AN ASSESSMENT OF THE EFFICACY OF SELECTED BIOPESTIDES AND SYNTHETIC CHEMICALS IN THE CONTROL OF THE CORN STRAIN OF THE FALL ARMYWORM.

BY

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ABSTRACT

The fall armyworm is a devastating pest of maize which has recently reached the African continent. Currently, there are no registered insecticides for FAW control in African countries, except applications allowed through an emergency label, suggesting an urgent need for insecticide screening. A study was carried out at the Department of Research and Specialist Service to investigate the efficacy of selected biopesticides and synthetic chemicals against the fall armyworm. The laboratory bioassays were carried out at the Entomology Section. Third instar stage fall armyworm larvae were exposed to the biopesticides (Achta, Delfin and Dynamo) and Synthetic Chemicals (Nemesis, Blanket and Vantex). This was done by placing four third instar FAW larvae in each perforated vial together with maize leaves as feed for the larvae and spraying each treatment into a vial at the manufacter's recommended rate. Recordings of mortality were taken for both biopesticides and synthetic chemicals within a four day period for the synthetic chemicals and a fourteen day period for the biopesticides. Two way ANOVA and Dunnet's test were used to analyse the results. The synthetic chemicals had an influence on larval mortality (p= 0.01). Nemesis was the most effective pesticide causing 93.8% mortality and the least effective was Vantex causing 60% mortality. Resistance of the larvae to Cyhalothrin was thought to be the main reason for its low efficacy. The biopesticides also had an influence on larval mortality (p= 0.417). Dynamo was the most effective causing 84.4% mortality and Achta was the least effective causing 78.1% mortality. The synthetic insecticides and biopesticides that showed high efficacy against FAW larvae can be used as components for integrated pest management (IPM) plans for FAW in Zimbabwe and elsewhere in Africa.

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Dedication

I dedicate this dissertation to my parents; I am most gratified by your efforts and support. For you I will undertake all that is desirable within my reach.

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CHAPTER ONE: INTRODUCTION

1.1 Background

The Fall Armyworm (FAW), *Spodoptera frugiperda* (J.E. Smith) is a devastating pest of maize. It is native to the tropical and subtropical regions of the western hemisphere from the United States of America to Argentina (Copyright, Chinwada, Mulila-Mitti, Luchen, Hove and Tanyongana, 2017). It derives its scientific name from the feeding habits of the larval stage, *frugiperda* meaning "lost fruit" in Latin, as the pest can cause damage to crops resulting in severe yield loss (IPPC, 2016; IITA, 2016).

In early 2016, the first reports of the fall armyworm on the African continent were made (Copyright *et al.*, 2017). Early reports were from West Africa: Nigeria, Benin, Togo and Sao Tome' and Principe (IPPC, 2016; 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 (Copyright *et al.*, 2017). By July 2017 all SADC states with the exclusion of Lesotho and the Island states had reported the existence of the pest (Copyright *et al.*, 2017).

Although the fall armyworm can feed on various kinds of food, with a host range of more than 80 plant species, its main preferences are grassy plants, in particular crops of economic importance such as maize, millet, sorghum, rice, wheat, and sugarcane (IITA, 2016). Other crops of key agricultural importance which are attacked by the pest include cowpea, peanut, potato, soybean, and cotton (IITA, 2016).

FAW can feed on both the reproductive structures of the crops as well as the vegetative structures. When the larvae feed on the leaves of maize, a yield loss does not necessarily occur because the plant is able to compensate for at least some loss of the leaf area. However, if the larvae attack the growth point, the result is a "dead heart", which is characterised by wilting and death of the unfurled leaves (Day, Bateman, Beale, Clottey, Cock, Colmenarez, Corniani, Abrahams, Early, Godwin, Gomez, Moreno, Murphy, Oppong-Mensah, Phiri, Pratt, Richards, Silvestri and Witt, 2017).

Older larvae of the FAW are generally difficult to control using spray applications as they tend to stay inside the maize funnel and are henceforth protected from spray applications to the foliage. On the other hand, young larvae tend to hide in the maize funnel during the day and emerge at night to feed on the leaves. For this reason, spray applications are more likely to be effective in controlling young larvae when undertaken at dawn or at dusk. It is important to time pesticide applications in such a way that they coincide with the presence of younger larvae as these are generally easier to control in comparison to older larvae (Day *et al.*, 2017).

The damage to the plant results from leaf feeding and defoliation as well as ear feeding. Injury may be cosmetic or it can cause grave economic loss. Nevertheless, the severity of the injury depends on the corn growth stage (Smith, Betran and Runge, 2004).

 FAW is likely to remain a significant agricultural pest across most areas of the Southern Saharan Region for the foreseeable future (Prasanna, Huesing, Eddy and Peschke, 2018). Henceforth it is essential to develop an effective and flexible approach to manage it across the African continent. Such an approach should be informed by scientific evidence which is built on past experiences (Prasanna *et al.*, 2018). An integrated pest management (IPM) approach is required for the

control of FAW. The IPM also encourages farmers to practice the use of regulated pesticides and ensuring that these pesticides are applied at the manufactures recommended rate (Day *et al.*, 2017).

1.2 Problem Statement

Currently, there are no registered insecticides for FAW control in African countries, except applications allowed through an emergency label, suggesting an urgent need for insecticide screening (Sisay, Tefera, Wakgari, Ayalew and Mendesil, 2019). It is therefore necessary to carryout efficacy trials so as to support a label extension for both biopesticides and synthetic chemicals which control the fall armyworm effectively but have not yet been registered (Nunda, 2018). Since the fall armyworm is new to the African continent, farmers are still unable to make an informed choice between biopesticides and synthetic chemicals as the effectiveness of both insecticides in this region is still unknown. The purpose of this study is to carry out a comparative analysis of the efficacy of biopesticides and synthetic pesticides.

1.3 Justification

The fall armyworm has caused devastating effects for farmers as yield losses of over 70% have been recorded (Hruska and Gould, 1997). So far losses due to confirmed and suspected infestations of FAW in maize, sorghum, rice and sugarcane in African countries have been estimated at USD13.38 billion (Copyright *et al.*, 2017) and the FAW in Africa has the potential to cause maize yield losses in ranging from 8.3 to 20.6m tonnes per annum, in the absence of any control measures (Day *et al*., 2017). The level of damage witnessed in the fields is likely to affect maize harvests across the region, which is expected to create more than two hundred million food-insecure people who depend on maize for food (Siamachira, 2018).

The moth poses a serious threat to African agriculture and food security as well as international trade through quarantine restrictions. Two hundred and eight million people are dependent on maize for food security in sub-Saharan Africa and maize also provides crucial income for small-holder farmers in the region. Therefore, the fall armyworm not only affects their food security but also affects their income generation and livelihood (Nunda, 2018). The capital costs for farmers are directly affected by the fall armyworm through increased labour needed and the type of knowledge required to deal with the pest and also through yield losses and the ability of agricultural lands to respond to shocks. The cost of production is also increased due to costs of control. It also indirectly affects households' social and physical capital (the household's assets).

The FAW also has massive implications on trade and this is because international trade carries the risk of introducing the pests to countries where they are not yet present. Thus, the arrival of FAW in Africa creates a new risk for countries importing from affected African countries if FAW is absent from the importing country. This includes countries in North Africa, Asia and Europe, although more focus is given to Europe as a major importer of agri-food products from Africa and for which good data is available (FAO, 2017). If consignments arriving in Europe are found to contain FAW, treatment may be required, import may be refused, or the consignment could even be destroyed, so there is a cost when contaminated consignments are intercepted by importing countries. To reduce the likelihood of this happening, additional measures may be required in the exporting country. This will also create an additional cost for the producers and the national plant protection organisation (FAO, 2017). Therefore, it is clearly evident that the fall armyworm is a major problem globally.

At this stage it is therefore necessary to find effective pesticides and other control strategies for the FAW so as to guard against or to minimize further damage by the pest. The use of a single

chemical to control FAW is greatly discouraged as resistance is likely to occur. It is therefore essential for farmers to rotate pesticides with different modes of action so as to guard against resistance.This study will provide researchers, farmers and other stakeholders with knowledge and evidence on the effectiveness of both biopesticides as well as synthetic chemicals. It will also give farmers a wide choice of pesticides to choose from as all of the insecticides in the study have different modes of action. The findings on the general performance of each pesticide will enable the farmer to make an informed decision when choosing the pesticide of choice. In this way the adoption of the most effective pesticides will consequently improve the livelihood of farmers.

1.4 OBJECTIVES

Main objective:

➢ to compare the effectiveness of biopesticides and synthetic chemicals on fall armyworm mortality.

Specific Objectives:

➢ to assess the effect of biopesticides on the mortality of the fall armyworm larvae,
➢ to assess the effect of synthetic chemicals on the mortality of fall armyworm larvae and
➢ to compare the overall effects of biopesticides and synthetic chemicals on fall armyworm larvae.

CHAPTER 2: LITERATURE REVIEW

2.1 Classification of the Fall Armyworm

Kingdom   : Metazoa

Phylum   : Arthropoda

 Subphylum   : Uniramia

Class   : Insecta

Order   : Lepidoptera

Family   : Noctuidae

Genus   : *Spodoptera*

Species   : *Spodoptera frugiperda*

2.2 Fall Armyworm life cycle and biology

The fall armyworm can have several generations per annum (Hardke, Lorenz and Leonard, 2015). Its life cycle consists of the egg, six to seven larval instar stages, the pupa and finally the adult stage (Luginbill, 1928). Completion of the life cycle normally takes 4 weeks; however, it can take up to 12 weeks at low temperatures (Vickery, 1929). The FAW does not possess the ability to diapause, consequently, its infestations occur continuously throughout the year in areas where the pest is endemic. In non-endemic areas, the migratory FAW tends to arrive when environmental conditions are favourable and may have as few as one generation before they become locally extinct (Prasanna *et al.,* 2018). The FAW generally lays eggs on the abaxial surface of leaves (Luginbill, 1928; Sparks, 1974; Ali, 1989). Upon eclosion, the neonates devour the egg mass from which they have hatched. Larvae will then disperse in all directions and begin to feed on vegetative tissue. Older instars prefer to feed on the reproductive structures of the

plants. Larval feeding and adult activity frequently occur at night; however, it can also occur late in the evening or early in the morning (Hardke *et al*., 2015). Instar stages can range from six to seven depending on the availability of food as well as the environmental conditions (Hardke *et al*., 2015). The last instar stage tends to consume a greater quantity of food than all the previous instars combined and hence causes more damage to the plant (Luginbill, 1928). Temperature and environmental conditions influence the rate of larval development (from hatching to pupation) which can range from 11 to 50 days (Luginbill, 1928; Hogg, Pitre and Anderson, 1982).

Mature larvae fall from the plant and burrow into the soil to a depth of 3 to 6cm and remain in a prepupal stage for about 2 to 4 days after which pupation will occur for about 7 to 10 days (Luginbill, 1928; Pitre and Hogg, 1983). The depth at which pupation occurs depends on factors such as soil texture, soil temperature and soil moisture (Sparks, 1974). As the moths emerge from the soil, they can either mate locally or fly for up to 480 kilometres before mating and ovipositing (Ashely, Wiseman, Davis and Andrews, 1989).

2.3 Host range of the Fall Armyworm

The FAW has a widespread host range, with more than 80 plants recorded (Prasanna *et al.*, 2018), although it clearly prefers grasses. It commonly feeds on field maize and sweet maize, sorghum, Bermuda grass, and grass weeds like the crabgrass (*Digitaria* spp) (Prasanna *et al.*, 2018). When the larvae are in large numbers, they tend to defoliate the ideal plants following which they acquire the typical "armyworm" habit, and then disperse in large numbers thus consuming nearly all vegetation in their path. Most of the host records reflect periods of abundance and do not truly indicate the oviposition and feeding behaviour of the larvae under normal conditions (Prasanna *et al.*, 2018). Field crops such as alfalfa, barley, Bermuda grass, buckwheat, cotton, clover, maize, oat, millet, peanut, rice, ryegrass, sorghum, sugar beet, Sudan

grass, soybean, sugarcane, tobacco, and wheat are frequently injured (Prasanna *et al.*, 2018) Sweet maize is frequently damaged among vegetable crops, but others are attacked occasionally. Crops such apple, grape, orange, papaya, peach, strawberry, and a number of flowers are sometimes injured. Bent grass, *Agrostis* ssp.; crabgrass, *Digitaria* spp.; Johnson grass, Sorghum halepense; morning glory, *Ipomoea* spp.; nutsedge, *Cyperus* spp.; pigweed, *Amaranthus* spp.; and sandspur, *Cenchrus tribuloides* are weeds which are known to serve as host plants (Prasanna *et al.*, 2018).

2.4 Fall Armyworm haplotypes

The fall armyworm comprises of two strains which are adapted to different host plants. One strain (the "maize strain") feeds chiefly on maize, sorghum and cotton whereas the other strain (the "rice strain") feeds mainly on rice and pasture grasses (Dumas, Legeai and Lemaitre, 2015). These two strains are morphologically alike but differ in the host range, pheromone compositions and mating behaviour. Mating between the two strains results in a viable offspring (Prasanna *et al.*, 2018)

Hardke *et al*. (2015) postulates that fall armyworms of the R-strain prefer rice, *Oryza sativa* (L.), and bermuda grass, *Cyndon dactylon* (L.) Pers. and other Graminaceae, whereas the C-strain prefers cotton and corn, *Zea mays* L. Mating between the two strains can occur but variability exists in mating preference (Hardke *et al*., 2015). The females of the R-strain prefer to accept C-strain males and this results in a mixed population whereas the C-strain females and R-strain males seem to be reproductively incompatible (Whitford, Quisenberry and Riley, 1988; Quisenberry, 1991). Allozyme variants and Genetic markers are used to differentiate the two strains (Nagoshi and Meagher, 2004). The differences between the strains have a profound effect

on the crop protection strategies and this is because of the differences in the life history strategies between the two strains. The differences in the development of larvae on host plants, mating behavior, use of food resources, resistance to pesticides as well as the variation in susceptibility to plants expressing *Bacillus thuringiensis* (Bt) proteins can influence management strategies (Veenstra, 1994; Nagoshi and Meagher, 2004).

2.5 Distribution of the Fall Armyworm in Africa

A study conducted by Day *et al.* (2017) indicates that so far 28 countries have officially reported the pest. Countries confirm the presence of the pest through a number of sources, including IPPC (eight reports), ministerial declarations, peer reviewed journals, and UN affiliated organization reports. An additional nine countries have carried out and some are still in the process of conducting surveys. This is because they either strongly suspect the presence of the FAW or are expecting official confirmation. Two countries have reported the absence of FAW from their country (Day *et al.*, 2017). It was impossible to obtain any information on FAW presence or absence in the remaining 15 countries.
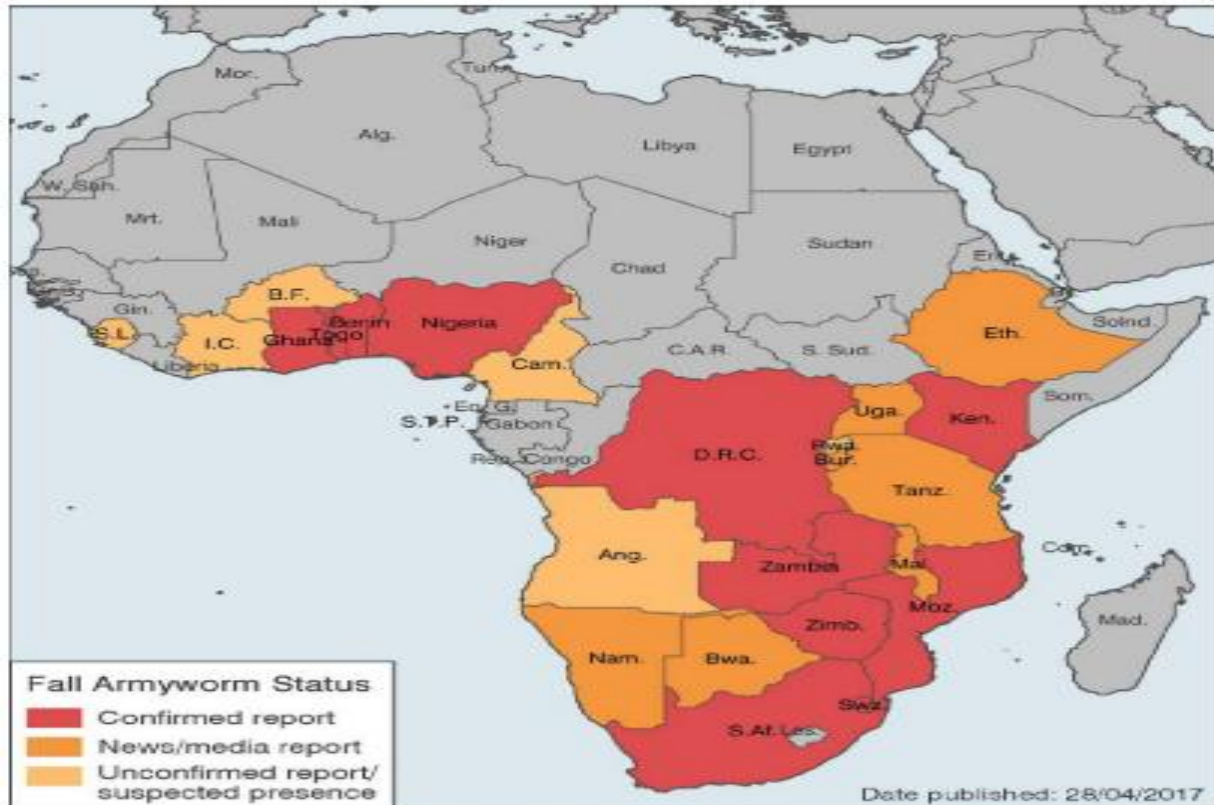
Figure 1: Distribution of the FAW in Africa (FAO, 2017).

2.6 Impact of the Fall Armyworm in Africa

FAW is most likely to have an effect on numerous diverse aspects of household livelihoods. The pest is most likely going to affect natural capital, through the loss of yields. International trade will possibly also be impacted by FAW as trading has the risk of introducing the pests to other countries where the pest will not have reached.

Consignments of food and agricultural products are a particular risk, henceforth countries in North Africa, Asia and Europe will possibly manage this risk by introducing other production or handling requirements and conditions on exports from the countries affected by FAW thus creating cost implications for the exporters. In June 2017, the first shipment (of roses) from Africa infested with the FAW was intercepted in Europe (Day *et al.*, 2017). Appropriate

measures have been taken by National Plant Protection Organisations (NPPOs) which have significant exports to Europe. Well organised NPPOs are most likely able to deal with the situation but in countries where export certification is weaker and in countries where the agri-food export sector is less developed it could be a big problem (Day *et al.*, 2017).

2.7 Prevention and control

2.7.1 Synthetic Chemicals

Nunda (2018) postulates that numerous synthetic pesticides are able to kill the fall armyworm, and producers and suppliers have been seeking registration of several diverse active ingredients. Efficacy trials for products which have already been registered for other uses may be required so as to support a label extension. A range of active ingredients and products have been recommended by different governments, for instance by April 2018, 8 pesticides were being recommended for the fall armyworm by the Kenyan government, in 2018 Ghana had recommended only "biorational" pesticides, together with numerous biopesticides. The various pesticides being used by farmers have modes of action which are significantly different. They contribute to many WHO categories including the highly hazardous (WHO class 1b) such as the monocrotophos. It is still unclear whether the Class 1 pesticides being made use of in Africa are registered for FAW, though they may be registered for other uses. Pesticides pose a risk to human health as they are regularly applied short of adequate safety measures being taken and this has become a key issue. Farmers with little or no resources are frequently reluctant or incapable of buying the appropriate safety equipment. It is important to note that Class 1 pesticides must never be used or registered for the control of FAW and class 2 pesticides must be avoided as much as possible.

Togola, Meseka, Menkir, Badu-Apraku, Bauka, Tamo and Djouaka (2018) have revealed that five insecticide compounds commonly used against FAW (cypermethrin, deltamethrin, lambda-cyhalothrin, permethrin, and chorpyrifos) persisted in soil samples where FAW had been treated. These compounds possibly have adverse effects on organisms which live in the soil and other non-targeted species. However, no residues of the compounds were found on the plants. Farmer surveys propose that synthetic pesticides are the most common control method used by farmers, however, users are not always satisfied (Togola *et al.*, 2018). In Ethiopia 46% and 60% of farmers in Kenya, believed that synthetic insecticides were highly ineffective (Kumela, Simivu, Sisay, Likhayo, Mendesil, Gohole and Tefera, 2018).

The wrong use of pesticides such as incorrect application rate, application of the pesticide at the wrong dosage, application of the pesticide after the damage has occurred are some of the reasons which can cause pesticides to be ineffective. However, sometimes pesticides are made ineffective because of fake labelling or adulteration. Some of the most commonly used pesticides on the African Continent fall into classes of mode of action to which resistance has developed in the Americas. No data is currently available to suggest that FAW has already developed resistance in any African country or that the populations were already resistant upon arrival hence the reports of insecticide ineffectiveness are most likely due to the wrong use or fake/adulterated products and not insecticide resistance. It is suggested that approaches for limiting repetitive exposure of consecutive generations of FAW to chemicals which possess the same mode of action be developed and executed to minimize the probability of pesticide resistance developing (Nunda, 2018).

2.7.2 Biopesticides

Biopesticides can be defined as products which are based on pathogens of the pest (Nunda, 2018). They may also be taken to contain other products which are biologically based such as extracts from plants (botanicals) biochemicals with different modes of action, and even parasites and predators (Nunda, 2018). A recent study assessing biopesticides which are potentially useful for FAW management (Bateman, Day, Luke, Edington, Kuhlman and Cock, 2018) reviewed products registered in 30 countries, 11 in FAW's native range and 19 in Africa. Fifty biopesticide active ingredients were identified. Twelve of these are already reported as being effective against FAW outside Africa, most of these being already registered in at least some African countries for other pests. However, there are safety concerns regarding four of these, which need to be assessed in a local context. The remaining eight active ingredients were recommended for immediate field testing in Africa, and some such tests are in progress.

2.7.2.1 *Beaveria bassiana*

The fall armyworm is susceptible to entomopathogens like fungi such as *Beauveria bassiana*. This widespread insect pathogen grows as an endophyte causing no lethal effect upon plants (Posada and Vega, 2006). The endophytic relations between the plants and the microbes are sometimes mutualistic (Schulz and Boyle, 2006). *B. bassiana* can colonize plants such as maize, coffee (*Coffea arabica L.*) seedlings (Posada and Vega, 2006), and bean (*Phaseolus vulgaris L.*) (Akutse, Maniania, Fiaboe, Van den Bery and Ekesi, 2013). Using entomopathogenic endophytes has been proposed as a probable substitute to the use of synthetic chemicals (Vega, Goettel, Blackwell, Chandler and Jackson, 2009). So far, the reports of insecticidal activity in plants, resulting from entomopathogenic endophytes, are inconsistent (Rodriguez and Pena, 2016).

2.7.2.2 *Bacillus thrungiensis*

The soil bacterium *Bacillus thuringiensis* (Bt) tends to produce crystals containing proteins that are lethal to some insects. These proteins are harmless to other organisms including humans,biota and most beneficial insects (Schnepf, , Crickmore, Van Rie, Lereclus, Baum, Feitelson, Zeigler, and Dean, 1998). *Bacillus thuringiensis* (*Bt*) has been considered as an alternative method of controlling FAW (Hofte and Whiteley, 1978). This bacterium acts in the gut of the insect due to cristals, composed by protoxins, discharged in the gut due to the alkaline pH that causes solubilization. These protoxins, in presence of digestive enzymes, are converted into toxic polypetides (delta-endotoxins). The activated toxins cross the peritrofic membrane, join to specific receptors in apical membrane of columnar cells of midgut, and insert themselves into the membrane (Fiuza, Nielsen-Leroux, Goze, Frutos and Charles, 1996; Hofte and Whiteley, 1978).

The formation of pores disrupts the ionic gradients and osmotic balance in the apical membrane, resulting in cell swelling and lysis. This phenomenon leads to massive destruction of the epithelium, causing death of larvae (Knowles, 1994). Whether the use of Bt sprays is a success or not greatly depends on having the Bt spray present on the foliage upon the first appearance of the larvae. Bt sprays are susceptible to UV degradation hence they are short lived and may require multiple sprays (Prassanna *et al*, 2018).

2.7.3 Botanicals

The use of plant-derived pesticides (commonly called "botanicals") in pest management is a cultural practice of most African farmers. It could provide a potential arsenal against the fall armyworm in Africa. The mode of action of botanical pesticides is broad and ranges from repellency, knock-down, larvicidal to anti-feedant, moulting inhibitors and growth regulation.

They have a broad-spectrum activity with generally little or no mammalian toxicity; however, some botanical pesticides are highly toxic not only to pests but also to natural enemies and to mammals including humans, for instance tobacco extracts. Pyrethroids will also affect natural enemies. Farmers generally extract bioactive compounds as a concoction after grinding plant materials using water. Essential oils from bioactive rich plants and powdered forms are also used to some extent (FAO, 2018).

2.7.3.1 Azadiractin

Many insect species including *Spodoptera frugiperda* can be controlled using the extract from the neem tree *Azadirachta indica* (Campos and Boica, 2012). Neem oils and extracts have an effect on insects because of the presence of limonoids in big quantities among which azadirachtin is the most complex and potent (Mordue and Nisbet, 2000). The antifeedant activity of azadirachtin is very strong and this is due to its effect on chemoreceptors, it affects ecdysteroid and juvenile hormone titers by blocking morphogenetic peptide hormone release, resulting in growth and molting aberrations, and it has direct histopathological effects on insect muscles, fat body, and gut epithelial cells (Mordue and Blackwell, 1993). Henceforth, neem tends to act as an insect growth regulator and as a feeding and oviposition deterrent (Mordue and Nisbet, 2000; Isman, 2006). The main disadvantage of using neem on a large scale is the high photosensitivity of azadirachtin, which tends to break down or isomerizes upon exposure to sunlight, thus making the residual effect of neem under field conditions significantly low (Riyajan and Sakdapipanich, 2009; Forim, Matos, Silva, Cass and Fernandes, 2010).

2.7.4 Cultural Control

The fall armyworm has a wide range of alternate hosts on which it feeds and multiplies. Most of these hosts are weeds and removing them from the fields will reduce the pest's breeding ground (Gibbs, 1992). Capinera (2017) proposes that the most significant cultural control method, which is commonly used in southern states, is the early planting method and also the planting of varieties that mature early. Harvesting the corn at an early stage permits several corn ears to escape the higher armyworm densities that tend to develop later in the season (Mitchell, 1978). Reduced tillage has a small effect on fall armyworm populations (All, 1988), even though delayed infestation by moths of fields with extensive crop residue has been observed, therefore postponing and reducing the necessity for chemical suppression (Roberts and All, 1993).

2.7.5 Host plant resistance

Resistance breeding programmes for the *Spodoptera* species have developed crop varieties with improved resistance; one example of such a crop is maize (Mihm, Smith and Deutsch, 1988). A resistance mechanism which seems to operate in maize is a thicker epidemis also known as the leaf thickness (Davis, Baker and Williams, 1995).

Transgenic maize which contains genes encoding delta-endotoxins from *Bacillus thuringiensis* Kurstaki have been commercialized in the USA and Brazil. Vegetative insecticidal proteins have been successfully isolated from *Bacillus thuringiensis* (Bt) during the vegetative phase of growth. These proteins show many activities against leptidopteran pests, especially the *Spodoptera* species (Estruch, Warren, Mullins, Nye, Craig and Koziel, 1996). *Spodoptera* spp seem to be successfully controlled by these toxins, however the development of resistance is a major concern (Moar, Pusztai-Carey, Hyan, Bosch, Frutos, Rang, Luo and Adang, 1995).

2.7.6 Genetically engineered Fall Armyworm

The use of genetically engineered fall armyworm has been proposed as another strategy for the management of the fall armyworm (Hardke *et al*., 2015). FAW males which contain a self-limiting gene are released in large quantities. These males' mate with the females and when this gene is passed on to the progenies, the females do not survive to adulthood. Intrexon (US) and Oxitec (UK) have been carrying out research on this method for many years and claim to be making good progress, however, a technology of this kind, may require regulatory approval as well a proper risk assessment before it can be used widely (Walton and Luginbil, 1916).

2.7.7 Common control strategies in Zimbabwe

Smallholder farmers in Zimbabwe are using methods which are at their disposal such as the use of botanicals, detergents, ash, soil and sugar (Mulilamitti, 2017). Other farmers have resorted to hand picking and crushing of eggs and larvae (Mulilamitti, 2017). In some cases, fish soup is used to attract natural enemies of the FAW like ants. Other control methods which are commonly used by smallholder farmers in Zimbabwe are mixed cropping practices, conservation agricultural practices, early planting and also the use of bio-pesticides (Mulilamitti, 2017). Synthetic insecticides have also been widely used as an emergency response to slow the spread of the pest and minimize damage to maize fields. It is, however, important to note that at this stage most of the insecticides being used are not registered for FAW control, except for applications which have been allowed through an emergency label, suggesting an urgent need for synthetic insecticide screening (Sisay *et al*., 2019). The objectives of this study, therefore, are to evaluate selected synthetic insecticides as well as selected biopesticides against FAW under laboratory conditions.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Study Site

The study was carried out at The Department of Research and Specialist Services (DR&SS) in the Entomology Section of the Plant Protection Research Institute (PPRI) in Harare. It is located 4km North East of the Harare CBD at latitude 17°48'23.7" S, longitude 31°03' 06.7" E with an altitude of 1479m above the sea level. The annual rainfall is between 800-1000mm and the average temperature is 32ºC in summer and 18º in winter. This site was chosen because the climatic conditions are generally favourable for the fall armyworm.

3.2 Experimental Design

The experiment was set up using a completely randomised design (CRD) with 4 replicates. The factors were the synthetic chemicals and the biopesticides. A positive control Ema Macten as well as the negative control (water) were included.

3.3 Sources of larvae feed

The maize leaves and stalks used as feed in the experiment were harvested from two weeks old maize plants which were planted in pots at the CBI glass houses. Young plants were used as feed because young larvae of *S. fruigiperda* prefer young plants as they are softer and hence easier to feed on.

3.4 Collection and rearing of the Fall Armyworm

The FAW larvae were collected from infested Crop Breeding Institute (CBI) fields and placed in aerated plastic containers and taken to the Entomology laboratory where the larvae were fed

with maize leaves and stalks until pupation occurred. Pupae were kept in petri dishes containing dampened filter paper so as to prevent desiccation from occurring as shown in Figure 2.

Figure 2: The pupae of the FAW in a petri dish.

Pupae matured to moths and the moths were transferred to rearing cages at a ratio of two females to one male. A two weeks old plant for the moths to oviposit on as well as a container containing syrup for the moths to feed on were also added to each rearing cage as shown in (*Figure 4*). The moths laid the eggs in batches and these hatched to produce the larvae which were used in this study.

Figure 3: FAW moths in a rearing cage (left) and the food as well as green plant foroviposition purposes (right).

3.5 Field application rates of synthetic chemicals

Table 1: A list of Synthetic Chemicals and their field application rates

BRAND NAME	ACTIVE INGREDIENT	APPLICATION RATE
Nemesis	Emamectin benzoate+Acetamiprid	250 ml/ha
Vantex	Cyhalothrin	100 ml/ha
Blanket	Indoxacarb	250 ml/ha
Ema Macten	Emamectin benzoate	300g/ha

Table 1 shows the active ingredients as well as their field application rates of synthetic chemicals which were used in the study.

3.6 Field application rates of biopesticides.

Table 2: A list of Biopesticides and their field application rates

BRAND NAME	ACTIVE INGREDIENT	APPLICATION RATE
Dynamo	*Beauvaria bassiana*	2.5kg/ha
Delfin	*Bacillus thuringiensis*	1kg/ha
Achta	Azadiractin	1kg/ha

Table 2 shows the active ingredients as well as the field application rates of the biopesticides which were used in the study.

3.7 Experimental procedure

A procedure similar to that described by Belay, Huckaba and Foster (2012) was used. Third instar larvae of *S. frugiperda* were used for the bioassays. Four third instar FAW larvae were placed in each perforated vial together with maize leaves as feed for the larvae. Each treatment was replicated four times and each replication had a total of four perforated vials which means each replication had 16 larvae (4vials x 4 larvae in each vial). The larvae of *S. fruigiperda* display cannibalstic properties if they cohabitate in large numbers, thus, all 16 larvae could not be placed in one vial. The synthetic chemicals (vantex, blanket and forceplus) as well as the biopesticides (dynamo, delfin and achta) were sprayed at the manufacturer's recommended rate. Ema Macten was used as the positive control for the study. Spraying was carried out using 500ml plastic hand sprayers and two shots were sprayed in each vial. This was done to provide adequate coverage of the feed so as to mimic the field spray coverage. The untreated control larvae were sprayed with an equal amount of water to avoid the effect of moisture differences in the perforated vials.

3.8 Data collection

The number of dead and live larvae were counted and recorded in each vial. Larvae mortality data was collected at 24, 48, 72 and 96 hours after exposure to synthetic chemicals and larvae mortality data was collected at 24, 72, 168, 240 and 336 hours after exposure to the biopesticides. The larva was considered dead if no motion was observed upon pricking with an inoculating needle.

3.9 Data analysis

Analysis of variance of mortality data was done to determine if any statistical differences between the means were present. There were two predictor variables which were the insecticide used and the number of hours after application and one response variable which was insect mortality. Dunnett's test was also used to assess the individual performance of each pesticide by comparing it with the positive control. SPSS version 21 was used to carry out the analysis as well as to plot the graphs.

CHAPTER 4: RESULTS

4.1 Larval mortality percentage rates for synthetic chemicals

The percentage larval mortalities for Ema Macten, Blanket, Vantex, Nemesis and the untreated control are presented in Table 3. The highest performance was shown by Ema Macten with 100% larval mortality, followed by Nemesis, Blanket and Vantex (93.8%, 92.5%, 60.9%), respectively. The untreated control showed the least performance with a recording of 5% mortality.

Table 3: Summary of the percentage mortality rates of larvae after exposure to synthetic chemicals.

Treatment	Sum of Dead 24hrs	Sum of Dead 48 hrs	Sum of Dead 72hrs	Sum of Dead 96hrs	% Mortality
Blanket	14	48	64	74	92.5
Ema Macten	54	75	79	80	100
Nemesis	56	69	74	75	93.8
Untreated_Control	1	3	4	4	5
Vantex	26	35	44	48	60

The synthetic chemicals influenced larval mortality (p=0.01) (Anova, Appendix 1). The number of hrs after application of the synthetic chemicals also had an influence on larval mortality (p=0.03) (Anova, Appendix 1). Dunnett's post hoc comparisons revealed that there were significant differences between the negative control (untreated control), Vantex and the positive control (Ema Macten) (P<0.05) (Dunnett, Appendix 2). However, the performance of all the

other treatments (Blanket and Nemesis) indicated that there were no significant differences with

the control ($p>0.05$) (Dunnet, Appendix 2).
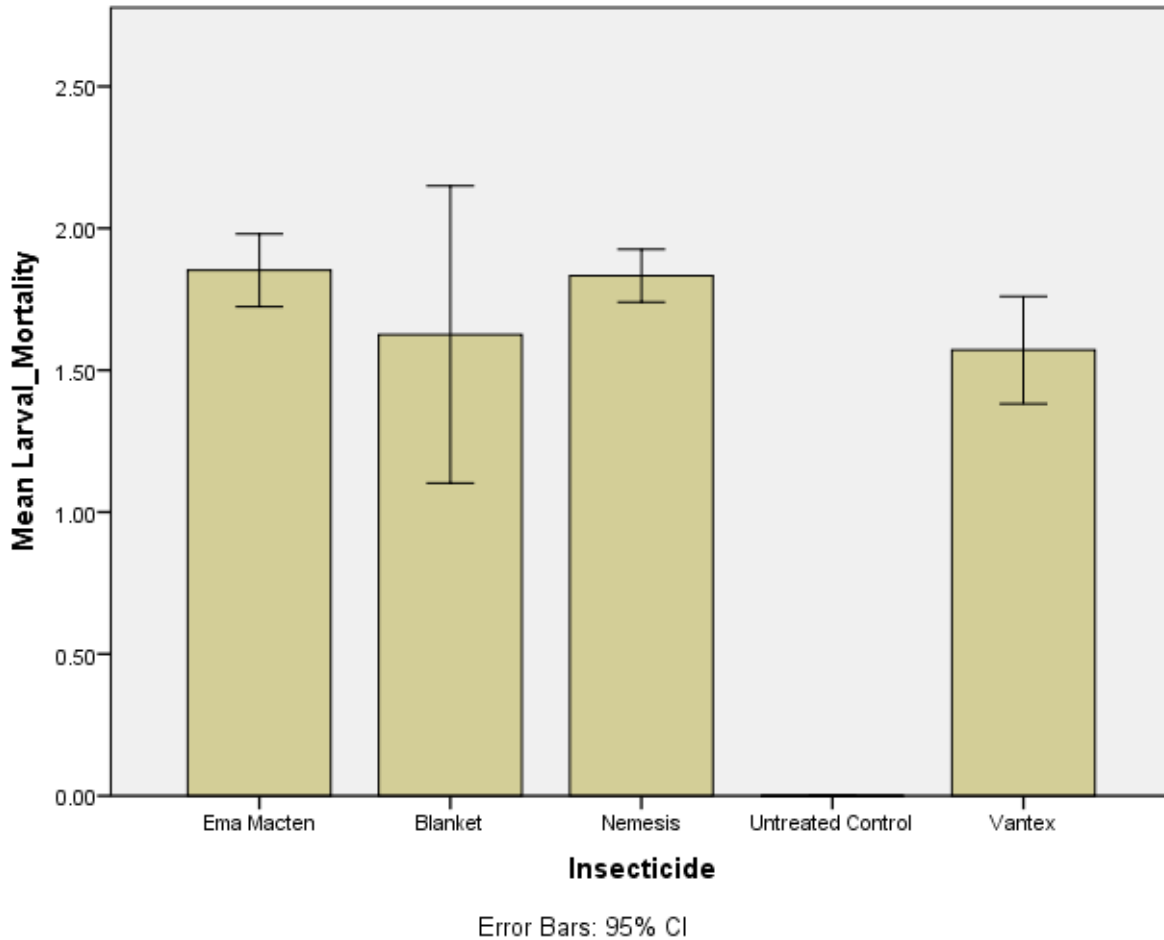
**Figure 4: Graphical presentation of the mean mortality of larvae after exposure to
synthetic chemicals.**

As shown in Figure 4 Ema Macten had the highest mean mortality and the untreated control had

the least mean mortality. The error bars indicate that the performance of Ema Macten, Blanket,

Nemesis and Vantex were not significantly different.

## 4.2 Larval mortality percentage rates for biopesticides

The percentage larval mortalities for the biopesticides are shown in Table 4. The highest larval mortality was caused by Ema Macten with a percentage of 98.4% followed by Dynamo, Delfin and Achta (84.3%, 82.8% and 78.1%), respectively. The lowest mortality (3.1%) was caused by the untreated control.

Table 4: Summary of the mortality of larvae after exposure to biopesticides.

Treatment	Sum of 72hrs dead	Sum of 24hrs dead	Sum of 168hrs dead	Sum of 240hrs dead	Sum of 336hrs dead	% Mortality
Achta	6	6	19	37	50	78.1
Delfin	2	2	17	36	53	82.8
Dynamo	18	20	23	44	54	84.4
EmaMacten	19	41	57	62	63	98.4
Untreated Control	0	0	0	1	2	3.1

The biopesticides had an influence on larval mortality ($p=0.046$) (Anova, Appendix 3). The number of days after application of the biopesticide showed no influence on larval mortality ($p=0.417$) (Anova, Appendix 3). Dunnett's post hoc comparisons indicated that there were significant differences between the performance of the untreated control and the positive control

(p=0.028) (Dunnett, Appendix 4). However, the performance of all other treatments (Achta, Delfin and Dynamo), had no significant differences with the control (p>0.05) (Dunnett, Appendix 4).
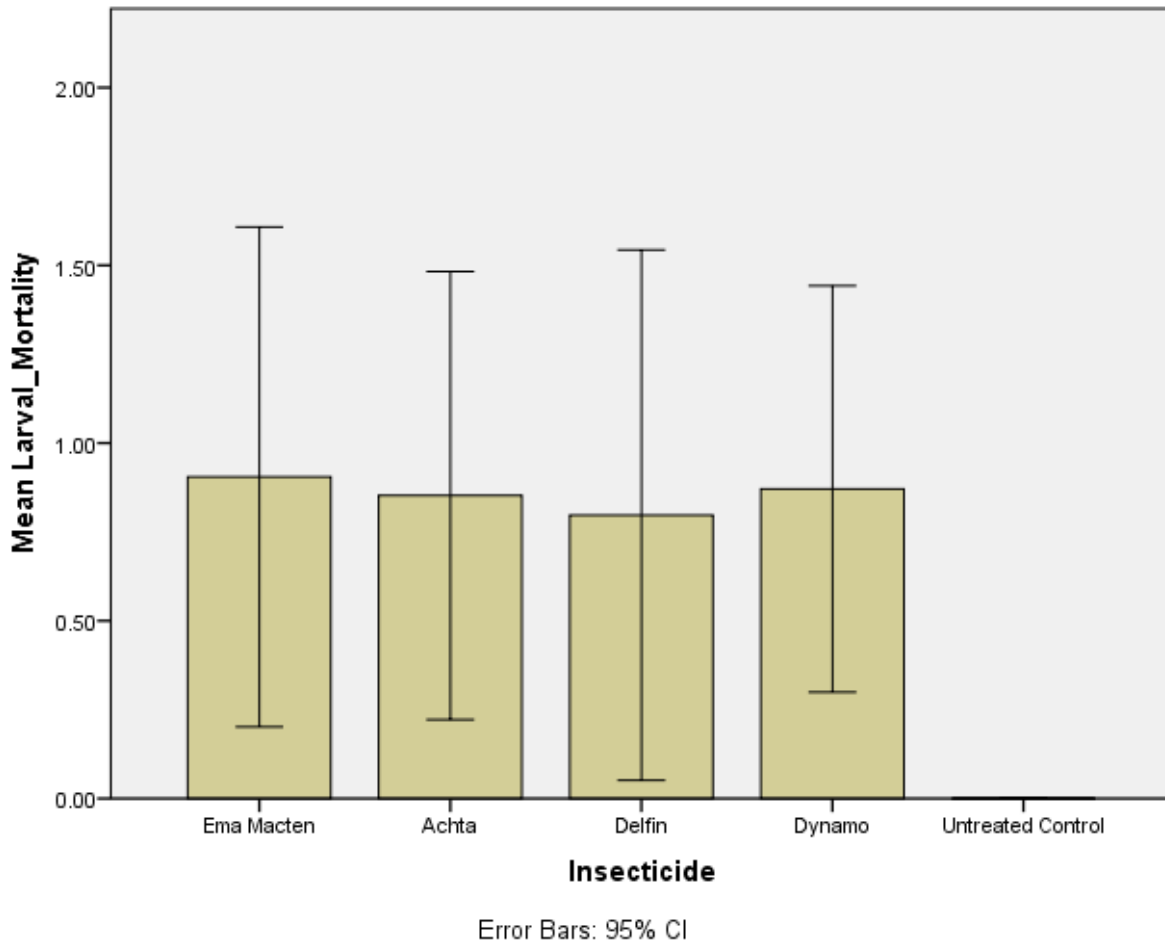
Fig 5: Graphical presentation of the mean larval mortality after exposure to abiopesticides.
As shown in Figure 5, the mean larval mortalities of the treatments EmaMacten, Achta, Delfin and Dynamo were almost equal. The error bars indicate that the performance of the treatments Ema Macten, Achta, Delfin and Dynamo were not significantly different.
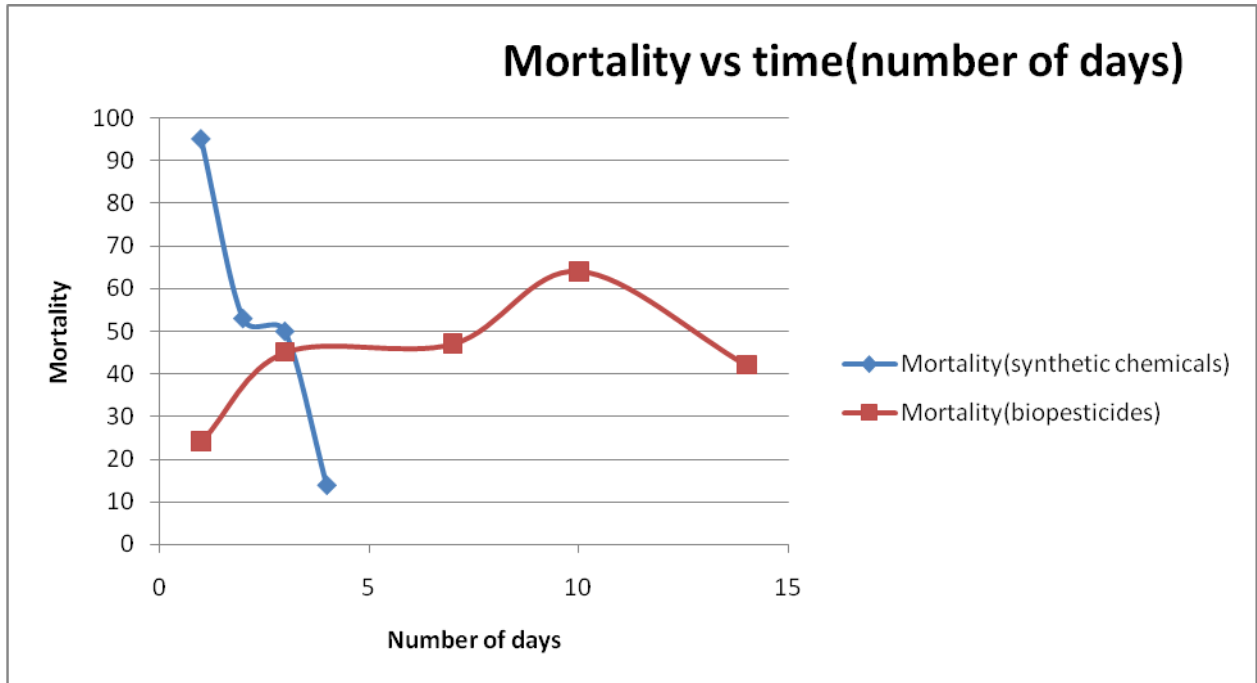
Figure 6: Effect of time on the mortality of larvae exposed to both biopesticides and synthetic chemicals

The synthetic pesticides killed the largest number of larvae on day 1 and the arval mortality recorded on each day decreased daily. For the biopesticides, The lowest number of larvae died on the first day and the mortality seemed to increase with time. However, the larval mortality decreased on the 14th day.
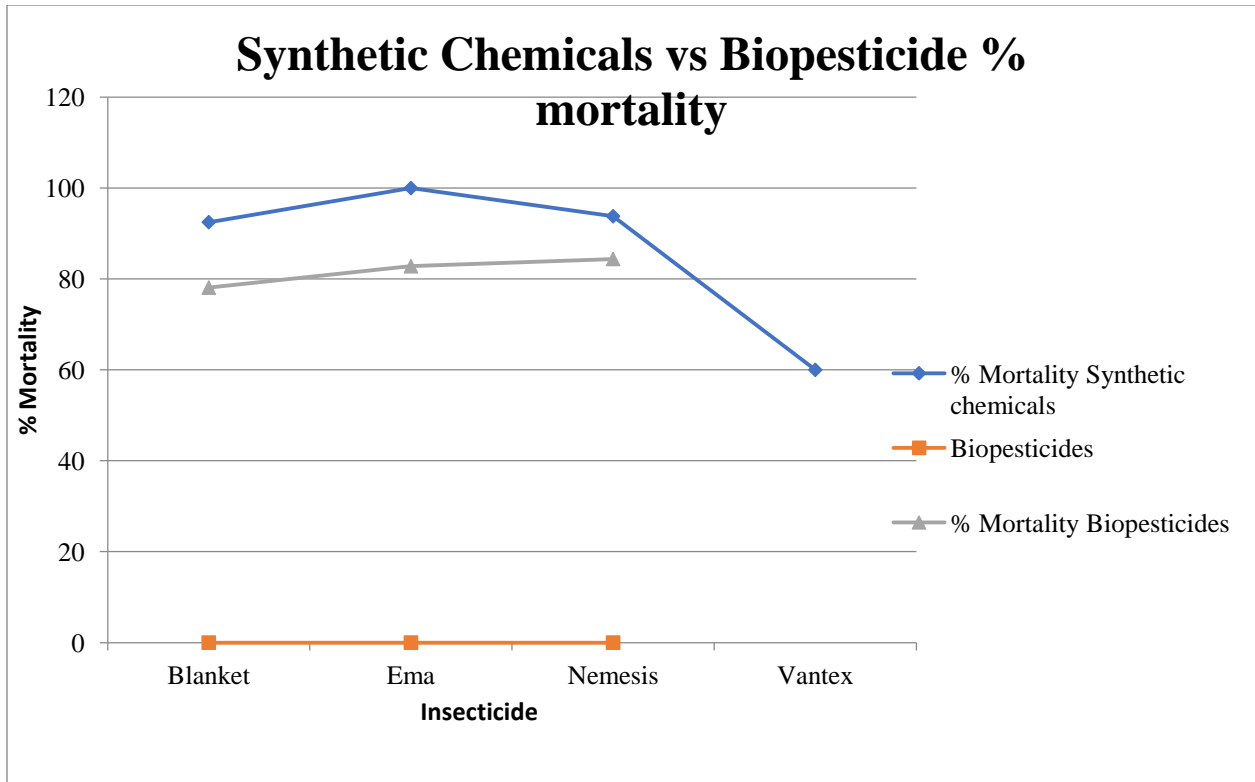
Fig 7: Synthetic Chemicals vs Biopesticides effectiveness

Fig 7 shows that all the other synthetic chemicals excluding Vantex were more effective than the

biopesticides.

CHAPTER 5: DISCUSSION

5.1 Synthetic chemicals

5.1.1 Vantex

The synthetic chemicals were very effective in killing the fall armyworm except for Vantex which showed a lower efficacy rate than the others, this could have been caused by resistance of some strains to the chemical. A study carried out by Yu (1991) revealed that a strain of the fall armyworm, *Spodoptera frugiperda* showed resistance to commonly used insecticides including resistance to the pyrethroid chemical Cyhalothrin which is the active ingredient of Vantex. His results also indicated that the broad spectrum of insecticide resistance observed in the field strain was due to multiple resistance mechanisms, including increased detoxication of these insecticides by microsomal oxidases and target site insensitivity such as insensitive acetylcholinesterase. The lower efficacy of Vantex could also be attributed by the concentration which was used. An increase in the concentration of the insecticide could relatively increase its efficacy.

5.1.2 Emamectin and Nemesis

Emamectin benzoate is a novel semi-synthetic insecticide that is derived from a natural fermentation product, avermectin B (Dybas and Babu, 1988). Laboratory bioassays have demonstrated that it is highly toxic to a broad range of Lepidoptera pest species at very low concentrations. In this study, Emamectin displayed the highest level of toxicity as it caused the highest mortality. A study conducted by El-Sayed and El-Sheikh (2015) indicated that Emamectin Benzoate showed high toxicity to 3rd instar stage larvae which is similar with the results of this study.

Nemesis contains Emamectin Benzoate and Acetamiprid as the active ingredients. Acetamiprid is an odourless, neonicotinoid insecticide composed of a synthetic organic compound. Neonicotinoid insecticides target the nervous system of insects causing paralyzation. Acetamiprid can be used combined with another pesticide with a different mode of action. This way, the developing of resistance by pest species can be prevented (US Environmental Protection Agency Office of Pesticide Programs, 2010). In this study, Nemesis was highly effective in controlling the fall armyworm.

5.1.3 Blanket

Indoxacarb is a non-systemic, synthetic organophosphate replacement insecticide used to control sucking insects (Moncara, 2003). It is used to control or suppress many insects including the fall armyworm (Dupont, 2002). Indoxacarb affects insects from direct exposure and through ingestion of treated foliage/fruit. Once indoxacarb is absorbed or ingested, feeding cessation occurs almost immediately. It kills by binding to a site on sodium channels and blocking the flow of sodium ions into nerve cells. The result is impaired nerve function, feeding cessation, paralysis, and death (Brugger, 1997). In this study, the Indoxarcab based insecticide was fairly toxic to the fall armyworm though it failed to cause 100% mortality. This could be an indication of resistance. A research conducted by Yu and McCord (2006) revealed that indoxacarb was quite toxic to *S. frugiperda* when applied topically and that it had lower contact toxicity. The inability of Indoxacarb to cause 100% mortality in this study could also be attributed by the fact that the insecticide was administered through contact feeding and not tropically. The reason for the low contact toxicity of indoxacarb is not known, but is possibly due to the poor cuticular penetration of the compound in the late instar of this insect (Yu and McCord, 2006).

5.2 Biopesticides

Biopesticides present a sustainable alternative to synthetic pesticides. However, lack of efficacy, inconsistent field performance and high cost has generally relegated them to niche products. Biopesticides are also important tools in integrated pest management (IPM) programmes and reducing the risk of resistance to chemical pesticides (Pretty and Bharucha, 2015). Current research efforts are focused on selecting native and exotic entomopathogens, which are highly virulent to arthropod pests, for developing efficient and environmentally sound bioinsecticides.

In this study, biopesticides were overall slow acting than synthetic chemicals and generally required more time for greater effectiveness. Direct correlations between insecticide and period of exposure were noted as the toxicity increased with an increasing period of exposure

It was also noted that even though they killed the FAW effectively, their efficacy was lower than that of synthetic pesticides. Mortality was observed in the controls indicating that other confounding effects were influencing the study however; the percentages were insignificant thus indicating that the confounding effects influenced the results to a lesser extent.

5.2.1 *Bacillus thrungiensis*

In biopesticides, Delfin, the *B. Thuringiensis*-based bioinsecticide was fairly effective in killing FAW. This entomopathogenic bacterium acts as a disruptor of the mesenterum, usually leading to death by septicemia and has proven to be effective against pests of different orders such as Lepidoptera, Coleoptera, and Diptera (Magalhaes, 2015).

Bacillus thrungiensis caused of 84.4% larval mortality. Polanczyk, Antonio, Rogério Fernando Pires da, and Fiuza (2000) conducted a similar experiment in which he exposed 2nd instar larvae to *B. thrungiensis*. A percentage mortality of 80.40% was obtained from his experiments and his findings are not significantly different from the results of this study. The small difference could

be attributed to the difference in larval stages which were used as 3rd instar stage larvae were used in the current study but 2nd instar stage larvae were used by (Polanczyk *et al*., 2000). Abiotic factors could have also played a role in the slight difference of the two results. Revelo (1963) concluded that environmental factors influence performance of *B. thuringiensis* in the control of insects. Studies conducted by Earle, Raun, Miguel and Revelo (1966) showed that Ultraviolet radiation had a drastic effect on the bacterium. The influence of gamma radiation on the growth rate of *B. thuringiensis* was studied in the laboratory by Smirnoff and Cantin (1967) and They found that survival of spores of *B. thuringiensis* diminished directly with an increase in radiation. All these studies can be evidence that weather factors exert stresses on *B.thrungiensis (*Frye, Scholl, Scholz and Funke, 1973). A study by Silva-Werneck (2000) also showed that *B. Thrungiensis* caused high mortality of S. *frugiperda* larvae.

5.2.*2* Azaradiractin

It appears that larvae of most lepidopterous pests are highly sensitive to neem. Neem blocks the larvae from feeding, although this effect is usually less important than the disruption of growth it causes, (National Research Council, 1992). A study conducted by Sisay *et al*. (2019) revealed that the extracts of *A. indica*, *P. dodecandra*, and *S. molle* consistently resulted in high larval mortality. Silva, Brogilo, Trindade, Ferreira, Gomes and Micheletti (2015) also reported high larval mortality of FAW using a seed cake extract of *A. Indica.* From the current study, the neem based biopesticide had a moderate performance. The reason being, that many biopesticides are highly sensitive to environmental conditions in space and in time (Sporleder and Lacey, 2013). The inconsistent performance of microbial pesticides has been attributed mainly to problems with formulation of entomopathogens, UV sensitivity, temperature and humidity (Dhillon, Gujar and Pak, 2010).

One problem with the use of neem on a large scale is the high photosensitivity of azadirachtin, which breaks down or isomerises under sunlight; thus, neem has a low residual effect under field conditions (Forim *et al*., 2010). Moreover, the lack of standardisation and quality control in neem-based formulations produced affect the reproducibility of the insecticide effect (Forim *et al*., 2010). Prates and Viana (2003), using an aqueous extract from neem leaves at 1%, found that the mortality level of *S. frugiperda* caterpillars was low during the first three days, after initial feeding, and high by 10 days, indicating that protocols for testing the efficacy of conventional pesticides may not be suitable for testing neem extracts (Day *et al*., 2018).

5.2.3 *Beaveria Bassiana*

The fall armyworm is susceptible to entomopathogens including fungi like *Beauveria bassiana* (Posada and Vega, 2006). The spores of *B. bassianna* attache to the insect cuticle, germinate and penetrate into the insect body. The hypahe, through enzyme action, proliferate in the insect body and cause mortality through a combination of chemical, mechanical, water loss and nutrient loss effects (Chadha, Mishra, Prasad, and Varma, 2014). *Beauveria bassiana* has been used in the control of *Spodoptera* (Maniania and Fargues, 1992). Compared to other lepidopteran pests, FAW larvae seem to be least susceptible to *Beauveria bassiana* (Wraight, Avery and Jaronski, 2010). In the current study, Dynamo, the *B. bassiana* based biopesticide was fairly toxic to the larvae causing 84.4% mortality. A study carried out by Akutse, Kimemia, Ekesi, Khamis, Ombura and Subramanian (2019) at ICIPE on second instar larvae of *S. fruigiperda* showed that *B. bassiana*caused moderate mortality of 30%. These differences are most likely to be due to enviromental constrants. Environmental constraints such as temperature and ultraviolet (UV) radiation, may limit field efficacy of the fungus. Laboratory studies suggest that low humidity does not limit the ability of the fungus to initiate disease. Sunlight is the major cause of mortality

of conidia on leaf surfaces (Goettel and Jaronski, 2012). In the current study, the temperatures were relatively warm (an average of 32^0C since it was summer) and the bioassays were carried out in a laboratory were sunlight was not accessible to the perforated vials containing the larvae. This could have contributed to the high mortality of the larvae as conditions which favour growth of the fungi were satisfied.

5.3 Conclusion

In conclusion, biopesticides and synthetic pesticides are both effective in controlling the fall armyworm. However, to achieve the best results, the two control methods should be used in combination rather than in isolation. The most effective biopesticides (Dynamo and Delfin) as well as the most effective synthetic chemicals (Emamectin, Blanket and Nemesis) are therefore recommended for the management of FAW in maize. However, an IPM approach is needed to control FAW as reliance on chemical control alone may, in the long run, increase the likelihood of FAW resistance to insecticides.

5.4 Recommendations

Additional research is necessary to determine the ecological effects of the persistent nature of these products in a row-crop ecosystem. Fieldwork is also needed to compliment these laboratory studies to determine the most effective rates of compounds given their respective residual properties. Research is also needed to understand the most appropriate timing for applications of these insecticides in order to maximize their effectiveness in various cropping systems. In the study the chemicals were only tested on third instar stage larvae, however, since toxicity is also influenced by the larval stage of an organism, further studies are needed to evaluate the efficiency the insecticide control according to the susceptibility and the stages of biological

development of the pest. The evaluation of insecticide susceptibility of FAW populations from different regions is important as the variability of insecticide resistance/susceptibility characteristics in FAW populations may assist in determining the origin of FAW infestations and in developing an appropriate management strategy.

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APPENDICES

APPENDIX 1

Tests of Between-Subjects Effects

Dependent Variable: Larval_Mortality

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	9.920[a]	7	1.417	88.064	.000
Intercept	37.884	1	37.884	2354.110	.000
Day	.204	3	.068	4.229	.030
Insecticide	9.716	4	2.429	150.940	.000
Error	.193	12	.016		
Total	47.997	20			
Corrected Total	10.113	19			

a. R Squared = .981 (Adjusted R Squared = .970)

APPENDIX 2

Multiple Comparisons

Dependent Variable: Larval_Mortality

Dunnett t (2-sided)a

(I) Insecticide	(J) Insecticide	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Blanket	Ema Macten	-.2263	.08970	.082	-.4781	.0255
Nemesis	Ema Macten	-.0192	.08970	.998	-.2710	.2326
Untreated Control	Ema Macten	-1.8520*	.08970	.000	-2.1038	-1.6002
Vantex	Ema Macten	-.2811*	.08970	.028	-.5329	-.0293

Based on observed means.

 The error term is Mean Square(Error) = .016.

*. The mean difference is significant at the 0.05 level.

a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

APPENDIX 3

Tests of Between-Subjects Effects

Dependent Variable: Larval_Mortality

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	3.914[a]	8	.489	2.142	.093
Intercept	11.465	1	11.465	50.181	.000
Day	.950	4	.238	1.040	.417
Insecticide	2.829	4	.707	3.096	.046
Error	3.655	16	.228		
Total	19.304	25			
Corrected Total	7.570	24			

a. R Squared = .517 (Adjusted R Squared = .276)

APPENDIX 4

Dunnett t (2-sided)a

(I) Insecticide	(J) Insecticide	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Achta	Ema Macten	-.0526	.30230	.999	-.8712	.7660
Delfin	Ema Macten	-.1076	.30230	.989	-.9262	.7110
Dynamo	Ema Macten	-.0337	.30230	1.000	-.8523	.7849
Untreated Control	Ema Macten	-.9049[*]	.30230	.028	-1.7235	-.0862

Based on observed means.

The error term is Mean Square(Error) = .228.

*. The mean difference is significant at the .05 level.