

**OPTIMIZATION OF NATURAL MANAGEMENT METHODS AGAINST FALL  
ARMYWORM (*Spodoptera frugiperda* (J.E. SMITH) (LEPIDOPTERA:  
NOCTUIDAE) IN MAIZE CROPS IN MOROGORO, TANZANIA**

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**A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE  
REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CROP  
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## EXTENDED ABSTRACT

Fall armyworm (FAW), *Spodoptera frugiperda* (J.E Smith) is a notorious pestiferous insect with wide host range, high dispersal ability and high fecundity that make it one of the most severe economic pests. It has been restricted to America and in 2016 was reported from various countries in Africa including Tanzania.

Objectives of this study were (i) Evaluation of natural management methods against FAW for improved maize productivity. (ii) To evaluate the effectiveness of biopesticides-enhanced push-pull systems against FAW in selected maize cropping systems (iii) To determine the influence of cropping systems and biopesticides on parasitism rate and abundance of Parasitoids of *S.frugiperda*. A completely randomized design (CRD) with four replications was adopted to determine developmental biology of the FAW using biopesticides *B. thuringiensis* (*Bt*), *M. anisopliae* and neem seed extract with lower dose of 2 mls, 2mls and (30g) per one litre of water respectively, tested against FAW in the laboratory. Results showed that developmental duration of FAW growth stages differed significantly among treatments. Egg incubation, larval duration, pupa stage duration and the total developmental duration were significantly longer on the biopesticides treated colony compared to untreated groups ( $p < 0.001$ ). The shortest developmental duration of FAW stages were observed on control colonies at  $2.1 \pm 0.18$ ,  $14.88 \pm 0.18$ , and  $27.7 \pm 0.34$  days for egg, larva and pupa stages respectively. The longest developmental duration was observed in colonies under *B. thuringiensis* treatment ( $3.5 \pm 0.37$  SE,  $22.03 \pm 0.59$  SE,  $12.68 \pm 0.23$  SE and  $37.7 \pm 0.54$  SE) days for egg, larva and pupa stages respectively.

The effectiveness of biopesticides-enhanced push-pull systems against FAW in selected maize cropping systems was evaluated. A factorial experiment with treatment

combination of  $5 \times 2$  was used. Factor A included biopesticides with five levels. Factor B cropping system with two levels. Results showed significant ( $p < 0.001$ ) effects of cropping system and biopesticide application on FAW egg masses, abundance of FAW larvae per plant, percent damaged plant and grain yield of maize crop. However, interaction of biopesticides  $\times$  sampled weeks and between cropping system  $\times$  biopesticides  $\times$  sampled week did not significantly influence egg masses. A total of 777 larval and 2241 egg-larval parasitoid species were collected during the 2020 – 2021 period of this study. To determine the influence of cropping systems and biopesticides on parasitism rate and abundance of Parasitoids of *S.frugiperda*, factor A included biopesticides with five levels (*B. thuringiensis*, *M.anispoliae*, neem seed extract, flubendiamide 480 Sc (positive control) and Maize sole crop (negative control). Factor B cropping system with two levels (Push pull technology and maize sole crop. The results showed significant effect on relative abundance of *Cotesia sp* ( $p < 0.001$ ) on plots treated with biopesticides. Results also showed significant effect of parasitism rate and relative abundance of one egg-larval parasitoid specie (*C. bifoveolatus*) and two larval-parasitoids species (*C. luteum* and *Cotesia sp*) ( $P < 0.05$ ) on cropping system. The results also showed significant effect ( $P < 0.05$ ) on parasitism rate and percentage relative abundance of one egg-larval parasitoid specie (*C. bifoveolatus*) and two larval-parasitoids species (*C. luteum* and *Cotesia sp*) on interaction between cropping system and biopesticides. The highest mean *C. bifoveolatus* was observed on maize sole crop cropping system treated with neem seed extract biopesticides and the lowest was observed on maize sole crop cropping system with no any biopesticides application, Also the highest mean of *Cotesia sp* was observed on Push pull technology (PPT) treated with *Bacillus thurigiensis* biopesticides and the lowest was observed on PPT treated with *Metarhizium anisopliae*. Then the highest mean of *C. luteum* was observed on Push pull technology treated with *Bacillus thurigiensis* biopesticides and the lowest was observed

on PPT treated with flubendamide as negative control. The results also showed significant effects of parasitism rate for *Chelonus bifoveolatus* and *Cotesia sp* on interaction between cropping system and biopesticides application, biopesticides and sampling week. The highest mean parasitism rate of *C. bifoveolatus* was observed on maize sole crop cropping system treated with neem seed extract biopesticides and the lowest was observed on maize sole crop cropping system with no any biopesticides application. The highest mean of *Cotesia sp* was observed on Push pull technology treated with *Bacillus thurigiensis* biopesticides and the lowest was observed on PPT treated with *Metarhizium anisopliae*. No emerged parasitoid species from eggs sampled from plots treated with Flubendiamide. Overall, both biopesticides and push pull technology demonstrated potential natural control methods for the invasive fall armyworm in maize fields in Morogoro Tanzania.

**DECLARATION**

I, **ERICK SIMON**, do declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work done within the period of registration and that it has neither been submitted nor concurrently being submitted for a degree award in any other institution.

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Date

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## **DEDICATION**

Dedicated to my lovable wife Betty, Damian Ndagara and my wonderful and incredible daughters Evelyne and Emilyne in gratitude for their support, sacrifices and time. May God bless them.

It is also dedicated with all respect to my parents Mrs. Imelda and Mr. Evodius Rwamtoga, my sisters and brothers for their constant encouragement.

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## TABLE OF CONTENTS

EXTENDED ABSTRACT .....	ii
DECLARATION .....	v
COPYRIGHT .....	vi
ACKNOWLEDGEMENTS .....	vii
DEDICATION .....	viii
TABLE OF CONTENTS .....	ix
LIST OF FIGURES .....	xvi
LIST OF ABBREVIATIONS AND SYMBOLS .....	xviii
CHAPTER ONE .....	1
1.0 GENERAL INTRODUCTION .....	1
1.1 Background Information .....	1
1.1.1 Origin and Distribution of FAW .....	3
1.1.2 FAW in Africa.....	4
1.1.3 Impact of FAW in Africa .....	5
1.1.3.1 Yield loss .....	5
1.1.3.2 Invasion of fall armyworm in Tanzania .....	5
1.1.3.3 Infestation caused by fall armyworm .....	6
1.1.3.4 Highlights of FAW in the Sub-Saharan Africa .....	6
1.1.3.5 Description of biology and lifecycle of fall armyworm .....	8
1.1.4 Injury symptoms of FAW .....	10
1.1.5 Conditions that favor survival of FAW .....	11
1.1.6 Pathways of spread of FAW.....	11
1.1.6.1 Environmental suitability in maize growing areas .....	12
1.1.6.2 Economic impact of fall armyworm in Africa .....	12

1.1.7	Management of FAW .....	13
1.1.7.1	Agronomic and cultural practices.....	13
1.1.7.2	Biological control and agents of fall armyworm.....	14
1.1.7.3	Advantages of using biological control of fall armyworm in Africa.....	15
1.1.7.4	Push pull technology (PPT) system.....	16
1.1.8	Description of <i>Azadirachta indica</i> A. Juss. (Meliaceae) as biopesticides .....	16
1.1.9	Description of <i>Bacillus thuringiensis</i> (Bt) .....	18
1.1.10	Description of <i>Metarhizium anisopliae</i> as biological control agent .....	19
1.2	Justification.....	19
1.3	Objectives .....	22
1.3.1	Overall objective .....	22
1.3.2	Specific objectives.....	22
1.4	References .....	23
CHAPTER TWO .....		35
EFFECTS OF BIOPESTICIDES ON DEVELOPMENTAL BIOLOGY OF FAW		
<i>Spodoptera frugiperda</i> (JE Smith) (Lepidoptera: Noctuidae) .....		35
Status: Submitted to TAJAS journal.....		35
2.1	Abstract.....	36
2.2	Introduction .....	37
2.3	Materials and Methods .....	39
2.3.1	Description of the study area.....	39
2.3.2	Laboratory bioassay of botanicals against FAW.....	39
2.3.2.1	Extraction of Azadirachtin from <i>Azadirachta indica</i> seeds ....	39

2.3.2.2	Thin layer chromatography (TLC) Confirmation .....	40
2.3.2.3	Identification of Azadirachtin compound from neem kernel powder .....	40
2.3.3	Maize planting.....	42
2.3.4	Establishment of FAW <i>Spodoptera frugiperda</i> (JE Smith) (Lepidoptera: Noctuidae) rearing unit.....	42
2.4.4	Experimental design.....	43
2.4	Data Collection.....	43
2.5	Data Analysis.....	44
2.6	Results .....	44
2.6.1	Effect of biopesticides on FAW egg incubation period .....	44
2.6.2	Effect of biopesticides on developmental duration of FAW Caterpillar....	45
2.6.3	Effect of biopesticides on developmental duration of FAW Pupa.....	46
2.6.4	Total developmental duration.....	47
2.7	Discussion.....	48
2.9	Conclusions and Recommendations.....	51
2.10	References .....	51
	CHAPTER THREE .....	59
	Paper two .....	59
	Effectiveness of Biopesticides-Enhanced Push-Pull Systems against FAW <i>Spodoptera frugiperda</i> (JE Smith) (Lepidoptera: Noctuidae) in Selected Maize Cropping Systems .....	59
3.1	Abstract.....	60
3.2	Introduction .....	61
3.3	Materials and Methods .....	62
3.3.1	Study area .....	62

3.3.2	Egg mass and larva collections .....	63
3.3.3	Experimental design .....	63
3.4	Data Collected .....	64
3.4.1	Infestation levels of Fall Armyworm and plant damage .....	64
3.4.2	Infestation level of FAW on maize crop .....	64
3.4.3	Maize grain yields assessment .....	65
3.5	Data Analysis.....	65
3.6	Results .....	65
3.6.1	Egg masses abundance in biopesticides and enhanced maize cropping system.....	65
3.6.2	FAW caterpillar abundance in biopesticides and enhanced maize cropping system.....	67
3.6.3	Infestation level of FAW on maize crop .....	69
3.6.4	Effects of FAW on Maize grain yield .....	70
3.7	Discussion.....	71
3.8	Conclusions and Recommendations .....	74
3.9	References .....	74
	CHAPTER FOUR.....	80
	Paper three .....	80
	Influence of Cropping systems and Biopesticides on parasitism rate and abundance of Parasitoids of FAW <i>Spodoptera frugiperda</i> (JE SMITH) (Lepidoptera: Noctuidae) in Maize Fields in Morogoro.....	80
4.1	Abstract.....	81
4.2	Introduction .....	82
4.3	Materials and Methods .....	83
4.3.1	Description of the Study Area .....	83

4.3.2	Experimental design .....	84
4.3.3	Rearing of fall armyworm .....	85
4.4.3	Identification of parasitoids species .....	86
4.4	Data collected .....	86
4.4.1	Parasitism rates .....	86
4.4.2	Relative abundance of parasitoids species .....	86
4.5	Data Analysis .....	87
4.6	Results .....	87
4.6.1	Total number of parasitoid species .....	87
4.6.2	Effect of cropping system and biobesticides in parasitism rate on recovered parasitoids species .....	88
4.6.2.1	Parasitism rate of <i>Chelonus bifoveolatus</i> Szépligeti .....	88
4.6.2.2	Parasitism rate of <i>Cotesia</i> sp .....	90
4.6.2.3	Parasitism rate of <i>C. luteum</i> .....	91
4.6.3	Relative abundance of recovered parasitoids species .....	93
4.6.3.1	Relative abundance of <i>Chelonus bifoveolatus</i> Szépligeti .....	93
4.6.3.2	Relative abundance of <i>C. luteum</i> .....	95
4.6.3.3	Relative abundance of <i>Cotesia</i> sp .....	97
4.7	Conclusions and Recommendations .....	102
4.8	References .....	102
	CHAPTER FIVE .....	110
5.0	GENERAL CONCLUSIONS AND RECOMMENDATIONS .....	110
5.1	Conclusions .....	110
5.2	Recommendations .....	111

## LIST OF TABLES

Table 3.1:	Description of the study areas .....	63
Table 3.3:	Analysis of variance for the effect of cropping system and biopesticides on FAW caterpillar abundance in Morogoro, Tanzania.....	68
Table 3.4:	Estimates for Poisson regression of infestation level of <i>Spodoptera frugiperda</i> as affected by cropping system, bio pesticides and Sampling weeks in Morogoro, Tanzania.....	69
Table 3.5:	Analysis of variance for the effect of cropping system and biopesticides on maize grain yield assessment on control of <i>Spodoptera frugiperda</i> in Morogoro, Tanzania .....	70
Table 4.1:	Description of the study areas .....	84
Table 4.2:	Parasitoid species collected in the maize crop field in late March, 2021 in Morogoro, Tanzania.....	88
Table 4.3:	Analysis of variance of Parasitism rate of <i>Chelonus bifoveolatus</i> Szépligeti on cropping system and biopesticides on maize crop in Morogoro, Tanzania .....	89
Table 4.4:	Analysis of variance for Parasitism rate of <i>Cotesia sp</i> on cropping system and biopesticides application plots on maize crop in Morogoro, Tanzania .....	90
Table 4.5:	Analysis of variance for Parasitism rate of <i>C. luteum</i> on the effect of cropping system and biopesticides application on maize crop in Morogoro, Tanzania .....	92
Table 4.6:	Analysis of variance for relative abundance of <i>C. bifoveolatus</i> on the effect of cropping system and biopesticides on maize crop in Morogoro, Tanzania .....	94

Table 4.7:	Analysis of variance for relative abundance of <i>C. luteum</i> on the effect of cropping system and biopesticides on maize crop in Morogoro, Tanzania .....	96
Table 4.8:	Analysis of variance on the relative abundance of <i>Cotesia sp</i> for the effect of cropping system and biopesticides on maize crop fields in Morogoro, Tanzania .....	98

## LIST OF FIGURES

Figure 1.1:	Presence of fall armyworm in Africa. ....	7
Figure 1.2:	Schematic representation of the agro-medicinal tree, <i>Azadirachta indica</i> indicating the potential of this tree as a biopesticides and therapeutic agent. ....	18
Figure 2.1:	TLC Plate on long UV length .....	41
Figure 2.2:	TLC plates of extracts under short UV light .....	41
Figure 2.3:	Effect of biopesticides on FAW egg incubation period .....	45
Figure 2.4:	Effect of biopesticides on FAW caterpillar duration .....	46
Figure 2.5:	Effect of biopesticides on pupae to adult duration.....	47
Figure 2.6:	Total developmental biology of FAW egg to adult.....	48
Figure 3.1:	Effect of interaction of cropping system and biopesticides on FAW egg masses in maize crop in Morogoro.....	67
Figure 3.2:	Effect of interaction of cropping system and biopesticides on FAW larvae abundances in maize crop in Morogoro .....	69
Figure 3.3:	Effect of cropping system and biopesticides on maize grain yield assessment for <i>Spodoptera frugiperda</i> control in Morogoro, Tanzania .....	71
Figure 4.1:	Effect of Parasitism rate of <i>Chelonus bifoveolatus</i> Szépligeti on cropping system and biopesticides on maize crop in Morogoro.....	89
Figure 4.2:	Effect of Parasitism rate of <i>Cotesia sp</i> on cropping system and biopesticides on maize crop in Morogoro. ....	91
Figure 4.3:	Effect of Parasitism rate of <i>C. luteum</i> on cropping system and biopesticides on maize crop in Morogoro.....	93
Figure 4.4:	Effect of relative abundance of <i>C. bifoveolatus</i> on cropping system and biopesticides on maize crop in Morogoro. ....	95

Figure 4.5: Effect of relative abundance of *C. luteum* on cropping system  
and biopesticides on maize crop in Morogoro. ....97

Figure 4.6: Effect of the relative abundance of *Cotesia sp* on cropping  
system and biopesticides on maize crop in Morogoro. ....99

**LIST OF ABBREVIATIONS AND SYMBOLS**

03	Ozone
<sup>0</sup> C	Degree Centigrade
Agriculture Seed	<i>Bacillus thuringensis</i>
Agency	
ANOVA	Analysis Of Variance
ASA	Agriculture Seed Agency
CABI	The Centre for Agriculture and Bioscience International
cm	Centimeter
CO <sub>2</sub>	Carbon dioxide
CRD	Completely randomized Design
DAP	Days after planting
DMRT	Duncan's Multiple Range Test
EPF	Entomopathogenic fungi
FAO	Food and Agriculture Organization
FAW	Fall armyworm
FE+VE	Flubendiamide as positive control
GDP	Gross Domestic Product
H	Hour
H <sub>2</sub> SO <sub>4</sub>	Sulphuric acid
HSD	Honestly Significant Difference
ID	Identification
IPM	Integrated Pest Management
kg	kilogram
km	kilometer

L	Litre
Ma	<i>Metarhizium anisopliae</i>
mm	Millimeter
MT	Metric tons
N	Total number of individuals of all parasitoid species
NE	Neem extract
NLGP	Neem leaf glycoprotein
OPV	Open pollinated variety
PL	Parasitized larvae
PP	Relative abundance
PPT	Push pull Technology
RCBD	Randomized Complete Block Design
Rf	Retardation factor
rh	Relative humidity
sat	Sustainable Agriculture Tanzania
se	Standard error
smc	Solomon Mahlangu Cumpus
SSA	Sub Saharan Africa
SUA	Sokoine University of Agriculture
TL	Total number of collected larvae
TLC	Thin layer Chromatography
TNEPP	Total number of egg batch per plant
US	United States
USD	United States Dollar
UV	Ultraviolet

## CHAPTER ONE

### 1.0 GENERAL INTRODUCTION

#### 1.1 Background Information

Fall armyworm (FAW) (*Spodoptera frugiperda*) (J.E. Smith) (Insecta: Lepidoptera: Noctuidae) is a major transboundary insect pest that has become a significant threat to food security and agricultural sustainability worldwide (FAO, 2020; Cokola *et al.*, 2021; Kleden and Simamora, 2021). It is a transboundary pest able to fly over 100 km in a single night (Bateman *et al.*, 2018). FAW, is a polyphagous pest native to tropical and sub-tropical regions of America. Most recently, the pest invaded Africa, with the first detections being reported in Central and Western Africa since early 2016 (Goergen *et al.*, 2016; Day *et al.*, 2017; Bateman *et al.*, 2018; Uzayisenga *et al.*, 2018 Harrison *et al.*, 2019; Kleden and Simamora, 2021). In late 2016 and 2017 FAW, spread to over 109 countries in parts of Southern (Goergen *et al.*, 2016; Day *et al.*, 2017), Eastern (Bateman *et al.*, 2018; De Groote *et al.*, 2020) and Northern Africa, and the Near East (Hruska, 2019; Cokola *et al.*, 2021) and Asia (Bateman *et al.*, 2018; De Groote *et al.*, 2020; Cokola *et al.*, 2021). Since then, it has become a very destructive invasive pest in sub-Saharan Africa. Its main impact is on maize crops, it attacks and affects the crop at different stages of growth, from early vegetative to physiological maturity (Bateman *et al.*, 2018; Hruska, 2019; Ngangambe and Mwatawala, 2020; FAO, 2020).

According to Malo and Hore (2020), FAW infest 186 host plant species in North and Central America. Pogue (2002), Bateman *et al.*, 2018 and Hruska (2019) reported that 353 host plant species principally Poaceae (maize, sorghum, rice, wheat, sugarcane, millet), Asteraceae (Sunflower), Malvaceae (cotton), Fabaceae (soybean), families, groundnuts, Potatoes, a number of fruit trees, ornamental plants, weed species, and

vegetable crops are also hosts to the FAW. According to Kleden and Simamora (2021), FAW that attack maize in the mid and late stages of maize growth can cause yield losses ranging from 15 to 73%, with a range of the number of plants affected by 55-100%. The reported losses vary depending on the age of the maize affected, the variety, and cultivation techniques employed. Its polyphagous nature presents challenges in management due to the presence of numerous alternative hosts outside the production season of main crops. In America, there are two races of FAW, namely the rice strain (R-strain), which is most consistently found in millet and grass species associated with pasture habitats, whereas the corn strain (C-strain) prefers maize and sorghum (Pogue, 2002; Hruska, 2019; De Groote *et al.*, 2020; Cokola *et al.*, 2021). The two strains of FAW have also been reported in Africa (Bateman *et al.*, 2018; Feldmann *et al.*, 2019; De Groote *et al.*, 2020).

Effective management of FAW through use of synthetic chemical pesticides faces challenges including improper use, unaffordability by smallholder farmers and development of resistance by the pest. Additionally, dispersal of FAW larvae into the lower maize plant canopy keeps them out of reach of topical insecticide sprays (Khan *et al.*, 2018). According to Khan *et al.* 2018, push-pull technology eliminate pesticide use and deploy natural processes are more suitable and cost-effective. Push-pull is a farming system intensification approach that involves attracting insect pests with trap plants (pull) such as *Brachiaria* grass, while driving them away from the main crop using a repellent intercrop (push), *Desmodium* spp., and attracting natural parasitoids and predators to the field (Midega *et al.*, 2018; Khan *et al.*, 2018).

The plant botanicals are among the promising alternatives to synthetic pesticide as it offered added advantages like repellent, and antifeedant and that's why, emphasized as an important tool in Integrated Pest Management (IPM) ( Keerth *et al.*, 2023).

Azadirachtin evolved in plant defense mechanisms against insects and that, when extracted and applied exogenously, can confer insecticidal, repellent, or antifeedant activities against the FAW larvae (Keerth *et al.*, 2023).

Fungi in the genus *Metarhizium* can establish endophytically inside plants and benefit them through growth promotion and pest suppression (Day *et al.*, 2017; Flonc *et al.*, 2021). Studies by Flonc *et al.*, 2021 reported that direct application of multiple strains of *M. anisopliae* caused significant and variable mortality up to 100% of eggs and neonate FAW.

*Bathillus thurigiensis* that express Cry proteins have been used effectively for control of the FAW in the United States, Canada, and several countries in South America and expected to use in several African countries (Botha. 2020).

### **1.1.1 Origin and Distribution of FAW**

FAW is pest that is currently terms as almost cosmopolitan in distribution. It was initially detected in Central and Western Africa in early 2016 (Benin, Nigeria, São Tome and Principe, and Togo), and within two years, it was reported to have invaded all sub-Saharan Africa including Tanzania, except Lesotho (Rwomushana *et al.*, 2018; Kasoma *et al.*, 2020). The pest has now been confirmed also in Asian countries including: India, Bangladesh, Sri Lanka, Thailand, Myanmar, China (including Taiwan Province of China), Indonesia, the Philippines, the Lao People's Democratic Republic, Malaysia, Viet Nam,

Cambodia, the Republic of Korea, Japan; and in the Near East, Sudan, Yemen and Egypt (Baudron *et al.*, 2019; Kasoma *et al.*, 2020).

The ability to spread rapidly into new areas, multiply and establish economic pest population levels quickly makes FAW a huge threat to food security and the rural livelihoods of millions of people in places where it has invaded. Before being first detected in Africa in 2016, FAW was already known to be a serious economic pest of maize especially, in tropical and subtropical areas of the Western Hemisphere. By crossing the Atlantic Ocean to the Eastern Hemisphere, and rapidly spreading through countries and continents, FAW's status quickly changed to become a global pest. It has huge impacts and implications the world (Bateman *et al.*, 2018).

The rate of spread and places of establishment in the new Hemisphere, in addition to its wide host-range, indicate that the risk of spread to even more places within countries, to new countries and regions in the Eastern Hemisphere is high. Without a coordinated and committed, large global intervention effort, FAW has proven it will prevent achievement of the 2030 Agenda for Sustainable Development Goals (SDGs). Already, its current distribution in Africa (including Tanzania), Asia and the Near East constitutes a threatening to food security and livelihoods of a global magnitude (Di Marco *et al.*, 2020).

### **1.1.2 FAW in Africa**

Rapid spread has continued and now 44 countries in Africa are affected (Rwomushana *et al.*, 2018). There are no reports from North Africa, but FAW has reached the Indian Ocean islands including Madagascar (Rwomushana *et al.*, 2018). Environmental suitability modelling suggests almost all areas suitable for FAW in sub-Saharan Africa

are now infested. Spread directly across the Sahara is unlikely. But if FAW does establish in the small suitable areas in North Africa, it would become a risk to Europe through migration (Rwomushana *et al.*, 2018; Bateman *et al.*, 2018; Baudron *et al.*, 2019).

### **1.1.3 Impact of FAW in Africa**

#### **1.1.3.1 Yield loss**

FAW attacks maize crop from seedling to ear development. The pest causes considerable yield losses in maize and in other key staple cereal crops, threatening food security and the livelihoods of hundreds of millions of smallholder farmers and consumers. It is estimated that, for 12 African countries alone, in Africa including Tanzania FAW could cause yield losses of 8.3 million to 20.6 million metric tons of maize annually, equivalent to economic loss value of between USD 2.5 billion to USD 6.2 billion, and enough to feed 40 million to 100 million people (FAO.2020). Under farmer level, the FAW can cause up to 100% yield loss (Day *et al.*, 2017; Toepfer *et al.*, 2019; Assefa and Ayalew, 2019; Naharkia *et al.*, 2020; Kleden and Simamora, 2021). According to Sisay *et al.* (2019), in Tanzania FAW percent of infested maize fields ranged from 93% to 100%.

#### **1.1.3.2 Invasion of fall armyworm in Tanzania**

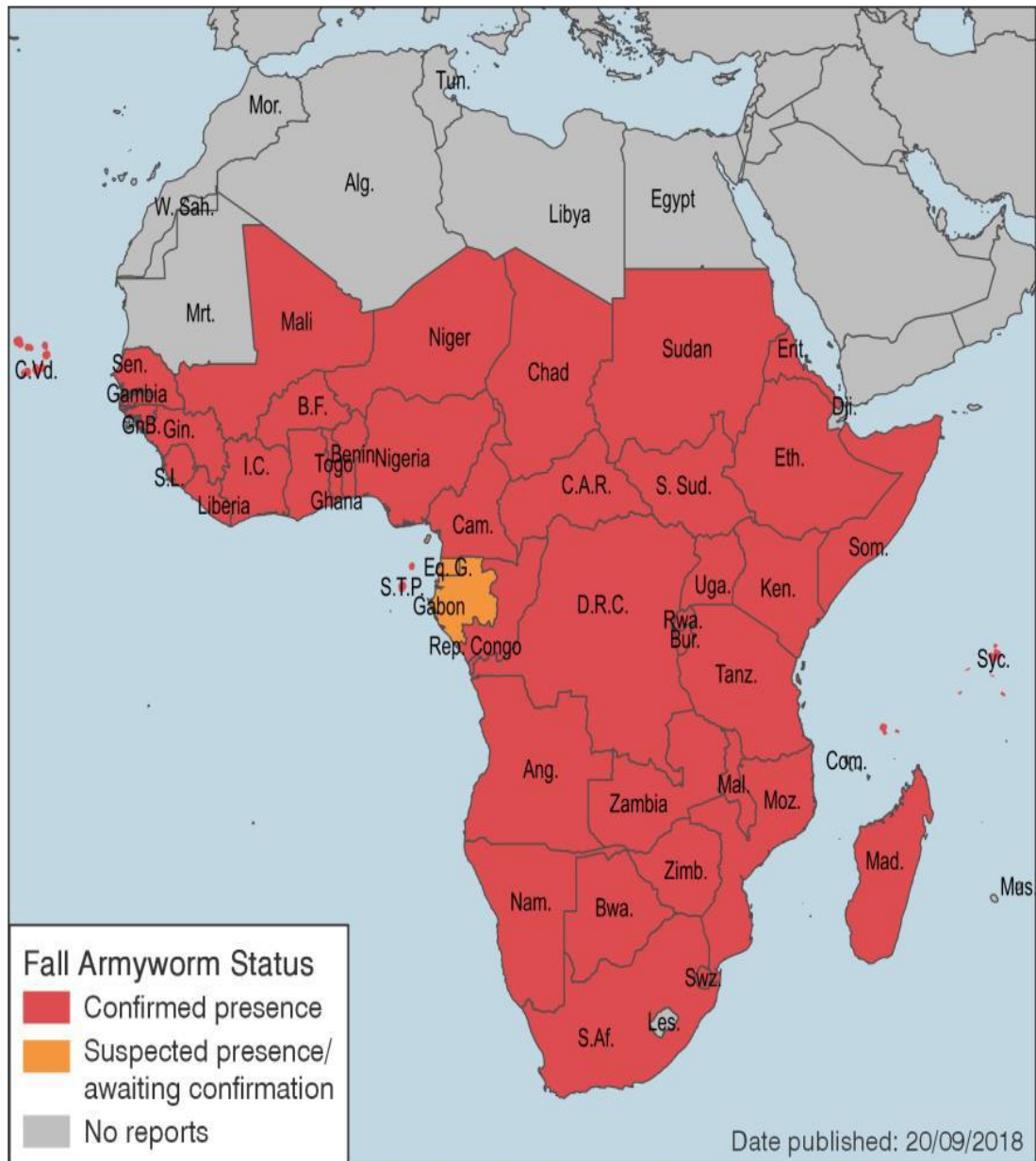
*Spodoptera frugiperda* (J.E. Smith) (Insecta: Lepidoptera: Noctuidae) is a strain of Fall Armyworm (FAW) native to South and Central America was initially confirmed by Tanzania authorities. According to Lobulu *et al.* (2019), the invasion of FAW started in February 2017 in Rukwa and thereafter reported in the border areas of Ruvuma and Mbeya, located in southern Tanzania. The fall armyworm is new in Africa and its caterpillars cause severe damage to more than 80 plant species especially cereal crops such as maize and rice at all stages and spreads very fast in the early stages (Suleiman and Kurt, 2015; Baudron *et al.*, 2019).

### **1.1.3.3 Infestation caused by fall armyworm**

In 2017, the percentage of infested fields ranged from 33% to 100% in Ethiopia, 93% to 100% in Tanzania and was 100% for the farms observed in Kenya (Sisay *et al.*, 2019; Hurska. 2019). In Ethiopia, the highest infestation was found in Shebe Senbo (62.3%), whilst the lowest infestation was recorded in Bahir Dar (5.7%). In Kenya, relatively high percentages of FAW infestation were observed, ranging from 77% in Mt Elgon to 100% in Webuye East and Tongaren in 2017(Sisay *et al.*, 2019). In Tanzania, Morogoro and Kilombero showed 72.7% and 95.7% infestation, respectively. Leaf damage score ranged from 1.8 to 7 in Ethiopia, 3.2 to 5.3 in Kenya and 3.7 to 5.2 in Tanzania in 2017 (Sisay *et al.*, 2019).

### **1.1.3.4 Highlights of FAW in the Sub-Saharan Africa**

Up to June 2017, FAW were already detected and officially confirmed in 19 Sub-Saharan countries where five countries are from east Africa.



**Figure 1.1: Presence of fall armyworm in Africa.**

Source: Rwomshana *et al.*, 2018)

### **1.1.3.5 Description of biology and lifecycle of fall armyworm**

A key feature of FAW biology is that, it does not diapause, hence several generations can overlap within a single crop cycle when conditions are suitable (Rwomushana *et al.*, 2019). In several African countries including Tanzania, FAW generations have been continuously observed throughout the year, wherever host plants are available, including off-season and irrigated crops (Prasanna *et al.*, 2018; Rwomushana *et al.*, 2018). In such areas buildup of the population is more likely, and main season crops are more likely to be infested early. Sharanabasappa *et al.* (2018) reported that male and females complete their lifecycle in 32-43 and 34-46 days, respectively at  $26\pm 2^{\circ}\text{C}$ , 75 to 80% RH and L12: D12 photoperiod. The number of generations occurring in an area varies with the appearance of the dispersing adults. Depending on the migrating adults and climate, FAW can have up to eight generations per year in maize fields in tropical areas (Busato *et al.*, 2005; Barros *et al.*, 2010; Montezano *et al.*, 2019). According to Johnson (1987), FAW did not enter into diapause. Seasonal migration is a major factor in the life history of FAW and it is considered one of the most mobile noctuid crop pests (Nagoshi and Meagher, 2008).

#### **Egg**

The egg is dome shaped; the base is flattened and the egg curves upward to a broadly rounded point at the apex. The egg measures about 0.4 mm in diameter and 0.3 mm in height. Eggs are laid at night mostly on the upper and sometimes on the lower surface of the leaves of the host plant, but occasionally they may lay on other parts of the host plants (Malo and Hore, 2020). Eggs are usually laid in masses of about 100-200 eggs, and total egg production per female averages about 1500 with a maximum of over 2000 (Rwomushana *et al.*, 2019). The eggs of FAW are white in color and later turn into brown. The newly hatched eggs are covered with a protective, felt-like layer of white scales

(setae) from the female abdomen and this gives a moldy appearance, but eggs laid by mature females are less or not covered and visible with naked eyes or through hand lens. Duration of the egg stage is only 2 to 3 days (20-30°C) (Sharanabasappa *et al.*, 2018).

### **Caterpillar**

The FAW typically has six larval instars. The young larvae feed where eggs were laid; the first two instars feed gregariously on the young leaves, causing a characteristic skeletonizing or 'windowing' effect. Later instar feed near the funnel and upper leaves leaving sawdust-like frass which can be an easily spotted sign of larval feeding (Day *et al.*, 2017). In a young crop, this feeding can kill the growing point, a symptom called 'dead heart' in maize, which prevents any cobs forming (Day *et al.*, 2017; Padhee and Prasanna, 2019). Young larvae hide in the funnel during the day but emerge at night to feed on the leaves (Day *et al.*, 2017). In young plants, the stem may be cut, providing evidence of injury. Older larvae stay inside the funnel and so are protected from insecticide spray applications and natural enemies. In older plants, the larger larvae can bore into the developing reproductive structures, such as maize cobs, reducing yield quantity and quality (Day *et al.*, 2017). Larger larvae are cannibalistic and have the ability to dominate interspecific competitors and reduce intraspecific rivals (Sokame *et al.*, 2021). Due to this high level of cannibalism, which commences in the third instar, only between one and three fully grown larvae remain per plant, in spite of very high initial numbers of neonates per plant (Day *et al.*, 2017; Toepfer *et al.*, 2019). Larval development takes 14-21 days at 26<sup>0</sup>C (Sharanabasappa *et al.*, 2018). Larger larvae are nocturnal. The larva of FAW can be identified by four characteristic spots on the second to last segment, forming a square or rectangle. Moreover, the head of the mature larva has an inverted "Y" mark (Day *et al.*, 2017; Toepfer *et al.*, 2019; Sharanabasappa *et al.*, 2018).

**Pupa**

Pupation normally occurs 2-8 cm deep in the soil, but also occurs in mature leaf bases or maize ears. If the soil is too hard, larvae may web together leaf debris and other material to form a cocoon on the soil surface. Duration of the pupal stage is about 9 to 13 days at 26°C (Sharanabasappa *et al.*, 2018; Malo and Hore, 2020; FAO, 2020).

**Adult**

Adults are nocturnal, and are most active during warm, humid evenings. Adults use their natural pre-oviposition period to fly for many kilometers before they settle to oviposit, sometimes migrating for long distances. After a preoviposition period of 3 to 4 days, the female moth normally deposits most of her eggs during the first 4 to 5 days, although some females have been reported to oviposit for up to 3 weeks (Johnson, 1987; Sharanabasappa *et al.*, 2018). Duration of adult life is estimated to average about 10 days, with a range of about 7 to 21 days (Capinera, 1999; Sharanabasappa *et al.*, 2018; Matti and Patil, 2019).

**1.1.4 Injury symptoms of FAW**

The pest can affect the crop at different stages of growth, from early vegetative to physiological maturity. When the plants are young and the leaf tissues are soft, first-instar FAW larvae produce clusters of pinhole-type injury or small, round “window panes”. Later on, as the leaf tissues mature and become more fibrous and tough, window panes may be scattered and elongated rather than clustered. The width of the window panes reflects the width of the larval head capsule. Whorl-feeding-injury results from damaged-leaves expanding out of the whorl, producing a horizontal series of holes across a “pinch” in the leaf. Later larval instars chew larger holes, causing ragged whorl leaves, and produce sawdust like larval droppings, while fresh feeding produces big lumps. Injury to

cobs may lead to fungal infection and aflatoxins, and loss of grain quality. When badly infested fields may look as if they have been hit by a severe hailstorm, they feed inside whorls and can destroy silks and developing tassels, thereby limiting fertilization of the ear. Larvae move to the ear zone and start feeding after tassel emergence because they get exposed to natural enemies (Rwomushana *et al.*, 2019; Matti and Patil, 2019; FAO, 2020; Malo and Hore, 2020).

### **1.1.5 Conditions that favor survival of FAW**

The rate at which FAW larvae develop is affected by diet, temperature and humidity. Rising temperatures increase rates of insect development and the number of pest generations. In cooler climates, development slows down to one or a few generations per year. The optimum temperature for larval development is 28°C (development can take place within a range of between 11°C and 30°C), although the egg stage and pupal stage require slightly lower temperatures. Frost kills the insect. Rainfall may wash off some of the immature stages of the insect and wind speed may aid the dispersal of moths. Higher carbon dioxide (CO<sub>2</sub>) and ozone (O<sub>3</sub>) concentrations may change natural enemy numbers and behavior that might affect host-finding and predation. While FAW can damage maize plants in nearly all stages of development, it will concentrate on later plantings that have not yet silked. FAW can only be effectively managed in early instars. Managing late instars is more difficult and costly (Sparks, 1979; Huang *et al.*, 2020).

### **1.1.6 Pathways of spread of FAW**

The rapid spread of FAW in Africa can be attributed to the strong flight capacity of the insect, though it is possible that it was already more widespread than realised when first detected, and the apparent rapid spread was in part due to the spread of awareness. The rapid spread to the Indian Ocean Islands is harder to explain by natural flight, so it is

possible that the frequent flights from the mainland to those countries could have played a part. Rwomushana *et al.* (2018) reported that potential pathways of spread included unaided dispersal by wind-assisted flight, as contaminants of traded commodities, and as stowaways on or in aircraft. Wind-assisted flight alone might not have been sufficient for FAW to cross the Atlantic, but once in Africa all the pathways listed could have occurred, including for the spread to the Indian Ocean Islands. It is still not clear whether there were multiple introduction events or a single event involving multiple individuals from both strains (Rwomushana *et al.*, 2018; Tambo *et al.*, 2020).

#### **1.1.6.1 Environmental suitability in maize growing areas**

Although large areas of sub-Saharan Africa are highly suitable, not all those areas are major maize-growing zones. Although the relationship between environmental suitability and population size which is similar to damage has not been determined. It is reasonable to assume that areas of maize production with a high environmental suitability index are those where the risk of economic loss is greatest (Rwomushana *et al.*, 2018).

#### **1.1.6.2 Economic impact of fall armyworm in Africa**

The invasion of FAW threatens the food security of more than 200 million people in Africa whose main staple crop is maize. Based on preliminary estimates in 12 African maize-producing countries, in the absence of proper control methods, *S. frugiperda* has the potential to cause maize yield losses of 8.3 to 20.6 million metric tons per year. This represents a range of 21-53% of the annual production of maize averaged over a three-year period in these countries. The value of these losses is estimated at between US \$2.5 to 6.2 billion (Day *et al.*, 2017).

## **1.1.7 Management of FAW**

### **1.1.7.1 Agronomic and cultural practices**

Agronomic practices including habitat management can suppress or avoid pest damage through a variety of mechanisms, including conserving and encouraging the proliferation of natural enemies. Planting promptly can in some cases allow the maize crop to escape FAW attack (Rwomushana *et al.*, 2019). According to Baudron *et al.* (2019), FAW damage was found to be significantly reduced by frequent weeding operations and by minimum and zero-tillage. However, native grasses and weeds may also host natural enemies of FAW (Hay-Roe *et al.*, 2016). Also Rivers *et al.* (2016) reported that Maize production under zero or minimum tillage tends to reduce FAW damage in the America because it favored population build up for predatory species.

Studies by Hailu *et al.* (2018), summarized that intercropping maize with edible legumes significantly reduced FAW and stem-borer infestation and damage. Yigezu and Wakgari (2020) indicated that non-host legumes, such as bean, when intercropped with maize, significantly reduced FAW infestation and injury on maize. Intercropping was also reported as one of the control strategies used by farmers in the America (Assefa and Ayalew, 2019; De Groote *et al.*, 2020). It is believed that intercropping of two or more crops, or inclusion of non-host crop plants in the field, can reduce FAW oviposition on the maize plant. Yigezu and Wakgari (2020) reported different cultural practices that have been utilized across SSA in managing and controlling FAW infestation and maize yield losses. These include handpicking and killing of larvae, placing sand or wood-ash in whorls of maize plants, drenching plants with tobacco extracts, deep plowing to kill overwintering pupae, early planting, destruction of ratoon host plants, burning infested crop residues after harvesting, intercropping with non-host plants, use of multiple

cultivars, and rotation with non-host crops. (Matova *et al.*, 2020; Yigezu and Wakgari, 2020).

### **1.1.7.2 Biological control and agents of fall armyworm**

According to Eilenberg *et al.* (2001), biological control or biocontrol is the use of living organisms to suppress the population density or impact of a specific pest organism, making it less abundant or less damaging than it would otherwise be. Natural parasitism levels of more than 44% have been recorded in fields without application of pesticides in the America (Hruska, 2019; De Groote *et al.*, 2020), which has implications for the development and recommendation of control strategies in SSA. A study by Sisay *et al.* (2018) in Ethiopia, Kenya, and Tanzania, identified five native species of parasitoids, some with parasitism levels as high as 45.3%. These include *Cotesia icipe* (Fernandez-Triana and Fiobe), *Palexorista zonata* (Curran), *Coccygidium luteum* (Brulle), *Charops ater* (Szépligeti), and *Chelonus curvimaculatus* (Cameron). Several other studies have also identified natural enemies of FAW, with the same high levels of parasitism. Kenis *et al.* (2019) reported the presence of *Telenomus remus* (Dixon) in at least five SSA countries. Agboyi *et al.* (2020) observed 10 species of parasitoids (*T. remus*, *Chelonus bifoveolatus* (Szpligeti), *Trichogramma sp.*, *C. luteum*, *C. icipe*, *Meteoridea cf. testacea* (Granger), *Charops sp.*, *Metopius discolor* (Tosquinet), *Pristomerus pallidus* (Kriechbaumer), *Drino quadrizonula* (Thomson) in Benin and Ghana.

Entomopathogens are pathogens that affect insects and naturally regulate FAW populations in the Americas (Molina-Ochoa *et al.*, 2003). These have also been observed to control FAW in some farmers' fields in Africa (Matova *et al.*, 2020). Studies by Assefa and Ayalew (2019) reported that FAW was susceptible, in the Americas, to 16 species of entomopathogens, mainly viruses and bacteria. They highlighted that the occurrence and distribution of bio-control agents, including entomopathogens, was dependent on their

habit and determined by geographical location, agricultural practices, and insecticide use. The FAW larvae killed by viruses and fungi are easy to identify. Larvae killed by viruses become soft, usually with head hanging down from the leaves, while those killed by fungi become hard, appearing frozen on leaves with a whitish or light green color (Matova *et al.*, 2020). Farmers in Central America recycle fungal spores and viroid particles through spraying strained entomopathogen filtrate of larvae killed by viruses and fungi into the whorls of maize plants infested by FAW (Matova *et al.*, 2020). This promotes continued infection and death of FAW caterpillar by entomopathogens, which results in reduced FAW populations in maize fields. Virus-based bio-pesticides have also been used in the Americas (Ruiz-Najera *et al.*, 2013; Matova *et al.*, 2020), but these may need testing and registration in Sub Saharan Africa (SSA). Bio-pesticides that have been tried and showed potential include viruses, such as nuclear polyhedrosis virus, bacteria, such as *Bacillus thuringiensis* (*Bt*) and fungi, such as *Metarhizium* and *Beauveria* spp (Matova *et al.*, 2020).

#### **1.1.7.3 Advantages of using biological control of fall armyworm in Africa**

The smallholder-based maize-production systems in Africa are diverse especially in terms of size, biodiversification/mixed cropping, seasonality, and other characteristics, rather than growing maize alone on huge area of land as it is practiced under the large-scale commercial monocropping systems of the developed world. Also, the use of pesticide sprays on maize at present are less in Africa compared to other parts of the world. These are ideal conditions for effective conservation of natural enemies to a large number and establishment in the field in long run and achieving the full benefits of biological control. Biological control, particularly classical and conservation biological control, is much cheaper and benefits smallholder production systems in Africa (Soul-kifouly *et al.*, 2016).

#### **1.1.7.4 Push pull technology (PPT) system**

The PPT is a novel cropping system that is based on intercropping maize with Greenleaf desmodium *Desmodium intortum* (Mill.). (Leguminaceae) (Push) and bordering the intercrop with *Brachiaria* 'Mulato II'. (Pull) (Midega *et al.*, 2018; Matova *et al.*, 2020). The desmodium intercrop emits cues that are repugnant to oviposition female moths thus acting as a 'push', while a grass such as *Brachiaria* 'Mulato II' emits attractive cues that 'pull' the moths towards it. These companion plants thus release behaviour-modifying plant chemicals to manipulate the distribution and abundance of stemborers, FAW and beneficial insects for management of the pests (Khan *et al.*, 2014). Midega *et al.* (2018) and Matova *et al.* (2020) reported that FAW infestation can be reduced by at least 80% in a field where the technology is being practiced. Studies by Hailu *et al.* (2018), reported FAW infestation levels of 36–38% on maize under PPT, which were significantly lower compared to 95% infestation observed under sole cropping. Push–pull technology reduced FAW infestation in maize better than maize–legume intercropping (Hailu *et al.*, 2018; Matova *et al.*, 2020).

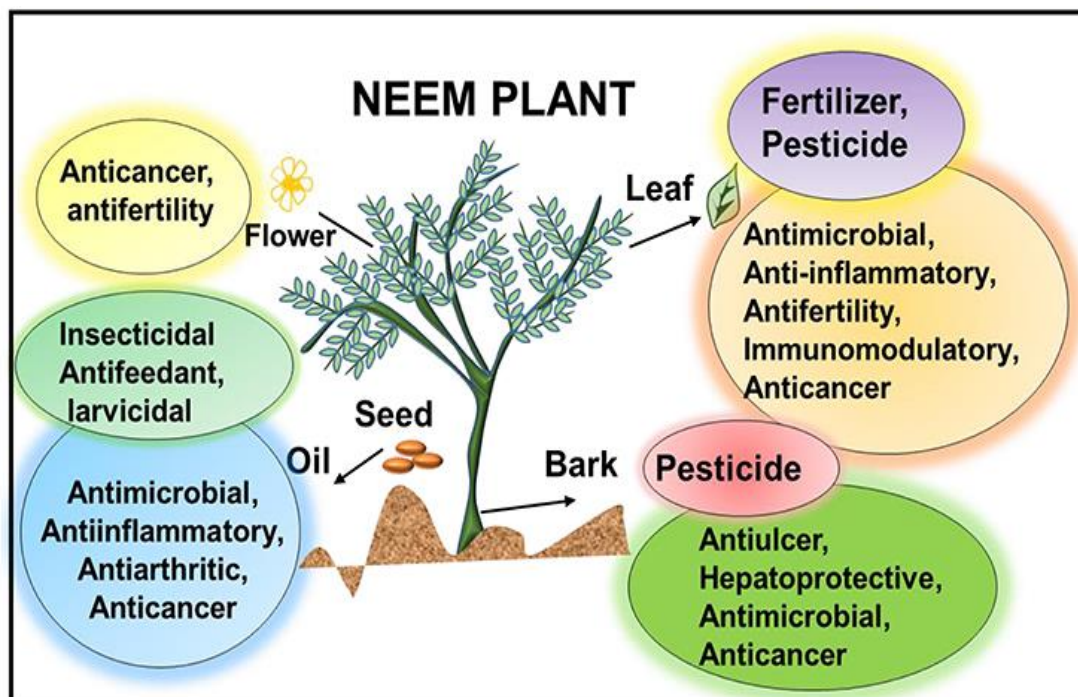
#### **1.1.8 Description of *Azadirachta indica* A. Juss. (*Meliaceae*) as biopesticides**

The use of synthetic pesticides has led to disturbances in the environment, causing pest resistance and toxicity to non-target organisms. In some cases, these synthetic pesticides have caused acute and chronic poisoning to farmworkers, applicators and even consumers, thus making it imperative to adopt alternative means (Chaudhary *et al.*, 2017; Keerth *et al.*, 2023) . One of the significant alternative strategies is employing botanical pesticides, which is the most efficient means to replace the wide use of synthetic pesticides. Among these, plant based biopesticides using plant extracts have proved to be the most efficient way of insect control (Keerth *et al.*, 2023). These herbal pesticides aid the agricultural yield, as they can be used as insecticides, fumigants, manures, urea

coating agent or soil conditioners. They can be used alone, or in combination with other herbs, so as to increase the insecticidal efficacy (Chaudhary *et al.*, 2017).

Neem has employed as a highly potent bio-pesticide. It is evergreen, fast-growing plant that offers immense antifeedant properties due to its efficacy in suppressing the feeding sensation in insects including FAW, at concentrations even less than 1 parts per million (Chaudhary *et al.*, 2017). It is a resistant tree that thrives in a sub-humid to sub-arid climate with an annual rainfall of 400–800 mm (Chaudhary *et al.*, 2017). It comprises of more than 200 allelochemical prevalent in variable concentrations in the different parts of the plant, providing a variety of pesticidal properties (Koul *et al.*, 2004; Raut *et al.*, 2014; Chaudhary *et al.*, 2017). Seeds from this tree comprises of 40% of oil with azadiractin as the major active ingredient that is mainly responsible for the insecticidal activity of neem (Chaudhary *et al.*, 2017). Additionally, neem leaves have been used for centuries against the stored grain pests due to its repellent properties (Chaudhary *al.*, 2017; Rioba and Stevenson, 2020). Collectively, all parts of this plant are known to exhibit by-products that inherently impart an internal chemical defense making neem free from the pest attack, which can also be exploited to develop an efficient pest control strategy.

Most importantly an active ingredient of neem known as NLGP has now evolved as a potent immunomodulatory agent (Chaudhary *et al.*, 2017), thus making it an ideal agro-medicinal plant. This unique attribute of neem makes it an ideal bio-pesticidal agent, as it does not cause non-specific toxicity to mammals (Chaudhary *et al.*, 2017).



**Figure 1.2: Schematic representation of the agro-medicinal tree, *Azadirachta indica* indicating the potential of this tree as a biopesticides and therapeutic agent.**

Source: (Chaudhary *et al.*, 2017)

### 1.1.9 Description of *Bacillus thuringiensis* (Bt)

*Bacillus thuringiensis* (Bt) are gram-positive spore-forming bacteria with entomopathogenic properties. Bt is a viable alternative for the control of insect pests in agriculture since are completely biodegradable (Bravo and Soberon, 2007). The application of *Bt* to combat invertebrates of human interest gained momentum with the growing demand for food free of chemical pesticides and with the implementation of agriculture methods that were less damaging to the environment (Roh, 2007).

### **1.1.10 Description of *Metarhizium anisopliae* as biological control agent**

The entomopathogenic fungus *Metarhizium anisopliae* (Metschn.) Sorokin is widely used for biocontrol of insects pest (Zimmermann, 2007; Barra-Bucarei *et al.*, 2016). The infection process of *M. anisopliae* is similar to other entomopathogenic fungi. The infection pathway includes several steps such as attachment of the spore to the cuticle, germination and formation of appressoria, penetration through the cuticle, overcoming of the host response and immune defense reactions of the host, spreading within the host by formation of hyphal bodies or blastospores, and outgrowing the dead host and production of new conidia. During the invasion of the whole insect body, nutrients in the hemolymph and the fat body are depleted. This is followed by the death of the insect and the end of the pathogenic process whereby sporulation occurs, and extrusion of the hyphae is observed when the green mycelium is formed on the carcass of the insect (Barra-Bucarei *et al.*, 2016; Rajula *et al.*, 2021)

## **1.2 Justification**

Tanzania has been ranked as the first and the fourth major maize producer for East Africa and sub Saharan Africa respectively (Makirita *et al.*, 2019). Apart from the importance of maize to feed the increasing population in Africa, its production is challenged with pest infestation among other factors (Suleiman and Rosentrater, 2015; Makirita *et al.*, 2019). Currently, the production is constrained with FAW (*Spodoptera frugiperda*) infestation which is a new invasive alien pest native to America (Goergen *et al.*, 2016; Makirita *et al.*, 2019). The pest is reported to cause massive crop damage in almost all African countries including Tanzania (Makirita *et al.*, 2019). FAW may cause up to 100 percent crop loss (Lobulu *et al.*, 2019).

Despite the number of biological control agents commercially available, the efficacy and safety of the products, and the many success stories using various biological control techniques, there is still a lack of uptake in biological control (Shah and Pell , 2003; van Lenteren, 2012; Barratt *et al.*, 2018; Messing and Brodeur, 2018). Although synthetic insecticides play an important role in FAW management, it also led to development of resistance to insecticide in FAW populations, environmental constraints, limited shortage of information, inaccessibility of appropriate and effective products and high costs of the products (Bateman *et al.*, 2018; Hailu *et al.*, 2018; Sisay *et al.*, 2019; Ngangambe and Mwatawala, 2020). Biopesticide use in Tanzania is not a new occurrence, but it is classified mainly as indigenous knowledge. Few institutions have been in the frontline in biopesticide research; these include Tanzania Pesticide Research Institute (TPRI), Sokoine University of Agriculture (SUA), Nelson Mandela Institute of Technology (NM-IT) and Tanzania Agricultural Research Institute- Naliendele (Moshi and Matoju, 2017). *Bathillus thrungiensis*, push pull using *Brachiaria cv Mulato II* and green desmodium and botanical extracts (Neem seeds) control packages have long been proposed as attractive alternatives to synthetic insecticides for pest management, thus they are eco-friendly, economical, usually target-specific, biodegradable and safer to environment (Bateman *et al.*, 2018; Hailu *et al.*, 2018 ; Kassie *et al.*, 2018; Cheruiyot *et al.*, 2018; Midega *et al.*, 2018; Sisay *et al.*, 2019; Feldmann *et al.*, 2019). It is imperative to use both biopesticides and cropping systems strategies for FAW, because are among of the promising alternative against FAW.

There is inadequate information on the efficacy of botanical extracts and EPFs to deter or kill FAW on maize in East Africa. Very little research is being carried out on the use of these methods (Hruska, 2019). According to Akutse *et al.* (2019), *Metarhizium anisopliae* and *Beauveria bassiana*, have also shown efficacy against eggs and second-instar larvae

of FAW, where *B. bassiana* caused moderate mortality of 30% to second-instar larvae, while *M. anisopliae* caused egg mortalities of 79.5-87.0% under laboratory conditions. Also, Ngangambe and Mwatawala (2020) reported that, in Tanzania, *M. anisopliae* and *B. bassiana* are likely to reduce FAW larval density and infestation by 55% and 69% respectively. Furthermore, infestation of maize by FAW can be lowered by 42% and 26% by *M. anisopliae* and *B. bassiana* treatments respectively.

According to Sisay *et al.* (2019), in Ethiopia, *Azadirachta indica* as among of botanical pesticides tested, resulted in the highest percentage larval mortality (>95%) 72 hours after application. According to Makate *et al.* (2018) and Kassie *et al.* (2018), over 176 000 smallholder farmers in eastern Africa where maize yields have increased from about 1 t/ha to over 3.5 t<sup>-1</sup>h., achieved with the use of botanical pesticides specifically neem seed extract. Economically, the Push-pull technology achieves higher factor productivity and returns on farmers' investments, which makes it both an affordable and sustainable IPM option for Africa. According to Bravo *et al.* (2007), upon sporulation, *Bt* produces insecticidal crystal inclusions that are formed by a variety of insecticidal proteins called Cry or Cyt toxins. These toxins show highly selective spectrum of activity killing a narrow range of insect species. *Bt* has been used to manage insects including testing for FAW pest (Yang *et al.*, 2022: Wang *et al.*, 2022). Therefore the overall objective of this study was evaluation of natural control methods against FAW for improved maize productivity. These information shall be the basis for implementing optimisation of biopesticides and cropping system of enhanced- push pull technology and botanical extracts against FAW in large scale experiments, which provides a novel alternative for management of insect pests in relation to environmental sustainability for both small and large scale agriculture.

### **1.3 Objectives**

#### **1.3.1 Overall objective**

Improve maize productivity through optimization of natural control methods against fall armyworm (*Spodoptera frugiperda*) for improved maize productivity.

#### **1.3.2 Specific objectives**

- i. To determine effects of biopesticides on developmental biology of FAW.
- ii. To evaluate effectiveness of biopesticides-enhanced push-pull systems against FAW in selected maize cropping systems.
- iii. To determine influence of Cropping systems and biopesticides on parasitism rate and abundance of Parasitoids of FAW *Spodoptera frugiperda* (JE SMITH) (Lepidoptera: Noctuidae) in Maize fields in Morogoro

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**CHAPTER TWO**

**Paper One**

**EFFECTS OF BIOPESTICIDES ON DEVELOPMENTAL BIOLOGY OF FAW**

*Spodoptera frugiperda* (JE Smith) (*Lepidoptera: Noctuidae*)

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## 2.1 Abstract

Fall armyworm (FAW, *Spodoptera frugiperda* (J.E. Smith), Lepidoptera: Noctuidae), is a highly mobile and polyphagous herbivore threatening crop production and the livelihoods of millions of smallholder farmers in the newly invaded areas in Africa including Tanzania. This study aim to determine the effect of biopesticides on developmental biology of FAW. A completely randomized design (CRD) with four replications was adopted to determine developmental biology of the FAW using biopesticides *B. thuringiensis* Bt, *M. anisopliae* and *A.indica* seed extract with lower dose of 2 mls, 2mls and 30g per one litre of water respectively, were tested against FAW in the laboratory. Results showed the developmental duration of FAW stages differed significantly among treatments. Egg incubation, larval duration, pupa stage duration and the total developmental duration were significantly longer ( $p < 0.001$ ) on the biopesticides treated colony compared to untreat ones. The shortest developmental duration of FAW stages were observed on control colonies at  $2.1 \pm 0.18$ ,  $14.88 \pm 0.18$ , and  $27.7 \pm 0.34$  days for egg, larva and pupa stages respectively. The longest developmental duration was observed in colonies under *B. thuringiensis* treatment ( $3.5 \pm 0.37$  SE,  $22.03 \pm 0.59$  SE,  $12.68 \pm 0.23$  SE and  $37.7 \pm 0.54$  SE) days for egg, larva and pupa stages respectively. These results confirmed that bio pesticides can work effectively to keep FAW levels under control, reducing the need to apply synthetic pesticides when used in conjunction with good crop management in Morogoro region.

**Keywords:** *B. thuringiensis*, bio pesticides, developmental duration, neem extract *M. anisopliae*, *Spodoptera frugiperda*.

## 2.2 Introduction

Fall armyworm (FAW), *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae), a highly transboundary migratory moth native to the America, was first detected in Central and Western Africa in early 2016 (Goergen *et al.*, 2016; FAO and CABI, 2019; Dorucher *et al.*, 2020; Ngangambe and Mwatawala, 2020), and since then it has spread across the African and Asian continents causing significant damage to maize, its principal food source (Rwomushana *et al.*, 2018; Li *et al.*, 2019; Sisay *et al.*, 2020; FAO, 2019; Cokola *et al.*, 2021).

Recent concern for the negative effects of synthetic insecticides on the environment and human health has provided the impetus for a reappraisal of the utility of *A.indica* as crop protectants (Vargas-Osuna 2001). Biopesticides has consequences in population dynamic of susceptible species (Vargas-Osuna 2001). Entomopathogens can suppress fall armyworm populations in at least three ways: 1) optimization of naturally occurring diseases, 2) introduction and colonization of pathogens into insect populations as natural regulatory agents, and 3) repeated applications of pathogens as microbial insecticides. Several microbial pathogens have been studied in hopes of utilizing them to control fall armyworm populations. Inconsistent results have been documented in field studies evaluating the use of entomopathogens to suppress fall armyworm on corn and cabbage (Hardke *et al.*, 2015; Ramanujam *et al.*, 2020; Tambo *et al.*, 2020). Hardke *et al.* (2015) reported that larvae of FAW required more time to pupate, while pupae required more time to develop when fed both *Bt* maize leaf tissues compared to that on non-*Bt* maize leaf tissue.

The fungi spores infect through the integument, multiply in various tissues within the insect body, and kill the insect due to destruction of tissues and by production of toxins.

Wraight *et al.* (2010) reported *Metarhizium anisopliae* (Metchnikoff) Sorokin (1883) is among of the common fungi with potential uses against insect pests including FAW. Prasanna *et al.* (2018) reported *Bacillus thuringiensis* (Berliner) as widely used biopesticides to control FAW population.

According to Rioba and Stevenson (2020), botanical pesticides caused significant larval deaths, slowed growth rate of the FAW larva due to ingestion of toxic substances present in *M. azedarach* and *A.indica*, extended pupation time, small pupae and deformed moths. Azadiractin is the most potent natural insect antifeedant discovered to date, suppressing insect feeding at concentrations of less than 1 part per million. It is also a potent insect growth regulator, which acts by disrupting molting and development, and interfering with reproduction in adult insects (Isman *et al.*, 1991). These actions have been observed in over 90% of the more than 200 species of pest insects tested to date including FAW (Ogendo *et al.*, 2013; Prasanna *et al.*, 2018; Rillich and Stevenson, 2019). Additionally, the bioassay indicated a static effect on the growth of FAW caterpillars, as most of them exhibited their exuviae in the terminal part of the body, incompletely releasing them and it limits the ability of the insects to feed by affecting the physiological functioning of ecdysis and in cellular processes, eventually causing insect death. This process takes some time and that is why comparatively, there is low larval mortality and high pupal mortality (Rioba and Stevenson, 2020). Therefore this study aimed to establish developmental biology of the fall armyworms on maize crop treated with different biopesticides as an arsenal in FAW management.

## **2.3 Materials and Methods**

### **2.3.1 Description of the study area**

All trials in this study were conducted in late December, 2020 at Sokoine University of Agriculture (SUA)-Entomology rearing unit laboratory Edward Moringe Campus (latitude 06°50'45.1'' south and longitude 037°39' 47.9'' east with 522 m above the sea level) and Solomon Mahlangu Campus - Chemistry and Physics lab (latitude 06°47'57.6'' south and longitude 037°37' 42.2'' east with 489 m above the sea level).

### **2.3.2 Laboratory bioassay of botanicals against FAW**

#### **2.3.2.1 Extraction of Azadirachtin from *Azadirachta indica* seeds**

Neem seeds were collected from Shinyanga Region in December 2020. These were juvenile, matured green-yellow fruits directly from the tree, and were healthy *Azadirachta indica* seeds. The seeds were air dried average temperature of between 25°C -30°C under shade for five days to avoid thermal and photo - decomposition of the active ingredients (Muro, 2010; Mwatawala *et al.*, 2015). Also seed were winnowed to remove debris and then grinded to a fine powder using an electronic blander at the department of soil and geological sciences SUA. 100 g of neem seed powder were extracted with 100 mL 95% ethanol for 72 hours under constant agitation for verification of azadiractin. Then, the solution was stirred repeatedly with a magnetic stirrer for 2 h to facilitate thorough mixing. The obtained extracts were filtered through a doubled muslin cloth and concentrated using a rotary evaporator, then cold centrifuged to remove suspended material and the supernatant was oven dried at boiling point of 78.37°C of the ethanol to obtain crude extracts (Susmitha *et al.*, 2013).

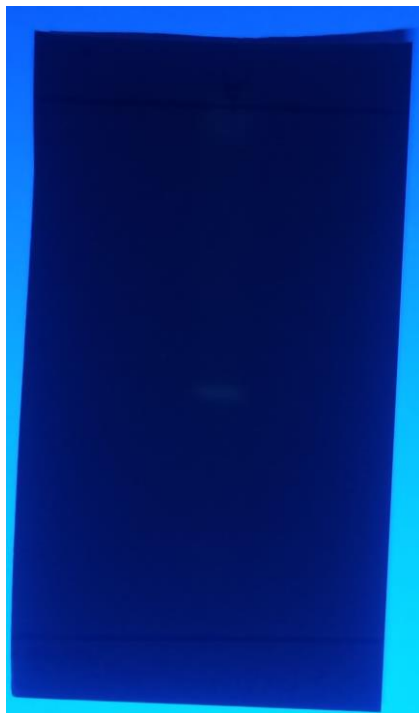
### 2.3.2.2 Thin layer chromatography (TLC) Confirmation

Thin layer chromatography (TLC) analysis was carried out with the purpose of verification of azadirachtin compound from the extracted crude sample using protocol described by Dougnon and Ito (2020). The extract was loaded onto 5 × 10 cm pre-coated silica gel plates (TLC grade, Merck, Darmstadt, Germany) using a capillary tube, and hexane/ethyl acetate (1:1) was used as the mobile phase system. The chromatograms were observed at UV 365 nm, UV 254 nm and after staining with Vanillin/H<sub>2</sub>SO reagent, and retardation factor (Rf) values were calculated using the formula;

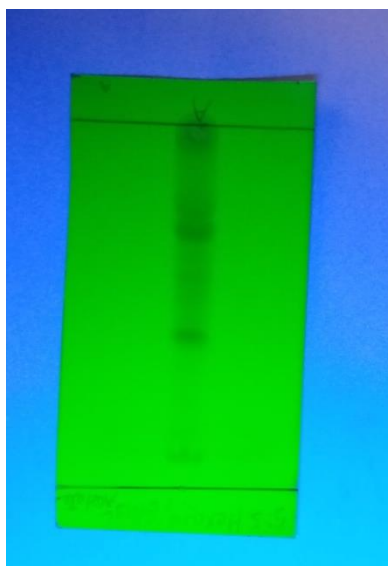
$$\text{Rf value} = \frac{\text{Distance travelled by compound}}{\text{Distance travelled by solvent system}} \dots\dots\dots (1)$$

### 2.3.2.3 Identification of Azadirachtin compound from neem kernel powder

Thin layer chromatography plates showed several spots for the seeds of *A. indica* using hexane/ethyl acetate (1:1) as a mobile phase under UV 365 nm and 254 nm. Azadirachtin was verified, similar retardation factor (Rf) value was found for *A. indica* seeds at 0.61. According to Mordue *et al.* (2005), azadirachtin accounts for maximum of 0.8% by weight of neem seed kernels. This is equal to 0.24g l<sup>-1</sup> active ingredient of azadirachtin in 30g of neem seed powder used for application per liter of water. The extracted 100g of neem seeds powder had 0.8 g l<sup>-1</sup> active ingredient of azadirachtin compound (Mordue *et al.* 2005).



**Figure 2.1: TLC Plate on long UV length**



**Figure 2.2: TLC plates of extracts under short UV light**

### **2.3.3 Maize planting**

The maize variety “STAHA” were grown under pesticide free conditions at the SUA Horticulture unit in the polythene bag to obtain maize tender leaves as the source of collected and reared FAW egg and larval diet and to necessitate the adult to lay eggs in the rearing cage so as to be easily collected.

### **2.3.4 Establishment of FAW *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae) rearing unit**

A FAW starter colony was collected from an unsprayed maize farm at Kasanga, and SUA-Crop Museum according to procedures described by Sisay *et al.* (2019). Approximately 500 larvae were collected; and the larvae were placed into ventilated rectangular plastic containers (10 cm×20.5 cm×25 cm) in the laboratory and fed with fresh and tender maize leaves collected from 15–30-day-old maize plants, variety “STAHA” cut to a 5-7cm length. The leaves were replaced after 2–3 days depending on freshness. The containers were kept in a room with an average temperature of 25°C and 60% relative humidity. The pre-pupal stage was transferred to a plastic container (14 cm × 6 cm) filled with sterilized sand as pupation media. The pupae were collected and placed in a moistened Petri dish in an oviposition cage (45 cm×45 cm×60 cm). Sterile cotton soaked in a honey solution was placed in a Petri dish inside the oviposition cage as a food source for the emerging adult moths. Maize planted in the polythene bags with 10 to 15cm height were placed in the cage for necessitating female adults to lay eggs and to be easy to collect them from plant leaves by cutting leaves with scissor. After approximately 1–2 days, egg batches were collected from the oviposition cages and placed in sterile plastic containers until a sufficient population was achieved to run the experiment. For emerged FAW moths, a cohort of 40 adults with 20 and 20 male and female respectively was established and placed in separate rearing cage for each cohort.

Rearing was done at room temperature of  $25\pm 3^{\circ}\text{C}$  and 50–60% RH, following protocol described by Prasanna *et al.*, 2018.

#### **2.4.4 Experimental design**

The experiment was laid out in a completely randomized design (CRD) with four replications. A cohorts of 100 fresh eggs batches placed on a moist filter paper in a plastic container (14 cm x 6 cm). In a set up similar to above, cohorts of 100 newly emerged larvae were collected and placed into rearing cages and fed with pieces of 15 - 20 days old tender maize leaves sprayed with biopesticides at lowest doses. Colony of 100 fresh pupae emerged for same day were collected and placed onto a moist filter paper in a Petridish with diameter of 14cm and deep for 10 second into lower doses of biopesticides, and then placed in the plastic container with length and width 14 cm x 6cm respectively, containing sterilized sand as pupation growth media. The eggs, larvae and pupa were exposed to biopesticides by spraying at the rate of 2mls, 2mls and 30g of *B.thurungiensis*, *M.anispoliae* purchased as commercial products from Real IPM (T) Ltd Arusha-Tanzania and neem seed extract respectively. Eggs, larvae, and pupae were observed following protocols described by Prasanna *et al.* (2018).

#### **2.4 Data Collection**

The data recorded at each of bio pesticide used and each developmental stage attained were; eggs incubation period, larvae to pupate and pupae to emerge into adult FAW moth. Finally, the total developmental duration of FAW from egg to adult moth emergence was established.

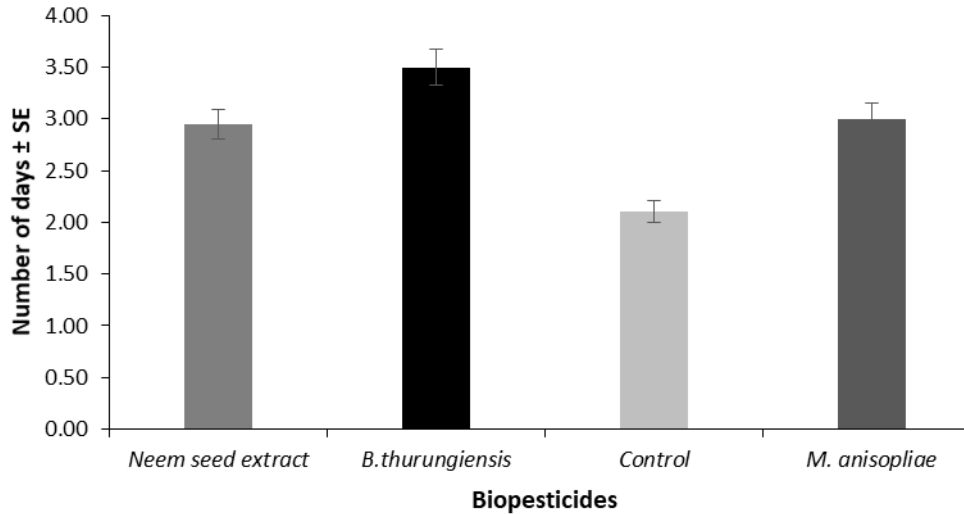
## 2.5 Data Analysis

One - way ANOVA was run and analyses were performed in R version 3.1.1 statistical software (R Core Team, 2014), to determine the effect of bio pesticides on FAW developmental biology, means were separated by *post hoc* Tukey's test. Data for egg incubation were log transformed to meet the normality assumptions. Before being transformed, Shapiro-Wilk normality test was used to test for normality on each variable. All statistical tests for significance were performed at  $P < 0.05$ .

## 2.6 Results

### 2.6.1 Effect of biopesticides on FAW egg incubation period

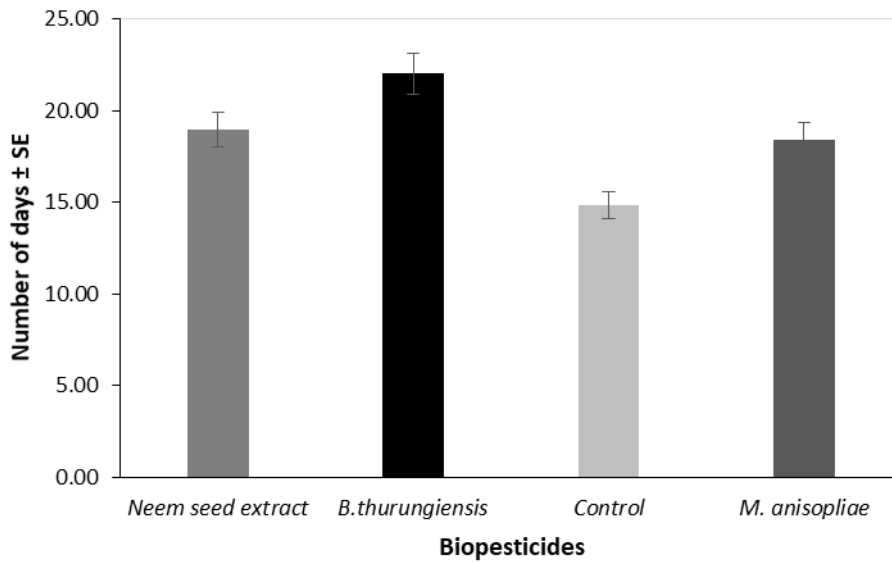
FAW eggs incubation period differed significantly ( $p < 0.001$ ) among treatments. The highest mean was observed in the egg colonies treated with *B. thuringiensis* followed by eggs colonies treated with *M. anisopliae*. The shortest mean was observed in untreated egg colonies followed by *A.indica* seed extract. The mean FAW egg incubation ranged from  $3.5 \pm 0.37$  to  $2.1 \pm 0.18$  (Fig. 2.3).



**Figure 2.3: Effect of biopesticides on FAW egg incubation period**

### **2.6.2 Effect of biopesticides on developmental duration of FAW Larvae**

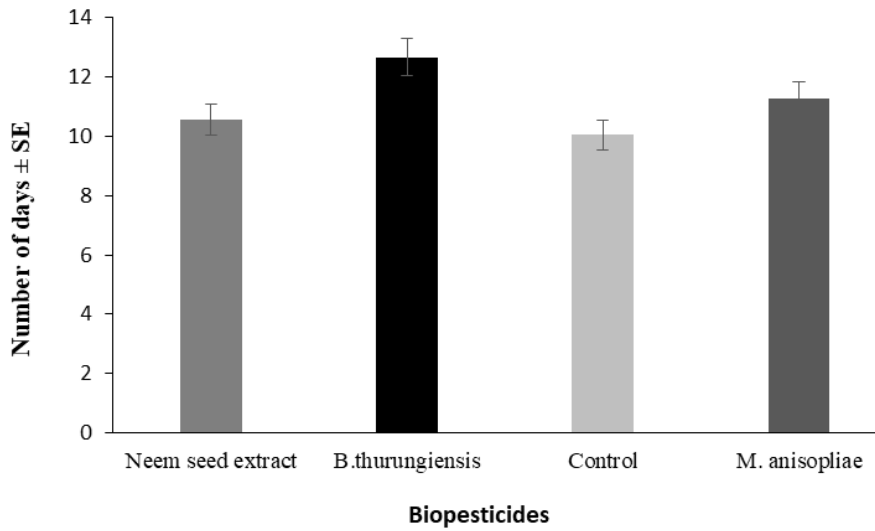
Results showed significant ( $P < 0.0001$ ) effects of treatments on FAW larval stage duration. Larval duration was significantly shorter in colonies exposed to biopesticides than the untreated colonies. The longest larval duration was observed in *B. thuringiensis* treated colonies followed by Neem seed extract treated colonies and *M.anispoliae* treated colonies. The shortest larval duration was observed in untreated colonies. Therefore the larval developmental duration ranged from  $14.88 \pm 0.18$  to  $22.03 \pm 0.59$  days Fig. 2.4.



**Figure 2.4: Effect of biopesticides on FAW larvae duration**

### 2.6.3 Effect of biopesticides on developmental duration of FAW Pupa

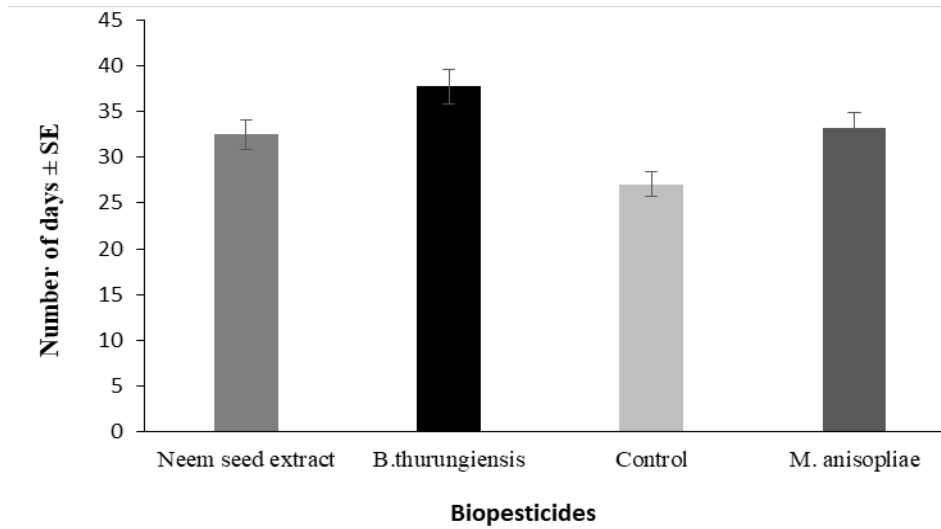
The pupal duration varied significantly ( $P < 0.001$ ) among treatments. The longest pupae duration of FAW was observed on *B. thuringiensis* treatment followed by *M. anisopliae* treated colonies. The shortest pupae duration was observed on untreated colonies followed by Neem seed extract treatment colonies respectively. However, there was no significant variation on pupa duration among *B. thuringiensis*, *M. anisopliae* treated pupae. The pupal duration of pupa stage ranged from  $10.05 \pm 0.2$  to  $12.68 \pm 0.23$  days in untreated and on *B. thuringiensis* treatment respectively (Fig. 2.5).



**Figure 2.5. Effect of biopesticides on pupae to adult duration**

#### **2.6.4 Total developmental duration**

Biopesticides significantly ( $p < 0.001$ ) affected total developmental duration of FAW. Total development duration was longer in *B. thuringiensis* treated colonies compared to untreated colonies. However, the variation in total developmental duration of FAW between *M. anisopliae* and neem extract treated colony was not significant. The total developmental duration period ranged from  $27.03 \pm 0.34$  to  $37.7 \pm 0.54$  days in untreated colony and *B. thuringiensis* treated colony respectively (Fig.2.6).



**Figure 2.6: Effect of biopesticides on total developmental duration of FAW**

## 2.7 Discussion

From this study, all tested biopesticides caused prolonged development duration of FAW growth compared to control. Growth and development is very important phenomenon for completion of life span of the insects and reproduction (Attardo *et al.*, 2005). Biopesticides are attracting global attention as new tools to kill or suppress pest populations including insects.

This study indicated that biopesticides caused increased developmental period of FAW egg, larval and pupal stages contrarily to that exposed to untreated control. Barbosa *et al.*, 2015 reported, Azadirachtin as biopesticides produced deformed pupae and adults as a result of its insect growth regulator properties. Also, Hussain *et al.* (2009) reported

*Metarhizium anisopliae* retarded the larval growth, which resulted in a prolonged developmental time of the infected larvae of *Ocinara varians*.

In the current study, biopesticides specifically *B.thuringiensis* and *M.anisploae* caused prolonged durations of FAW life stages compared to untreated control. This study is in consistence with Wang and Jaal. (2005) who observed prolonged developmental duration of *Aedes aegypti* from egg to adult when treated with *B, thuringiensis*.

Studies by Zhou *et al.* (2020) reported azadirachtin as biopesticides mixed in an artificial diet prolongs the developmental duration of larvae and decrease the larval survival rate and pupal weight of *Bactrocera dorsalis*. Further, Alouani *et al.* (2009) observed in the laboratory, a prolonged larval development of mosquito larvae when treated with neem seed extract. Similarly, Silva *et al.* (2015) reported prolonged larvae duration and high larval mortality of FAW using seed cake extract of *A. indica*.

Furthermore, among tested biopesticides, results showed *Bt* appeared across all FAW developmental stages enhance prolonged developmental period. Prasanna *et al.* (2018) reported that *B. thuringiensis* as the amongst the biopesticides that are widely used for insect control including pest suppression. *Bt* maize has been demonstrated to decrease damage from Fall armyworm. *Bacillus thuringiensis Bt* is ubiquitous, soil-dwelling gram-positive bacterium that is characterized by producing parasporal crystal proteins named delta-endotoxins with insecticidal activity (Cry toxins) during sporulation. *Bt* is considered an almost ideal agent for pest management because of its combination of insecticidal specificity and lack of toxicity to humans and nontarget organisms. Most *Bt*-based insecticides are formulated mixtures of delta-endotoxin crystals and *Bt* spores. The *Bt* spores synergize the toxicity of the crystalline proteins (Narva *et al.*, 2014;

Lacey *et al.*, 2015). Maize has been genetically engineered by incorporating genes from the bacterium *Bacillus thuringiensis* (*Bt*) that produce insecticidal proteins that kill important crop pests. The use of *Bt* maize has resulted in some cases in reduced insecticide use, pest suppression, conservation of beneficial natural enemies and higher farmer profits (Wallner *et al.*, 1983; Gujar *et al.*, 2001; Catarino *et al.*, 2001; Akutse *et al.*, 2019).

In addition to this study, effects of *Bt* as natural biopesticides have been investigated on *H. armigera*. For example, in a study to assess sub-lethal effects of *B. thuringiensis* var. *kurstaki* Berliner (Bacillales: Bacillaceae) (Btk) on *H. armigera* (Sedaratian *et al.*, 2013).

Based on the developmental duration of FAW stages on this study, the shortest egg incubation period and the longest incubation period observed were the same reported by Naharkia *et al.* (2020). Also the result is comparable to that reported by CABI and FAO (2019). Furthermore, the study observed FAW shortest larva stage duration on control treatment and the longest larva stage duration recorded on *B. thuringiensis* which both comparable to be within the range of 14 – 30 days as it was reported by FAO (2020). The shortest pupa stage duration recorded in this study on the control treatment where by the longest pupa stage duration recorded in this study, are within the range of 9 – 13 days as reported by Prasanna *et al.* (2018). Furthermore, the longest total developmental time was recorded on *B. thuringiensis* and the shortest total developmental period recorded on the control, this were within the comparable range of 21 - 30 days which can extend up to 60 to 90 days when the temperature drops down (CABI and FAO, 2019). Also, studies by Dively. (2018) reported the total developmental duration of FAW range of 21-40 days which is the same as that observed in the control of this study.

## 2.9 Conclusions and Recommendations

Given that biopesticides such as *B. thuringiensis* and *Metarhizium anisopliae* had significant effect on the developmental stages of the FAW except and neem seed extract that shown significant effect only on larva duration, these are considered to be lower risk options for pest management and are a promising avenue for exploration. When used in conjunction with good crop management, they can help to keep pest levels under control, Prolonged growth durations which have impact on FAW generations in which FAW developmental duration affected with biopesticides will have prolonged generations compared to that not affected, thus result to the successfully reducing the need to apply other pesticides. This study provides a basis for designing interventions to make biopesticides more useful for FAW control in Morogoro region and other part of Tanzania.

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**CHAPTER THREE**

**Paper two**

**Effectiveness of Biopesticides-Enhanced Push-Pull Systems against FAW *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae) in Selected Maize Cropping Systems**

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### 3.1 Abstract

FAW is an invasive polyphagous pest that causes damage to economically important crops and has recently been reported in Africa including Tanzania. The aim of this study was to evaluate effectiveness of biopesticides- enhanced push pull systems against FAW in selected maize cropping systems. A factorial experiment with two factors ( $5 \times 2$ ) was used. Factor A included natural pesticides with five levels (*B.thurungiensis*, *M.anispoliae*, neem seed extract, flubendiamide 480 Sc (negative control) and untreated maize unit plot (positive control). Factor B cropping system with two levels (push pull technology, *Desmodium intortum* (Mill.) Urb. (Leguminosae), and *Brachiaria cv Mulato II*, and maize sole crop. Results showed significant ( $p < 0.001$ ) effects of cropping system and biopesticide application on FAW egg masses, abundance of FAW larvae per plant , percent injury plant and grain yield of maize crop. Results on interaction of Cropping system and biopesticides showed significant effect ( $P < 0.001$ ) as well. However, interaction of biopesticides  $\times$  sampled weeks and between cropping system  $\times$  biopesticides  $\times$  sampled week did not significantly influence egg masses, FAW larval abundance and FAW injury on maize crop for cropping season. Overall, the biopesticides and push pull technology demonstrated effectiveness as viable sustainable alternative managemnet measures for the invasive fall armyworm in maize fields in Morogoro Tanzania.

**Key words:** Fall armyworms, infestation, biopesticides, push pull technology rearing.

### 3.2 Introduction

Fall Armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith), is a pest originating from America, but it has spread to various parts globally, including Tanzania. The species may have come Tanzania through flight from neighboring Zambia (Goergen *et al.*, 2016; Early *et al.*, 2018; Deole and Paul, 2018; Tambo *et al.*, 2020; Navik *et al.*, 2021) According to Johnson (1987) cited by FAO (2020), FAW moths have both a migratory habit and a more localized dispersal habit. In the migratory habit, moths can migrate over 500 km before oviposition. FAW that attack maize in the mid and late stages of maize growth can cause yield losses ranging from 15 to 73%, with a range of the number of plants affected by 55-100%. The reported losses vary depending on the age of the maize affected, the variety, and cultivation techniques employed. When the destructive pests attack maize, it can lead to 100 percent crop loss (Lobulu *et al.*, 2019; Sun *et al.*, 2021). Based on preliminary estimates in 12 African maize-producing countries, in the absence of proper control methods, *S. frugiperda* has the potential to cause maize yield losses of 8.3 to 20.6 million metric tons per year. This represents a range of 21-53% of the annual production of maize averaged over a three-year period in these countries. The value of these losses is estimated at between US \$2.5 to 6.2 billion (Day *et al.*, 2017).

Management of the fall armyworm has been mainly effected through use of synthetic insecticides (Cook *et al.*, 2004; Midega *et al.*, 2018; Ngangambe and Mwatawala, 2020). Use of biopesticides as a pest management tool for small scale farmers in Africa is minimal, largely due to shortage of information, inaccessibility of appropriate and effective products, and high costs (Midega *et al.*, 2012). Among of the most effective ways of managing these pests is through the use of a companion cropping system ‘push-pull’, the technology uses the drought tolerant Greenleaf desmodium, *Desmodium intortum* (Mill.) Urb. (Leguminosae), and *Brachiaria cv Mulato II* (Poaceae) as the ‘push’

and ‘pull’ crops and biopesticides (Chamberlain *et al.*, 2006; Cook *et al.*, 2007; Hassanali *et al.*, 2008; Murage *et al.*, 2012; Khan *et al.*, 2014).

Studies by Molina-Ochoa *et al.*, 2003 reported that, *B. thuringiensis*, *M. anisopliae* and *B. bassiana* are among of the pathogens that can cause significant mortality in FAW populations and help to reduce leaf defoliation in crops. Also Capalbo *et al.* (2001) and Dhobi *et al.* (2020) observed mortality of neonate larvae to be 100 per cent within two days of spraying of *B. thuringiensis* (*Bt*) and *M. anisopliae* and neem seed extract and all larvae were found dead on leaves. Studies by Silva *et al.* (2015) found that, the neem seed cake more effectively controlled FAW infestations. Little information is known on proper natural biopesticides dosage that had been documented for control of FAW moths in the field. This study focused to evaluate the effectiveness of natural biopesticides (*M. anisopliae*, *Bacillus thuringiensis*, botanical (Neem seed extract) enhanced cropping system (climate-smart PPT) in Morogoro for the management of FAW in maize, and ultimately with the aim of developing recommendations that can be used at large scale.

### **3.3 Materials and Methods**

#### **3.3.1 Study area**

Studies were conducted from mid- January to July 2021 in the Morogoro region, in three selected farms within campuses of the Sokoine University of Agriculture (SUA), as described in Table 3.1. Distance between the farms (replication) was at least 1.5 - 6 kms.

**Table 3.1: Description of the study areas**

<b>Farm/ Location</b>	<b>Coordinates</b>	<b>Altitude</b>
<b>Crop Museum, Edward Moringe Campus</b> (1.2 acre)	37 <sup>0</sup> 39' 23.6'' E and 60 <sup>0</sup> 50'57.5''S	537 m.a.s.l
<b>SUGECO farm, Edward Moringe Campus</b> (1.2 acre)	37 <sup>0</sup> 38' 39.3''E and 60 <sup>0</sup> 50' 25''S	505 m.a.s.l
<b>Horticulture area, Solomon Mahangu Campus</b> (1.2 acre).	37 <sup>0</sup> 38' 9.2'' E and 60 <sup>0</sup> 47' 28''S	476 m.a.s.l

### 3.3.2 Egg mass and larva collections

After maize emergence, each site was visited weekly for collecting FAW eggs and larvae until tasseling and silking stages. FAW larvae were collected starting from 28 days after maize emergence. Each plant was assessed thoroughly for egg masses and larvae in a non-destructive manner until each subplot covered to allow continuous collection over the crop growth stage. Hidden larvae in whorls or between the leaves were collected using a forceps to avoid damaging the maize plants.

### 3.3.3 Experimental design

A factorial experiment with two factors (5 × 2) was used. Maize, Staha variety, from Agriculture Seed Agency (ASA) was planted in 20 m × 20 m plots (experimental units), at 75 cm × 30 cm spacing. Proper agronomic practices for maize crops were adopted. Distance between plots was 5 m. Total experimental area was 14,400 m<sup>2</sup> (1.44 Ha). Space of about 1.5 - 6 km between one to other replication.

The factors, biopesticide and cropping system were laid in Randomized Complete Block Design (RCBD) with three replications (Sahu, 2013). Biopesticides included neem (*Azadirachta.indica*) seed extracts 30g in one litre of water, *Metarhizium anisopliae* (Metsch.) Sorokin (1 × 10<sup>7</sup> cfu/ml, application rate 10mls/16L, *Bacillus thuringiensis* (Bt)

( $1 \times 10^7$  cfu/ml, application rate 10mls/16L Insecticide Belt® (flubendiamide 480SC application rate 4 ml in 20L sprayer was used as negative control, all were applied in weekly basis.

The cropping systems were the Push pull technology and maize sole crop. In the farm, maize was intercropped with Greenleaf desmodium (*Desmodium intortum* (Mill.) Urb) (East African Seed Company/ SAO Hill Company) and Brachiaria hybrid cv. Mulato II (East African Seed Company). Desmodium was intercropped with maize a spacing of 50 × 50 cm. Brachiaria (was planted as boarder crop at 50cm× 25cm spacing, at rate of 7-10 seeds per hole. Sole maize was planted at a spacing of 75cm × 30cm. The experiments were conducted from mid January 2020 to late November 2021 where it experience both short and long rainy durations.

### **3.4 Data Collected**

#### **3.4.1 Infestation levels of Fall Armyworm and plant damage**

Infestation levels of FAW pest were assessed non-destructively using methodologies as adapted from Midega *et al.* (2015). In each unit plot, a 5 m - wide four transect lines were demarcated diagonally across the field and 30 maize plants randomly selected from within the transect line. Each plant was then visually examined and larvae and egg masses on the plant collected. Both egg masses and larval data from the examined plants were summed, then divided by the total number of plants and expressed as number of egg masses and larvae per plant (Midega *et al.*, 2018).

#### **3.4.2 Infestation level of FAW on maize crop**

FAW infestation level, defined as the extent of infestation on maize plants in the field was calculated by expressing the number of infested plants as a percentage of the total number

of plants in the plot. It was recorded and estimated as percentage infestation (Nono-Womdim *et al.*, 2003).

### **3.4.3 Maize grain yields assessment**

Maize grain yields were determined at the crop's full physiological maturity when all the cobs on the maize plants in each plot were harvested except guard rows and sun-dried separately for each treatment plot. The cobs were shelled and maize grain sun-dried to 15% moisture content, confirmed using a moisture meter (Multi-grain tester, Dickey-John corporation, Auburn, USA), and weights individually taken for each treatment plot using hanging digital weighing balance. The grain weights were recorded.

### **3.5 Data Analysis**

Data on fall armyworm egg and larva abundance per plant and grain yields of maize were normalized by log transformation. Shapiro–Wilk tests (Shapiro and Wilk, 1965) was used to check if the assumptions of normality were met before further analysis. Two-way analyses of variance (ANOVA) was used to determine the effects of biopesticides and Cropping systems on FAW egg and larval per plant and maize grain yield. Means were separated using Tukey's HSD test at  $\alpha = 0.05$  level of confidence. To check the effects of biopesticides, cropping systems and sampling week on infestation level of FAW, Poisson regression was performed. All analysis were performed using R statistical software version 4.1 (R Development Core Team, 2020).

### **3.6 Results**

#### **3.6.1 Egg masses abundance in biopesticides and enhanced maize cropping system**

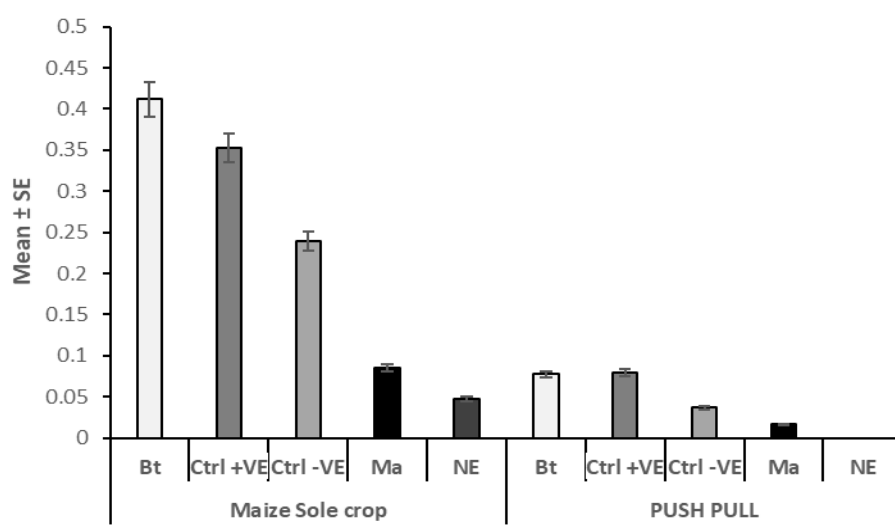
There were significant effects ( $P < 0.001$ ) of cropping systems, biopesticides and sampling weeks on number of eggs per plant of FAW (Table 3.2). The effects of cropping system  $\times$

sampling week as well cropping system  $\times$  biopesticide were also significant ( $p < 0.001$ ). Further examination of the significant system  $\times$  biopesticide showed that all combinations with push pull technology had significantly lower number of eggs ( $P < 0.05$ ). Untreated maize sole crop had significantly higher number of egg masses than all other treatments. Significantly, lower number of eggs masses were recovered in push pull plot sprayed with *Meterhiizium anispolae* than in all other plots. Examination of the cropping system  $\times$  sampling week showed the highest number of egg per plant was observed in maize sole crop cropping system on week 1, and the lowest was observed in push pull cropping system in week 8 followed by maize sole crop cropping system in week 8. Number of eggs ranged from  $0.41 \pm 0.07$  SE in Maize sole crop treated with *Bacillus thuringiensis* biopesticides to  $0.17 \pm 0.008$  SE in push pull technology and *Meterhizium anispolae* (Figure 3.1).

**Table 3.2:** Analysis of variance for the effect of cropping system and biopesticides on FAW egg masses in Morogoro, Tanzania

Factor	Statistics		
	<i>d.f</i>	<i>F</i>	<i>P</i>
Cropping system (CS)	1	131.631	< 0.001
Biopesticides (BIOP)	4	6.198	0.0000927
Sampling week after emergence (SWK)	7	45.967	< 0.001
CS $\times$ BIOP	4	4.239	0.002475593
CS $\times$ SWK	7	23.153	< 0.001
BIOP $\times$ SWK	28	0.532	0.9756 ns
CS $\times$ BIOP $\times$ SWK	28	0.729	0.841 ns

d.f., degree of freedom; ns. Not significant.



**Figure 3.1: Effect of cropping system and biopesticides on FAW egg masses in maize in Morogoro**

**Key:** Ma = *Metarhizium anisopliae*, Bt = *Bacillus thuringiensis*, Ne = *Neem extract*, Ctrl-ve = maize sole plot with no any application used as negative control, Ctrl+ve = maize plot treated with flubendiamide used as positive control

### 3.6.2 FAW caterpillar abundance in biopesticides and enhanced maize cropping system

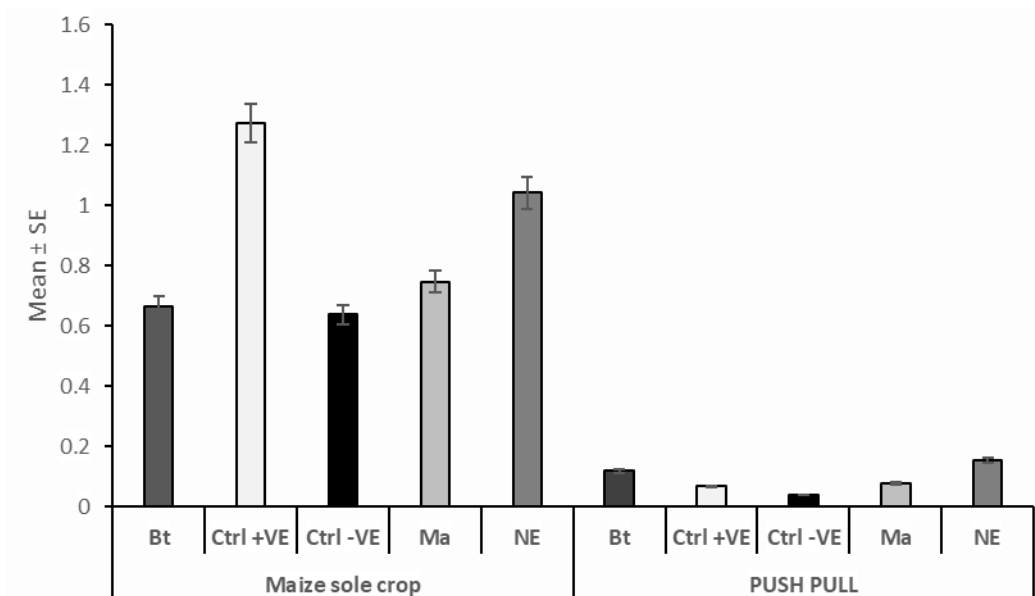
There were significant effects ( $p < 0.001$ ) of cropping systems, biopesticides and sampling weeks on number of caterpillar per plant of FAW (Table 3.2). The effects off cropping system  $\times$  sampling week as well cropping system  $\times$  biopesticide were also significantly ( $p < 0.001$ ). Further analysis of the cropping system  $\times$  biopesticide showed significantly low number of eggs in all combinations with push pull technology. Untreated maize sole plots had significantly higher number of larvae than all other treatments. Significantly, lower number of caterpillar were collected from push pull plots sprayed with Flubendamide than in all other plots. Number of larvae ranged from  $1.28 \pm$

0.25 SE in Maize sole crop with no any application of biopesticide as negative control to  $0.04 \pm 0.016$  SE in push pull technology plots treated with Flubendiamide as positive control (Figure 3.2).

**Table 3.3: Analysis of variance for the effect of cropping system and biopesticides on FAW larvae abundance in Morogoro, Tanzania**

Factor	Statistics		
	d.f	<i>F</i>	<i>P</i>
Cropping system (CROS)	1	165.0025	< 0.001
Biopesticides (BIOP)	4	4.7385	< 0.001
Sampling week after emergence (SWK)	7	42.7769	< 0.001
CS:BIOP	4	4.2642	0.00234
CS:SWK	7	32.1524	< 0.001
BIOP:SWK	28	0.4610	0.991 ns
CS:BIOP:SWK	28	0.5109	0.981ns

d.f., degree of freedom; ns., not significant.



**Figure 3.2: Effect of cropping system and biopesticides on FAW caterpillar abundances in maize crop in Morogoro**

**Key:** Ma = *Metarhizium anisopliae*, Bt = *Bacillus thuringiensis*, Ne = *Neem extract*, Ctrl-ve = maize sole plot with no any application used as negative control, Ctrl+ve = maize plot treated with flubendiamide used as positive control.

**3.6.3 Infestation level of FAW on maize crop**

The results further showed significant association ( $P < 0.001$ ) between cropping systems, biopesticides and sampled weeks on FAW infestation. Compared with biopesticides flubendiamide used as positive control, untreated plot used as negative control was likely to have significant infestation level of *S. frugiperda* (0.607) ( $P < 0.001$ ) than maize plot treated with *B. thuringiensis* biopesticides. In contrary, biopesticides flubendiamide used as positive control was likely to have significant lower infestation rate than *M. anisoplae* biopesticides (-0.239) ( $P < 0.001$ ). Infestation rate of FAW was likely to be higher under untreated plot used as negative control (0.607) ( $P < 0.001$ ) than treated plot with biopesticides. Compared to sampling weeks, sampling week 2 after maize emergence showed significant association with infestation rate of *S. frugiperda* (0.053) ( $P > 0.001$ ) than sampling week 8 after emergence (-19.745) ( $P > 0.001$ ) (Table 3.4).

**Table 3.4: Estimates for Poisson regression of infestation level of *Spodoptera frugiperda* as affected by cropping system, bio pesticides and Sampling weeks in Morogoro, Tanzania**

Terms	Estimate	Std.Error	Z value	P - Value
Intercept	3.8016	0.04703	80.84	< 0.001
BIOPCtrl -VE	-0.89553	0.06945	-12.895	< 0.001
BIOPCtrl +VE	0.60703	0.04648	13.059	< 0.001
BIOPMa	-0.239	0.05635	-4.242	< 0.001
BIOPNE	0.20194	0.05041	4.006	< 0.001
CSPUSH PULL	-0.70074	0.03465	-20.222	< 0.001
SWKW002	0.05257	0.04296	1.224	0.221 ns
SWKW003	-0.54062	0.05072	-10.658	< 0.001
SWKW004	-0.95836	0.05845	-16.397	< 0.001
SWKW005	-1.04335	0.06029	-17.305	< 0.001

SWKW006	-1.89334	0.08507	-22.257	< 0.001
SWKW007	-3.35133	0.16725	-20.037	< 0.001
SWKW008	-19.74476	361.86064	-0.055	0.956 ns

**Key:** Ma = *Metarhizium anisopliae*, Bt = *Bacillus thuringiensis*, Ne = *Neem extract*, Ctrl-ve = maize sole plot with no any application used as negative control, Ctrl+ve = maize plot treated with flubendiamide used as positive control, SWK= Sampling week.

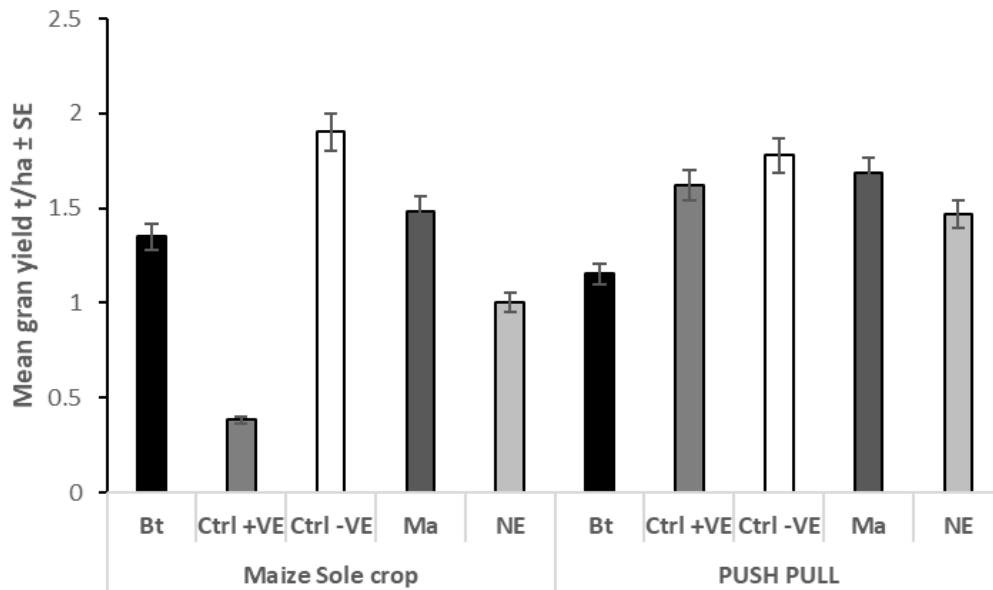
### 3.6.4 Effects of FAW on Maize grain yield

Results further showed significant effects of cropping system  $\times$  biopesticides on maize grain ( $P < 0.001$ ) (Table 3.5). Results on figure 3.4 shows that untreated sole maize plots gave significantly lower yield compared to treated plots. On the other hand, highest yield was obtained from sole maize crop treated with flubendiamide. All push pull combinations had significantly higher yield than untreated sole maize crop. The mean maize grain yield ranged from  $1.90 \pm 0.26$  SE tons per hactre to  $0.38 \pm 0.26$  SE tons per hactre.

**Table 3.5: Analysis of variance for the effect of cropping system and biopesticides on maize grain yield assessment on control of *Spodoptera frugiperda* in Morogoro, Tanzania**

Factor	Statistics		
	<i>d.f</i>	<i>F</i>	<i>P</i>
Cropping system (CS)	1	25.79989	< 0.001
Biopesticides (BIOP)	4	22.25262	< 0.001
CS:BIOP	4	17.2865	< 0.001

d.f., degree of freedom.



**Figure 3.3: Effect of cropping system and biopesticides on maize grain yield assessment for *Spodoptera frugiperda* control in Morogoro, Tanzania**

**Key:** Ma = *Metarhizium anisopliae*, Bt = *Bacillus thuringiensis*, Ne = *Neem extract*, Ctrl-ve = maize sole plot with no any application used as negative control, Ctrl+ve = maize plot treated with flubendiamide used as positive control.

### 3.7 Discussion

Results of the current study indicate the effective reduction of infestation by fall armyworm with both biopesticides (*M. anisopliae*, *B. thuringiensis*) and *A. indica* neem seed extract (NE)) and enhanced push-pull system, which resulting in significantly lower injury levels on maize field trials compared to maize sole crop plot trials. The highest mean average count of number of caterpillar per plant was recorded on maize sole crop plots with no any biopesticide application. This finding was within the same line of

studies by Kleden and Simamora (2021) who reported the caterpillar population was relatively high, ranging from 1 to 28 per plant with an average of 6.65 in West Solor Sub-district, while in Ile Mandiri Subdistrict, it ranged from 1 to 7 caterpillar per plant with an average of 2.55 per plant on heads in East Nusa Tenggara Pr Yovince, Indonesia. Studies by Midega *et al.* (2018), reported the average number of caterpillar per maize plant ranged from 0.23 to 2.07 in Uganda. The lowest mean average count of number of caterpillar per plant was recorded on PPT plot trial applied with flubendiamide insectides. Push–Pull systems presented lower incidence of fall armyworm compared to maize sole crop. These results concur with those of several field effectiveness studies (against Fall Armyworm) of the successful Push–Pull system designed by Khan *et al.* (2017), Midega *et al.* (2018) and Khan *et al.* (2018) reported reductions of 82.7% in the average number of caterpillar per plant and 86.7% in the injury of plants per plot in the Push–Pull plots made up of *Brachiaria* cv Mulato II and *Desmodium intortum* (Mill.) Urb in Uganda, Kenya and Tanzania. They were decrease of number of larvae per plant from second observation to fourth observation or after second spray, whereby no number of caterpillar observed after third spray.

Moreover, studies by Hailu *et al.* (2018) reported that push-pull technology performed best in reducing stemborer, FAW, and striga infestation over all the phenological stages of maize in Uganda. According to Day *et al.* (2017), FAW feed more in tender young leaves stage than other stages. Hence fall armyworm population abundance as low as 0.2 - 0.8 caterpillar per plant during the late whorl stage may be sufficient to reduce yields (Cruz *et al.*, 1999). The number of egg masses batch per plant across all treatment differed significantly. This study found that, the number of egg mass per plant was not the factors which could influence the reduction of maize grain yield. According to Sokame *et al.* (2020), the number of eggs per batch was not the factors which could

influence all caterpillar movements and cause significant yield loss. Also, studies by Murúa *et al.* (2006) found that the distribution of FAW caterpillar and eggs varied according to the phenological stage of the maize. According to Khan *et al.* (2018) maize grain yields were significantly higher, approximately 2.7 times, in the push-pull plots in Kenya, Uganda and Tanzania.

According to Bateman *et al.* (2018), biopesticides are generally help to keep pest levels under control. This result is governed with the mode of action of biopesticides on the pest and could be either due to the efficacy of product used to control the pest. The high percentage of infested plants appeared on neem seed extract application could be due to changing of weather. Forim *et al.* (2010) suggested that for neem-based sprays, a major problem is the high photosensitivity of azadirachtin, which breaks down or isomerizes under sunlight; thus, neem has a low residual life under field conditions. Biopesticides that performed well included *M.anisopliae* and *B.thuringiensis*. The current result is in line with Molina- Ochoa *et al.* (2003), Assefa and Ayalew (2019) and Gebreziher (2020) who reported *Bacillus thuringiensis*, *M. anisopliae* and *Beauveria bassiana* that can cause significant mortality in FAW populations and help to reduce leaf defoliation in crops. The PPT plots × Flubendiamide showed highest performance and high yield over all treatment. Moreover, for biopesticides, maize plot treated with *M. anisopliae* showed highest performance including maize grain yield over the control of FAW for cropping season compared to plots treated with *B.thuringiensis* and neem seed extract. Studies by Kaushik and Dutta (2016) showed that infestation of aphid, *Toxoptera aurantii*; tea mosquito bug, *Helopeltis theivora*; termite, *Odontotermes obesus*; red spider mite, *Oligonychus coffeae* and carpenter worm, *Prionoxystus robiniae* was found less in the plot treated with *M.anisopliae* as compared to the untreated control, which indicating potential as a biopesticide control agent against the insect pest.

### 3.8 Conclusions and Recommendations

This study demonstrated that biopesticides *M.anisopliae* is very essential tools used effectively to control fall armyworm in maize farming systems in Morogoro, Tanzania. These management aspects have potential for expansion in the country and African continent at large to manage key pests affecting cereal production in the region. The ability of *M.anisopliae* to manage such a devastating pest indicates its stability and resilience, and confirms that they are ecologically sustainable to pest management.

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**CHAPTER FOUR**

**Paper three**

**Influence of Cropping systems and Biopesticides on parasitism rate and abundance of Parasitoids of FAW *Spodoptera frugiperda* (JE SMITH) (Lepidoptera: Noctuidae) in Maize Fields in Morogoro**

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#### 4.1 Abstract

In order to evaluate the diversity of parasitoids attacking *Spodoptera frugiperda*, a total of 777 larval and 2241 egg-larval parasitoid species were collected during the 2020 – 2021 period in Morogoro Tanzania. The objective of this study was to determine the influence of cropping systems and biopesticides on parasitism rate and abundance of Parasitoids of *S.frugiperda*. Factor A included biopesticides with five levels (*B.thurungiensis*, *M.anisopliae*, neem seed extract, flubendiamide 480 Sc (positive control) and Maize sole crop (negative control). Factor B was cropping system with two levels (Push-pull systems and maize sole crop). The identification of parasitoid species, percent parasitism and relative abundance of the parasitoids species were estimated. Total of 3 parasitoid species one egg-larval (*Chelonus bifoveolatus* Szépligeti) and two larval (*Coccygidium luteum* and *Cotesia* sp) parasitoid species were identified. The results showed significant effect ( $P<0.001$ ) only on relative abundance of *Cotesia* sp on biopesticides. Results also showed significant effect ( $P<0.05$ ) on parasitism rate and relative abundance of one egg-larval parasitoid specie (*C. bifoveolatus*) and two larval-parasitoids species (*C. luteum* and *Cotesia* sp) on sampling week. The results also showed significant effect ( $P<0.05$ ) on parasitism rate and percentage relative abundance of one egg-larval parasitoid specie (*C. bifoveolatus*) and two larval-parasitoids species (*C. luteum* and *Cotesia* sp) on interaction between cropping system and biopesticides. The results also showed significant effects of parasitism rate for *Chelonus bifoveolatus* and *Cotesia* sp on interaction between cropping system and biopesticides application, biopesticides and sampling week. No emerged parasitoid species from eggs sampled from plots treated with Flubendiamide. Parasitoid diversity was different possibly relating to the kind of treatment being examined. The diversity of parasitoids in maize field crop under biopesticides and enhanced push pull technology is one of the first steps in developing a comprehensive management program for an invasive insect pest in Morogoro Tanzania.

**Key words:** Climate smart PPT, Diversity of parasitoids, biopesticides, parasitoid species, percentage parasitism, relative abundance.

## 4.2 Introduction

Native to the America, the Fall Armyworm “FAW” *Spodoptera frugiperda* (Lepidoptera, Noctuidae) a pest that feeds on leaves and stems of more than 80 plant species, but with a preference for poaceous crops, and maize in particular, was first reported as present on the African continent in January 2016 (Baudron *et al.*, 2019; De Groote *et al.*, 2020; Tanyi *et al.*, 2020; Siazemo, 2020; Overton *et al.*, 2021). According to Molina-Ochoa *et al.* (2003), FAW has a diverse complex of natural enemies.

FAW is attacked by over 150 parasitoid species (Molina-Ochoa *et al.*, 2003; Firake and Behere, 2020) and by diverse taxa of insect predators (Braman *et al.*, 2002; Harrison *et al.*, 2019). Studies by Sisay *et al.* (2018) recorded five parasitoid species causing up to 50% parasitism of FAW in Ethiopia. Similarly, seven parasitoid species and three entomopathogens were reported attacking FAW in México (Ríos-Velasco *et al.*, 2011). Despite a huge diversity, the occurrence and distribution of natural enemies varies among habitats. Factors including geographical location, agricultural practices, and insecticide use can also affect the occurrence of natural control agents that could potentially impact FAW populations (Murua *et al.*, 2006; Firake and Behere, 2020). Molina- Ochoa *et al.* (2003) and Wyckhuys and O’Nei, (2006) reported that, natural enemy complex associated with FAW varies among localities.

Natural enemies as a part of biological control including predators, parasitoids, parasites and pathogens, play a major role in the natural regulation of insect populations. Parasitoids are biological agents for which at least one of their life stages is intimately associated with specific life stages of the pest and with greater levels of specificity. The larvae of parasitoids always kill their host as the outcome of their development (Bernasconi Ockroy *et al.*, 2001; Tschardtke and Brandl, 2004; Mailafiya *et al.*, 2010;

Durocher *et al.*, 2020). Parasitoids are among the most widely used natural enemies in biological control. Various biotic and abiotic factors such as temperature, rainfall, altitude, landscape characteristics, food and shelter sources, sink habitats, host density, volatile emissions and seasonality can influence the diversity, abundance and parasitism rates in the field (Bernasconi Ockroy *et al.*, 2001; D'Alessandro *et al.*, 2009; Mailafiya *et al.*, 2010; Tscharrntke and Brandl, 2004; Durocher *et al.*, 2020). These factors are important to evaluate and understand when searching for availability of potential biological control agents as they will influence the spatial and temporal activities of the natural enemy populations. According to Firake and Behere (2020), Native natural enemies of related existing pest species are the first defense against invasive pest species. Hence this study focused to determine diversity of parasitoids of FAW among different practices particularly cropping system push- pull technology and biopesticides (*M.anisopliae*, *Bt*, and botanical insecticides) on the maize field for FAW management in Morogoro, Tanzania.

### **4.3 Materials and Methods**

#### **4.3.1 Description of the Study Area**

The Experiment were conducted in Morogoro region in three selected farms, namely crop Museum and SUGECO farm, both located at Edward Moringe Campus (EMC) and Solomon Mahlangu Campus (SMC) (Table 4.1).

**Table 4. 1 Description of the study areas**

<b>Farm/ Location</b>	<b>Coordinates</b>	<b>Altitude</b>
<b>Crop Museum, Edward Moringe Campus</b> (1.2 acre)	37° 39' 23.6'' E and 60° 50' 57.5'' S	537 m.a.s.l
<b>SUGECO farm, Edward Moringe Campus</b> (1.2 acre)	37° 38' 39.3'' E and 60° 50' 25'' S	505 m.a.s.l
<b>Horticulture area, Solomon Mahangu Campus</b> (1.2 acre).	37° 38' 9.2'' E and 60° 47' 28'' S	476 m.a.s.l

### 4.3.2 Experimental design

A factorial experiment with two factors ( $5 \times 2$ ) was used. Factor A included biopesticides *Bacillus thuringiensis*, *Meterhizium anispolae* purchased as commercial product from Real IPM (T) Ltd Arusha-Tanzania, neem seed extract flubendiamide and untreated plot used as negative control, and Factor B was cropping systems which included Maize sole crop and Climate adapted push pull technology. Maize, Staha variety, from Agriculture Seed Agency (ASA) was planted in 20 m  $\times$  20 m plots (experimental units), at 75 cm  $\times$  30 cm spacing. Proper agronomic practices for maize crops were adopted. Distance between plots was 5 m. Total experimental area was 14,400 m<sup>2</sup> (1.44 Ha). The distance between one to other replication was about 1.5 to 6 km.

The factors, biopesticide and cropping system were laid in Randomized Complete Block Design (RCBD) with three replications (Gomez and Gomez, 1984). Biopesticides included neem (*Azadirachta.indica*) seed extracts 3% in one litre 60% in 20L sprayer, *Metarhizium anisopliae* (Metsch.) Sorokin (2 mls/L) equivalent to  $2 \times 10^6$  cfu/L, *Bacillus thurigiensis* (Bt) ( $2 \times 10^4$  cfu/ml, application rate 10mls/16L Insecticide Belt® (flubendiamide 480SC application rate 4 ml in 20L sprayer was used as positive control, all were applied in weekly basis after 3 weeks.

The cropping systems were the Push pull technology and maize sole crop. In the farm, maize was intercropped with Greenleaf desmodium (*Desmodium intortum* (Mill.) Urb) (East African Seed Company/ SAO Hill Company) and Brachiaria hybrid cv. Mulato II (East African Seed Company). Desmodium was intercropped with maize a spacing of 50 × 50 cm. Brachiaria (East was planted around the intercrop at 50cm × 25cm spacing, at rate of 7-10 seeds per hole. Sole maize was planted at a spacing of 75cm × 30cm.

The experiments were conducted from mid- January 2020 to late November 2021 where it experience both short and long rainy durations. The interaction was between cropping system and biopesticides, cropping system with sampling week, biopesticides with sampling week and cropping system with biopesticides and with sampling week was tested on diversity of parasitoids species.

#### **4.3.3 Rearing of fall armyworm**

FAW Egg batches and caterpillar were collected from the experimental maize fields 4 weeks after maize emergence. Rearing was done in aerated plastic containers with measurement of (20 cm × 10 cm × 25cm) diameter, height and length respectively with piece of cloth with tiny hole placed to prevent escape of parasitoids (Laminou *et al.*, 2020). Each container was given a sample label for each treatment from different replication. The insects were routinely reared at SUA entomology laboratory located at Horticulture unit using the method described by Prasanna *et al.* (2018).

Caterpillar were reared on an established artificial diet where untreated maize young leaves were used as source of diet, which was changed every 2-3 days until emergence of the parasitoids or adult moths. Egg and larvae were observed every day, and the

developed parasitoids were collected by using insect collecting putter. Development of parasitoids or FAW moths was daily recorded.

#### 4.4.3 Identification of parasitoids species

All parasitoids species obtained during this study were morphologically identified using various identification keys and collections of insects gathered during previous studies on *S. frugiperda* parasitoids (Prasanna *et al.*, 2018; Agboyi *et al.* 2019; Ngangambe and Mwatawala, 2020).

#### 4.4 Data collected

##### 4.4.1 Parasitism rates

The parasitism rate of each parasitoid species was determined and converted to percent values. Gregarious parasitoids emerging from a single larva were considered as being only one. Parasitism rate of the egg masses was not calculated as none were parasitized

$$\%Pp = \frac{Lp}{TL} \times 100 \dots\dots\dots (1)$$

Where; Pp = Parasitism rate of each parasitoid species, Lp= parasitized larvae and  
TL = Total number of collected larvae

##### 4.4.2 Relative abundance of parasitoids species

The relative abundance of each parasitoid species (RA) obtained after FAW rearing was determined by dividing the number of individuals of a given parasitoid species (ni) by the total number of individuals of all parasitoid species obtained from a rearing cage (N) and converted to percent values.

$$\%RA = \frac{ni}{N} \times 100 \dots\dots\dots (2)$$

## 4.5 Data Analysis

Data on percentage of parasitism rate and relative abundance of parasitoid species were averaged across all treatments. Normality was tested using Shapiro–Wilk tests (Shapiro and Wilk, 1965) before being subjected to three-way analyses of variance (ANOVA). In case where the data were not normally distributed, they were further log transformed ( $\log_{10}x + 1$ ) before analysis. Whenever treatments were found to be significantly different ( $p < 0.05$ ), means were separated using Tukey’s HSD test at  $\alpha = 0.05$ . All data analyses were performed using R (version 4.1) statistical software packages (R Development Core Team, 2020).

## 4.6 Results

### 4.6.1 Total number of parasitoid species

Overall, 777 larval and 2241 egg-larval parasitoid species were recorded from FAW eggs and larvae collected and reared on the laboratory in late March 2021. Table 4.2 shows three different parasitoid species emerged from field-collected FAW larvae and egg masses across all treatments. The two most common species *Coccygidium luteum* (Brullé) and *Cotesia* sp was the only larva parasitoids recorded across all treatment. The species *Coccygidium luteum* (Brullé) was the most abundant. The parasitoids species *Chelonus bifoveolatus* Szépligeti was the only egg-larval parasitoids recorded.

**Table 4.2: Parasitoid species collected in the maize crop field in late March, 2021 in Morogoro, Tanzania**

Treatment	Number of Parasitoids species/traits recovery			Parasitoids species in percentage (%)		
	<i>C.bifoveolatus</i> - Egg-larval	<i>C.luteum</i> - Larva	<i>Cotesia icipe</i> Fernández <i>sp</i> - Larva	<i>C.bifoveolatus</i> - Egg- larval	<i>C.luteum</i> - Larva	<i>Cotesia icipe</i> Fernández <i>sp</i> - Larva
Bt+VE	31	6	0	1.38	1.98	0.00
FE+VE	9	0	0	0.40	0.00	0.00
Bt+P	96	43	69	4.28	14.19	4.56
P+FE	77	0	0	3.44	0.00	0.00
P+M	125	9	0	5.58	2.97	0.00
P+Ne	168	7	23	7.50	2.31	4.85
P+VE	168	38	0	7.50	12.54	0.00
M+VE	187	5	1	8.34	1.65	0.21
Ne+VE	416	69	97	18.56	22.77	20.46
VE+VE	964	126	284	43.02	41.58	59.92
<b>Total</b>	<b>2241</b>	<b>303</b>	<b>474</b>	<b>100.00</b>	<b>100.00</b>	<b>100.00</b>

**Key:** Ma = *Metarhizium anisopliae*, Bt = *Bacillus thuringiensis*, Ne = *Neem extract*, Ctrl-ve = maize sole plot with no any application used as negative control, VE+VE = maize plot treated with flubendiamide used as positive control

#### 4.6.2 Effect of cropping system and biopesticides in parasitism rate on recovered parasitoids species

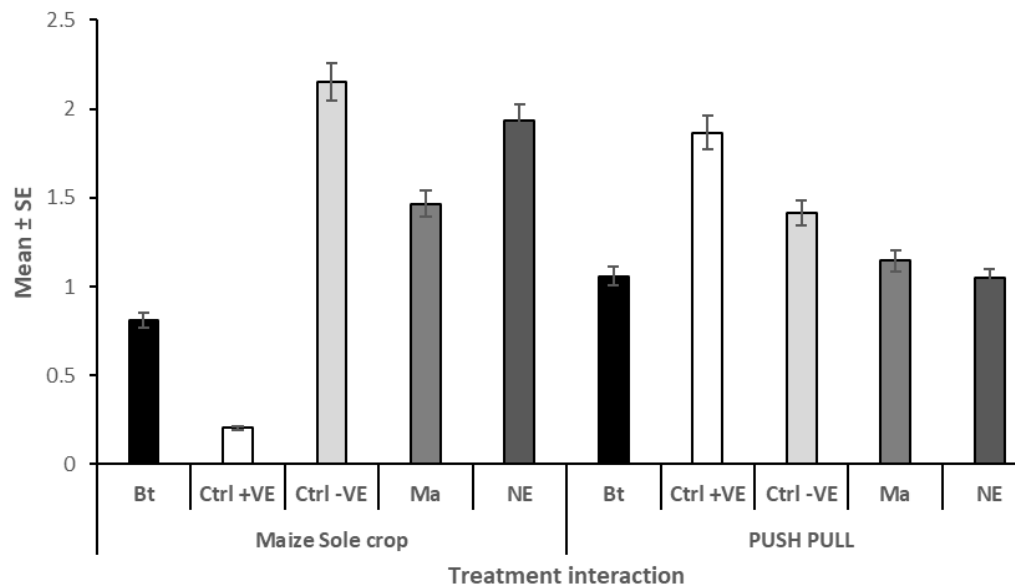
##### 4.6.2.1 Parasitism rate of *Chelonus bifoveolatus szépligeti*

There was significant effect of sampling week, cropping system  $\times$  biopesticide, cropping system  $\times$  sampling week and cropping system  $\times$  biopesticide  $\times$  sampling week on the parasitism rate of *C. bifoveolatus* (Table 4.3). Further analysis of the cropping system  $\times$  biopesticide  $\times$  sampling week showed highest mean was observed on maize sole crop cropping system with untreated maize and the lowest was observed on maize sole crop cropping system with treated with flubendiamide as positive control. Parasitism rate ranged from  $2.15 \pm 0.57$  SE to  $0.206 \pm 0.57$  SE. The highest mean was observed in untreated maize sole unit plot t a rate of  $1.31 \pm 0.3$  SE. Also the highest mean of PRCBE was observed in sampled week 1 (Figure 4.1).

**Table 4.3: Analysis of variance of cropping system and biopesticides on parasitism rate of *Chelonus bifoveolatus* in maize crop in Morogoro, Tanzania**

Factor	Statistics		
	<i>d.f</i>	<i>F</i>	<i>P</i>
Cropping system (CS)	1	0.0005	0.9820
Biopesticides (BIOP)	4	1.1283	0.3438
Sampling week after emergence (SWK)	7	37.2579	0.0000
CS:BIOP	4	2.8767	0.0236
CS:SWK	7	0.4942	0.8384
BIOP:SWK	28	1.6854	0.0204
CS:BIOP:SWK	28	1.7327	0.0155

d.f., degree of freedom



**Figure 4.1: Effect of cropping system and biopesticides in parasitism on maize crop in Morogoro.**

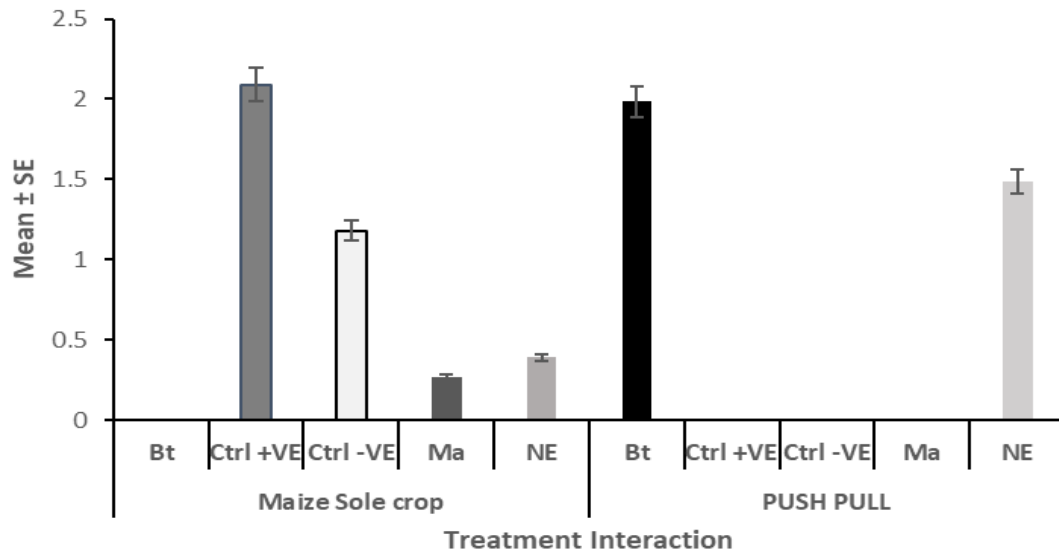
**Key:** Ma = *Metarhizium anisopliae*, Bt = *Bacillus thuringiensis*, Ne = *Neem extract*, Ctrl-ve = maize sole plot with no any application used as negative control, Ctrl+ve = maize plot treated with flubendiamide used as positive control.

#### 4.6.2.2 Parasitism rate of *Cotesia* sp

The results showed no significant effects of sampling week, cropping system  $\times$  biopesticide and cropping system  $\times$  biopesticide  $\times$  sampling week on parasitism rate of *Cotesia* sp (Table 4.4). The cropping system  $\times$  biopesticide showed significant effect ( $p < 0.001$ ) (Figure 4.2). In interaction of cropping system  $\times$  sampling week, biopesticide  $\times$  sampling week there were no significant effect. The highest mean was recorded in maize sole crop cropping system followed by PPT both in week 1 ( $3.57 \pm 0.66$  SE and  $3.1 \pm 0.66$  SE) respectively, and the lowest observed on PPT in week 8. The mean ranged from  $3.57 \pm 0.66$  SE to  $-7.10e-15 \pm 0.66$  SE. The highest mean was observed on *Bacillus thurigiensis* biopesticides ( $4.32 \pm 1.08$  SE) followed by maize sole crop ( $4.23 \pm 1.08$  SE) in week 1 and that of *Bt* in week 2 ( $4.14 \pm 1.08$  SE) and the lowest was observed on *Bacillus thurigiensis* biopesticides ( $1.18e-14 \pm 1.08$  SE). The mean ranged from  $4.32 \pm 1.08$  SE to  $1.18e-14 \pm 1.08$  SE.

**Table 4.4: Analysis of variance of cropping system and biopesticides on Parasitism rate of *Cotesia* sp on maize crop in Morogoro, Tanzania**

Factors	Statistics		
	d.f	F	P
Cropping system (CS)	1	0.16008	0.68944
Biopesticides (BIOP)	4	2.17988	0.07195
Sampling week after emergence (SWK)	7	14.25882	0.00000
CS:BIOP	4	10.16941	0.00000
CS:SWK	7	0.07606	0.99930
BIOP:SWK	28	0.76767	0.79569
CS:BIOP:SWK	28	3.56367	0.00000



**Figure 4.2: Effect of cropping system and biopesticide on Parasitism rate of *Cotesia* sp on maize crop in Morogoro.**

**Key:** Ma= *Metarhizium anisopliae*, Bt = *Bacillus thuringiensis*, NE= *Neem extract*, Ctrl+ve = maize sole plot with no any application used as positive control, Ctrl-ve = maize plot treated with flubendiamide used as negative control.

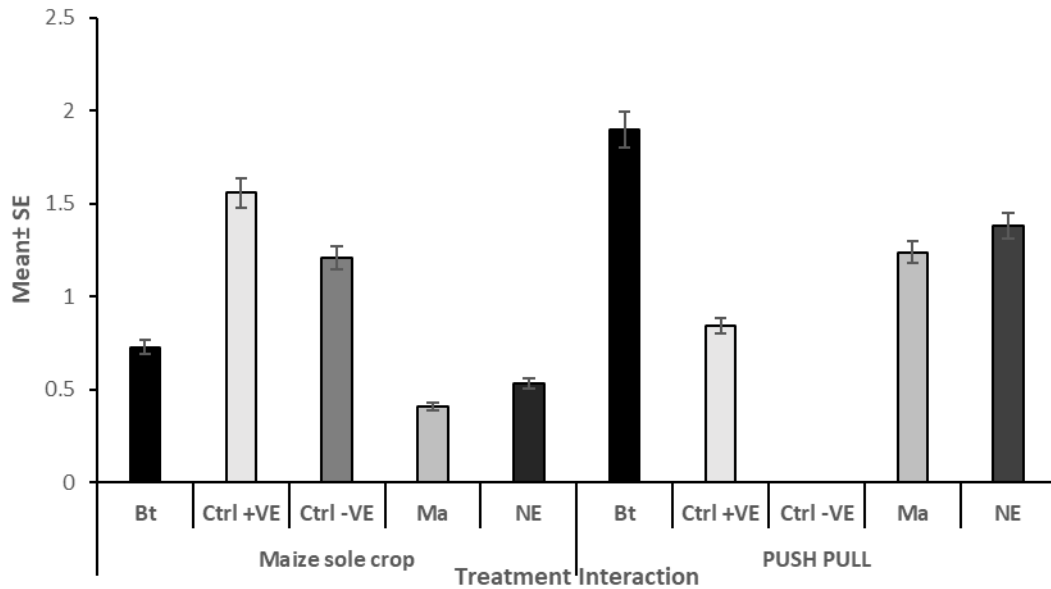
#### 4.6.2.3 Parasitism rate of *C. luteum*

The results further showed significant effect ( $P < 0.001$ ) on interaction between cropping system and biopesticides application. The highest mean was observed on Push pull technology treated with *Bacillus thuringiensis* biopesticides and the lowest was observed on PPT treated with flubendiamide as negative control (Table 4.5) and (figure 4.3). The mean ranged from  $1.9 \pm 0.62$  SE to  $1.70e-14 \pm 0.62$  SE. However, results on interaction between cropping system  $\times$  sampling week, biopesticides  $\times$  sampled week and that of between cropping system  $\times$  biopesticides  $\times$  sampled week did not show significant effects

on presence of *number of C. luteum*. The highest mean was observed in maize sole crop cropping system, *Bt* biopesticide application sample and sampled week 1 with average of  $0.880 \pm 0.42$  SE,  $1.297 \pm 0.5$  SE and  $3.333 \pm 0.57$  SE respectively.

**Table 4.5: Analysis of variance of cropping system and biopesticides on Parasitism rate of *C. luteum* on maize crop in Morogoro, Tanzania**

Factors	Statistics		
	<i>d.f</i>	<i>F</i>	<i>P</i>
Cropping system (CS)	1	0.5198	0.4716
Biopesticides (BIOP)	4	0.9017	0.4636
Sampling week after emergence (SWK)	7	15.2385	0.0000
CS:BIOP	4	3.3134	0.00115
CS:SWK	7	0.2400	0.9749
BIOP:SWK	28	0.3054	0.9998
CS:BIOP:SWK	28	1.1924	0.2389



**Figure 4.3: Effect of cropping system and biopesticides on Parasitism rate of *C. luteum* on on maize crop in Morogoro.**

**Key:** Ma = *Metarhizium anisopliae*, Bt = *Bacillus thuringiensis*, Ne = *Neem extract*, Ctrl-ve = maize sole plot with no any application used as negative control, Ctrl+ve = maize plot treated with flubendiamide used as positive control.

#### 4.6.3 Relative abundance of recovered parasitoids species

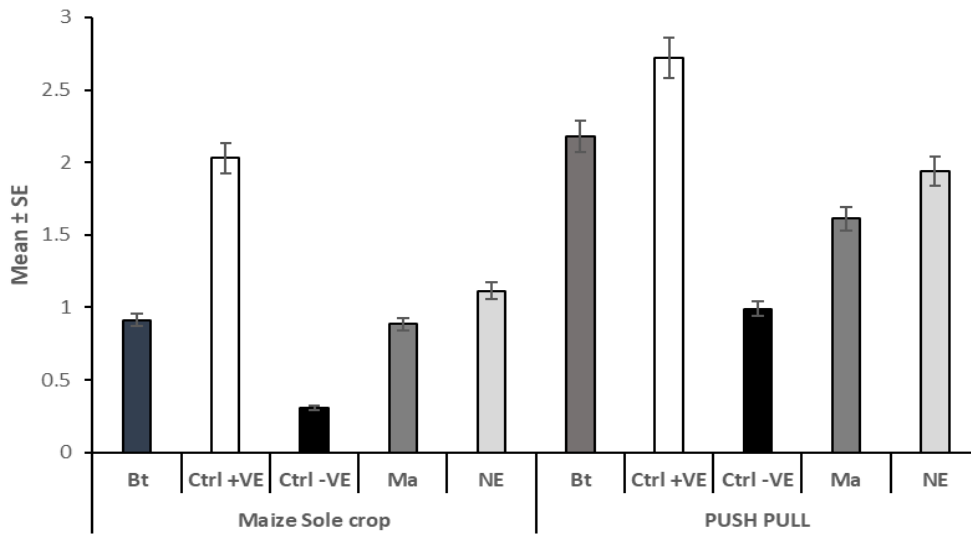
##### 4.6.3.1 Relative abundance of *Chelonus bifoveolatus* Szépligeti

The results showed significant effect ( $P < 0.05$ ) on interaction between cropping systems and sampling week with the highest mean percentage  $10.11 \pm 0.39$  SE on push pull technology in sampling week 1 and lowest on maize sole crop in sampling week 4, and that of between biopesticides and sampling week with the highest mean percentage

11.74± 0.597 SE on maize sole crop with no biopesticides application followed by neem biopesticides Bt and *Meterhizium anisopliae* in sampling week 1 and the lowest was observed in maize sole crop treated with flubendamide in sampling week 6, 7 and 8. However, results on interaction between cropping system × sampling week and that of between cropping system × biopesticides × sampled week did not show significant effects on relative abundance of *C. bifoventatus* species (Table 4.6) and (Figure 4.4). The highest mean was observed in climate- adapted PPT cropping system, maize sole cropping system and sampling week 1 with average mean of 11.96 ± 0.95 SE, 2.37 ± 0.25 SE, and 7.58 ± 0.29 SE respectively.

**Table 4.6: Analysis of variance of cropping system and biopesticides on relative abundance of *C. bifoventatus* on maize crop in Morogoro, Tanzania**

Factor	Statics		
	<i>d.f</i>	<i>F</i>	<i>P</i>
Cropping system (CS)	1	36.08370205	< 0.001
Biopesticides (BIOP)	4	14.26490465	< 0.001
Sampling week after emergence(SWK)	7	163.175652	< 0.001
(CS:BIOP)	4	0.815963784	0.51601
CROS:SWK	7	17.88698075	< 0.001
BIOP:SWK	28	5.53112369	< 0.001
CS:BIOP:SWK	28	0.384776508	0.9981



**Figure 4.4: Effect of relative abundance of *C. bifoveolatus* on cropping system and biopesticides on maize crop in Morogoro.**

**Key:** *Ma* = *Metarhizium anisopliae*, *Bt* = *Bacillus thuringiensis*, *Ne* = Neem extract, *Ctrl-ve* = maize sole plot with no any application used as negative control, *Ctrl+ve* = maize plot treated with flubendiamide used as positive control

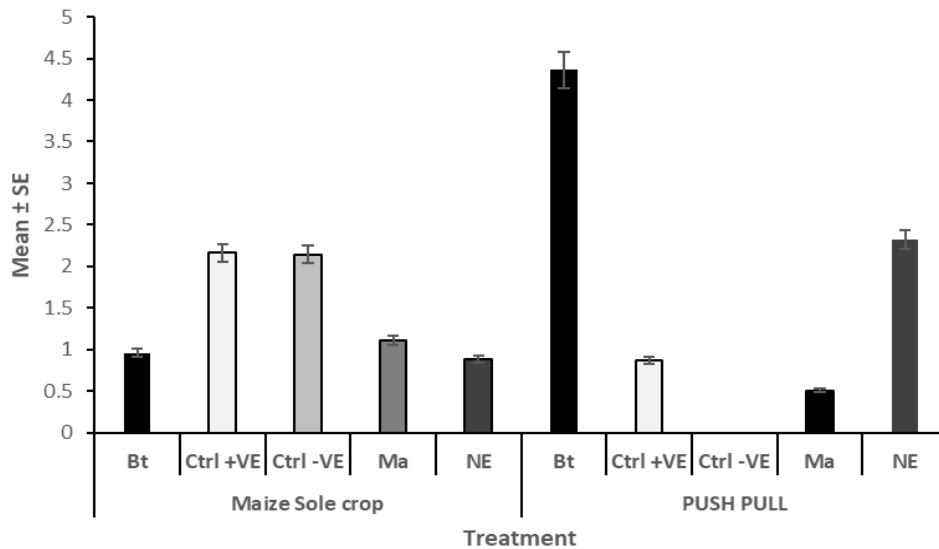
#### 4.6.3.2 Relative abundance of *C. luteum*

There was significant effect ( $P < 0.05$ ) of sampling week on the abundances *C. luteum* species. The results showed no significant effect of cropping systems and biopesticides treatment. The results also showed significant effect ( $P < 0.05$ ) on interaction between cropping system  $\times$  biopesticides application and that of between cropping system  $\times$  biopesticides  $\times$  sampled week (Table 4.7). The highest mean was observed on Push pull technology treated with Bt biopesticides and the lowest was observed on PPT treated with flubendamide ranged from  $4.36 \pm 1.09$  SE. Also the highest was observed on *Bt* biopesticides application and the lowest was observed on *Metarhizium anisopliae*

biopesticide application ranged from  $2.66 \pm 0.86$  SE to  $0.801 \pm 0.86$  SE. Then, the highest observed on *Bt* biopesticides in sampling week 1 and the lowest was observed on maize sole cropping system with no any biopesticide application in week 6. The mean ranged from  $13.47 \pm 1.98$  SE to  $-2.75e14 \pm 1.98$  SE. However, results on interaction between cropping system  $\times$  sampling week and that of between biopesticides  $\times$  sampled week did not show significant effects on relative abundance of *C. luteum* species. The highest mean was observed in PPT cropping system, *Bt* biopesticide application sample and sampling week 1 with average mean of  $1.61 \pm 0.68$  SE,  $2.66 \pm 0.85$  SE, and  $8.009 \pm 1$  SE respectively (Figure 4.5).

**Table 4.7: Analysis of variance of cropping sytem and biopesticides on relative abundance of *C. luteum* on maize crop in Morogoro, Tanzania**

Factors	Statistics		
	<i>d.f</i>	<i>F</i>	<i>P</i>
Cropping system (CS)	1	0.1089	0.7417
Biopesticides (BIOP)	4	1.6701	0.1577
Sampling week after emergence (SWK)	7	16.6337	0.0000
CS:BIOP	4	4.1552	0.0028
CS:SWK	7	0.0621	0.9996
BIOP:SWK	28	0.6288	0.9286
CS:BIOP:SWK	28	1.6398	0.0265



**Figure 4.5: Effect of cropping system and biopesticides on relative abundance of *C. luteum* on maize crop in Morogoro.**

**Key:** Ma = *Metarhizium anisopliae*, Bt = *Bacillus thuringiensis*, Ne = *Neem extract*, Ctrl-ve = maize sole plot with no any application used as negative control, Ctrl+ve = maize plot treated with flubendiamide used as positive control

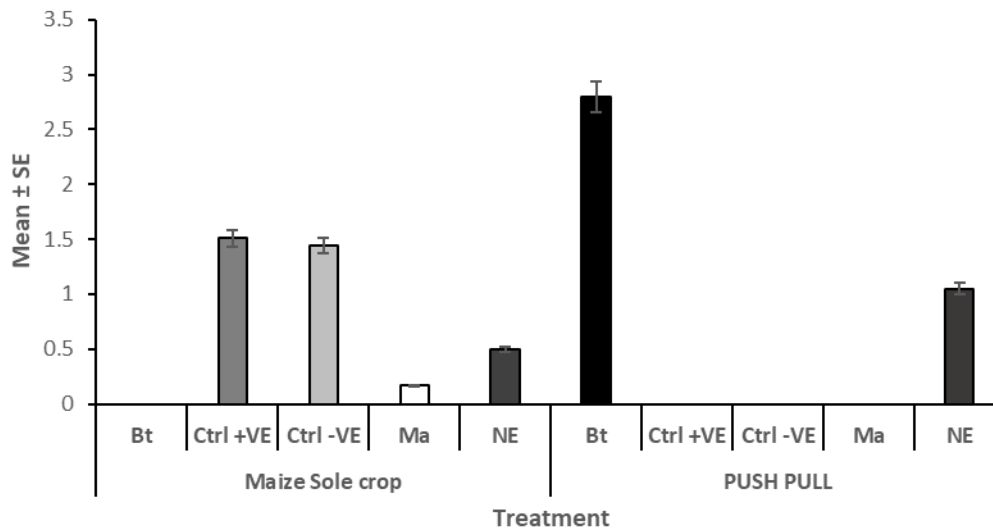
#### 4.6.3.3 Relative abundance of *Cotesia sp*

There was significant effect ( $P < 0.05$ ) on biopesticides application and sampling week on the percentage relative abundances of *Cotesia sp*. The results showed no significant effects of cropping systems. The results also showed significant effect ( $P < 0.001$ ) on interaction between cropping system and biopesticides application and that of between cropping system  $\times$  biopesticides  $\times$  sampled week. The highest percentage relative abundances of *Cotesia sp* was observed on climate adapted Push pull technology treated with *Bacillus thurigiensis* biopesticides and the lowest was observed on maize sole

cropping system treated with *Bt* biopesticides which ranged from  $2.8 \pm 0.567$  SE to  $2.44e-15 \pm 0.567$  SE. Also the highest was observed on *Bt* biopesticides application in sampling week 1 and the lowest was observed on maize sole crop treated with flubendamide in week 8. The mean ranged from  $6.41 \pm 0.96$  SE to  $-2.26e-16 \pm 0.96$  SE. Then, the highest observed on PPT followed by maize sole crop cropping system and the lowest was observed in maize sole cropping system in sampling week 4 which ranged from  $3.504 \pm 0.664$  SE to  $1.63e15 \pm 0.664$  SE. However, results on interaction between cropping system  $\times$  sampling week and that of between biopesticides  $\times$  sampled week did not show significant effects on percentage relative abundance of *Cotesia sp* (Table 4.8). The highest mean was observed in PPT cropping system, *Bt* biopesticide application and sampling week 1 with average mean of  $0.77 \pm 0.45$  SE,  $1.4 \pm 0.47$  SE and  $3.333 \pm 0.529$  SE respectively (Figure 4.6).

**Table 4.8: Analysis of variance of cropping system and biopesticides on the relative abundance of *Cotesia sp* on maize crop fields in Morogoro, Tanzania**

Factors	Statistics		
	<i>df</i>	<i>F</i>	<i>P</i>
Cropping system (CS)	1	0.0395	0.8426
Biopesticides (BIOP)	4	3.2404	0.0130
Sampling week after emergence (SWK)	7	14.7042	0.0000
CS:BIOP	4	11.6798	0.0000
CS:SWK	7	0.0395	0.9999
BIOP:SWK	28	1.1545	0.2773
CS:BIOP:SWK	28	4.1417	0.0000



**Figure 4.6: Effect of cropping system and biopesticides on the relative abundance of *Cotesia* sp on maize crop in Morogoro.**

**Key:** Ma = *Metarhizium anisopliae*, Bt = *Bacillus thuringiensis*, Ne = *Neem extract*, Ctrl-ve = maize sole plot with no any application used as negative control, Ctrl+ve = maize plot treated with flubendiamide used as positive control

#### 4.6 Discussion

In this study cropping system and biopesticides influence parasitism rate. Three species of parasitoids were recorded from FAW eggs and larvae collected in Morogoro Tanzania. Results of this study are consistent with recent findings of Ngangambe and Mwatawala

(2020) who reported that *Coccygidium luteum* (Brullé) and *Cotesia* sp were the only dominant larva parasitoids recorded with parasitism rate ranging from 9.5% to 12.3%. *Chelonus bifoveolatus* Szépligeti was the only dominant egg-larva parasitoids recorded with parasitism rate ranged from 2.56%  $\pm$ 2.56 to 18.20%  $\pm$  2.81. Studies by Sisay *et al.* 2018 reported *Cotesia* sp was the dominant larval parasitoid, with parasitism ranging from 33.8% to 45.3% in Ethiopia. However, in the present study, percent of parasitism by *C. luteum* and *Cotesia* sp were lower compared to the previous year's results reported by Ngangambe and Mwatawala (2020).

The *Chelonus bifoveolatus* Szépligeti was the primary egg-larva parasitoid species, which its parasitism rate ranged from 2.56% $\pm$ 2.56 to 18.20%  $\pm$ 2.81. *C. luteum* and *Cotesia* sp was the most common larva parasitoids recovered across the treatment for both cropping season. According to Sisay *et al.* (2018) larva parasitoids was found in Kenya and Tanzania, with parasitism ranging from 6% to 12% and 4% to 8.3%, respectively. Studies by Ngangambe and Mwatawala (2020) also found that the both egg-larva and larva parasitoid species across the Maize field in Tanzania. Further, one additional egg parasitoids, namely *Chelonus bifoveolatus* Szépligeti was recovered in 2021. The highest level of egg parasitism was 6.7 % $\pm$ 0.5 SE. Parasitoids species found in the present study have been also recovered from FAW eggs and larvae in East and West Africa (Kenis *et al.*, 2019; Cruz *et al.*, 2018). Five different species of parasitoids recovered from FAW eggs and caterpillar, *Cotesia icipe* (Hymenoptera: Braconidae) was among and the main parasitoid recorded in Ethiopia, with a percent parasitism rate of 37.6% (Sisay *et al.*, 2020).

The *Chelonus bifoveolatus* was the most abundant parasitoid collected from larvae in both cropping system and biobesticides. *Chelonus* spp are typical of egg-larval solitary

endoparasitoids that attack Noctuidae and Pyralidae (Murúa *et al.*, 2009) by ovipositing into host eggs. In the Western Hemisphere, *Chelonus* spp. appear to be the most geographically dispersed parasitoid of fall armyworm (Meagher *et al.*, 2016) and were reported to be present in 12 countries of the Caribbean, and South and Central America (Molina-Ochoa *et al.*, 2003). In many areas, *Chelonus* spp were reported to be the most common species collected (Rios-Velasco *et al.*, 2011; Virgen *et al.*, 2013). *Chelonus bifoveolatus* found in 7 of the 10 regions was recorded in all agroecological zones of Ghana cropping system and biopesticides farms. These results suggest this species is adapted to all sub-Saharan Africa agroecological zones (Koffi *et al.*, 2020).

According to Durocher-Granger *et al.* (2021) reported the presence of *Chelonus bifoveolatus* Szépligeti and *C. luteum* in Zimbabwe. Further, some of the parasitoids recovered in this study have been reported as parasitizing other insect species; for example, *Cotesia* spp, which is a new species from eastern Africa, was reared in Kenya as a solitary parasitoid from *Spodoptera littoralis* (Sisay *et al.*, 2019; Koffi *et al.*, 2020). Appropriate conducive conditions provided in the field such as farm practices, biodiversity of the natural flora, shelter, food source, no or limited use of synthetic pesticides are important for the survival of diverse natural enemies and their attraction to and colonization of the host habitat location (Ratnadass *et al.*, 2012). The above-mentioned conditions might constitute a “Push-Pull” strategy that led to the ecological niche occupation of the natural enemies found in this study. Also, the majority of the parasitoids were collected in maize plants with signs of FAW damage relative to the undamaged and slightly damaged (Koffi *et al.*, 2020; Ogunfunmilayo *et al.*, 2021). Parasitism of fall armyworm will increase as the pest continues to be present. However, high applications of chemical insecticides will negatively affect the natural enemies (Molina-Ochoa *et al.*, 2004; Figueiredo *et al.* 2006; Koffi *et al.*, 2020).

Due to occurrences and availability of both egg-larva and larva parasitoids species in both cropping system and biopesticides plots, which seems to encourage the life and development of such species, therefore, this can favor growth and development of parasitoid species in cropping system and biopesticides applied fields, which ultimately can be available and to be used as to for implementation as biological control of fall armyworm in Africa.

#### **4.7 Conclusions and Recommendations**

This study confirm the existence of a complex of native parasitoids of FAW in Morogoro Tanzania, some of which are among the most common larval parasitoids of FAW such as *C. luteum* can be used as the basis for the design of sustainable and farmer-friendly methods to control FAW. The availability of egg parasitoids of FAW such as *Chelonus bifoveolatus Szépligeti* which already occurs in most part Africa countries and *C. luteum* with the already occurring larval parasitoids should be considered for a significant reduction of FAW population. For an optimization of the impact of these parasitoids, it is crucial to manage farms using both cropping system and biopesticides approach.

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## CHAPTER FIVE

### 5.0 GENERAL CONCLUSIONS AND RECOMMENDATIONS

#### 5.1 Conclusions

The study showed that biopesticides caused prolonged developmental duration periods of life stages of *Spodoptera frugiperda*.

- a) Among all natural biopesticides used in this study, the longest developmental duration period was recorded on *B. thuringiensis* showed prolonged developmental duration among of all tested biopesticides where by the shortest developmental duration period were recorded on untreated FAW colony as it was carried out under laboratory conditions.
  
- b) Among of 777 larval and 2241 egg-larval parasitoid species collected in mid of March 2021 in Morogoro Tanzania from the fields, the study showed existing of only three parasitoids species one being egg-larval parasitoid species (*C. bifoveolatus*) and two being larva parasitoids species (*C. luteum* and *Cotesia sp*).
  
- c) The study demonstrated that Biopesticide namely *M.anisopliae* managed effectively fall armyworm in maize crop field, thus has potential for expansion in the African continent to manage key pests affecting cereal production in the continent

## 5.2 Recommendations

- a) Further studies on establishment of mass rearing of parasitoids as biological control of FAW for field release.
- b) Field trials to validate the laboratory results along with mass production and release of these biological control agents to keep the FAW pest below economically damaging population levels.
- c) Biopesticides (*M. anisopliae*, *B.thuringiensis* and *Azadirachta indica*) and push pull technology were effectively control FAW across all field trials. Emphasize should be made to the farmer on the use of it for sustainable and profitable maize production.
- d) Emphasize on Mass cultivation of *Brachiaria cv Mulato 11* and silver desmodium farm seeds should be made to the farmer to acquire enough seeds at low cost.
- e) Emphasize on tested botanical pesticidal plant (Neem plant) afforestation to farmers should be encouraged, this will enable them to get ecofriendly, biodegradable and low cost biopesticides for FAW control in Maize production.
- f) The government should ensure availability of registered and mass production of biopesticides to the market with affordable price.
- g) Further study should be conducted assessing durability and storage of harvested neem seeds for future use.