Effects of Biopesticides on Developmental Biology of Fall Armyworm (*Spodoptera frugiperda* (JE Smith) (*Lepidoptera: Noctuidae*) in Maize Crops in Morogoro, Tanzania

Simon, E.[1]* , A.B. Kudra[1] and M.W. Mwatawala[1]

Department of Crop Science and Horticulture, Sokoine University of Agriculture.
P.O. Box 3005 CHUO KIKUU Morogoro. Tanzania

*Corresponding Author Email address: *ericksimon45@yahoo.com*, Phone: +255765738812

Abstract

The Fall armyworm (Spodoptera frugiperda (J.E. Smith), 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. 353 host plant species, principally Poaceae (maize, sorghum, rice, wheat, sugarcane, millet), Asteraceae (sunflower), Malvaceae (cotton), Fabaceae (soybean), families, groundnuts, potatoes, several fruit trees, ornamental plants, weed species, and vegetable crops are also hosts to FAW. This study aims to determine the effect of biopesticides on the developmental biology of FAW. A completely randomized design (CRD) was used to establish an experiment involving biopesticides; Bacillus thuringiensis Bt, Metarhizium anisopliae and Azadirachta indica seed extract each applied at a lower dose of 2 millimeters, 2 millimeters and 30gram per liter of water respectively, and tested against FAW in the laboratory. Each treatment was replicated four times. Results showed the developmental duration of FAW stages differed significantly between treatments. The time for each growth stage; egg incubation, larval, pupation and the total developmental were significantly longer ($p < 0.001$) in the biopesticides-treated colony compared to the untreated ones. The shortest developmental duration of FAW stages was observed on control colonies at 2.1 ± 0.18, 14.88 ± 0.18, and 27.7 ± 0.34 days for egg, larva and pupa stages respectively. The longest developmental duration of 3.5 ± 0.37, 22.03 ± 0.59, 12.68 ± 0.23 and 37.7± 0.54 days for egg, larva and pupa stages respectively was observed in colonies under B. thuringiensis treatment. These results confirmed that biopesticides can work effectively to keep FAW levels under control. Therefore, these are promising biocontrol alternatives to be included in the integrated pest management scheme.

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

Introduction

Fall armyworm (FAW), *Spodoptera frugiperda* (JE Smith), a highly transboundary migratory moth native to America, was first detected in Central and Western Africa in early 2016 (Goergen *et al.,* 2016; Bateman *et al.,* 2018; 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 (Li *et al.,* 2019; Sisay *et al.,* 2020: 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 a crop protectant (Ogunnupebi *et al.,* 2020). Biopesticides have consequences in the population dynamic of susceptible species (Sarwar, 2015). 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. 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 the direct application of multiple strains of *M. anisopliae* caused significant and variable mortality in 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 are expected to be used in several African countries (Botha. 2020). 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 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 the destruction of tissues and by production of toxins. Wraight *et al.* (2010) reported Metarhizium anisopliae (Metchnikoff) Sorokin (1883) is among the common fungi with potential uses against insect pests including FAW. Prasanna *et al.* (2018) reported *Bacillus thuringiensis* (Berliner) as a widely used biopesticide to control the 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, 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 limiting 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 determine the effect of biopesticides on the developmental biology of FAW on maize crops treated with different biopesticides as an arsenal in FAW management.

Materials and Methods

Description of the study area

All trials in this study were conducted from late December 2020 to early February 2021 at Sokoine University of Agriculture (SUA)-Entomology rearing unit laboratory Edward Moringe Campus (latitude 06050′45.1′′ south and longitude 037039′ 47.9′′ east with 522 m above the sea level) and Solomon Mahlangu Campus - Chemistry and Physics laboratory (latitude 06047′57.6′′ south and longitude 037037′ 42.2′′east with 489 m above the sea level). The experiments were carried out under Laboratory conditions.

Laboratory bioassay of botanicals against FAW

Preparation of Neem Seed Extract from Azadirachta indica seeds

Neem seeds were collected from Shinyanga Region in December 2020. These were juvenile, matured plain absolute yellow fruits directly from the tree, spread on the plastic sheet not to come in contact with the soil and the danger of fungus attack. These were healthy *Azadirachta indica* seeds. The seeds pulp was removed and air-dried at the average temperature of between

25°C-30°C under shade for five days to avoid thermal and photodecomposition 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 grain blander mill powder machine (3000W 110V) at the Department of Soil and Geological Sciences SUA. 100 g of neem seed powder was 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 a boiling point of 78.37°C of the ethanol to obtain crude extracts (Susmitha *et al.,* 2013).

Thin layer chromatography (TLC) Confirmation

Thin layer chromatography (TLC) analysis was carried out with the purpose of verification of azadiractin compound from the extracted crude sample using the 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/ H2SO reagent, and retardation factor (Rf) values were calculated using the formula;

$$Rf\ value = \frac{Distance\ traveled\ by\ compound}{Distance\ traveled\ by\ solvent\ system}$$

Identification of aAzadirachtin 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 a maximum of 0.8% by weight of neem seed kernels. This is equal to $0.24gl^{-1}$ 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 $0.8\ gl^{-1}$ active ingredient of azadirachtin compound (Mordue *et al.* 2005).

The eggs, larvae and pupa were exposed to biopesticides by spraying at the rate of 2mls and 2mls of *B. thurungiensis*, *M. anispoliae* purchased as commercial products from Real IPM (T) Ltd Arusha-Tanzania.

Rearing of *Spodoptera frugiperda* (JE Smith) under Laboratory Conditions

Insect colony

A FAW starter colony was collected from different farmers at an unsprayed maize farm at Kasanga, and SUA-Crop Museum according to procedures described by Sisay *et al.* (2019). 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" purchased from Agricultural Seed Agency (ASA) Morogoro, 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 room 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 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 scissors. 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 cages for three cohorts (egg, larva and pupa). Rearing was done at room temperature of 25+3°C and 50–60%

RH, following protocol described by Prasanna *et al.,* 2018.

Experimental design

The experiment was laid out in a completely randomized design (CRD) with four replications. A cohort of 100 fresh egg batches was placed on moist filter paper using forceps in a plastic container (14 cm x 6 cm). In a setup 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 the lowest doses of 2mls where the highest dose was 5mls. A colony of 100 fresh pupae that emerged on the same day were collected and placed onto a moist filter paper in a Petri dish with a diameter of 14cm and deep for 10 seconds into lower doses of biopesticides, and then placed in the plastic container with length and width of 14 cm x 6cm respectively, containing sterilized sand with Sodium chlorate 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).

Data collection

The data recorded at each biopesticide used and each developmental stage attained was; the developmental duration for eggs to hatch, larvae to pupate and pupae to emerge into adult FAW moths. Finally, the total developmental duration of FAW from egg to adult moth emergence was established.

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 biopesticides 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, the Shapiro-Wilk normality test was used to test for normality on each variable. All statistical tests for significance were performed at $P < 0.05$.

Results

Developmental duration of FAW egg incubation

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 egg 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 ± 0.37 to 2.1 ± 0.18 (Table 1).

Developmental duration of FAW Larvae

Table 1: Effect of biopesticides on FAW egg incubation period

Treatment	Mean/days
Bacillus thuringiensis	3.5a
Metarhizium anisopliae	3b
Neem seed extract	2.95b
Untreated control	2.1c
CV	10.72
SE	0.09583
P	0.00096

Means followed by the same letter are not significantly different at P≤0.05

Results showed significant ($P<0.0001$) effects of treatments on FAW larval stage duration. Larval stage duration was significantly shorter in colonies exposed to biopesticides than in 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 ± 0.18 to 22.03 ± 0.59 days (Table 2).

Table 2: Effect of biopesticides on FAW larvae duration

Treatment	Mean/days
Bacillus thuringiensis	22.03a
Metarhizium anisopliae	18.98b
Neem seed extract	18.45b
Untreated control	14.88c
CV	5.51
SE	1.05
P	0.00004

Means followed by the same letter are not significantly different at P≤0.05

Developmental duration of FAW Pupa to adult stage

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 in pupa duration among *B. thuringiensis, M. anisopliae* treated pupae. The pupal duration of the pupa stage ranged from 10.05 ± 0.2 to12.68 ± 0.23 days in untreated and on *B. thuringiensis* treatment respectively (Table 3).

Table 3: Effect of biopesticides on FAW pupae to adult duration

Treatment	Mean/days
Bacillus thuringiensis	12.68a
Metarhizium anisopliae	11.28b
Neem seed extract	10.5c
Untreated control	10.05d
CV	0.65
SE	0.0053
P	0.00

Means followed by the same letter are not significantly different at P≤0.05

Total developmental duration

Biopesticides significantly (p<0.001) affected the total developmental duration of FAW. Total development duration days were 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 ± 0.34 to 37.7 ± 0.54 days in the untreated colony and *B. thuringiensis* treated colony respectively (Table 4).

Table 4: Effect of biopesticides on total developmental duration of FAW

Treatment	Mean/days
Bacillus thuringiensis	37.7a
Metarhizium anisopliae	33.23b
Neem seed extract	32.48b
Untreated control	27.03c
CV	3.48
SE	1.286
P	0.0

Means followed by the same letter are not significantly different at P≤0.05

Discussion

From this study, all tested biopesticides caused prolonged development duration of FAW compared to control. Developmental duration is a very important phenomenon for the completion of life span of insects and reproduction (De Loof. 2011). Biopesticides are attracting global attention as new tools to kill or suppress pest populations including insects.

This study indicated that biopesticides caused increased developmental periods of FAW egg, larval and pupal stages contrary to that exposed to untreated control. Barbosa *et al.,* 2015 reported, that 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 larval growth, which resulted in a prolonged developmental time of the infected larvae of *Ocinara varians*.

In the current study, biopesticides specifically *B. thurungiensis* 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 biopesticide mixed in an artificial diet prolongs the developmental duration of larvae and decreases 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 enhancing prolonged developmental period. Prasanna *et al.* (2018) reported that *B. thuringiensis* as 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 a ubiquitous, soil-dwelling gram-positive bacterium that is characterized by producing parasporal crystal proteins named delta-endotoxins with insecticidal activity (Cry toxins) during sporulation. It is considered an almost ideal agent for pest management because of it combines insecticidal specificity and lack of toxicity to humans and non-target 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 (Lacey *et al.,* 2015: Osman *et al.,* 2015: Fatoretto *et al.,* 2017). 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; Akutse *et al.,* 2019).

In addition to this study, the effects of Bt as biopesticides have been investigated on *H. armigera.* For example, in a study to assess the 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 in this study, the shortest egg incubation period and the longest incubation period observed were the same as reported by Naharkia *et al.* (2020). Furthermore, the study observed FAW's 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 whereas by the longest pupa stage duration recorded in this study, is 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 was recorded on the control, this was within the comparable range of 21-30 days which can extend up to 60 to 90 days when the temperature drops down (Acharya *et al.,* 2020; Naharki *et al.,* 2020). 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.

Conclusion and Recommendation

Given that biopesticides such as B. thuringiensis, Metarhizium anisopliae and neem seed extract had significant effects on the developmental stages of the FAW, 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 have an impact on FAW generations in which FAW developmental duration affected with biopesticides will have fewer generations compared to that not affected, thus resulting in the successfully reducing the need to apply other pesticides. Further field study on the effective control of FAW using biopesticides such as

B. thuringiensis, Metarhizium anisopliae and neem seed extract is recommended. Therefore, the study will provide a basis for designing interventions to make biopesticides more useful for FAW control in the Morogoro region and another part of Tanzania.

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Conflicts of Interest

The author(s) declare(s) that there is no conflict of interest regarding this work.

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