Hydrological Processes*, 24(25), 3747–3758. <https://doi.org/10.1002/hyp.7893>
- Thakur, K., Sharma, A., & Sharma, K. (2021). Management of agricultural insect pests with physical control methods. *The Pharma Innovation*, 10(6), 306–314.
- Thomas, G., Rusman, Q., Morrison, W. R., Magalhães, D. M., Dowell, J. A., Ngumbi, E., Osei-Owusu, J., Kansman, J., Gaffke, A., Pagadala Damodaram, K. J., Kim, S. J., & Tabanca, N. (2023). Deciphering Plant-Insect-Microorganism Signals for Sustainable Crop Production. *Biomolecules*, 13(6), 925–997. <https://doi.org/10.3390/biom13060997>
- Trade Map. (2020). *List of exporters for the selected product (edible vegetables and certain roots and tubers)* (pp. 1–2). Trade statistics for international business development. <https://www.trademap.org/Country>
- Trinh, D. N., Ha, T. K. L., & Qiu, D. (2020). Biocontrol Potential of Some Entomopathogenic Fungal Strains Against Bean Aphid *Megoura japonica* (Matsumura). *Agriculture*, 10(4), Article 4. <https://doi.org/10.3390/agriculture10040114>


- Tsimbiri, P. F., Moturi, W. N., Sawe, J., Henley, P., & Bend, J. R. (2015). Health Impact of Pesticides on Residents and Horticultural Workers in the Lake Naivasha Region, Kenya. *Occupational Diseases and Environmental Medicine*, 3(2), Article 2. <https://doi.org/10.4236/odem.2015.32004>
- Ullah, R. Muhammad. K., Gao, F., Sikandar, A., & Wu, H. (2023). Insights into the Effects of Insecticides on Aphids (Hemiptera: Aphididae): Resistance Mechanisms and Molecular Basis. *International Journal of Molecular Sciences*, 24(7), 6750–6758. <https://doi.org/10.3390/ijms24076750>
- United Nations Economic Commission for Europe, (UNECE). (2017). *Standard FFV-06 concerning the marketing and commercial quality control of beans*. United nations. <https://unece.org/standard/Beans>
- Uwiringiyimana, T., Kusolwa, M. P., Mamiro, D. P., Umuhozariho, M. G., & Niyonzima, J. P. (2020). Performance of snap beans varieties in lowland of Morogoro in Tanzania. *Rwanda Journal of Agricultural Sciences*, 2(1), Article 1.
- Varenhorst, A., Rozeboom, P., & Wagner, P. (2021). Sunflower Insect Pests. Insect pests of crops in South Dakota (1st ed., Vol. 1, pp. 11–26). South Dakota State University. <https://extension.sdstate.edu/sites/default/files/2022-03/P-00205-11-v2>.
- Villamizar, L. F., Barrera, G., Hurst, M., & Glare, T. R. (2021). Characterization of a new strain of *Metarhizium novozealandicum* with potential to be developed as a biopesticide. *Mycology*, 2(1), 1–18. <https://doi.org/10.1080/21501203.2021.1935359>
- Wallace, L., Arkwazee, H., Vining, K., & Myers, J. (2018). Genetic Diversity within Snap Beans and Their Relation to Dry Beans. *Genes*, 9(12), 587. <https://doi.org/10.3390/genes9120587>
- Wamonje, F. O., Donnelly, R., Tungadi, T. D., Murphy, A. M., Pate, A. E., Woodcock, C., Caulfield, J., Mutuku, J. M., Bruce, T. J. A., Gilligan, C. A., Pickett, J. A., & Carr, J. P. (2020). Different Plant Viruses Induce Changes in Feeding Behavior of Specialist and Generalist Aphids on Common Bean That Are Likely to Enhance Virus Transmission. *Frontiers in Plant Science*, 10(1), 1–1811. <https://doi.org/10.3389/fpls.2019.01811>
- Wan, N.-F., Cai, Y.-M., Shen, Y.-J., Ji, X.-Y., Wu, X.-W., Zheng, X.-R., Cheng, W., Li, J., Jiang, Y.-P., Chen, X., Weiner, J., Jiang, J.-X., Nie, M., Ju, R.-T., Yuan, T., Tang, J.-J., Tian, W.-D., Zhang, H., & Li, B. (2018). Increasing plant diversity with border crops reduces


- insecticide use and increases crop yield in urban agriculture. *eLife*, 7(1), 35–103. <https://doi.org/10.7554/eLife.35103>
- Wang, Z., Zhao, X., Wu, P., Gao, Y., Yang, Q., & Shen, Y. (2017). Border row effects on light interception in wheat/maize strip intercropping systems. *Field Crops Research*, 214, 1–13. <https://doi.org/10.1016/j.fcr.2017.08.017>
- War, A. R., Paulraj, M. G., Ahmad, T., Buhroo, A. A., Hussain, B., Ignacimuthu, S., & Sharma, H. C. (2012). Mechanisms of plant defense against insect herbivores. *Plant Signaling & Behavior*, 7(10), 1306–1320. <https://doi.org/10.4161/psb.21663>
- Warra, A. A., & Prasad, M. N. V. (2020). Chapter 16—African perspective of chemical usage in agriculture and horticulture—Their impact on human health and environment. M. N. V. Prasad (Ed.), *Agrochemicals Detection, Treatment and Remediation* (pp. 401–436). Butterworth-Heinemann. <https://doi.org/10.1016/B978-0-08-103017-2.00016-7>
- Waweru, B. W., Rukundo, P., Kilalo, D. C., Miano, D. W., & Kimenju, J. W. (2021). Effect of border crops and intercropping on aphid infestation and the associated viral diseases in hot pepper (*Capsicum* sp.). *Crop Protection*, 145(01), 105–623. <https://doi.org/10.1016/j.cropro.2021.105623>
- Wei, W., Liu, T., Shen, L., Wang, X., Zhang, S., & Zhang, W. (2022). Effect of Maize (*Zea mays*) and Soybean (*Glycine max*) Intercropping on Yield and Root Development in Xinjiang, China. *Agriculture*, 12(7), Article 7. <https://doi.org/10.3390/agriculture12070996>
- Xu, Y., Qiu, W., Sun, J., Müller, C., & Lei, B. (2019). Effects of wheat/faba bean intercropping on soil nitrogen transformation processes. *Journal of Soils and Sediments*, 19(4), 1724–1734. <https://doi.org/10.1007/s11368-018-2164-3>
- Xu, Z., Li, C., Zhang, C., Yu, Y., van der Werf, W., & Zhang, F. (2020). Intercropping maize and soybean increases efficiency of land and fertilizer nitrogen use; A meta-analysis. *Field Crops Research*, 246, 107661. <https://doi.org/10.1016/j.fcr.2019.107661>
- Yang, Z.-K., Qu, C., Pan, S.-X., Liu, Y., Shi, Z., Luo, C., Qin, Y.-G., & Yang, X.-L. (2023). Aphid-repellent, ladybug-attraction activities, and binding mechanism of methyl salicylate derivatives containing geraniol moiety. *Pest Management Science*, 79(2), 760–770. <https://doi.org/10.1002/ps.7245>
- Yeshe, K., Crayn, D., Ritmejerytè, E., & Wangchuk, P. (2022). Plant Secondary Metabolites Produced in Response to Abiotic Stresses Has Potential Application in Pharmaceutical

- Product Development. *Molecules*, 27(1), Article 1.
<https://doi.org/10.3390/molecules27010313>
- Youn, W. B., Hernandez, J. O., & Park, B. B. (2021). Effects of Shade and Planting Methods on the Growth of *Heracleum moellendorffii* and *Adenophora divaricata* in Different Soil Moisture and Nutrient Conditions. *Plants*, 10(10), Article 10.
<https://doi.org/10.3390/plants10102203>
- Zaller, J. G. (2020). What Is the Problem? Pesticides in Our Everyday Life. In J. G. Zaller (Ed.), *Daily Poison: Pesticides—An Underestimated Danger* (pp. 1–125). Springer International Publishing. https://doi.org/10.1007/978-3-030-50530-1_1
- Zeder, M. A. (Ed.). (2006). *Documenting domestication: New genetic and archaeological paradigms* (2nd ed., Vol. 1). University of California Press.
- Zekeya, N., Dubois, T., Smith, J., & Ramasamy, S. (2022). Field effectiveness of *Metarhizium anisopliae* and pheromone traps against *Phthorimaea absoluta* on tomato in Tanzania. *Crop Protection*, 156(1), 105940–105942. <https://doi.org/10.1016/j.cropro.2022.105942>
- Zhang, Y., Han, M., Song, M., Tian, J., Song, B., Hu, Y., Zhang, J., & Yao, Y. (2021). Intercropping With Aromatic Plants Increased the Soil Organic Matter Content and Changed the Microbial Community in a Pear Orchard. *Frontiers in Microbiology*, 12, 616932. <https://doi.org/10.3389/fmicb.2021.616932>
- Zhao, J., Wang, Z., Li, Z., Shi, J., Meng, L., Wang, G., Cheng, J., & Du, Y. (2020). Development of lady beetle attractants from floral volatiles and other semiochemicals for the biological control of aphids. *Journal of Asia-Pacific Entomology*, 23(4), 1023–1029. <https://doi.org/10.1016/j.aspen.2020.08.005>
- Zhu, J., Werf, W. van der, Anten, N. P. R., Vos, J., & Evers, J. B. (2015). The contribution of phenotypic plasticity to complementary light capture in plant mixtures. *New Phytologist*, 207(4), 1213–1222. <https://doi.org/10.1111/nph.13416>
- Zhu, J.-K. (2016). Abiotic Stress Signaling and Responses in Plants. *Cell*, 167(2), 313–324. <https://doi.org/10.1016/j.cell.2016.08.029>

APPENDICES


Appendix A: Research permit NACOSTI


REPUBLIC OF KENYA


NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY & INNOVATION

Ref No: **166195** Date of Issue: **01/July/2022**


RESEARCH LICENSE




This is to Certify that Mr.. Anthony Emaru of Egerton University, has been licensed to conduct research in Nakuru on the topic: EFFECTS OF METARHIZIUM ANISOPLIAE AND COMPANION CROPPING ON BEAN APHID POPULATION, GROWTH, YIELD, AND QUALITY OF FRENCH BEAN for the period ending : 01/July/2023.

License No: **NACOSTI/P/22/18396**

166195
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.

Appendix B: Ethical Research Permit of Egerton University

EGERTON

TEL: (051) 2217808
FAX: 051-2217942



UNIVERSITY

P. O. BOX 536
EGERTON

**EGERTON UNIVERSITY INSTITUTIONAL SCIENTIFIC AND ETHICS
REVIEW COMMITTEE**

EU/RE/DVC/009

Approval No. EUISERC/APP/197/2022

6th September, 2022

Anthony Emaru
Egerton University
P.O.Box 536.
Egerton
Telephone: +256777292701/+254112864727
E-mail: emaruathony@gmail.com

Dear Anthony,

RE: ETHICAL APPROVAL: EFFECTS OF ENTOMOPATHOGENIC FUNGUS (Metarhizium anisopliae) AND COMPANION CROPPING ON BEAN APHID (Aphis fabae) POPULATION, GROWTH, YIELD AND QUALITY OF FRENCH BEAN (Phaseolus vulgaris L.)

This is to inform you that **Egerton University Institutional Scientific and Ethics Review Committee** has reviewed and approved your above research proposal. Your application approval number is **EUISERC/APP/197/2022**. The approval period is **6th September, 2022 –7th September, 2023**.

This approval is subject to compliance with the following requirements;

- i. Only approved documents including (informed consents, study instruments, MTA) will be used.
- ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by **Egerton University Institutional Scientific and Ethics Review Committee**.
- iii. Death and life-threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to **Egerton University Institutional Scientific and Ethics Review Committee** within 72 hours of notification
- iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to **Egerton University Institutional Scientific and Ethics Review Committee** within 72 hours.

"Transforming Lives through Quality Education"

- v. Clearance for Material Transfer of biological specimens must be obtained from relevant institutions.
- vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
- vii. Submission of an executive summary report within 90 days upon completion of the study to **Egerton University Institutional Scientific and Ethics Review Committee**.

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) <https://oris.nacosti.go.ke> and also obtain other clearances needed.

Yours sincerely,

A handwritten signature in black ink, appearing to be "R. Ngunjiri".

Prof. R. Ngunjiri
**CHAIRMAN, EGERTON UNIVERSITY INSTITUTIONAL SCIENTIFIC AND ETHICS
REVIEW CTTEE**
RMN/BK/



Appendix C: Publications

RESEARCH ARTICLE · Volume 10, Issue 13, e33037, July 15, 2024 · Open Access

Integrating *Metarhizium anisopliae* entomopathogenic fungi with border cropping reduces black bean aphids (*Aphis fabae*) damage and enhances yield and quality of French bean

Anthony Emaru  · Jane G. Nyaanga · Mwanarusi Saidi

Affiliations & Notes  Article Info 

 Download PDF  Cite  Share  Set Alert  Get Rights  Reprints



 Previous article  Next article 

» Abstract

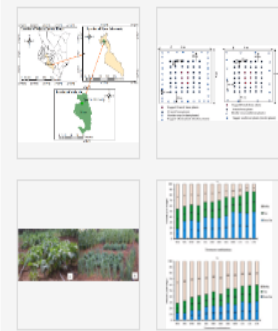
Show Outline

French bean growers, rely mainly on pesticides for pest management. The acceptable tolerance for pesticides residue in French beans is a major concern and has led to several tonnes of the crop continuously rejected and listed as unsafe for human consumption. There is growing demand for alternative approaches and products that are effective at managing pests without the side-effects associated with reliance on pesticides. A field study to determine the combined effects of *Metarhizium anisopliae*, (*Metaril* WP E9 and *Biomagic*) biopesticides and border crops (Sunflower and wheat) on aphid population, damage severity, growth, yield and quality of French bean. A two-factor experiment was conducted at the Egerton University, Kenya. First factor included two border crops (sunflower and wheat) and no border crop (control). Second factor included spraying *Metaril* WPE9 (2×10^8 cfu/g), *Biomagic* (2×10^8 cfu/ml) biopesticides, alpha-cypermethrin (synthetic insecticide) and water. Data on growth, yield and quality parameters were collected and analyzed using the SAS version 9.4M8. Results showed that *M. anisopliae* and border crop significantly ($p < 0.0005$) enhanced growth, yield and quality of French bean in both seasons. French bean grown with wheat or sunflower borders showed a significant reduction in aphid population ($p < 0.0001$) and damage severity ($p < 0.0001$) when sprayed with various treatments compared to the control. Plots with wheat border caused an increase in collar diameter of French bean. The plots (*Metaril* and wheat border) caused a 4% and 5% increase in marketable yield, a 2% and 12% reduction in non-marketable yield. To exploit the benefits of biopesticides, the study recommends their integration with and border crops. Thus, French bean growers could benefit more from fungal-based biopesticides in aphid-IPM approach, as it reduces pre-harvest intervals and residues compared to synthetic insecticides.

Keywords

Figures (4)

Figure Viewer



Article metrics

8

Captures



Open Access



Efficacy of the entomopathogenic fungus *Metarhizium anisopliae* (Clavicipitaceae: Hypocreales) against the black bean aphid (*Aphis fabae* Scopoli, 1763) under laboratory conditions

Anthony Emaru^{1*}; Jane. G. Nyaanga¹ & Mwanarusi Saidi¹

¹Department of Crop, Horticulture and Soil (CHS), Faculty of Agriculture, Egerton University, P.O Box, 536-20115, Njoro Nakuru, Kenya

*Corresponding author, E-mail: emaruanthony@gmail.com

Copyright © 2024, Emaru et al. | Published by LENA/ IFA-Yangambi | [License CC BY-NC-4.0](https://creativecommons.org/licenses/by-nc/4.0/)



Received: 17 December 2023

Accepted: 27 April 2024

Published : 02 May 2024

ABSTRACT

Black bean aphid (BBA) is a serious insect pest that severely significantly impacts French bean production. Over-reliance on synthetic chemicals to reduce economic losses caused by aphids poses serious environmental and human health threats. Biopesticides containing entomopathogenic fungi (EPF) have potential as viable alternatives that can be incorporated into integrated to manage such insect pests. The efficacy of *Metarhizium anisopliae* against BBA was assessed in the laboratory under a complete randomized design (CRD) design with five replicates. Data was analysed using SAS 9.4 (2021). In part one, *Metarhizium anisopliae* products caused bean aphid mortality between 60.1% and 95.5%. Mazao supreme (4×10^8 spores/ml) and Metarril (4×10^8 spores/ml) caused the highest mortality (95.5%) and the lowest concentration of irrespective product caused the lowest mortality (60.1%) after treatment application. In part two, alpha-cypermethrin caused the highest aphid mortality (99.3%), followed by Metarril (84.2%), Mazao (80.7%) and biomagic had the lowest (68.4%) mortality after treatment application. Alpha-cypermethrin had the lowest lethal time ($L.T_{50}$), causing 50% aphid mortality, followed by Metarril, Mazao supreme, and biomagic. Different treatments had varying effects on nymph numbers. Alpha-cypermethrin caused 50% aphid mortality, followed by Metarril, Mazao supreme, and biomagic, with varying effects on nymph numbers. The study recommends incorporating *Metarhizium anisopliae*-based biopesticides, particularly Metarril by small holder farmers to offer a cost-effective integrated pest management approach for managing BBA (adults and nymphs) in French bean production while reducing reliance on synthetic chemicals.

Keywords: Black bean aphid, biopesticides, French beans

Review

Use of Entomopathogenic Fungi as Biopesticides to Manage Insect Pest: A Review

Anthony Emaru, Jane Gesimba Nyaanga and Saidi Mwanarusi

Department of Crop, Horticulture and Soil (CHS), Faculty of Agriculture, Egerton University, Njoro Nakuru, Kenya

Article history

Received: 06-12-2023

Revised: 23-04-2024

Accepted: 06-05-2024

Corresponding Author:

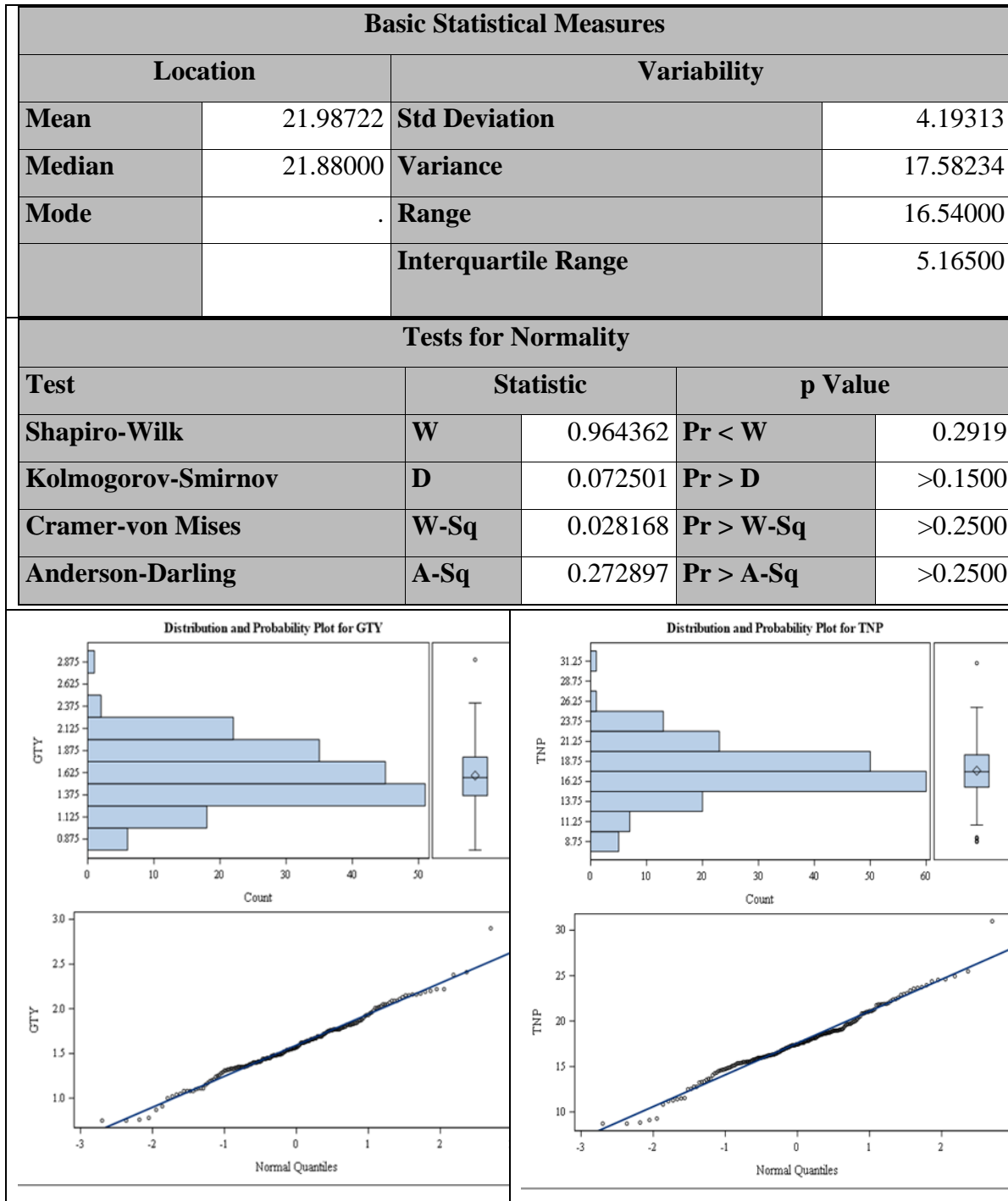
Anthony Emaru
Department of Crop,
Horticulture and Soil (CHS),
Faculty of Agriculture, Egerton
University, Njoro Nakuru,
Kenya

Email: emaru.0903320@student.egerton.ac.ke

Abstract: Entomopathogenic Fungi (EPF) biopesticides are more environmentally friendly and are an alternative to toxic synthetic chemicals. Due to EPF's pathogenic nature to various arthropod pests, it is thus considered a primary candidate for an integrated biological approach due to its numerous invaluable effects. However, an urgent need is to develop an environmentally safer, more sustainable, and practical approach to managing insect pests. Incorporating fungal biopesticide in an Integrated Pest Management (IPM) approach offers an opportunity to reduce the unselective and continuous use of synthetic chemicals to manage insect pests. There are limited reviews on biopesticides in developing countries concerning research questions. The review aimed to provide an understanding of the use of entomopathogenic fungal biopesticides to manage insect pests, majorly aphids in horticultural crops such as French beans." The study uses an inclusive search approach, identifying 1046 articles and reports from 2010-2022 from relevant sources like Web of Science, dimension, Google Scholar, and Google. Out of these, 85 original papers and grey literature were selected and were related to fungal biopesticide use in Kenya and aimed at improving comprehensive knowledge on the benefits and use of EPF biopesticide against insect pests, their action, and how they kill the target pest. The current review presents information on the use of EPF, *Metarhizium anisopliae*, and *Beauveria bassiana* as biopesticides that are dominantly used in Kenya and attributed to their pathogenicity, registry, accessibility, and secondary metabolites produced, thus, increased demand with more than 132,980 hectares in 2019 in Kenya under biopesticides. However, their use in biocontrol processes is still underestimated due to a lack of knowledge. Thus, this study review recommends integrating EPF with other measures for enhanced fungal biopesticide formulation, pathogenicity, and increased shelf-life.

Keywords: Microbial Biopesticides, IPM, *Metarhizium anisopliae* Aphids

Appendix D: The normality test analysis sample for different variables data before being subjected for ANOVA test.



Appendix E: Analysis of variance for Metarril and Mazao supreme different concentration on *Aphis fabae* mortality under laboratory bioassay

Source of variation	DF	Chisq	P value
Product	1	1.6268	0.2021
Concentration	2	0.00842	0.000209***
Product*Conc	4	3.6637	0.4552
Error	24	8.0494	

Appendix F: Analysis of variance for Metarril and Mazao supreme different concentration on *Aphis fabae* median lethal time (LT₅₀) bioassay

Source of variation	DF	Sum of square	Mean square	F- value	P value
Product	1	0.408	0.4084	1.820	0.18158
Expt	2	4.303	2.1514	9.587	0.0000204***
Concentration	2	5.935	2.9676	13.223	0.0000128***
Product*Conc	4	0.252	0.1262	0.562	0.5723
Product*Expt	2	1.362	0.6811	13.223	0.8293
Expt*Product*Conc	4	0.201	0.0501	0.223	0.9245
Error	72	16.158	0.2244		

Appendix G: Analysis of variance for different products on *Aphis fabae* mortality under laboratory bioassay

Source of variation	DF	Chisq	P value
Expt	2	5.5894	0.06113
Product	3	12.882	0.00489**
Expt*Product	6	9.5825	0.143371
Error	24		

The different products were; metarril, mazao, Biomagic, alpha-cypermethrin and water

Appendix H: Analysis of variance for different products on *Aphis fabae* LT₅₀

Source of variation	DF	Chisq	P value
Expt	2	4.472	0.1069
Product	3	51.261	4.305e ^{11***}
Expt*Product	6	9.157	0.1649
Error	24		

Appendix I: Mean separation effect of different products on aphid mortality and LT₅₀

Product	Mortality	Number	Sd	Se
Biomagic	68.4b	15	30.5	7.88
Mazao	80.7ab	15	31.5	8.14
Metarril	84.2ab	15	24.9	6.43
Alpha-cypermethrin	99.3	15	2.8	0.72
	LT ₅₀	Number	Sd	Se
Biomagic	4.3a	10	1.5	0.5
Mazao	3.9a	12	1.5	0.4
Metarril	3.8a	14	1.6	0.4
Alpha-cypermethrin	1.0b	15	0.2	0.1

Standard deviation (Sd), Standard error and median lethal time (LT₅₀)

Appendix J: Analysis of variance for the aphid population and damage severity

Source of variation	Df	Aphid population S1	Aphid population S2	Damage S1	Damage S2
Model	13	5.27***	7.40***	3.26***	1.88***
Block	2	0.04Ns	1.29Ns	0.01Ns	0.18Ns
Biopesticides	3	20.04***	24.74***	11.89***	5.53***
Border crop	2	3.20***	7.19***	1.21Ns	2.26***
Biopesticide*Border crop	6	1.58*	2.13*	0.70Ns	1.50**
Error	166	0.52	0.59	0.48	0.30
Cv	-	24.17	28.43	29.08	25.93

*, **, ***, treatments significant at ($p \leq 0.05$), ($p \leq 0.005$), ($p \leq 0.0005$), respectively;

CV=Coefficient of variation; d.f= degree of freedom, Ns- not significant, S1- season one and S2 – season two.

Appendix K: Analysis of variance for the growth parameters

Source of variation	Season one				Season two		
	DF	Plant height	Collar diameter	Number of branches	Plant height	Collar diameter	Number of branches
Model	61	140.64**	0.35***	17.92***	837.92***	0.06***	19.49***
Block	2	49.40	0.10	0.47	70.87	0.00	0.01
Border	2	548.89***	0.03	0.80	149.50**	0.05***	23.21***
Pesticide	3	445.44***	0.02	0.54	19.98	0.07***	8.00***
Day After Planting (DAP)	4	10775.00***	4.95***	268.63***	12182.9***	0.73***	256.04***
Border*Pesticide	6	113.46***	0.01	0.36	140.28***	0.02***	4.43***
Border*DAP	8	35.45	0.08***	0.22	17.78	0.01**	1.32**
Pesticide*DAP	12	88.37***	0.03	0.23	11.51	0.01***	0.63
Border*Pesticide*DAP	24	10.92	0.02	0.32	32.37*	0.004**	0.57
Error	118	20.66	0.01	0.39	17.14	0.002	0.40
Cv	-	22.38	18.16	12.92	10.04	8.59	13.17

*Means are significantly difference at $p < 0.05$, ** means are significantly different at $p < 0.005$ and *** means are significantly different at $p < 0.0005$, Cv- Coefficient of variation, DF- degree of freedom,

Appendix L: Analysis for number of pods, average pod weight and total pod weight yield

Source of variation	df	Season one			Season two		
		Number of pods	Average pod weight	Total pod yield	Number of pods	Average pod weight	Total pod yield
Model	13	36507.05*	0.68**	32.71**	38716.65***	3.270ns	43.71**
Block	2	7441.36	0.05	0.40	5414.25	4.99	25.15
Border	2	577.03	0.04	1.59	24413.08*	4.30	36.05*
Pesticide spray	3	305324.75**	1.81***	97.61***	99734.74***	3.98	90.84***
Border*pesticide	6	153230.17	0.33	21.40*	7834.36	1.99	8.88*
Error	22	15000.31	0.19	10.38	6468.61	2.36	10.06
cv		23.58	19.40	26.17	17.84	12.17	23.87

*Means are significantly difference at $p < 0.05$, ** means are significantly different at $p < 0.005$ and *** means are significantly different at $p < 0.0005$, Cv- Coefficient of variation, DF- degree of freedom.

Appendix M: Analysis of variance for pod grade, quality, non-marketable and marketable yield

Source of Variation	Df	Season one						Season two				
		Extra Fine	Fine grade	Bobby grade	Non-Marketable	Export	Marketable	Extra F	Fine	Boby	Markable	Non-Markable
Model	13	9.27***	3.83**	1.26*	380.59**	24.43***	459.68***	5.92***	3.72**	7.59	1.51	18.
Block	2	1.21	0.79	0.05	48.37	2.14	64.17	3.58	1.67	32.01	0.31	3.1
Border	2	2.19	0.34	0.15	274.29	3.46	226.08	1.94	2.74	6.32	1.24	8.8
Pesticide	3	33.44***	14.87***	2.76**	1474.40***	92.92***	1556.97***	20.26***	11.43***	4.22	1.80	62.
Border* Pesticide	6	12.23*	9.48*	1.27	92.94	14.59*	140.75*	15.86*	8.87*	1.88	1.85	13.
Error	22	1.84	1.17	0.44	1.17	5.11	75.30	1.07	0.96	421	0.88	3.1

*Means are significantly difference at $p < 0.05$, ** means are significantly different at $p < 0.005$ and *** means are significantly different at $p < 0.0005$, Cv- Coefficient of variation, DF- degree of freedom.