EFFECTS OF BIOPESTICIDES ON DEMOGRAPHIC PARAMETERS OF FALL ARMYWORM *Spodoptera frugiperda* J.E Smith (LEPIDOPTERA: NOCTUIDAE) IN MOROGORO TANZANIA

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A DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CROP SCIENCE OF SOKOINE UNIVERSITY OF AGRICULTURE. MOROGORO, TANZANIA.

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EXTENDED ABSTRACT

Fall armyworm (FAW) *Spodoptera frugiperda* J.E Smith is a new insect pest in Africa causing severe damage to maize (*Zea mays*) at its growth leading to yield loss as between 8.3 to 20.6 million metric tonnes per year (Day *et al.*, 2017). The pest is native to America with a wide host range of about 80 plant species that makes it difficult to manage (Prasanna *et al*., 2018). This study aimed at establishing the population dynamics of FAW in maize treated with biopesticides. Developmental biology and demographic parameters of FAW were determined using biopesticides (*Beauveria bassiana, Metarhizium anisopliae* and *Bacillus thuringiensis)* in a commercialized maize variety (DK9089, Monsanto Company, Nairobi, Kenya). The study was conducted at the entomology laboratory of Sokoine university of Agriculture in Morogoro, Tanzania. Experiments were set in a completely randomized design (CRD) and the FAW cohorts were maintained at 26^{o}C and 75% relative humidity. Results on the developmental biology of FAW showed significant variations in developmental durations of immature stages of FAW among treatments. Treated cohorts had significantly longer developmental durations than untreated cohorts. Likewise, results on demographic parameters of FAW differed significantly among treatments. In life table parameters, treated cohorts had low survivorships ($p < 0.001$), life expectancy ($p < 0.001$) and high probability of dying ($p < 0.001$) and mortality rates ($p < 0.001$) than untreated cohorts. Biopesticides also showed significant variation on population parameters of FAW between treated and untreated cohorts. Treated cohorts had low net reproductive rates ($p < 0.003$), intrinsic rate of increase ($p < 0.001$), finite rate of increase ($p < 0.001$) and long doubling time ($p < 0.001$) as well as the mean generation time ($p < 0.001$) contrary to untreated cohorts. Results obtained from this study can be used in implementing sustainable management options against FAW populations in Tanzania and rest of the world.

DECLARATION

I, Maduka Joseph Maduka, do hereby declare to 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 being concurrently submitted in any other institution.

…… ……

Maduka Joseph Maduka **Date**

MSc. Crop Science Candidate

The above declaration is confirmed by

…… ……

Prof. M. W. Mwatawala **Date**

Supervisor

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LIST OF ABREVIATIONS AND SYMBOLS

^{0}C	-	Degree Centigrade
ANOVA	-	Analysis of Variance
CABI	-	The Centre for Agriculture and Bioscience International
DK	-	Dekalb
EPF	-	Entomopathogenic fungi
FAO	-	Food and Agriculture Organization
FAOSTAT	-	The Food and Agriculture Organization Corporate Statistical Database
FAW	-	Fall armyworm
H	-	Hour
HSD	-	Honestly Significantly Difference
IITA	-	International Institute of Tropical Agriculture
IPM	-	Integrated Pest Management
CRD	-	Completely randomized Design
SUA	-	Sokoine University of Agriculture
USA	-	United States of America

CHAPTER ONE

1.0 GENERAL INTRODUCTION

1.1 Background

Maize (*Zea mays* L.) is the primary staple food crop for majority of Tanzanians and one of the priority crops to the Government of Tanzania. The key maize growing areas are Iringa, Mbeya, Ruvuma, Rukwa, Tanga, Kilimanjaro, Kagera, Morogoro, Manyara and Arusha (Rowhani *et al.*, 2011). Tanzania is among the major producers of maize in Sub-Saharan Africa, also ranked the 24th among the top 25 maize producing countries in the world (Suleiman and Kurt, 2015; FAO, 2016). Maize is an important source of calories, contributing 33% of the total household consumption and also the major source of income and food for majority of smallholder farmers (Amare *et al*., 2012; Suleiman and Kurt, 2015). Maize production is mainly affected by both biotic factors (pest and diseases) and abiotic factors such as temperature, wind, rainfall, soil type and soil p^{H}.

Among insect pests of maize, Fall armyworm (*Spodoptera frugiperda* J.E Smith) is currently the most devastating pest originated from America with a wide host range of 80 different crops such as cotton (*Gossypium hirsutum* L.), groundnut (*Arachis hypogaea* L.), sorghum (*Sorghum bicolor* (L.) Moench), wheat (*Trticum aestivum* L.), potato (*Solanum tuberosum* L.), soybean (*Glycine max* (L.) Merr) and sugarcane (*Saccharum officinarum* L.) (Day *et al*., 2017; IITA, 2016). Its polyphagous nature is a challenge to control due to the presence of numerous alternative hosts outside the production season of main crops (Silva *et al*., 2017). FAW has several generations per year, whose life cycle consists of egg, six larval instars, pupa, and adult (IITA, 2016). Completion of the life cycle usually takes about 4 weeks in warmer production regions, but it can take as long as 12 weeks during periods of low temperatures (Hardke *et al*., 2015).

Eggs of FAW are laid at night on the leaves of the host, stuck to the lower surface of the lower part of the lower leaves, in tight clusters of 100 - 300 and sometimes in two layers, usually covered with a protective layer of abdominal bristles. Hatching requires 2 - 10 days (usually 3 - 5) (IITA, 2016). The young larvae feed deep in the whorl and the first two instars feed gregariously on the underside of the young leaves that cause a characteristic skeletonizing or windowing effect, and the growing point can be killed (Day *et al.*, 2017). The rate of larval development through the six instars is controlled by a combination of diet and temperature conditions, and usually takes 14 - 21 days (Prasanna *et al*., 2018). Pupation takes place inside a loose cocoon in the soil, or rarely between leaves on the host plant, and 9 - 13 days are required for development. Adults emerge at night, and they typically use their natural pre-oviposition period to fly for many kilometers before they settle to oviposit. On average, adults live for 12 - 14 days (Prasanna *et al*., 2018).

Information about life table parameters of FAW such as survivorships, mortality rates, longevity, fecundity, life expectancy, probability of dying and the population parameters such as net reproductive rate, intrinsic rate of increase, finite rate of increase, doubling time and mean generation time is limited. FAW can migrate long distances on prevailing winds, but it can also reproduce continuously in areas that are climatically suitable (Prasanna *et al*., 2018).

FAW was reported for the first time to be in African continent early 2016. Early 2017, the pest was confirmed to be in East Africa (Kabede, 2018). Since identification FAW has already caused an estimated crop loss of 236 500 tonnes in Tanzania (Day *et al*., 2017).

In the absence of control measures, the pest can cause up to 100% yield loss (Abrahams *et al*., 2017). Chemical control is the widely used control approach. Application of available biocontrol methods is very limited.

1.4 Justification

Insect population dynamics have fundamental different characteristics depending on the strength and form of exogenous (density- independent) and endogenous (density-dependent) forces (Murúa *et al.*, 2006). Competition, natural enemies, resources and management practices are the factors which affects population abundance of the insect. The wide distribution of the FAW depends on environmental factors such as temperature, wind, moisture and soil type, but also the plant genotype, agricultural practices, and the crop phenology influences development and survival of the pest (Murúa *et al.*, 2006).

The polyphagous nature of FAW makes it difficult to control (Silva *et al*., 2017). Entomopathogens (fungi, bacteria, viruses, and nematodes) are among biological control measures that recently gained more attention for sustainable management of FAW (Prasanna *et al*., 2018; Day *et al*., 2017). These methods optimize naturally occurring diseases, through introduction and colonization of pathogens into insect populations as natural regulatory agents (Hardke *et al*., 2015). Developmental biology of FAW on maize has been previously reported (IITA, 2016). Studies showed the use of biopesticides extended developmental durations, reduced survival rates, fecundity, longevity, life expectancy and increased mortality rates of other lepidopteran and non-lepidopteran insect pests such as a tomato leaf minor *Tuta absoluta* (Meyrick), *Cyclocephala lurida* (Bland), and *Helicoverpa armigera* (Hübner) (Alikhani *et al.*, 2019; Wu *et al*., 2016; Lawo *et al*., 2008).

Information related to effect of biopesticides on developmental rates, survivorships, mortality rates, probability of dying, life expectancy, fecundity and longevity as well as population parameters such as net reproductive rate, intrinsic rate of increase, finite rate of increase, doubling time and mean generation time of FAW in maize is limited. There is a great need to establish the developmental biology and demography of FAW on maize treated with biopesticides as a strategy for development of sustainable management program.

1.5 Objectives

1.5.1 Overall objective

The overall objective of this study was to establish population dynamics of FAW in maize treated with bio pesticides

1.5.2 Specific objectives

i. To determine the developmental biology of the FAW in maize under different bio pesticide treatments
ii. To determine the demographic parameters of FAW in maize as affected by bio pesticides

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CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Historical background and distribution of maize in Tanzania

2.1.1 The history of maize in Tanzania

Maize (*Zea mays* L.) was introduced to Africa along the western and eastern coasts in the 16th century by the Portuguese and Arab explorers (Suleiman and Kurt, 2015). The crop was first received in the coastal areas (Pemba Island) by the Portuguese as food for themselves and their slaves (Urassa, 2010). Later, maize was introduced to Tanzania mainland (Tanganyika) in the 17th and spread to other parts by mid 19th century. Currently, it has spread as an important cereal crop all over the country (Urassa, 2010; Suleiman and Kurt, 2015).

2.1.2 Production trends and consumption of maize in Tanzania

Tanzania is the major maize producers in Sub-Saharan Africa and ranked the 24th among the top 25 maize producing countries in the world (Suleiman and Kurt, 2015; FAO, 2016). It is estimated that the annual per capita consumption of maize in Tanzania is 112.5 kg; national maize consumption is estimated to be three million tons per year (Suleiman and Kurt , 2015). Maize is grown in all regions of Tanzania on an average of two million hectares or about 45% of the cultivated area in Tanzania (Lyimo *et al*., 2014; Rowhani *et al*., 2011). Among all regions, most of maize is produced in the Southern Highlands, the Lake zone, and the Northern zone. Dar es Salaam, Lindi, Singida, Coast, and Kigoma are maize deficit regions (Amare *et al*., 2012; Suleiman and Kurt, 2015). The current national yield on maize production is reported to be low between 1.0 - 1.5 t/h, compared to the estimated potential yield of 4 - 5 t/h (Suleiman and Kurt , 2015).

2.1.3 Economic importance of maize in Tanzania

Maize is not only a staple crop but also a cash crop to farmers in the country. It has been identified as a key crop to enhance food production, income, poverty alleviation and food security (Rowhani *et al*., 2011). Maize contributes 60% of dietary calories and more than 50% of utilizable protein to Tanzanian consumers (Suleiman and Kurt, 2015).

2.1.4 Major insect pests affecting maize production in Tanzania

The major constraints to maize production in Tanzania include insect pests. Maize is attacked by many insect pests during all stages of growth from seedling to storage (Shiferaw *et al.*, 2011). The most economically important insect pests of maize in Tanzania can be categorized into field pests such as stalkborer *Busseola fusca* (Fuller), leafhoppers *Cicadulina mbila* (Latreille) and mole crickets *Gryllotalpa pluvialis* (Saussure). Others include African bollworm *Helicoverpa armigera* (Hübner), African armyworm *Spodoptera exempta* (Walker), and cutworms *Agrotis ipsilon* (Hufnagel), FAW *Spodoptera frugiperda* (J.E Smith) and storage pests like the maize weevil *Sitophilus zeamais* (Motschulsky), larger grain borer *Prostephanus truncatus* (Hon), red flour beetle *Tribolium castaneum* (Herbst), and dried bean beetles *Callosobruchus maculatus* (Fabricius) and Indian moths *Plodia interpunctella* (Hubner) (Suleiman and Kurt, 2015).

2.2 FAW geographical distribution

Fall armyworm is native to tropical and subtropical regions of the Americas. In 2016 it was reported for the first time from the African continent, in Nigeria, Sao Tomé, Benin and Togo (IITA, 2016).

It has now been confirmed in more than 30 African countries (Prasanna *et al*., 2018). In 2018, the pest was reported from the Indian subcontinent in Karnataka and Andhra

Pradesh. The pest has also been reported in Bihar, Chhattisgarh, Gujarat, Maharashtra, Odisha, Tamil Nadu, Telangana and West Bengal. Furthermore, FAW has also been reported in Bangladesh, China, Myanmar, Sri Lanka, and Thailand (IITA, 2016). Globally, the presence of the pest has been confirmed in the continents of Africa, North America, Central America, South America, and recently detected in Asia (figure 2.1) (CABI, 2019).

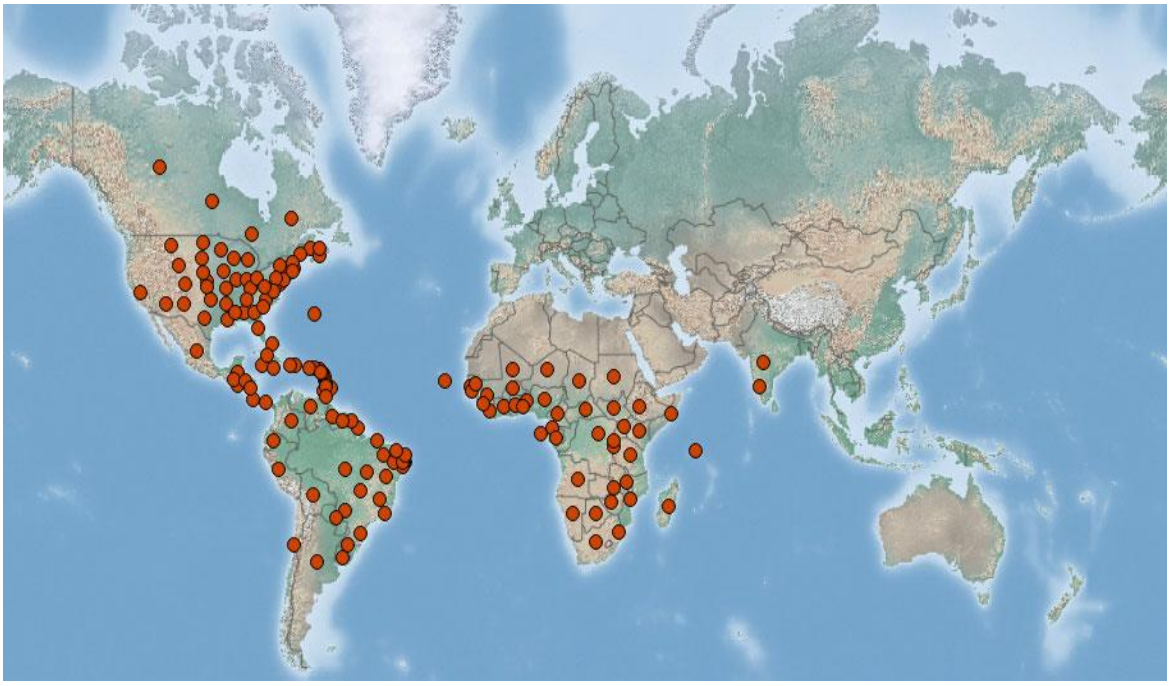

Figure 2.1: Map of Global FAW distribution

Source: (CABI, 2019)

2.3 FAW host range

Fall armyworms have wide host range over 80 different crops as listed below (Table 2.1) (CABI, 2019).

Table 2.1: List of main host plants of FAW

Common name	Scientific name	Family name
Banana	*Musa sapientum* L.	Musaceae
Bell pepper/sweet pepper	*Capsicum annuum* L.	Solanaceae
Cabbages, cauliflowers)	*Brassica oleracea* L.	Brassicaceae
Carnation	*Dianthus caryophyllus* L.	Caryophyllaceae
Chrysanthemum (florists)	*Chrysanthemum morifolium* L.	Asteraceae
Common bean	*Phaseolus vulgaris* L.	Fabaceae
Cotton	*Gossypium hirsutum* L.	Malvaceae
Cucumber	*Cucumis sativus* L.	Cucurbitaceae
Cucurbits	*Cucurbita maxima* L.	Cucurbitaceae
Egg plant	*Solanum melongena* L.	Solanaceae
Ginger	*Zingiber officinale* (Roscoe)	Zingiberaceae
Groundnut	*Arachis hypogaea* L.	Fabaceae
Lucerne/Alfaalfa	*Medicago sativa* L.	Fabaceae
Maize	*Zea mays* L.	Poaceae
Potato	*Solanum tuberosum* L.	Solanaceae
Rice	*Oryza sativa* L.	Poaceae
Sorghum	*Sorghum bicolor* L.	Poaceae
Soybean	*Glycine max* (L.) Merr.	Fabaceae
Spinach	*Spinacia oleracea* L.	Chenopodiaceae
Sugar beet var. saccharifera	*Beta vulgaris* L.	Chenopodiaceae
Sugarcane	*Saccharum officinarum* L.	Poaceae
Sweet potato	*Ipomoea batatas* L.	Convolvulaceae
Tobacco	*Nicotiana tabacum* L.	Solanaceae
Tomato	*Solanum lycopersicum* L.	Solanaceae
Turnip	*Brassica rapa* L.	Brassicaceae

Source: (CABI, 2019)

2.4 FAW description of life history and damage

2.4.1 Egg

FAW eggs are spherical in shape and have 0.75 mm diameter. At the time of oviposition they are green and become light brown prior to eclosion. Egg incubation period is 2 to 3 days at temperature range between 20°C to 30°C.

They are usually laid in masses of 150 - 200 eggs, laid in two to four layers deep on the upper surface of the leaf. The egg mass is usually covered with a protective, felt-like layer of grey-pink scales (setae) from the female abdomen. The total female fecundity is more than 1000 eggs (CABI, 2019).

2.4.2 Larva

Fall armyworm larvae are a light green to dark brown with longitudinal stripes. They have eight prolegs and a pair of prolegs on the last adbominal segment. After egg hatching, larvae are green with black lines and spots. Furthermore, they may turn into brown or remain green as they grow with black dorsal and spiracular lines. Large larvae are characterized by an inverted Y-shape on the head, black dorsal pinaculae with long primary setae (two each side of each segment within the pale dorsal zone) and four black spots arranged in a square on the last abdominal segment. Large larvae have cannibalism tendency over small larvae. Larva developmental stage has six instars whose length ranges from 1.68 mm for first instars to 34.15 mm for sixth instars. The head capsule width ranges from 0.314 mm for first instars to 2.78 mm for sixth instars (Prasanna *et al*., 2018; CABI, 2019).

2.4.3 Pupa

Fall armyworm pupae are brown in colour and turn darker as it grows. Pupation takes place inside a loose cocoon in the soil, or rarely between leaves on the host plant, and 9 - 13 days are required for pupal stage development (Prasanna *et al*., 2018).

2.4.4 Adult

Fall armyworm adults have a wing span of about 3.81cm (1.5 inches). The upper portion of the forewings are a mottled dark gray, with a distinctive white spot near the dorsal tip of the wing, while the lower portion of the forewings is a light gray to brown colour. The hind wings appear light gray to white. Adult male body length is 1.6 cm and wingspan 3.7 cm while adult female body length is 1.7 cm and wingspan 3.8 cm. The moths have filiform (threadlike) antennae and are active at night (Hardke *et al*., 2014).

2.5 FAW biology and ecology

Eggs are laid at night on the leaves of the host, stuck to the lower surface of the lower part of the lower leaves, in tight clusters of 100 - 300 and sometimes in two layers, usually covered with a protective layer of abdominal bristles (IITA, 2016). Hatching requires 2 - 10 days. The young larvae feed deep in the whorl; the first two instars feed gregariously on the underside of the young leaves causing a characteristic skeletonizing or windowing effect, and the growing point can be killed (Day *et al.*, 2017). Larger larvae become cannibalistic and thus one or two larvae per whorl are usual. The rate of larval development through the six instars is controlled by a combination of diet and temperature conditions, and usually takes 14 - 21 days (Prasanna *et al*., 2018).

Larger larvae are nocturnal unless they enter the armyworm phase when they swarm and disperse, seeking other food sources. Pupation takes place inside a loose cocoon in an

earthen cell, or rarely between leaves on the host plant, and 9 - 13 days are required for development. Adults emerge at night, and they typically use their natural pre-oviposition period to fly for many kilometers before they settle to oviposit, sometimes migrating for long distances. On average, adults live for 12 - 14 days (Prasanna *et al*., 2018).

Fall armyworm is adapted to warmer parts of the world, the optimum temperature for larval development is reported to be 28°C, but it is lower for both oviposition and pupation. In the tropics, breeding can be continuous with four to six generations per year, but in low temperature regions only one or two generations develops (IITA, 2016). This is because, at lower temperatures, activity and development cease, and when freezing occurs all stages are usually killed. Sandy-clay or clay-sand soils are suitable for pupation and adult emergence. Above 30°C the wings of adults tend to be deformed. Pupae require a threshold temperature of 14.6°C to complete their development (Day *et al*., 2017).

2.6 Economic impact of the FAW

Damages imposed by FAW at large pest population results to severe defoliation and eventually yield losses. According to Abrahams *et al*. (2017), yield losses due to FAW in Africa range from 8.3 to 20.6 million metric tonnes per year. Authors further reported that, yield loss under farmer level, can reach to 100% in the absence of control measures. Moreover, Day *et al.* (2017) reported yield losses in twelve maize producing countries in Africa (Table 2.2).

Table.2.2: Mean yield losses in the twelve maize producing countries in Africa.

Country	Yield loss (tonnes)
Benin	530 400
Cameroon	687 400
Democratic Republic of Congo	484 200
Ethiopia	2 735 200
Ghana	824 300
Malawi	1 380 300
Mozambique	514 700
Nigeria	3 838 900
Uganda	1 134 100
Tanzania	2 365 600
Zambia	1 154 000
Zimbabwe	455 600

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

2.7 Management of FAW

Different management options has been reported to control the damages resulted from FAW infestations. These are grouped into, cultural control, biological control, host plant resistance, chemical control and the use of pheromones (Day *et al.*, 2017).

2.7.1 Cultural control

The cultural and agronomic practices involves habitat management that can suppress or avoid pest damage through a variety of mechanisms, including conserving and encouraging the proliferation of natural enemies (Abrahams *et al.*, 2017). Early planting, intercropping, crop rotations, mulching, fertilizer application, use of Ash, field sanitation and push pull technique are reported to control FAW populations (Prasanna *et al.*, 2018).

Intercropping involves growing two or more crops in the same field at the same time, crops may be planted without regard to rows (mixed intercropping), in alternating rows, or with different crops alternating within the same row (Smith and McSorley, 2000).

Intercropping maize with food legume crops can reduce FAW damage levels by 30% with bean, 21% with soybean and 31% with groundnut (Hailu *et al*., 2018).

Push pull technique involve combination of intercropped companion plants that have repellant effect on pests and attractant trap crops round the edge of the field. The push pull is reported to reduce damage due to various pests in maize including FAW (Midega *et al*., 2018). The author further reported reductions of 82.7% in the average number of larvae per plant and 86.7% in plant damage per plot in climate-adapted push-pull compared to maize monocropped plots. According to Hailu *et al*. (2018) in push pull technology maize is intercropped with Silverleaf desmodium, *Desmodium uncinatum* (Jacq), to repel FAW moths and Napier grass, *Pennisetum purpureum* (Schum), a susceptible attractant crop, is planted surrounding the plot to attract repelled FAW moths. Results on the fields of farmers who adopted push pull technology in Kenya, Uganda and Tanzania indicated reduction of FAW larvae per plant and subsequent reduction in plant damage (Prasanna *et al*., 2018).

2.7.2 Biological control

Biological control is the action of living organisms (parasites, parasitoids, predators, and pathogens) introduced by human intervention for regulating the population of another organism at densities less than those that would occur in their absence (Prasanna *et al*., 2018). Parasitoids are biological agents for whom at least one of their life stages is intimately associated with specific life stages of the pest and with greater levels of specificity. Some of the parasitoids reported to parasitize egg of the FAW are species belongs to *Trichogramma* and *Telenomus* species (Day *et al*., 2017). Apart from that, Sisay *et al*. (2018) found parasitoids including *Cotesia* spp (ICIPE), *Palexorista zonata* (Curan), *Charops ater* (Szépligeti, 1910), and *Coccygidium luteum* (Brullé) with up to

45%, 12.5%, 12% and 8.3% field parasitism, respectively in surveys conducted in Kenya, Tanzania and Ethiopia.

Predators are insects such as ladybird beetles, earwigs, and sap sucking insects are reported as predators on various life stages of FAW (Prasanna *et al*., 2018). The earwig *Doru luteipes* (Scudder) is an important mortality factor for FAW (Sueldo *et al*., 2010). Luginbill, (1928) reported that, *Orius insidiosus* (Say, 1832) is a primary predator of the FAW, preying upon both eggs and larvae.

2.7.3 Biopesticides

Entomopathogenic Fungi (EPF): These have a broad spectrum of action with the ability to infect several species of insects and different stages, causing epizootics under natural conditions (Alves *et al*., 2014). The use of EPF brought more attention as a sustainable control in regulating insect pest population without harming the non-target insects (Lacey and Shapiro-Ilan, 2008). The fungi penetrate through cuticle of the insect by physical or enzymatic mechanisms and release fungal spores (Shahid *et al*., 2012). Degrading enzymes such as proteases, esterase, lipases and chitinases which modify the insect cuticle surface for spore penetration and attachment, for spore germination under favorable conditions (Khan *et al*., 2012; Shahid *et al*., 2012). The fungus multiplies rapidly using its host nutrients leading to physiological disruption leading to death (Lacey and Shapiro-Ilan, 2008).

Beauveria bassiana Bals. (Vuill), *Metarhizium anisopliae* (Metchnikoff), and *Nomuraea rileyi* (Farl.) are the common fungi with potential uses against insect pests including FAW (Wraight *et al*., 2010). Cruz-Avalos *et al.* (2018) reported high mortalities on eggs and

neonate larvae of FAW when exposed to *B. bassiana* and *M. anisopliae* isolates than untreated control.

Bacteria: *Bacillus thuringiensis* (*Bt*) Berliner biopesticides are the most widely used in America. These are ubiquitous, soil-dwelling, gram-positive bacteria that produce crystal proteins named delta-endotoxins, which are insecticidal. These endotoxins have relative levels of specificity to specific groups of insects. Among the various strains of *Bt*, FAW is more susceptible to *B. aizawai* and *B. thuringiensis* (Polanczyk *et al*., 2003; Da Silva *et al*., 2004). Variations among populations of FAW in their susceptibility to different Cry toxins are reported (Monnerat *et al*., 2006).

According to Prasanna *et al*. (2018), seven *Bt* strains were recorded highly effective, and can cause 100% mortality of FAW larva on 7 days post treatment of a lethal concentration. Apart from the Cry toxins, FAW is also susceptible to some of the vegetative insecticidal proteins found in the *Bt* culture supernatants (Polanczyk *et al*., 2000).

Entomopathogenic nematodes: have been reported to be alternatives to chemical pesticides in controlling many soil-dwelling insect pests in America, Asia and Europe (Kaya *et al*., 2006). Garcia *et al.* (2008) reported that 280 infective juveniles of *Steinernema* spp. were required to kill 100% of third-instar FAW in petri dishes, as compared to 400 infective juveniles of the *Heterorhabditis indica* Poinar, nematode to obtain 75% FAW control. Furthermore, Negrisoli *et al.* (2010a) reported that several commercial insecticides are compatible with three species of EPNs including *H. indica*, *Steinernema carpocapsae* (Weiser, 1955) and *Steinernema glaseri* Steiner, under laboratory conditions. It was also reported that the efficacy of *H. indica* was enhanced against FAW when mixed with an insecticide, Lufenuron (Negrisoli *et al.*, 2010b).

Viruses: Entomopathogenic viruses are obligate intracellular parasites having either DNA or RNA encapsulated into a protein coat known as capsid to form the virions or nucleocapsids. These viruses have proved to be very effective in managing populations of certain pests such as Lepidoptera and Hymenoptera pests in Europe, USA and Canada (Kalha *et al*., 2014). According to Barrera *et al.* (2011) virus-based insecticides are mostly in the Baculovirus group, have been identified having the highest potential for development as bio insecticides due to specificity, high host virulence, and the highest safety to vertebrates.

Two types of *Baculovirus* have been studied for the control of FAW, namely granulovirus (SfGV) (Betabaculovirus) and multiple nucleopolyhedrovirus (SfMNPV) (Alphabaculovirus). However, SfMNPV has greater potential for use in the management of FAW (Haase *et al.*, 2015). SfMNPV is specific to only FAW larvae and under natural conditions, the pest is infected orally by ingesting the contaminated food and multiplies in the nucleus of the host and spreads to the body cavity leading to infection of other tissues such adipose tissue, epidermal, tracheal matrix and even salivary glands, malpighian tube, and blood cells, causing its death from 6 to 8 days after ingestion (Gómez *et al.*, 2013). Hamm and Shapiro (1992) reported high mortality of FAW larvae from infection by nuclear polyhedrosis virus enhanced by a fluorescent brightener.

2.7.4 Host plant resistance in maize

The use of transgenic or genetically modified (GM) crop varieties that express lepidopteran resistance is reported to effectively control FAW damage in maize (Prasanna *et al*., 2018). In America, the use of GM crops containing *Bt* genes is popular and provides control of more than one pest species; however the approach is not cost effective in Africa (Fischer *et al*., 2015). Williams *et al*. (1998) reported that, resistant maize varieties to

FAW showed less leaf damage, and larvae feeding on resistant maize grew more slowly. The author further reported that, susceptible and resistant varieties shows less damage as the plant grew older, but resistant varieties completed the transition from juvenile to adult plant earlier than susceptible varieties.

Early maturing maize varieties has less damage than late maturing varieties (Pitre, 2015). Host plant resistance in maize occurs through antibiosis, resulting in reduced survival, reduced feeding rate resulting in reduced size and fitness, and lower attractiveness of the plants, resulting in reduced oviposition (Viana and Potenza, 2000).

2.7.5 Pheromone traps

Female FAW moths attract males by emitting a pheromone. The chemical composition of this has been determined, and synthetic pheromone can be used as a lure in a trap to monitor the moth population (Abrahams *et al*., 2017). However, not all the chemical components of the natural pheromone are required to make an effective control. The trap should be placed in the centre of the planting area and used at a density of one trap for every five hectares of crop also should be hung approximately 1.5 meters above the ground. If the planted seed has not been treated, monitoring should start soon after emergence (Prasanna *et al*., 2018). Trapping reduces the male population to such an extent that females are unable to mate. Gilson *et al*. (2018) reported the use of traps made from plastic bottles with a pheromone lure. The information on pheromone traps against FAW males to reduce damage is limited.

2.7.6 Chemical control

Chemical control involves the application of poisons to the pest and/or crop that kill the FAW through a variety of mechanisms, including on contact or through ingestion (Day *et*

al., 2017). Most commonly, the pesticides are diluted with water and sprayed on growing plants at around 200 – 400 litres per hectare, though this can vary considerably with the age of the plant and the application method (Prasanna *et al*., 2018). Commonly used insecticides against FAW include cypermethrin, deltamethrin, lambda-cyhalothrin, permethrin, and chorpyrifos (Togola *et al*., 2018). According to Kumela *et al*. (2019), 46% of farmers in Kenya use insecticides for controlling FAW; similarly 60% of farmers in Ethiopia use insecticides against FAW.

Insecticides are used in Africa as an immediate control option against FAW (Sisay *et al*., 2018). Prassana *et al*. (2018) reported that, early pest detection enhances insecticide effectiveness on young larvae and spraying should target the middle portions of plants leaves where the pest hides and lays eggs. Moreover, Hardke *et al*. (2014) observed high mortality rates of FAW larvae on cotton treated by insecticides chlorantraniliprole (Coragen 200 SC), flubendiamide (Belt 480 SC), lambda-cyhalothrin (Karate Z 250 EC), novaluron (Diamond 100 EC) and spinetoram (Radiant 120 SC) than untreated cotton.

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CHAPTER THREE

3.0 DEVELOPMENTAL BIOLOGY OF FALL ARMYWORMW IN MAIZE

3.1 Abstract

Fall armyworm (FAW) *Spodoptera frugiperda* is one of the most devastating polyphagous field pests in many parts of the world, including Tanzania. Developmental biology of the FAW was determined by using commercial formulations of bio pesticides *Bacillus thuringiensis, Beauveria bassiana* and *Metarhizium anisopliae*. Durations of developmental stages of FAW on maize (*Zea mays* L.) treated with lower dose of 2 ml/litre of water were determined in the laboratory at Sokoine university of Agriculture in a completely randomized design (CRD) with four replications. Cohorts of FAW were established from maize plots around the study area. Results showed that, duration of immature stages of FAW differed significantly among treatments. Egg incubation period, larval stage duration, pupa stages duration and the total developmental time were significantly longer ($P \leq 0.05$) on bio pesticides treated cohorts than on the control. The lowest developmental thresholds of immature stages were estimated at 2 ± 0.2, 10 ± 0.4, and 9 ± 0.41 days for egg, larval and pupal stages respectively. These results further confirmed that bio pesticides can work effectively to control FAW population in Morogoro region and other areas of Tanzania.

Keywords: *Spodoptera frugiperda*, *Bacillus thuringiensis*, *Metarhizium anisopliae*, *Beauveria bassiana*, immature stage, duration, fall armyworm, bio pesticides

3.2 Introduction

The fall armyworm (FAW) *Spodoptera frugiperda* J.E Smith (Lepidoptera: Noctuidae) is a devastating pest of maize which is native to America (Day *et al.*, 2017). The pest was reported for the first time on the African continent in early 2016 (Amare *et al*., 2012;

IITA, 2016). By May 2017 the pest had spread to 14 East and Central African countries and 11 out of the 15 countries of the Southern African Development Community (SADC) region (Sisay *et al.*, 2019). According to Day *et al.* (2017), yield losses due to FAW in Africa range from 8.3 to 20.6 million metric tonnes per year in the absence of any control methods. Under farmer level, the insect can cause up to 100% yield loss (Abrahams *et al.*, 2017).

The pest attacks over 80 different plant species including major crops such as cotton (*Gossypium hirsutum* L.), groundnut (*Arachis hypogaea* L.), sorghum (*Sorghum bicolor* (L.) Moench), wheat (*Trticum aestivum* L.), potato (*Solanum tuberosum* L.), soybean (*Glycine max* (L.) Merr) and sugarcane (*Saccharum officinarum* L.) (IITA, 2016). The polyphagous nature of FAW is a challenge in management due to the presence of numerous alternative hosts outside the production season of main crops (Silva *et al.*, 2017).

Fall armyworm has several generations per year, with the life cycle consisting of egg, six larval instars, pupa, and adult. Completion of the life cycle usually takes about 4 weeks in warmer production regions, but it can take as long as 12 weeks during periods of low temperatures (Hardke *et al.*, 2015). Eggs of FAW are laid at night on the leaves of the host, stuck to the lower surface of the lower part of the lower leaves, in tight clusters of 100-300 and sometimes in two layers, usually covered with a protective layer of abdominal bristles. Hatching requires 2-10 days (usually 3-5) (IITA, 2016). The young larvae feed deep in the whorl and the first two instars feed gregariously on the underside of the young leaves that cause a characteristic skeletonizing or windowing effect, and the growing point can be killed (Day *et al.* , 2017).

The rate of larval development through the six instars is controlled by a combination of diet and temperature conditions, and usually takes 14-21 days (Prasanna *et al*., 2018). Pupation takes place inside a loose cocoon in the soil, or rarely between leaves on the host plant, and 9-13 days are required for development. Adults emerge at night, and they typically use their natural pre-oviposition period to fly for many kilometers before they settle to oviposit, sometimes migrating for long distances. On average, adults live for 12-14 days (Prasanna *et al*., 2018).

Chemical control has been the main management option, with limited biological control measures. However, among biological control measures reported, entomopathogens (fungi, bacteria, viruses, and nematodes) gained more attention as a sustainable management option, to suppress FAW populations. These methods optimize naturally occurring diseases, through introduction and colonization of pathogens into insect populations as natural regulatory agents (Hardke *et al.*, 2015). Several studies have reported the effect of entomopathogens on developmental durations of insect pests (Wu *et al*., 2016; Wakil *et al.*, 2017; Alikhani *et al.*, 2019). Information on effects on entomopathogens on development of FAW is limited. 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. *Beauveria bassiana* Bals. (Vuill), *Metarhizium anisopliae* (Metchnikoff) Sorokin (1883), and *Nomuraea rileyi* (Farl.) Samson are the common fungi with potential uses against insect pests including FAW (Wraight *et al.*, 2010). The bacteria, *Bacillus thuringiensis* (Berliner) is also the widely used biopesticides to control FAW population (Prasanna *et*

al., 2018). These are ubiquitous, soil-dwelling, gram-positive bacteria that produce crystal proteins named delta-endotoxins, as well as vegetative proteins which are insecticidal. These endotoxins have relative levels of specificity to specific groups of insects (Da Silva *et al.*, 2004; Polanczyk *et al*., 2000). The current study is aimed at establishing the developmental biology of the fall armyworms on maize treated with bio pesticides.

3.3 Materials and methods

3.3.1 Study location

The studies were conducted under laboratory conditions at the Sokoine University of Agriculture (SUA), situated in Morogoro, (Eastern Central Tanzania).

3.3.2 Establishment of cohort of FAW

Fall armyworm larvae were collected from farmers maize fields around Morogoro municipality and brought to the laboratory at SUA. The larvae were then placed in the rearing cages of dimensions of 100 cmx 50 cm x 50 cm with a perforated lid to allow ventilation and prevent individuals from escaping. The cages were kept at an average temperature of 26^{o}C and 75% relative humidity. Pieces of tender, fresh maize leaves were introduced daily into the cages to feed the larvae. FAW larvae were maintained until adult emergence. Emerged adults were fed with honey on petri dishes (diameter = 14cm). A cohort of 30 adults at ratio of 15 male: 15 female was formed from individuals that emerged. Each cohort was placed in a rearing cage. A cohort was established following the protocols described by Prasanna *et al*. (2018), and maintained for three generations.

3.3.3 Establishment of egg incubation period, larval and pupa stage duration

Maize variety DK9089 (Monsanto Company, Nairobi, Kenya) was treated with biopesticides, Mazao prevail® (*B. bassiana* 1×10^{9}cfu/ml), Mazao achieve®,

(*M. anisopliae* 1×10^{9}cfu/ml) and Real thuringiensis® (*B. thuringensis* 1×10^{7}cfu/ml) all from Real IPM Company (K) Limited, Kenya. Biopesticides were applied at the lowest recommended application rate of 2 mls/L (highest being 5mls/L) equivalent to 2×10^{6}cfu/L (for *B. bassiana* and *M. anisopliae*) and 2×10^{4}cfu/L (for *B. thuringensis*). The experiment was conducted in a completely randomized design (CRD) with four replications. Honey was placed in a petridish (diameter = 14cm) and introduced into a rearing cage containing a cohort of 30 adult FAW (ratio 1: 1) aged 14 days for feeding. The adults were allowed to mate and eggs were collected daily and counted under a microscope. A camel's hairbrush was used to place on a moist filter paper in a petridish (diameter = 14cm), cohorts of 100 fresh eggs that were exposed to entomopathogens by spraying to make a direct contact of eggs and pathogens at the rate of 2 ml/L of water for hatching. Egg hatching was observed under a light microscope twice a day; 12 h after extractions. Number of hatched eggs and duration of hatching were recorded.

In a set up similar to above, a cohorts of 50 newly emerged larvae were collected and placed into rearing cages and fed with pieces of maize leaves sprayed with at lower doses of biopesticides. The cages had sterilized sand as pupation media. Larvae were examined daily for symptoms of infections from entomopathogens for the period of 14 days. The duration of larva development was recorded. Cohorts of 30 fresh pupae (24 h after emergence) were placed onto a moist filter paper in a petridish (diameter = 14cm) and sprayed to lower doses of entomopathogens. The pupae were placed in rearing cages containing sterilized sand as a growth media. Pupae were examined daily for a period of 10 days, and the duration for pupa development was recorded. Eggs, larvae, and pupae were observed following protocols described by Prasanna *et al*., (2018), Samuels *et al*., (2002), and Schneider *et al*., (2013).

3.4 Data collected

The data recorded at each bio pesticide and each developmental stage was, times taken for eggs to hatch, larvae to pupate and pupae to emerge into adult FAW. Also, the number of newly emerged adults was recorded. Then, the total developmental duration of FAW from egg to adult emergence was established.

3.5 Data analysis

One - way ANOVA was run using Genstat software 15th edition to determine the effect of bio pesticides, followed by *post hoc* Tukey's Honest Significance Difference (HSD). All statistical tests for significance were performed at $P \leq 0.05$.

3.6 Results

3.6.1 Egg incubation

Results showed that incubation period of FAW eggs varied significantly ($F_{3, 12} = 8.23$, $p = 0.003$) among treatments. Biopesticides caused significantly longer incubation period compared to the control. FAW eggs sprayed with *B. thuringiensis* hatched at slowest rate, while untreated eggs hatched at fastest rate (2 ± 0.2 days). Incubation period of eggs treated with *B. bassiana* and *M. anisopliae* did not differ significantly (Figure 3.1). Incubation period ranged from 2 ± 0.2 to 2.8 ± 0.04 days.

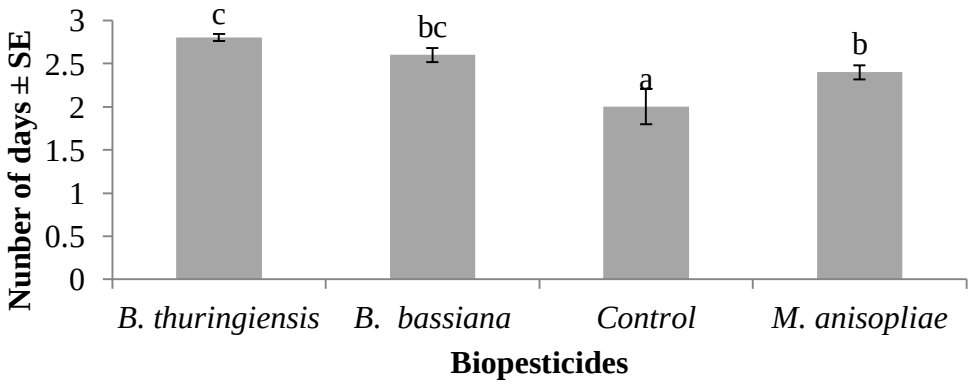

Figure 3.1: Effect of biopesticides on egg incubation period

3.6.2 Larval duration

Results also showed significant ($F_{3, 12}$ = 10.4, p = 0.001) effects of treatments on FAW larval duration. Cohorts of FAW larvae exposed to biopesticides developed significantly slower than the untreated cohorts. The longest larval duration was recorded in *B. thuringiensis* treated cohorts. Untreated cohorts had shortest larval duration. However, there was no significant difference in duration of *B. bassiana* and *M. anisopliae* treated larvae (Figure 3.2). Larval duration ranged from 10 ± 0.41 to 15 ± 0.38 days.

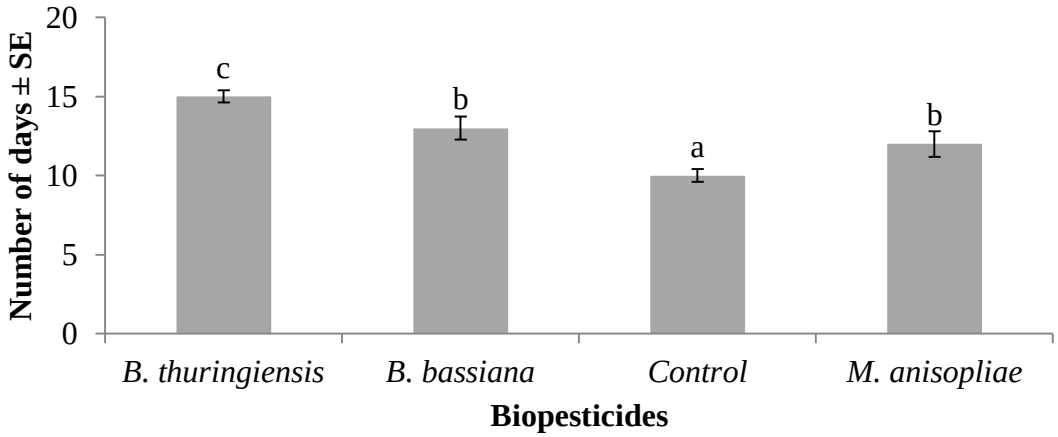

Figure 3.2: The effect of biopesticides on larva stage duration

3.6.3 Pupa stage duration

Pupa stage duration also varied significantly ($F_{3, 15}$ = 7.57, p = 0.004) among treatments. Pupae exposed to *B. thuringiensis* developed at slowest rate. However, there was no significance variation on pupa duration among *B. thuringiensis*, *B. bassiana* and *M. anisopliae* treated pupae. The duration of pupal stage ranged from 9 ± 0.4days in untreated cohorts to 12.75 ± 0.6 days in *B. thuringiensis* treated cohorts (Figure 3.3).

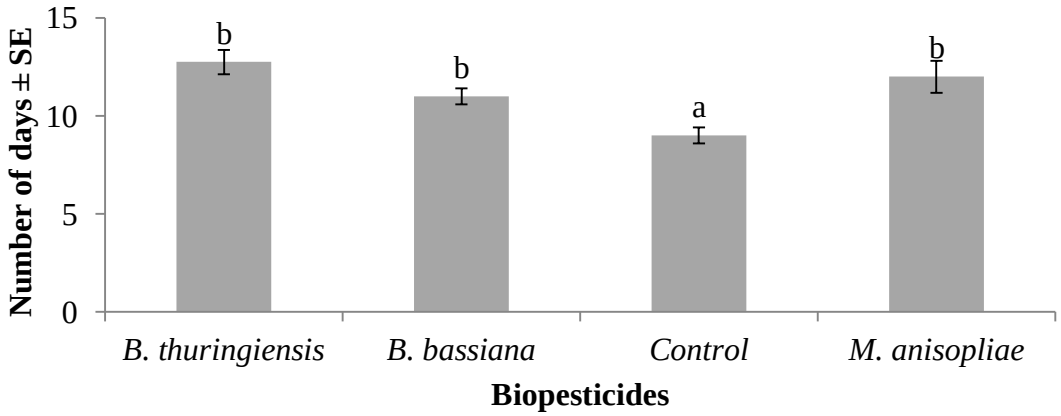

Figure 3.3: The effect of biopesticides on pupa stage duration

3.6.4 Total developmental duration

It was observed that biopesticides significantly ($F_{3, 12}$ = 1 1.75, $p < 0.001$) increased the total development period (egg to adult) of FAW. Total developmental duration was longest on *B. thuringiensis* treated cohorts and shortest in the untreated control. However, the variation in total developmental period of FAW between *B. bassiana* and *M. anisopliae* treated cohorts was not significant. Total development period ranged from 21 ± 0.6 days in untreated cohorts to 30.55 ± 1.07 days in *B. thuringiensis* treated cohorts (figure 3.4).

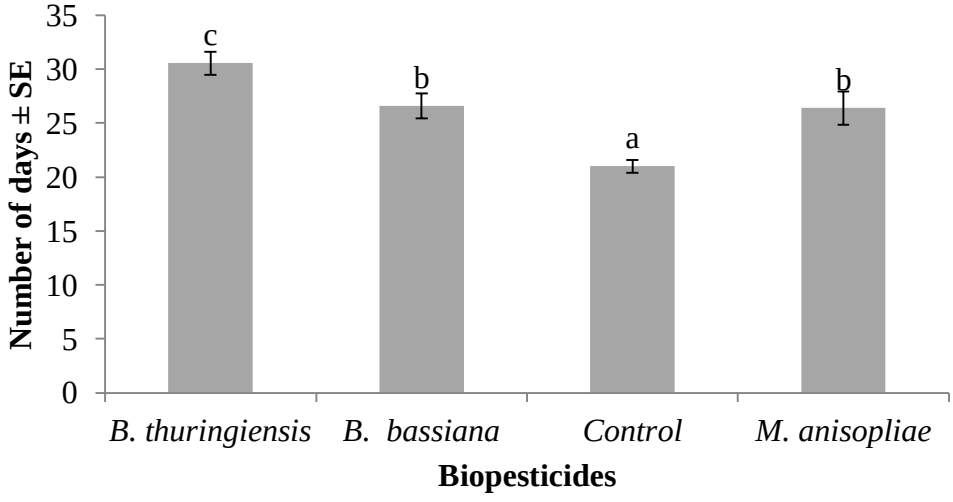

Figure 3.4: The effect of bio pesticides on total development period

3.7 Discussion

The results showed significant variations in developmental times of FAW among biopesticides and the control. Biopesticides are one of the biological factors that affect developmental stages of insect pests that include FAW (Murua *et al.*, 2006). Growth and development are necessary for successful completion of the insect's life - cycle and reproduction. Any delay may render the insect susceptible to biotic and abiotic factors (such as natural enemies or environmental regimes) that would ultimately limit their growth and development (Wakil *et al.*, 2017).

In the current study, entomopathogens infected the immature stages of FAW which affected their physiological growth that caused prolonged durations of life stages and eventually failure to attain the next stage of growth. This was contrary to the untreated control. Wakil *et al.* (2017) observed prolonged durations of larva growth stage and pupa growth stage of *Rhynchophorus ferrugineus* (Olivier) when treated with *B. bassiana* and *M. anisopliae.*

The authors further observed similar trend on the combined effect of *Heterorhabditis bacteriophora* (Poinar) and *B. bassiana*, as well as *H. bacteriophora* and *M. anisopliae.* Similarly to that, Bokonon-Ganta *et al.* (2003) observed prolonged larval and pupal durations of FAW on transgenic maize incorporated with *B. thuringiensis* than on conventional maize. Also, Alikhani *et al.* (2019) observed delayed/ prolonged egg incubation period, larva developmental time, and pupa developmental time on the tomato leaf miner *Tuta absoluta* (Meyrick, 1917) when treated with *M. anisopliae* on sub lethal to lethal concentrations. Apart from that, Hardke *et al.* (2015) reported that larva of FAW required more time to pupate as well as pupa required more time to develop when fed both *Bt* maize leaf tissues compared to that on non - *Bt* maize leaf tissue. Moreover, Wu *et al.*

(2016) observed prolonged pupa duration of *Cyclocephala lurida* (Bland) when treated with *B. bassiana* and *M. brunneum* on sub lethal doses. Similar trend was observed in the current study on all the developmental durations of FAW.

However, the bacteria *B. thuringiensis* caused extended durations of FAW life stages than all bio pesticides tested. Among the various biopesticides used for insect control, *B. thuringiensis* is the most widely used (Prasanna *et al*., 2018). The bacteria produce crystal proteins named delta-endotoxins, which are insecticidal. The endotoxins have relative levels of specificity to specific groups of insects. Apart from the Cry toxins, FAW is also susceptible to some of the vegetative insecticidal proteins found in the *Bt* culture supernatants (Polanczyk *et al*., 2000).

The current study confirmed that, entomopathogens have significant importance on FAW population control. Delayed/prolonged immature stages developmental durations of the FAW were attributed to the effects of biopesticides to infect eggs, larva, and pupa, which affected the physiological growth and development of the FAW. An insect can stay longer on a population while infected with no impact on the population just because it cannot survive to the next growth stage due to reduced feeding, growth and development hence become susceptible to biotic and abiotic factors (Wakil *et al*., 2017).

The minimum egg incubation period recorded on the control (2 ± 0.2 days) and the highest egg incubation period (2.8 ± 0.04 days) recorded during this study were within the range of 2 – 3 days reported by Prasanna *et al.* (2018). The longest larva stage duration (15 ± 0.38 days) recorded on *B. thuringiensis* are within the range of 14 – 30 days reported by Day *et al*. (2017). Furthermore the shortest pupa stage duration (9 ± 0.4 days) recorded in this study on the control and the highest pupa stage duration (12.75 ± 0.63) recorded in

this study, are within the range of 9 – 13 days as reported by Prasanna *et al.* (2018). Furthermore, the highest total developmental time recorded on *B. thuringiensis* (30.55 ±1.07 days) and the lowest total developmental period (21 ± 0.6 days) recorded on the control, are within the range of 21-30 days as reported by Prasanna *et al.* (2018).

3.8 Conclusion

Generally, biopesticides have a significant effect on the developmental stages of the FAW; this implies to a great advantage on the management options against FAW. Prolonged growth durations have impact on FAW generations, infected FAW will have fewer generations compared to non-infected FAW. This reduces the population of FAW and hence sustainable management can be attained. Biological controls should be emphasized to the farmers because it is sustainable. From the results, this study confirmed that biopesticides are important and recommended control option against fall armyworm.

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CHAPTER FOUR

4.0 DEMOGRAPHIC PARAMETERS OF FALL ARMYWORM IN MAIZE

Abstract

Fall armyworm (*Spodoptera frugiperda* J.E Smith) is among the most important pests damaging maize crops in Tanzania. Life table and population parameters of FAW when exposed to biopesticides were compared in an experiment laid in Completely Randomised Design (CRD) with four replications. Commercial formulations of *Bacillus thuringiensis*, *Beauveria bassiana* and *Metarhizium anisopliae* were applied at lower doses on a maize (*Zea mays* L.) variety DK9089 and exposed to developmental stages of FAW in a laboratory at Sokoine University of Agriculture (SUA), Morogoro, Tanzania. Results showed that, there were significantly reduction in survivorship ($p < 0.001$) and life expectancy ($p < 0.001$). On the contrary, biopesticides significantly increased mortality rate ($p < 0.001$) and the probability of dying ($p < 0.001$) but lowered net reproductive rate ($P < 0.003$), intrinsic rate of increase ($p < 0.001$) and the finite rate of increase ($p < 0.001$). Biopesticides also significantly reduced doubling time (DT) ($p < 0.001$) and the mean generation time ($p < 0.001$). These results showed great potential of use of biopesticides to control FAW in maize.

Key words: biopesticides, *B. thuringiensis, B. bassiana*, *M. anisopliae*, *Spodoptera frugiperda*, life table, survivorship, life expectancy, mortality

4.1 Introduction

Fall armyworm (FAW) *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae) is a fairly new pest of maize in Africa. The pest is native to tropical and subtropical regions of the western hemisphere from the United States of America to Argentina (Day *et al.* , 2017). Currently, FAW has spread to several counties in Africa, that include East and

Central African countries and caused significant yield losses on maize (*Zea mays* L.) of around 8.3 to 20.6 million metric tonnes per year under the absence of control methods (Abrahams *et al.*, 2017). The pest has a wide host range of over 80 different plant species (Prasanna *et al*., 2018; Day *et al*., 2017; IITA, 2016).

Chemical control methods against FAW are the most dominant (Da Silva *et al*., 2017). However, chemical control options are not sustainable and there is a great need to explore biological control options for sustainable management of FAW. Different studies have reported on life table parameters (such as survivorships, mortality rates, probability of dying and life expectancy) and population parameters such as net reproductive rate, intrinsic rate of increase, finite rate of increase, doubling time and the mean generation time of different pests including lepidopterans insects when exposed to entomopathogens (Hardke *et al.*, 2015; Wu *et al.*, 2016; Cruz-Avalos *et al.*, 2018; Alikhani *et al.*, 2019).

However, there is limited information on effects of entomopathogens on life table and population parameters of FAW. Virla *et al.* (2008) reported high mortality rate and low survival rate of FAW larvae when exposed to artificial diet treated with *Bacillus thuringiensis* (Berliner). Similarly, Cruz-Avalos *et al.* (2018) reported high mortalities on eggs and neonate larvae of FAW when exposed to *Beauveria bassiana* Bals. (Vuill), and *Metarhizium anisopliae* (Metchnikoff) Sorokin (1883), isolates. Alikhani *et al.* (2019) reported low reproductive rate, low intrinsic rate of increase, finite rate of increase and long doubling time and the mean generation time of the tomato leaf miner *Tuta absoluta* (Meyrick, 1917) when treated with *M. anisopliae*. Hardke *et al.* (2015) reported that, entomopathogens (fungi, bacteria, viruses and nematodes) suppress insect pest populations by optimization of naturally occurring diseases. These results into reduced feeding, reduction on fecundity or reproduction potential, decreased longevity and reducing the rate

of development as well as survival chances of the pest in the population (Wu *et al.*, 2016). Entomopathogenic fungi (EPF) have a broad spectrum of action with the ability to infect several species of insects and different stages, causing epizootics under natural conditions (Prasanna *et al.*, 2018; Alves *et al.*, 2014). The fungus 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. Induction of epizootics depends on climatic factors such as wind, rain or frequency of contact among the insects. Diseased insects stop feeding, become discolored (cream, green, reddish or brown) and ultimately die.

Beauveria bassiana, *M. anisopliae*, and *Nomuraea rileyi* (Farl.) Samson are common potential fungi used against insect pests including FAW (Wraight *et al.*, 2010). The bacteria, *B. thuringiensis* (*Bt*) is also the widely used biopesticides to control FAW population (Prasanna *et al.*, 2018). It has been reported that, FAW is more susceptible to *B. thuringiensis* subsp *aizawai* and *B. thuringiensis* strains (Da Silva *et al.* 2004; Prasanna *et al.*, 2018). *Bt* strains produces endotoxins (Cry toxins) but also vegetative proteins found in the *Bt* culture supernatants which have insecticidal effect against FAW (Polanczyk *et al.*, 2000). FAW was first recorded in Tanzania in the first quarter of 2017 (Amare *et al.*, 2012; IITA, 2016). The pest has already caused an estimated crop loss of 2 365 600 tonnes in the country since identification (Day *et al.*, 2017). There are no reliable management options against the FAW in Tanzania. The current study is aimed at establishing the demography of FAW on maize treated with biopesticides. The information generated from this study will facilitate management of FAW based on biological control in Morogoro region and other parts of Tanzania.

4.2 Materials and methods

4.2.1 Study location

The studies were conducted in the laboratory conditions at the Sokoine University of Agriculture (SUA), Morogoro, Tanzania. The temperature in the lab was maintained at an average temperature of 26^{o}C and relative humidity of 75%.

4.2.2 Establishment of cohort of FAW

Fall armyworm larvae collected from farmers maize fields were placed in the rearing cages (100 cm x 50 cm x 50 cm in dimensions) with perforated lids to allow ventilation, to prevent individuals from escaping. The larvae were examined daily for growth and development. The larvae were fed with fresh tender maize leaves that were cut into small pieces of 10 cm long. The leaves were changed daily. Emerging adults were fed with honey on petri dishes placed in the rearing cages. Adults that emerged on the same day were counted and isolated into cohorts of 30 cohort individuals at a ratio of 15:15 (male: female) and placed in the rearing cages. A cohort was established following the protocols described by Prasanna *et al*. (2018) and maintained for three generations.

4.2.3 Establishing survival rates of life stages of FAW

The experiment was laid in a completely randomized design (CRD) with four replications. A cohort of adults was introduced in a rearing cage containing a petridish (diameter = 14cm) with honey for feeding. The adults were allowed to feed and mate for 14 days before eggs collection.

Eggs were collected daily and counted by using light microscope. A camel's hairbrush was used to place on a moist filter paper in a petridish (diameter = 14cm).

A cohort of 100 eggs were sprayed with lower recommended doses of commercial formulations of entomopathogens *B. bassiana* (Mazao prevail®, Real IPM Company, Nairobi, Kenya), *M. anisopliae* (Mazao achieve®, Real IPM Company, Nairobi, Kenya) and *B. thuringiensis* (Real thuringiensis®, Real IPM Company, Nairobi, Kenya) at the rate of 2 ml/L of water for hatching (Samuels *et al*., 2002; Wu *et al*., 2016). Egg hatching was observed under a microscope twice a day; 12 h after extractions and the number of hatched eggs and duration of hatching were counted and recorded. In similar set to above, a cohort of 50 newly emerged larvae were collected and placed into a rearing cage.

Pieces of maize leaves 10cm long, (variety DK9089, from Monsanto Company, Nairobi, Kenya) treated with biopesticides at lower recommended dose were placed on a petridish (diameter = 14cm) and introduced into the rearing container with sterilized sand as pupation media. Larvae were examined daily for symptoms of infections from entomopathogens for the period of 14 days and the number of dead larvae was recorded until pupa emergence. Cohorts of 30 fresh collected pupae (24 h after emergence) were placed onto a moist filter paper in a petridish (diameter = 14cm) and sprayed to lower doses of entomopathogens (Schneider *et al*., 2013). The petridishes (diameter = 14cm) with pupae were introduced in the rearing unit containing sterilized sand as a growth media. Pupae were examined daily for a period of 10 days, and the number of dead pupa and survived pupa were recorded until adult emergence. Eggs, larvae, and pupae were observed following protocols described by Prasanna *et al*. (2018), Samuels *et al*. (2002), and Schneider *et al*. (2013).

4.2.4 Adult demographic parameters

Adult life-history traits were assessed using a cohort of 30 individuals emerged from infected pupae placed in rearing cages, and adults were fed with honey and sugar (Modolon *et al.*, 2017).

Maize leaves were wrapped in the containers to facilitate oviposition. Eggs were gently removed and counted under a light microscope. Number of dead and surviving adults was also recorded daily.

4.2.5 Establishing a standard life table and population parameters

Life table (Survivorships, mortality rates, probability of dying and life expectancy) and population parameters such as net reproductive rate, intrinsic rate of increase, finite rate of increase, doubling time and mean generation time of FAW were calculated as per data collected in 4.3, using the formula which were also used by Sansan *et al.* (2011) and Alikhani *et al.* (2019) as detailed in the table below:-

Table 4.1: Life table and population parameters

Parameter	Symbol	Formula
Net reproductive rate	Ro	$\sum_{\times=\propto}^{\beta} I_x m_x$
Intrinsic rate of increase	R	$Ln(Ro)/T$
Finite rate of increase	ƛ	e^r
Doubling time	DT	$Ln(2)/r$
Mean generation time	T	$\sum_{x=\propto}^{\beta} x I_x m_x \Big/ \sum_{x=\alpha}^{\beta} I_x m_x$
Survivorship	I_x	N_x/N_o
Mortality rate	d_x	$I_x - I_{x+1}$
Probability of dying	q_x	d_x/I_x
Life expectancy	E_x	$\sum L_x/N_x$

α = Age at which reproduction starts; β = age at which reproduction stops; I_x= proportional of original cohort surviving at age x and x+1; m_x= number of offspring per individual at age x, Nx= total number of individuals at age X, N_o = total number of individuals in the cohort, L_x = mean number of alive individuals.

4.3 Data Recorded

At each biopesticides treatment, the following data were recorded; number of laid eggs per day per female, numbers of eggs hatched, emerged larvae, pupae, and adults, daily mortality of adults, daily mortality of immature stages and fecundity of adults. These were used to establish life table parameters and compute fecundity, mortality rates, survival rates, probability of dying and life expectancy.

4.3.1 Data analysis

One way ANOVA was run using Genstat software 15th edition to determine the effect of bio pesticides, also Tukey‘s honest significance difference was used for means separation. All statistical tests for significance were performed at $P \leq 0.05$.

4.4 Results

4.4.1 Life table parameters

4.4.1.1 Survivorships

Egg survival rate varied significantly ($F_{3, 12} = 114.24$, $p < 0.001$) among treatments (Table 4.2). The highest egg survival rate was significantly higher in untreated than in biopesticides treated cohorts. The lowest egg survival rate was 30.94 ± 4.08% recorded in *B. thuringiensis* treated cohort while the highest was 93.95 ± 1.17% recorded in untreated control. Likewise, larval survival rate significantly ($F_{3, 12} = 29.5$, $p < 0.001$) changed with treatments. Larva survival rate was higher in the control treatment compared to cohorts exposed to biopesticides. Results further showed significantly ($F_{3, 12} =149.89$, $p < 0.001$) higher survival rates of pupae in control cohorts compared to cohorts of pupae treated with biopesticides. The highest pupa survival was recorded on the control (96.00 ± 1.63%) and the lowest on *B. thuringiensis (*37.00 ± 2.86%).

Survival rate of untreated adults was significantly ($F_{3, 12} = 82.02$, $p < 0.001$) higher than in treated adults cohorts. Adult survival rate ranged from 49.00 ± 3.67% in *B. thuringiensis* exposed cohorts to 86.00 ± 0.82% in untreated control. Among all treatments, the lowest survival rates on life stages of FAW were recorded on *B. thuringiensis* while the highest survival rates were recorded on the control treatment. The survivorship trend was as shown on figure 4.1

Table 4.2: Percentage survival rates of growth stages of FAW

Stage	Treatment			
	B. thuringiensis	***B. bassiana***	***M. anisopliae***	**Control**
Egg	30.94 ± 4.08a	46.90 ± 0.06b	42.89 ± 3.00b	93.95 ± 1.17c
Larva	33.33 ± 5.44a	49.43 ± 5.99b	43.24 ± 1.36ab	89.43 ± 3.81c
Pupa	37.00 ± 2.86a	41.10 ± 0.40ab	43.00 ± 1.22b	96.00 ± 1.63c
Adult	49.00 ± 3.67a	51.03 ± 0.41a	50.40 ± 1.22a	86.00 ± 0.82b

NB: Values in a row followed by similar letters are not statistically different at $P \leq 0.05$

4.4.1.2 Mortality rates

Egg mortality rate varied significantly ($F_{3, 12} = 113.94$, $p < 0.001$) among treatments (Table 4.3). The highest egg mortality rate was inflicted by *B. thuringiensis* (69.06 ± 4.09%) while lowest egg mortality rate was recorded on the control treatment (6.05 ± 1.17%). There was no significant different difference in egg mortality rate among cohorts exposed to *B. bassiana* and *M. anisopliae*. Mortality of larvae treated with biopesticides was significantly ($F_{3, 12} = 29.5$, $p < 0.001$) higher than the control. Larva mortality rate was highest in *B. thuringiensis* treated cohorts and lowest in untreated cohorts. Larva mortality rates recorded on cohorts treated *B. bassiana* and *M. anisopliae* were not significantly different.

Pupa mortality rate varied significantly ($F_{3, 12} = 249.89$, $p < 0.001$) among treatments. The highest pupa mortality was recorded on *B. thuringiensis* treated cohorts and the lowest was recorded in untreated control. Results further showed that pupa mortality rates recorded among *B. bassiana* and *M. anisopliae* treated cohorts were not significantly different. Mortality rates of adults exposed to biopesticides were significantly higher ($F_{3, 12} = 82.02$, $p < 0.001$) than the control. The highest adult mortality rate was recorded on *B. thuringiensis* (51.00 ± 3.67%) and the lowest on the control treatment (14.00 ± 0.82%).

However the variations were not significantly different among cohorts treated with *B. bassiana* and *M. anisopliae.* Among all treatments, the lowest mortality rates on life stages of FAW were recorded on the control while the highest mortality rates were recorded on *B. thuringiensis.*

Table 4.3: Percentage mortality rates of growth stages of FAW

	Treatments			
Stage	***B. thuringiensis***	***B. bassiana***	***M. anisopliae***	**Control**
Egg	69.06 ± 4.09c	53.10 ± 0.06b	57.10 ± 3.00b	6.05 ± 1.17a
Larva	66.76 ± 5.44c	50.67 ± 5.99b	56.66 ± 1.36bc	10.67 ± 3.81a
Pupa	63.00 ± 2.86c	59.00 ± 0.41b	57.00 ± 1.22bc	4.00 ± 1.63a
Adult	51.00 ± 3.67b	49.00 ± 0.41b	50.00 ± 1.23b	14.00 ± 0.82a

NB: Values with similar letters has no significance difference at P≤0.05

4.4.1.3 Probability of dying and age specific life expectancy

The probability of adults to die was significantly ($F_{3, 12}$ = 25.89, p < 0.001) changed by treatments (Table 4.4). Cohorts exposed to *B. thuringiensis* and *M. anisopliae* showed highest probability of dying. Among all treatments, the lowest probability of dying of adult FAW was observed on the control. The probability of dying ranged from 0.1631 ± 0.011 on untreated cohorts to 1.00 ± 0.16 and 1.00 ± 0.05 on *B. thuringiensis* and *M. anisopliae* treated cohorts respectively. Untreated adults were expected to live significantly ($F_{3, 12}$ = 82.02, p < 0.001) longer than treated individuals. Among all treatments, the lowest life expectancy was recorded in *B. thuringiensis* (4.98 ± 0.018) while the highest life expectancy was observed on the control (5.72 ± 0.008). The life expectancy trend was as shown in figure 4.3

Table 4.4: Probability of dying and life expectancy

Parameter	Treatments			
	B .thuringiensis	***B. bassiana***	***M.anisopliae***	**Control**
Pd	1.00 ± 0.16b	0.96 ± 0.02b	1.00 ± 0.05b	0.16 ± 0.01a
e_x	4.98 ± 0.02a	5.02 ± 0.00a	5.00 ± 0.01a	5.72 ± 0.01b

NB: Values in a row followed by similar letters are not statistically different at $P \leq 0.05$
Pd = probability of dying, e_x = life expectancy

4.4.1.4 Adult longevity and total fecundity

Adult longevity was significantly reduced by bio pesticides than untreated control on males ($F_{3, 12} = 23.86$, $p < 0.001$) and female FAW ($F_{3, 12} = 44.29$, $p < 0.001$). Male longevity ranged from 6.25 ± 0.5 days on *B. thuringiensis* treated cohorts to 11.0 ± 0.42 days in untreated cohorts. Likewise, female longevity ranged from 6.7 ± 0.13 days on *B. thuringiensis* treated cohorts to 12.48 ± 0.36 days in untreated cohorts. Treatments significantly ($F_{3, 12} = 8.34$, $p < 0.003$) affected total fecundity of adults. The lowest fecundity rate (787.60 ± 99.54 eggs) was recorded on *B. thuringiensis* treated cohorts and the highest fecundity rate (1200 ± 48.99 eggs) was recorded on untreated cohorts (Table 4.5). Fecundity trend for adult FAW was as shown in figure 4.2.

Table 4.5: Adult longevity and total fecundity

Parameter	Treatment			
	B. thuringiensis	***B. bassiana***	***M. anisopliae***	**Control**
M. longevity	6.25 ± 0.50a	8.10 ± 0.41a	7.33 ± 0.39a	11.00 ± 0.42b
F. longevity	6.70 ± 0.13a	8.63 ± 0.38ab	7.82 ± 0.33b	12.48 ± 0.36c
T. fecundity	787.60± 99.54a	950.00±37.86ab	1028.00±19.60bc	1200.00±48.99c

NB: Values in a row followed by similar letters are not statistically different at $P \leq 0.05$
M = male, F = female, T = total

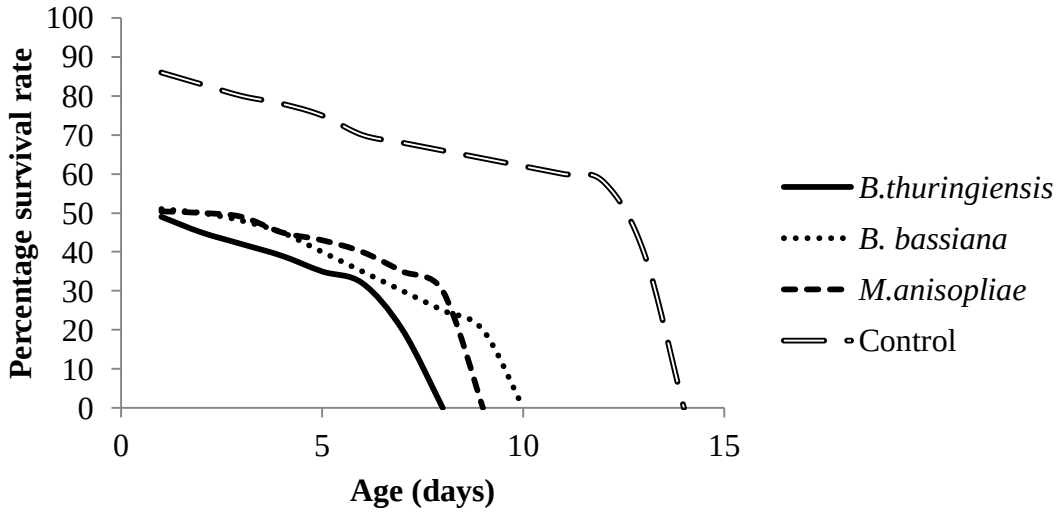

Figure 4.1: Survivorship trend of adult FAW under biopesticide treatments

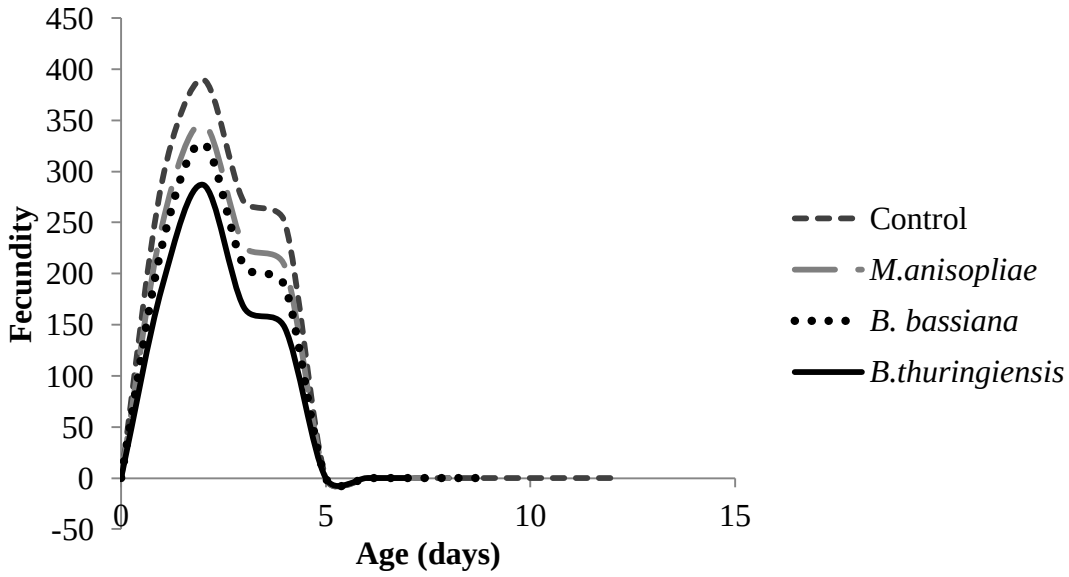

Figure 4.2: Fecundity trend of adult FAW under biopesticides treatments

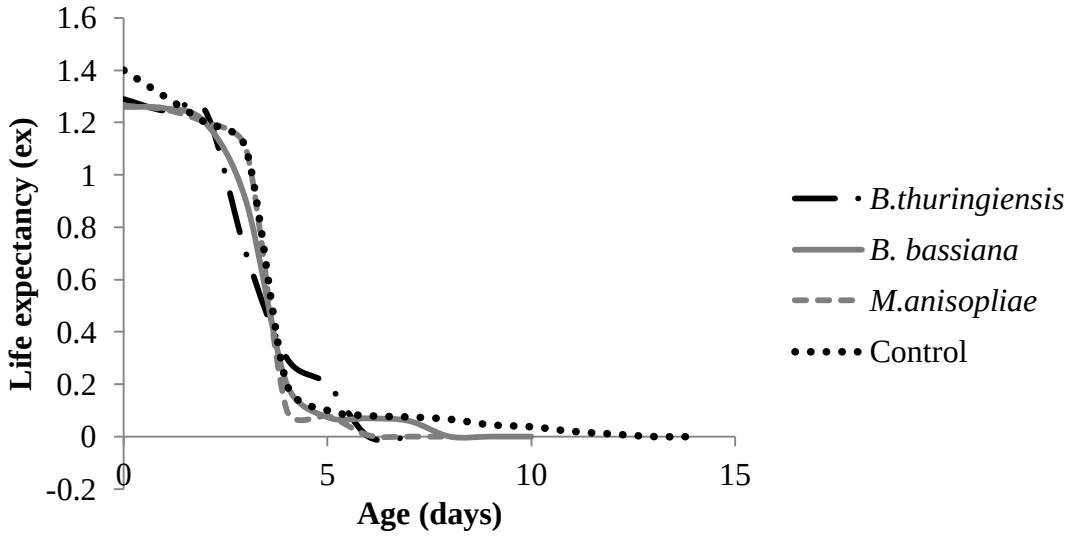

Figure 4.3: Life expectancy trend of adult FAW under biopesticides treatments

4.4.1.5 Population parameters

Treatments significantly ($F_{3, 12} = 8.32$, $p < 0.003$) affected the net reproductive rate (Ro) between among treatment. Untreated cohorts had highest net reproduction rate, that rate ranged from 196.9 ± 24.88 eggs in *B. thuringiesis* treated cohorts to 300.0 ± 12.25eggs in untreated cohorts. This study also found that the intrinsic rate of increase was significantly ($F_{3, 12} = 20.25$, $p < 0.001$) higher in the untreated cohorts compared to individuals exposed to biopesticides. Intrinsic rate of increase varied from 0.1729 ± 0.008 in *B. thuringiensis* treated to 0.2726 ± 0.006 in untreated cohorts. Furthermore, the finite rate of increase was significantly ($F_{3,12} = 22.69$, $p < 0.001$) higher in the untreated control than in biopesticides exposed cohorts, ranged from 1.190 ± 0.010 in *B. thuringiensis* treated to 1.315 ± 0.009 in untreated cohorts.

Likewise, the doubling time (DT) was significantly ($F_{3, 12} = 15.68$, $p < 0.001$) shorter in the control than biopesticides treated cohorts. The highest doubling time was 4.033 ± 0.178 days recorded in *B. thuringiensis* treated cohorts and the lowest was 2.545 ± 0.051days recorded in untreated cohorts. The mean generation time was significantly

($F_{3, 12} = 11.75$, $p < 0.001$) changed by treatments. The longest mean generation time (30.55 ± 1.071 days) was recorded in cohorts exposed to *B. thuringiensis* while the shortest (21.00 ± 0.612 days) was recorded in untreated cohorts. Cohorts exposed to *B. bassiana* and *M. anisopliae* did not differ significantly in all population parameters (Table 4.6).

Table 4.6: The effect of biopesticides on population parameters of FAW

Parameter	*B. thuringiensis*	*B. bassiana*	*M. anisopliae*	Control
Ro	196.90±24.88a	237.50±9.46ab	257.00±9.80b	300.00±12.25c
R	0.17±0.01a	0.21±0.01b	0.21±0.01b	0.27±0.01c
ƛ	1.19±0.01a	1.23±0.01b	1.24±0.01b	1.32±0.01c
DT	4.03±0.18c	3.37±0.16b	3.39±0.18b	2.55±0.05a
T	30.55±1.07c	26.60±1.14b	26.40±1.55b	21.00±0.61a

NB: Values with similar letters along the row has no significance difference at P≤0.05
Ro = net reproductive rate, r = intrinsic rate of increase, ƛ = finite rate of increase,
DT = doubling time, T = mean generation time.

4.5 Discussion

The results showed significant variations on life table parameters of FAW among biopesticides and the control treatment. Entomopathogens including bacteria and fungi are among biopesticides that infect and cause diseases in insects. According to Prasanna *et al*. (2018), fungi such as *M. anisopliae* and *B. bassiana* and bacteria such as *B. thuringiensis,* infect FAW and cause cessation of developmental activities and eventually death. However, this also impairs the survival and reproduction rates of the FAW. Results of the current study showed that survival rates of FAW egg, larva, pupa and adult were low when exposed to biopesticides as compared to the control treatment. Furthermore, the mortality rates of biological stages of FAW exposed to biopesticides were higher than untreated insects.

This was due to high virulence of entomopathogens bacteria (*B. thuringiensis)* and fungi (*B. bassiana* and *M. anisopliae*) that infected FAW. Virla *et al.* (2008) reported high mortality rate and low survival rate of FAW larvae when exposed to artificial diet treated with *B. thuringiensis* than untreated control. Similarly, Cruz-Avalos *et al.* (2018) reported high mortalities on eggs and neonate larvae of FAW when exposed to *B. bassiana* and *M. anisopliae* isolates than untreated control.

Also, Lezama-Gutiérrez *et al.* (2001) on the study of occurrence of entomopathogens of FAW in Mexico reported high larva mortalities on maize treated with *B. thuringiensis, B. bassiana* and *M. anisopliae*. Apart from the studies reported about FAW, there are other studies reported similar trends in different lepidopteran and non- lepidopteran insects. Lawo *et al.* (2008) reported high mortality rates of *Helicoverpa armigera* Hübner larvae on transgenic chickpeas incorporated with *B. thuringiensis.* The authors further reported that the mortality rate of larvae was high when exposed to *M. anisopliae* then untreated control. Alikhani *et al.* (2019) reported low age specific survivorship on the tomato leaf miner *Tuta absoluta* (Meyrick, 1917) when treated with *M. anisopliae* at sub lethal doses than on untreated control. Similarly to that, Contreras *et al.* (2014) reported high mortality rates and low survival rates of *T. absoluta* at pupal stage when treated with *M. anisoplia*e at lethal doses. Apart from that, Scorsetti *et al.* (2017) reported low survival rates and high mortality of larva and pupa of aphid treated with a single lethal dose. Moreover, Yoder *et al.* (2017) reported low egg and larva survival of a winter tick when exposed to *B. bassiana* and *M. anisopliae* than on the untreated control.

The results also showed significant variations on population parameters of FAW among bio pesticides and the control treatment. The probability of dying on life stages of FAW was high when exposed to biopesticides than on the control treatment. This was attributed

to the infection caused by the entomopathogens. This study also found that life expectancy, longevity and fecundity of untreated FAW were higher compared to those exposed to entomopathogens. Alikhani *et al.* (2019) reported low life expectancy, fecundity and longevity on *T. absoluta* when treated with *M. anisopliae* than the control treatment. These results confirmed that biopesticides could regulate FAW populations.

Results on population parameters in the current study are comparable. The net reproductive rate (Ro), intrinsic rate of increase and the finite rate of increase were high on the control treatment than on biopesticides. This was due to the impact of biopesticides on physiological growth of insect pests as reported by Senthil-Nathan, (2015). Alikhani *et al.* (2019) reported low reproductive rate of *T. absoluta* when treated with *M. anisopliae* the untreated control. However, the doubling time (DT) increased among biopesticides and decreased on the control treatment. This implied that, a population required a long time to double when exposed to biopesticides and shorter time to double when unexposed to biopesticides. The significant of this is that, as the population require long time to double, it is easy to control the population because the number of individuals in a population is small. Similarly, the mean generation increased among biopesticides and decreased on the control treatment.

The population when treated with biopesticides required more time to complete one generation as compared to untreated population. The significance of this is that, population control is possible because the pest will have fewer generations. Alikhani *et al.* (2019) reported a similar trend on *T. absoluta* when treated with *M. anisopliae*. From the current study, the bacteria *B. thuringiensis* caused high effects on life table parameters and population parameters than *B. bassiana* and *M. anisopliae*.

The bacteria *B. thuringiensis produces* crystal proteins named delta-endotoxins or cry toxins, which are highly insecticidal (Silva *et al.*, 2017; Senthil-Nathan, 2015). According to Prasanna *et al*. (2018), Seven *Bt* strains were recorded highly effective, and can cause 100% mortality of FAW larva on 7 days post treatment of a lethal concentration. Apart from the Cry toxins, FAW is also susceptible to some of the vegetative insecticidal proteins found in the *Bt* culture supernatants (Polanczyk *et al.*, 2000).

4.6 Conclusion

Generally, biopesticides have a significance effect on the demographic traits of the FAW; this implies to a great advantage on the management options against FAW in Morogoro region of Tanzania. In order to attain sustainable management of FAW, biological control is essential. This study confirmed that biopesticides are important in the regulation of FAW populations.

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CHAPTER FIVE

5.0 GENERAL CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

a) The study showed that biopesticides extended developmental durations of life stages of FAW

b) Among all bio pesticides used in this study, the longest developmental duration was recorded on *B. thuringiensis* treated cohorts and the shortest duration was recorded on untreated cohorts.

c) Bio pesticides reduced survivorships, longevity, total fecundity, life expectancy, net reproductive rate, intrinsic rate of increases, finite rate of increase, while increased mortality rates and probability of dying of FAW.

d) *B. thuringiensis* treated cohorts showed the lowest survivorships, longevity, fecundity, life expectancy and highest mortality rates as well as the probability of dying than all tested treatments

5.2 Recommendations

a) For sustainable management of FAW, biopesticides should be recommended because they are safe to environment

b) This study focused on entomopathogens only. Further studies on bio pesticides apart from those used in the current study, such as botanicals are required.

c) The current study used one variety as food for larvae; further studies are encouraged on different varieties (open pollinated and hybrids) as source of variations against FAW population.

d) The government and private sectors should emphasize on the availability of biopesticides to the market at affordable price for both low scale and large scale farmers.

e) The current study reported the effect of biopesticides under laboratory conditions, there is a need for field testing so that the effects of biopesticides under field conditions can be evaluated.