TARGET-SITE MUTATIONS, BASELINE SUSCEPTIBILITY AND CROSS-RESISTANCE EVALUATION OF FALL ARMYWORM INFESTING MAIZE IN KENYA

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A Thesis Submitted in Partial Fulfillment of the Requirements for the Degree of Masters of Science in Molecular Biology of Masinde Muliro University of Science and Technology

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DECLARATION

This thesis is my original work prepared with no other than the indicated sources and support and has not been presented elsewhere for a degree or any other award.

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CERTIFICATION

We the undersigned certify that we have read and hereby recommend for acceptance of Masinde Muliro University of Science and Technology a thesis entitled ***"Target-Site Mutations, Baseline Susceptibility and Cross-Resistance Evaluation of Fall Armyworm Infesting Maize in Kenya".***

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DEDICATION

I dedicate this thesis to my beloved mother, Jane Waithera Gichere a strong and gentle soul who has been on my side to encourage and support me immensely. To my beloved husband, Benson Kimani who has been my strongest pillar, and our son Raymond Muiruri Kimani, my source of inspiration. To my brothers, sisters, and cousins for the care and priceless love. Teresia Nyambura I dedicate this study to you, for the guidance, prayers, and love you have shown me. Above all I dedicate this study to our Almighty God for the wisdom he has bestowed upon me, protection and guidance.

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ABSTRACT

The fall armyworm, *Spodoptera frugiperda,* is a pest of gramineous crops known worldwide. It is resistant to various insecticides presenting a serious challenge in its wake. Sodium channels that are voltage-gated, ryanodine receptors and acetylcholinesterase's active site transmit electrical signals in excitable cells. The sites are targets of various synthetic insecticides. The current study sought to establish baseline susceptibility and investigate the cross-resistance of fall armyworm to a range of nine insecticides,determine its molecular tolerance mechanisms via gene expression, and model target-site mutations in sodium channels. A complete randomized design was used in selection of test insect samples and leaf discs subjected to different insecticides. *Spodoptera frugiperda* larvae, fourth to the sixth instar were collected from different geographical sites in infested maize plantations. They were then reared in the laboratory under ambient conditions allowing them to mass mate. F2 third larva instar was subjected to leaf-dip bioassay to determine baseline susceptibility. A software named Polo Plus was utilized to determine the median lethal concentrations of the pest populations. The resistance ratio value was determined via division of the median lethal concentration (LC_{50}) value of each field population by the corresponding LC_{50} value of the susceptible strain. Cross-resistance pattern was determined through Pearson's correlation analysis. Quantitative PCR were conducted to determine gene expression in the 3 target sites to validate their involvement in molecular tolerance. I*n silico* approach was conducted to locate the existing docking sites on pest's sodium channels along with the interactions between study toxicants. The results demonstrated a low presence of resistance (1 to 4-folds) to the insecticides tested. Abamectin was the least potent with a ratio of 1 while spinosyns were the most potent (spinetoram, 11188, spinosad, 7079). Lambda cyhalothrin showed weak correlations to the 8 insecticides tested hence a lack of cross-resistance to them. Secondly, voltage-gated sodium channel, acetylcholinesterase's active site, ryanodine receptors had 13.59, 34.93 and 4.90- fold higher expression than the untreated samples, respectively. The genes in these regions were up regulated in the wild type than in the knock down genes due to the positive fold changes. Thirdly, residue $Serine^{1873}$ exhibited the most frequent interactions with the 6 insecticides used forming close binding contacts (<4 Å) with the insecticides. This hasn't been implicated previously in mutations that cause knockdown resistance in this pest. Cartap exhibited the highest number of binding sites. Its binding capability to this site has not been reported previously. Indoxacarb had 3 different binding amino acids namely Serine 1873, Tyrosine 1927, and Asparagine 1045, different from mutations that have been previously attributed to its resistance. In conclusion, spinosyns, and lufenuron exhibited high toxicity to FAW while imidacloprid and abamectin were the least potent. Lower quantities of relative transcripts and the positive fold changes in expression validates molecular tolerance. Residue Ser^{1873} had the most interactions hence should be considered in making of more efficacious insecticides.

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ABBREVIATIONS AND ACRONYMS

AChE	Acetylcholinesterase
ADP	Adenosine diphosphate
ATP	Adenosine triphosphate
Bt	*Bacillus thuringiensis*
cDNA	Complementary deoxyribonucleic acid
Cry	Crystal endotoxins
Ct	Cycle threshold
DDT	Dichlorodiphenyltrichloroethane
GMQE	Global model quality estimation
GSTs	Glutathione -S-transferases
IFM	Isoleucine-Phenylalanine-Methionine
IPM	Integrated pest management
IRAC	Insecticide resistance action committee
K*dr*	Knockdown resistance gene
LC_{50}	Lethal concentration 50
MFM	Methionine-Phenylalanine-Methionine
MFO	Mixed function oxidases
MMUST	Masinde Muliro University of Science and Technology

nAChRs	Nicotinic acetylcholine receptors
Nav	Sodium channel
q-PCR	Quantitative PCR
RH	Relative humidity
SCBIs	Sodium channel binding inhibitors
VGSCs	Voltage-gated sodium channels

DEFINITION OF TERMS

Below are the commonly used terms in this study with their definition;

Agro-ecological zones: A land resource mapping zone

Baseline Susceptibility: Data obtained from a pest which has no history of selection with an insecticide/ toxicant

Lethal Concentration 50: Concentration of a toxicant that leads to the deaths of 50% of the dosed population.

Cross-resistance: A phenomenon in which resistance to one toxicant causes insensitivity to another toxicant

Selected Strains: Proportion of resistant insects to a compound from generation to generation

Probit Analysis: A specialized regression model of binomial response variables.

***In Silico*:** Prediction of a phenomenon using computational approaches

Insect Population: A subset of insects of one species occupying a particular geographical area

Insecticide Resistance: Reduced sensitivity/total insensitivity of a pest to an insecticide

Molecular Docking: Identification of a molecule orientation to a second one to form a stable complex.

CHAPTER ONE

INTRODUCTION

1.1 Background Information

Food security is one of the key pillars of Kenya's economy. The fall armyworm, *Spodoptera frugiperda* originates from American sub-tropical and tropical zones. The pest is a migratory polyphagous pest and the adult moth is able to move 100 km per night (Srikanth, *et al*., 2018). These species have over 80 crop species as hosts, damaging cultivated cereals like maize, vegetable crops, sorghum, legumes, and sugarcane (Capinera, 2017; FAO, 2017). In 2016, fall armyworm was reported in West Africa which later spread to the East African countries like Kenya (CABI, 2017).

Maize is an economic crop and part of key food security in most African countries. As per Sisay *et al*. (2019), consumption of maize per capita annual is 153, 168, and 181 kg in Zimbabwe, Zambia, and Malawi respectively. In countries like Malawi, South Africa and Zambia, the daily mean consumption of maize per person is 252.7 g hence it is the most vital staple crop in the region (Grant *et al*., 2012). In Kenya, we have 6 different agro-ecological regions where maize is cultivated (Hassan *et al*., 1998; De Groote *et al*., 2020). The agro-ecological zones include moist mid-altitudes, highland tropics, dry mid-altitudes, lowland zones, moist transitional and dry transitional regions. The western and central regions referred to as the highland zones, and moist transitional zone bordering on the west and east produce more than 2.5 t/ha, producing approximately half of quantity of this crop in countrywide. The lowland tropics within the coastal region, the dry mid-altitude tropics and dry transitional regions produce low yields of around 1 t/ha. Lastly, the zone surrounding Lake Victoria, a moist mid-altitude region, produces 1.5 t/ha yields (De Groote

et al., 2020). This study focused on sampling farms located in most of these maize growing zones.

Fall armyworm (FAW) infestation and damage are attributed to low maize yields leading to adverse losses in household incomes and negatively impacting on the gross domestic product of a nation by reducing market access and regional trade (Otipa *et al*. 2017; Kasoma *et al*., 2021). This pest attacks plants from seedling emergence (Vegetative emergence stage), early and late whorl stage (Vegetative stage 1-7), tasseling stage, silking stage, blister and maturity stage (Reproductive stage 1-6). They may damage all leaves killing young plants, damage whorls resulting in yield losses, or feed on the ear resulting in poor grain quality and yield reductions (Kasoma *et al*., 2021). According to Sisay *et al*. (2019), FAW infestation has been aired in 44 countries in African. If left uncontrolled, the pet has a high potential of leading to maize yield losses in Africa valued at US$ 2.4–6.2 billion that is, 8 to 21 million tones (21–53% of total production) per annum (Cock *et al*., 2017). Kumela *et al*. (2019) recorded that farmers from Ethiopia and Kenya estimated maize infestation by the FAW as a range of 24.1% to 39.4% and 38% to 53.9%, respectively. In addition, the farmers expressed their concerns on how infestation by the FAW would cause reduction in maize yield, estimated as of about 934 kg/ha and 1381 kg/ha in Ethiopia and Kenya respectively. The potential economic losses occurring as a result of uncontrolled existence of FAW in annual basis are expected to be to US$6.1 billion (CABI, 2017).

In most countries in Latin American, FAW is a pest in cotton, with a possibility of directly affecting crop productivity by causing significant crop damage to the plant's reproductive parts (Blanco *et al*., 2016). In Mexico, FAW is said to exist throughout corn vegetative

development (V2-12), however, it may also infest at both blister and silking stages of maize plant (Blanco *et al*. 2014). In case its left uncontrolled, FAW can reach 100% losses in some tropical areas (Blanco *et al*., 2016). Fall armyworm is the major pest infesting maize in Brazil, leading to losses of US $400 million yearly (Kumela *et al*., 2019).

Insecticides are utilized as key components of Integrated Pest Management (IPM) recommendations to control fall armyworm because of its ability to feed on a broad host range and migrate long distances making other control methods less successful. Even though synthetic insecticides present efficient control of pests including *Spodoptera frugiperda,* full dependence on insecticides results into existence of insensitivity to most toxicants (Belay *et al*., 2012). Carvalho *et al.* (2013) demonstrated that the pest has developed resistance to organophosphates and pyrethroids. Resistance has also been previously reported in carbamates, organophosphates, pyrethroids, and *Bacillus thuringiensis* (Huang *et al*., 2014). It is against this background that investigating emerging resistance development at a molecular level and determining baseline susceptibility of this pest will go a long way in ensuring food security in the country.

FAW's susceptibility to many insecticides has greatly reduced because of ovipositional preference and larval behavior within a host. Adults may deposit eggs all over the plant canopy with preference to the cotton plant lower two-thirds or in corn and sorghum whorls (Hardke *et al.*,2011). Additionally, indiscriminate use of insecticides and genetically modified plants in its control has resulted to evolvement of resistance and selection pressure. The very initial report of insecticide resistance development was to carbaryl. Field-relevant resistance of Cry1F has developed both in Brazil and the United States (Storer *et al*., 2010; Farias *et al.,* 2014b). Diez-Rodrigues (2001) reported resistance to

pyrethroids (lambda- cyhalothrin) in Brazil having almost 13-fold resistance ratio. The evolvement of resistance mechanisms heightens the need to ascertain baseline susceptibility of the pest populations, to recentlyapplied toxicants to establish the baseline toxic concentrations at which the pest will be susceptible.

The recently used insecticides against this pest exhibit cross-resistance which affects their efficiency. Resistant strains to tebufenozide exhibit high cross-resistance to abamectin, but a selection of the resistant strain with abamectin exhibit no cross-resistance to tebufenozide (Qian *et al*., 2008). Indoxacarb is effective against most lepidopterans, however, pyrethroid and organophosphate resistant strains exhibit positive cross-resistance to indoxacarb (Nehare *et al.,* 2010).

Insecticide resistance mechanisms majorly are mediated by reduced target-site insensitivity. Voltage-gated sodium channels (VGSCs) sites are vital for signaling and function as a molecular target for neurotoxins. A mutation in the gene structure of this channel affects the binding efficiency of blocker insecticides (Araujo *et al*., 2011). Domain II, Segment 4 to 6 regions of the para-type sodium channel contain sites mutations known to cause knockdown gene resistance. The L1014F mutation is the mostly reported knockdown resistance (kdr) type mutation conferring resistance in pyrethroids in various arthropods (Davies and Williamson, 2009). The T929I mutation is a super knockdown resistance mutation, identified first in *Plutella xylostella* strains that are pyrethroids resistant and later reported in lepidopterous insects (Araujo *et al*., 2011). Rı´os-Dı´ez and Saldamando-Benjumea, (2011) studied the genetics behind FAW's lambda-cyhalothrin and carbamate methomyl resistance, indicating he involvement of multiple recessive genes.

Many studies report resistance cases related to pyrethroid insecticides targeting inhibitors of acetylcholinesterase (AChE), and VGSC. Currently as per the global Arthropod Pesticide Resistance Database (APRD), globally there are 144 insecticide resistance cases in fall armyworm. Among this cases 26% are due to insecticides targeting VGSCs, 19% to those targeting AChE and less than 10% targets RyR (Boaventura *et al*.,2020). Since the VGSCs has been reported by previous studies to be highly involved in molecular resistance in FAW and also having the highest percentage as per APRD, this study majored on modelling only the VGSC protein to predict the interaction of the study insecticides with this target site using *in silico* methods. The low cost and frequent applications of pyrethroids (organophosphates and carbamates) may also have contributed to this resistance cases in FAW. Even though it is unclear of whether in Africa the FAW pests are already resistant to the older active chemicals (Day *et al*., 2017; Boaventura *et al*.,2020), the increasing complains from farmers on low efficacy of these compounds in fields is very alarming. A314S, G340A, and F402V point mutations at acetylcholinesterase confer insecticide resistance.

Diamide insecticides are the most recently introduced chemical class into the insecticides market. They are of broad spectrum and high efficacy against various pests (Boaventura *et al*.,2020). These newest insecticides kill pests by acting on pest's ryanodine receptors (RyR). Nevertheless, several studies have reported the existence of a target site mutation (I4734M) at ryanodine receptor (RyR) in laboratory-selected FAW strain (Zhao *et al*., 2020). As per studies by Boaventura *et al*. (2020), G4946E mutations has also conferred resistance in FAW. This diamide resistance may due to frequent diamide applications and use of high rates than the recommended field rates which have a high possibility of

resulting to RyR target site mutations thus affecting the binding of diamides (Richardson *et al*., 2020). This study was conducted to validate existence of mutations in the VGSCs, AChE, RyR target sites of FAW populations through relative quantification of gene expression and via modelling of the VGSC protein as the target site which has been previously reported to highly influence molecular resistance in FAW than RyR and AChE sites do.

1.2 Statement of the Problem

Maize is a staple crop in Kenya but is highly infested by FAW leading to high economic losses and hunger. Although FAW has been successfully managed by using synthetic insecticides, the pest has become resistance to most of these insecticides (Benardi *et al*., 2015). The high rate of infestation by FAW led to over reliance on use of chemical insecticides in controlling damage in the host plants (Carvalho *et al.,* 2013). The extensive use of these synthetic insecticides prompted development of resistance mechanisms to most insecticides (Gutierrez *et al.,* 2019). Newer insecticides having novel mode of action are now replacing the old formulations because of their high target specificity, low mammalian toxicity, safety of beneficial insects and their residue persistence is short. However, the development of these new compounds to match the evolution of resistance is becoming exceedingly difficult due to limited number of target sites in the pest. This has made it difficult to control the mass destruction of maize crops caused by this pest. Although researchers have demonstrated that FAW resistance is due to both detoxification action of metabolic enzymes and insensitivity of the modified site at the target regions (Yu *et al*.,2003; Carvalho *et al.,* 2013), few have established its baseline susceptibility to classes of insecticides recently used against this pest and the cross-resistance pattern.

This study investigated fall armyworm resistance to selected pesticides by determining baseline susceptibility of field populations and evaluating cross-resistance of these insecticides and validating the involvement of VGSCs, AChE and RyR target-site mutations in molecular tolerance mechanisms of FAW using gene expression via Quantitative PCR. In addition, VGSCs protein was modelled using *in silico* methods to predict the interaction of test insecticides with this protein to identify the binding positions and any presence of mutations on the VGSCs.

1.3 Justification of the Study

Maize and other economically valuable crops have been infested by FAW which is difficult to control using synthetic insecticides which instead has led to evolution of resistant strains (Belay *et al*., 2012). This has caused a reduction in maize yields in most of the maize-growing agro-ecological zones, impacting on Kenyan economy negatively (FAO, 2017). There is, therefore, need to determine and understand the emerging resistance mechanisms used by the pest at a molecular level so as to develop effective control strategies. This study validated the action of the most commonly used synthetic insecticides against this pest, provided valuable information in understanding molecular resistance mechanisms and helped in development of rational management strategies to effectively manage resistant pests in the field.

1.4 Objectives

1.4.1 General Objective

To determine baseline susceptibility and evaluate cross-resistance, determine molecular tolerance mechanisms and model target-site mutations of *Spodoptera frugiperda*, infesting maize in Kenya's agro-ecological regions.

1.4.2 Specific Objectives

1. To determine the baseline susceptibility and cross-resistance of the *Spodoptera frugiperda* populations from different agro-ecological regions, to a range of nine insecticide classes based on IRAC classification.
2. To determine the fall armyworm molecular tolerance mechanisms against a range of insecticides.
3. To model the target-site mutations in the voltage-gated sodium channels of *Spodoptera frugiperda* populations within Kenya's agro-ecological regions.

1.5 Research Questions

1. What is the baseline susceptibility and cross-resistance of the *Spodoptera frugiperda* populations from Kenya's agro-ecological regions, to a range of nine insecticide classes based on IRAC classification?

2. What are the fall armyworm molecular tolerance mechanisms against a range of insecticides?

3. What are the target-site mutations of the voltage-gated sodium channels of *Spodoptera frugiperda* populations within Kenya's agro-ecological regions?

1.6 Significance of the Study

The findings of determination of baseline susceptibility and target site mutations of FAW in establishing the resistance mechanism affecting the newly introduced chemicals may be of help in developing effective management strategies to control the pest. The study availed a detailed understanding of development of resistance at a molecular level for effective design of control strategies by developing insecticides that will have multiple target sites for increased binding efficiency and chemicals that may not cause cross resistance that

increases selection pressure nor kill non-target organisms. These insecticides will be effective against both susceptible and resistance *Spodoptera frugiperda* strains. Secondly, the study was of great importance in pointing out the most and least potent insecticides which can be of help to farmers in screening for effective chemicals in the field for the better management of fall armyworm. Thirdly, the findings of this study can be employed in development of a rotational program for application of recently used insecticides minimizing cross resistance. This was geared towards mitigating crop damage caused by the pest countrywide and globally at large ensuring that food security was achieved through increased crop yields.

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Fall armyworm infests gramineous crops. Increased infestation has led to intensive application of synthetic insecticide to control pests. Consequently, this has contributed to development of populations resistant to these chemical insecticides and *Bt* infested crops posing a challenge in their management (Chandrasena *et al.*, 2018). Fall armyworm evolves different mechanisms to resist and defend itself against the new synthetic insecticides. These mechanisms include metabolic, behavioral and alteration of molecular sites hence reduce the efficacy of these new chemistries. FAW survival in unfriendly chemical environment depends on its capability to degrade these detrimental chemicals thus keep on evolving mechanisms as it colonizes new plant hosts or upon encountering newer insecticides.

2.2 Spread of Fall Armyworm

Fall armyworm is a transboundary pest that is a native of tropical regions of America. FAW, in Brazil, is the most known destructive pest of maize (Cruz *et al*., 2012). In early 2016, Infestation on the African continent was for the first time reported in Nigeria, Togo, Benin, and Sao Tome' and Principle (IITA, 2016; IPPC, 2016). The pest had invaded Central and East African countries and most countries of the Southern African Development Community (SADC) region by May 2017(Figure 1). Analysis of how they spread to Africa suggests a successful transfer as stop ways on a direct flight, either in cargo containers or airplane holds (Cock *et al*., 2017; Tambo *et al*., 2020). There is high probability (>90%) that invasion of fall armyworm to Africa was from the Florida strain,

restricted to the Caribbean islands and the eastern seaboard of the USA (Day *et al*., 2017). It is anticipated that the numerous flights from the mainland to countries in the Indian Ocean Islands could have caused the rapid spread (Rwomushana *et al*., 2018). In March 2017, the pest was first reported in Western Kenya. Initial counties infested were Busia, Trans-Nzoia, Nandi, Bungoma, and Uasin-Gishu (FAO, 2017). Recently it has been reported in India and Yemen in Asia (Shylesha *et al*., 2018). Although the distribution of this pest does well in warm climates, it covers large geographic areas because of adults' dispersal ability hence enable it to invade numerous host species (Carvalho *et al*., 2013).

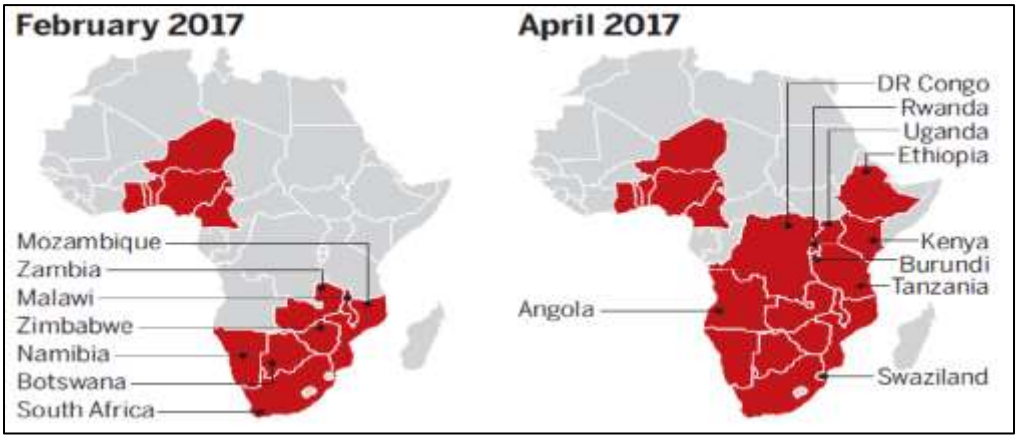


Figure 1: Spread of Fall Armyworm in African Continent (FAO, 2017)

2.3 Host Range of Fall Armyworm

Fall armyworm is a voracious insect and polyphagous in nature hence its accidental introduction in the African continent can cause a long lasting threat to economically vital crops. As shown in table 1, its host range is wide with recorded 80 plant species in 23 families (Pashley, 1988; Pogue 2002; Goergen, 2016). The pest utilizes important cultivated graminaceous plants as hosts like sugar cane, maize, rice, millet, wheat, sorghum and can reach pest status on several of them (Capinera, 2002; Barros *et al*., 2010). Fall

armyworm has a capability of causing more harm to maize crops than to other species in same genus. To manage this pest a better understanding of its host use within and between crop seasons, including pest reservoir plants that are either cultivated or uncultivated, is of great importance. Secondly, understanding the resistance mechanisms of this pest to synthetic insecticides, at molecular level, could help in developing new chemicals with increased efficacy to eradicate it.

Table 1: 80 Host Plant Species for Fall Armyworm

Maize	Alfalfa	Sudan grass	Pigweed
Rice	Onion	Timothy grass	Barley
Sorghum	Guinea grass	Tobacco	Kales
Sugarcane	Millet	Rye grass	Capsicum
Cabbage	Tomato	Oats	Ginger
Beet	Potato	Wheat	Spinach
Groundnut	Up-land cotton	Bent grass	Lemon
Soybean	Banana	Crab grass	Bermuda grass
Johnson grass	Hay	Red cloves	Nut sedge
Sand spur	Pumpkin	Asparagus	Cucumber
Peanuts	Strawberry	Teosinte	Para grass
Sunflower	Peach	Kentucky bluegrass	Buckwheat
Eggplants	Apples	Pearl millet	Pigeon pea
Potatoes	Dehuh	Violets	White cloves
Tall fescue	Grapes	Rutabaga	Cowpeas
Purslane	Wild morning glory	Turnip	Seville orange
Hollyhock	Cocklebur	Pea	Red peppers
Watermelon	Lettuce	Velvet beans	Cocklebur
Papaya	Slash pine	Kudzu	Broom sedge
Napier grass	Garlic	Chick pea	Oats

2.4 The Biology of Fall Armyworm

Fall armyworm is a moth in the noctuid family, undergoes four stages of development comprising of eggs, six instars, pupa and adults depending on food availability and the environmental factors (FAO, 2017; Assefa and Ayalew, 2019). The life cycle takes about

30-45 days but in cooler temperatures, it may take 60-90 days (Padhee and Prasanna, 2019). The optimum temperature for larval development is 28^0C, however the temperature may be lower for laying of eggs and pupation (CABI, 2017). The maximum total egg production for the adult female moth is over 2000 with an average of about 1500 eggs. They lay eggs on foliage of the host plant. The egg base appears flattened; it is curved upward and at the apex, eggs are broadly rounded point. Eggs are usually oviposited in groups on the emerging leaves. Each group contains about 300 to 400 eggs (Kumela *et al*., 2018). The eggs measure between 0.3 and 0.4 mm and form a mass consisting of two layers. A protective layer consisting of silk from the female abdomen covers the egg mass, hence a furry appearance (Figure 3). The egg stage takes 2 to 10 days depending on the temperature, but at optimum temperatures hatching takes 2-3 days. After the eclosion neonates consume the egg mass and the larvae dislocate to start feeding on different vegetative tissues. The larval phase consists of six larval instars (stages) before pupation as shown in figure 2, which varies in color from light tan to green to black. The larval stage may last for 14 to 30 days depending on the temperature, weather conditions, and humidity. Young larvae are green in appearance and have a blackish head. The dorsal body surface of the second and third instar turns brownish and forms lateral white lines. The fourth to sixth instars are reddish brown, mottled with white sub-dorsal and lateral lines. They have elevated dorsal spots that are dark and bear spines. As the larvae of *S. frugiperda* develop, they present 4 pinaculas in a square pattern on the dorsal side of 8^{th} abdominal segment (Figure 3). Full-grown larvae are 30-40 mm long. Older larvae possess a distinct white inverted "Y" in the cephalic capsule (Hardke *et al*., 2015; Deole and Paul, 2018). Fully grown larvae then burrow into soil where they pupate.

Pupation happens at a depth of 2 to 8 cm in the soil. If nymphosis occurs in loose soil, the larva forms a loose cocoon made of soil particles with silk. In too hard soil, it may tie debris together with other materials to make a cocoon (Tendeng *et al*., 2019). The pupa is reddish brown in color, about 4.5mm in width and 14-18mm in length (Figure 2). Pupal period takes about 6-8 days and adult moths emerge. In the male adult moth, the forewing is gray and brown in color, and at the tip and also near the center of the wing are distinct white spots (Tendeng *et al*., 2019). However, in females, the forewing is less distinctly marked with a uniform grayish brown color (Deole and Paul, 2018). In both sexes, the hind wing is iridescent silver-white having narrow dark boarders. The lifetime of adults is 31 days on average. The moths can either mate locally or migrate miles away before ovipositing and mating (Jarrod *et al*., 2015).

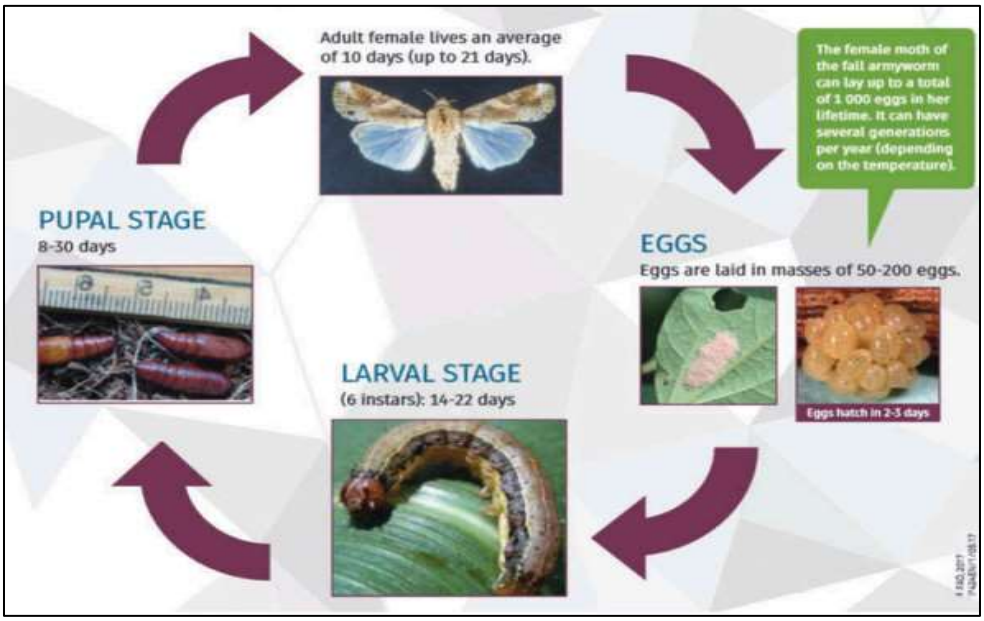
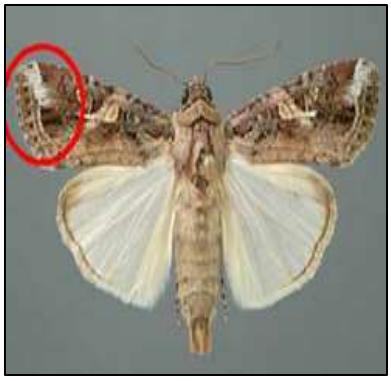
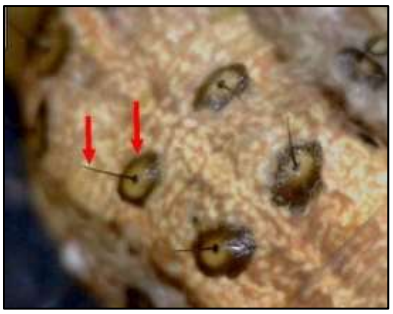


Figure 2: The lifecycle of fall armyworm (FAO, 2017; Assefa and Ayalew, 2019)

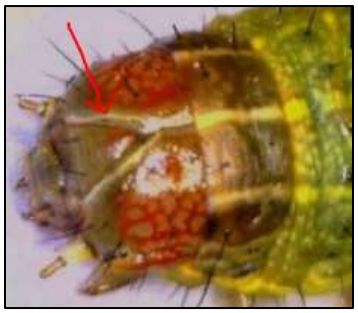
A. Egg mass protective silk layer



B. White patches on adult male forewings



C. Pinacula arranged in a square



D. White inverted 'Y' at Cephalic capsule

Figure 3: Key features of fall armyworm (Tendeng *et al*., 2019)

2.5 Host Damage

Fall armyworm is a highly destructive that for more than a century has been prevalent in the America. In the last two years, the pest has affected the livelihoods of several millions of smallholder farmers by causing devastating damage to 1.5 million ha of maize crop and more, in Africa according to the assessment carried out by the Consultative Group on International Agricultural Research (CGIAR) Research Program on Maize (MAIZE) led by the International Maize and Wheat Improvement Center (CIMMYT). Fall armyworm affects the grain basket of Kenya by attacking crop leaves at 3 to 4 weeks while still young. Enormous losses and damage are caused by larvae which can go until the total destruction

of crops. The longer the duration of the larval stage, the higher the losses because of its high consumption more so during the last few days before pupation (Flanders *et al*., 2017). Instantly after hatching, the larvae being consumption of the host plant until being of nymphosis. The mandibles of the fall armyworm caterpillars unlike most of *Spodoptera* species have stronger, serrated cutting edges, which enable them to feed on plants containing high silica with ease (Pogue, 2002; Goergen *et al*., 2016).

According to Goergen *et al*. (2016), FAW may cause damage on the whole host plant. Full grown caterpillars function as cutworms where they entirely section the stem base of maize plantlets (Figure 4). Tendeng *et al*. (2019) reported that constant feeding leaves plants with skeletonized leaves and windowed whorls having larval frass (Figure 4). Additionally, larvae attack reproductive organs on grown maize plants by feeding on tassels or may bore into the ears. Foliar damage is identified by ragged feeding, presence of moist sawdust-like frass near the leaf whorl and upper leaves (Figure 4). Tassel feeding can lead to pollination problems. Ear feeding in corn grain is associated with high levels of the aflatoxin and fumonisin (Chilcutt *et al.*, 2014). Ingestion quantity increases with the increase in growth of the larvae.

Seedlings and young leaves are more susceptible to larvae damage. Plants with 6-10 leaves are prone to attack resulting to more severe and harmful damages (Tendeng *et al*., 2019). Older larvae are mostly housed in the whorls blocked with frass hence protected against predators, parasitoids and some chemicals (Prasanna *et al*., 2018).

A. Ear damage (Prasanna *et al*., 2018) B. Skeletonized leaves (Goergen *et al*., 2016)

C. Frass (Padhee and Prasanna, 2019) D. Cutting and tearing (Prasanna *et al*., 2018)

Figure 4: Damage caused on maize plant by fall armyworm

2.6 Prevention and Management of Fall Armyworm

Blanco *et al*. (2016) demonstrated that in the absence of control methods, fall armyworm can cause losses that can reach 100% in some tropical areas of American continent. The estimated economic losses in a year in the African continent as a result of FAW infestation is estimated to be about US$6 billion (CABI, 2017). Therefore, implementation of management methods is critical in minimizing negative adverse economic impacts.

Early detection of fall armyworm infestations before the occurrence of huge damages is very crucial in their management. The proper timing is vital for successful pest control,

taking into consideration the larval stages cycle and the application day time, of these management strategies. These strategies include monitoring and scouting, cultural practices, genetic modification of crops, biological methods, botanicals, chemical methods and integrated pest management. Some farmers have used these methods singly while others try and combine two or more of these methods which seem to be more effective in bringing its population to manageable levels.

2.6.1 Monitoring and Scouting

Monitoring is done to track the presence, density, movement of a pest within a specified geographical area. The activity is often conducted by a trained personnel or village farmers that have been trained. Pheromone traps are used, which contain pheromones secreted by female insects to lure adult male moths. The trapped fall armyworm adult moths are counted and data is recorded to inform appropriate action (Prasanna *et al*., 2018). The pheromone traps are hanged in a manner that they are 1.5 m off the ground and the distance should be 50 m between any 2 traps (Malo *et al*., 2013; Jing *et al*., 2021). Scouting is recommended to begin soon after seedling emergence. It requires the scout in charge to have an understanding of the pest and the agroecosystem and mitigation measures. “W” or “Ladder” pattern is the commonly used methods for scouting. Scouting is done away from the boarders(5m) to avoid possibility of edge effects, accessing 10-20 plants for each chosen location (Prasanna *et al*., 2018).

2.6.2 Cultural Practices

The use of cultural methods is more recommended for the small-scale farmers because of the low costs involved. Most these farmers in Africa do not use synthetic insecticides, which are costly, but do apply cultural control methods that deter insect activity or kill

pests. Kumela *et al.* (2018) reported that 14% and 39% of the farmers in Ethiopia and Kenya respectively, practiced cultural methods like handpicking and intercropping**.** These methods include intercropping maize with non-host crops (sunflower, ground nuts, soybeans and bean), deep ploughing, push and pull approach, handpicking, manually killing caterpillars, use of wood ashes, sawdust and soils to infested leaf whorls to kill young larvae (Tambo,2018; Tambo *et al*.,2020). Handpicking, crashing of eggs and killing of caterpillars reduces pest build-up**.** Deep ploughing prior sowing helps in exposing pupae in the ground to predatory birds, cutting off the life cycle of fall armyworm thus decrease its population (Prasanna *et al*., 2018; Jing *et al*., 2021). In "push" and "pull" strategy, maize crops are intercropped with plants (e.g., *Desmodium* spp.) that are pest repellent (push) and border them with plants (e.g., Napier grass) that are pest attractive(pull) (Prasanna *et al*., 2018). Both early and/or timely planting increases chances of evading infestation, as maize ears would have already been heavily attacked by a higher fall armyworm population, in delayed planting. Intercropping and companion cropping may interrupt egg laying, reduce mobility of larvae between host plants, increase the spectrum of natural enemies of the pest, improve crop health and provide shelter (Pichersky and Gershenzon ,2002; Prasanna *et al*.,2018).

2.6.3 Biological Control

Biological control is considered as an important alternative control measure to synthetic insecticides because it's safe to the environment, human health and plants. A better understanding of the adaptation and establishment of the used biological control agents in agroecosystem is a necessity for a successful outcome (Assefa and Ayalew, 2019). These biological agents include parasitoids, predators and entomopathogens. Predators help in

management of pests by feeding on them as prey often with less specificity (e.g., ladybird beetles, earwigs, and sap-sucking insects attack larvae of fall armyworm) (Prasanna *et al*., 2018). According to Sisay *et al*. (2018), *Cotesia icipe, Palexorista zonata* and *Coccygidium luteum* were the parasitoids that emerged from the sampled fall armyworm in Ethiopia. The most common parasitoid that emerged was Cotesia icipe with parasitism ranging from 33.8% in Awash-Melkasa to 45.3% in Jimma. A tachinid fly, *Archytas marmoratus* was the main parasitoid found in Kenya with 12.5% parasitism. *Charops ater* and *Coccygidium luteum* were the most prevalent parasitoids in Kenya and Tanzania ranging from 6–12% and 4–8.3% parasitism respectively (Sisay *et al*., 2018; Assefa and Ayalew, 2019). The parasitized larvae may not be killed immediately but seem to continue with their normal development until the exit of the parasitoid. During emergence, the mobility and feeding ability of the host larvae is ceased. The parasitoid remains attached to the larvae track on the outside and continues to suck the hemolymph after which it detaches (Tendeng *et al*., 2019).

Fall armyworm is susceptible to attack and damage by entomopathogens such as bacteria (e.g., *Bacillus thuringiensis*), fungi (e.g., *Beauveria bassiana*), protozoans, nematodes, or viruses (e.g., Spodoptera frugiperda multiple nucleopolyhedrovirus, SfMNPV) that infects and causes diseases in insects (Prasanna *et al*., 2018). For baculoviruses, the pest larvae ingest occlusion bodies which dissolve in the mid-gut fluid, virions are released thus the primary infection is established. The virion envelope consists a minimum of nine *per os* infectivity factors (PIFs), entry complex into the epithelial cells. (Song *et al*., 2016; Boogaard *et al*., 2017). Upon entry into the cell nuclei, the virus replicates, initiating a

secondary virus infection which results to infection of organs causing larval death gradually.

2.6.4 Integrated Pest Management (IPM)

Integrated Pest Management is considered to be the arsenal to pest management. The strategy is globally embraced by international bodies such as the UN Food and Agriculture Organization (FAO). Successful implementation of this process requires one to have adequate knowhow of agronomic and pest management tools. The main objective of IPM is to suppress pest populations at a low cost by using an integration of various prevention and control techniques that are safe to the environment, animals and people (Prasanna *et al*., 2018). A combination of these techniques includes biological control, synthetic and biopesticides, host plant resistance varieties, cultural control. According to Day *et al*. (2017), IPM is practiced at large in Latin America by the small-scale farmers than in the large monocultures where they utilize transgenic crops and/ or calendar spraying.

2.6.5 Genetically Modified Crops

Use of genetically modified (GM) crop varieties expressing lepidopteran resistance genes can be effective in controlling fall armyworm damage in maize. Over 20 years, Bt maize crops expressing different cry genes have been commercialized. e.g., CrylA, CrylAb, and CrylF. However, in Puerto Rico fields, FAW has developed resistance mechanisms to Cry1F, Cry1Ac and Cry1Ab (Storer *et al*., 2010; Blanco *et al*., 2010). These protein crystals bind to the epithelial cells in the mid-gut and lyse it once they have inserted into the membrane hence form pores. The Cry proteins convert, in the alkaline gut, into membrane-inserted oligomers, from inactive crystal inclusion pro-toxins (Bravo *et al*., 2007). Bt varieties also produces Vegetative Insecticidal Proteins (VIP), lepidopteran-

specific proteins, encoded by *vip* genes**.** The *vip3A* gene is the most utilized VIP used to confer resistance (Padhee and Prassana, 2019). These Vip proteins also function by forming pores in the pest's mid-gut (Bentivenha *et al*., 2019).

2.6.6 Chemical Control

For decades, use of synthetic insecticides has been a crucial tool for controlling fall armyworm. Proper timing for the application of chemicals is critical for effective pest control. It is recommended to spray at night or dawn when the larvae have emerged to feed (Day *et al.,* 2017). Insecticides like Spinosad, chlorantraniliprole, thiodicarb, spinetoram and flubendiamide have been reported to be highly effective at the recommended field rates both in Puerto Rico and Santa Isabel (Belay *et al*. 2012; Gutierrez *et al*., 2019). Indoxacarb and metaflumizone are a new class of sodium channel-targeting insecticides. They are sodium channel blocker insecticides thus inhibit sodium current (Wing *et al*., 2010). However, application of chemical insecticides has its own implications such as high cost, may cause harm to non-target species and humans, increase insect resistance, potential environmental contamination **(**Colborn, 1995; Crowe & Booty, 1995**;** Assefa and Ayalew, 2019). Over reliance has led to FAW resistance in fields. Knockdown resistance is a major mechanism of resistance, reported globally in both agriculturally and medically significant arthropod pests (Rinkevich *et al*., 2013).

2.6.7 Botanical Pesticides

Botanicals are plant derived-pesticides which have recently displayed good performance in insecticidal activity. They are preferred than the synthetic insecticides which may lead to environmental disturbance, increase in pest resistance and increase in user cost. The botanicals are affordable and easily available to farmers, based on the frequent occurrence

of the pesticidal plants in the ecosystem thus has been used for long to control both stored yields and field crops (Schmutterer, 1985; Assefa and Ayalew, 2019). Additionally, the botanical pesticides are environmentally friendly, biodegradable, has less health impacts to both humans and natural enemies hence recommended for use (Prasanna *et al*., 2018). Botanicals that have been effective in control of insect pests including fall armyworm are plant extracts of plants like Neem (*Azadirachta indica*), *Argemone ochroleuca*, Boldo (*Peumus boldus*), Jabuticabeira, *Myrciaria cauliflora* (Alves *et al*.,2014; Silva *et al*.,2015; Martinez *et al*.,2017; Sisay *et al*.,2019). Some of these botanical pesticides have been used against fall armyworm, for instance, Lin *et al*. (2020) estimated indoor toxicity and effectiveness of *azadirachtin* against it, in a maize field. They found out that it had toxicity and antifeedant activity, and that seven days after treatment the insecticidal effect was at the peak. Application of seed cake extract of the Neem plant has a potential to cause high fall armyworm larval mortality as reported by Silva *et al*. (2015). However, the bio pesticides extracted from Neem have low residual life in the field due to azadirachtin poses high photosensitivity characteristics of which either isomerizes or breaks down. Extracts of many other plants are potential botanical bio pesticides against fall armyworm but relatively few have been successfully commercialized (Silva *et al*., 2015).

2.7 Insecticide Resistance

The intensive utilization of insecticides leads to insecticide resistance evolvement in pests and is thought to be the greatest example of micro-evolution. Studies have shown more than 500 different pest species that have evolved insecticide resistance to insecticides (Khan *et al*., 2015). Resistant insects overcome the adverse effect of insecticides by adopting mechanisms ranging from cuticular thickening, nerve penetration, enhanced

excretion, target site insensitivity and production of modified metabolic enzymes (Khan *et al*., 2015). According to Gutiérrez-Moreno *et al*. (2019) corn farmers in Mexico report how difficult it is to manage fall armyworm, using up to 3,000 tons annually, of synthetic insecticides. In Africa, Day *et al*. (2017) reported that estimated national mean loss of maize in Ghana was 45% (range 22–67%), and in Zambia 40% (range 25–50%) based on the survey conducted. Consequently, fall armyworm has a presented itself as an economic pest and threats food security worldwide. This calls for determination of baseline susceptibility of current pest field populations to understand the status of susceptibility FAW to various insecticides. In addition, this will also help in informing on dosages that can only kill the fall armyworm without killing non-target pests as most insecticides are formulated in a manner that kills indiscriminately threatening biodiversity/pests.

2.7.1 Mechanisms of Insecticide Resistance

2.7.1.1 Penetration and Behavioral Resistance

Behavioral resistance involve behavior that inhibits an insect's contact with toxic compounds or enable it to survive in a toxic and fatal environment. This resistance is stimulus dependent based on hypersensitivity. In evolution of behavioral resistance, avoidance is the first step (Saha and Mukhopadhyay, 2013). This entails mechanisms like reluctance to feed if they detect insecticides on their diet, avoiding sprayed leaves, flying away from the target plants/areas or hiding in whorls (Zalucki and Furlong, 2017). Resistant insect may also have modified exoskeleton that inhibit insecticide penetration. The cuticle serves as the first and major barrier that prevents penetration of external compounds protecting insects. Its structure is generally well preserved among insect species (Balabanidou *et al*., 2019). Cuticular changes causing insecticide resistance involve

either the thickness or composition of the cuticle. According to Balabanidou *et al*. (2019) a multi-resistant *Anopheles gambiae* mosquito population, exhibiting remarkable tolerance to multiple insecticide, had thicker leg cuticles enriched with deposition of hydrocarbons to their epicuticle. This decreased penetration creates ample time for the detoxification of insecticides by metabolic enzymes.

2.7.1.2 Metabolic resistance

Resistance strains may possess modified metabolic pathways. This type of resistance is mediated by specialized enzymes that biochemically detoxify insecticides into less toxic metabolites. Insecticide's detoxification is essential in enabling pests to tolerate applied insecticides (Khan *et al*., 2020). Detoxification is divided into 2 phases. Phase I reactions comprises of hydrolysis, oxidation, and lastly reduction process. Though, the phase 1 metabolites are may be polar enough to be excreted or be converted in phase II reactions where they are conjugated with a variety of endogenous compounds before excretion (Berenbaum and Johnson, 2015). Phase I reactions decrease the biological activity of toxins. This biotransformation is important in decreasing the lipophilicity of insecticides, for easier excretion (Li *et al*., 2007). The major detoxification system conferring insecticide resistance in insects consist of three enzyme systems. These enzymes include cytochrome P450-dependent monooxygenases, esterases, and glutathione-S-transferases. For instance, resistance to indoxacarb in *Spodoptera exigua* was attributed to glutathione S-transferases, carboxylesterases, and a Leu-1014-Phe mutation in the voltage-gated sodium channel (Gao *et al*., 2014). Enhanced metabolism of pyrethroids, organophosphates and carbamates are frequently associated with esterases through gene upregulation, amplification, mutations by coding sequence, or a combination of all these mechanisms (Cui *et al*., 2015).

2.7.1.3 Altered Target -Site Mutation

The target sites of insecticides undergo genetic modification changing the sequence of amino acid within the binding region (Silver *et al*., 2014). These modifications are a critical problem in the chemical control of many insects leading to evolved resistance. A mutation may change the structure of a gene leading to structural change in its product (Khan *et al*., 2020). This may cause resistance by reducing the capability of the insecticide to bind to target site. In several insect species, the K*dr* resistance is a common mechanism rendering a reduced sensitivity to DDT and pyrethroids because of mutation of protein targeted by the insecticides in the voltage-gated sodium channel (Silver *et al*., 2014). According to Zhang *et al*. (2020) resistance mechanisms of FAW to active compounds of insecticides compose of metabolic mechanism for detoxification and the target-site resistance mechanism.

2. 8 The Voltage- Gated Sodium Channel

2.8.1 The Voltage- gated Sodium Channel Structure

Transmembrane voltage-gated sodium channels help in generation and propagation of action potentials in most excitable cells. The current comprehensive knowledge on the function and structure of VGSCs is generated from extensive molecular and functional analysis of sodium channels in mammals (Catterall, 2000; Rinkevich *et al*., 2013). The Mammalian sodium channels is composed of several small auxiliary β -subunits and one complex pore-forming α-subunit. The pore-forming α-subunit expression is adequate to sustain functionality of sodium channel while the auxiliary subunits mainly regulate the kinetics and voltage dependence of the channel gating and /or protein expression (Dong *et al*., 2007). In insects there are no orthologs of mammalian β-subunits. However, the non-

orthologous proteins TipE and four TipE-homologs (TEH1–4), in *D. melanogaster* (three to four orthologs in other insect species) seem to serve as auxiliary subunits of sodium channels in vivo (Silver *et al*.,2014). The α-subunit is composed of four serially homologous domains, consisting of 6 spanning segments (S1–S6) linked by extracellular or intracellular loops of amino acids (Chahine,2018). The S1–S4 segments in each domain function as the voltage-sensing domains, whereas the S5 and S6 segment, and the P-loops connecting them compose the pore-forming domains (Silver *et al*., 2014). The pore-forming and the voltage-sensing domain are joined together by a small intracellular linker connecting the S4 and S5 segments (Rinkevich *et al*., 2013). Each S4 segment harbors five to eight evenly spaced positively charged residues arginine or lysine hence act as voltage sensors (Figure 5). Amino acids present in P-loops between segments 5and 6, makes a filter that is ion-selectivity. In eukaryotic voltage-gated sodium channels, the pore signature is Asparagine/Glutamine/Lysine/Alanine (D/E/K/A) present in the loops connecting Segment 5 and 6 of domains I, II, III, and IV, respectively (Dong *et al*., 2014). In contrast, the residues determining ion selectivity in bacterial channels are identical in each protomer in the homotetrameric channels (Ren *et al*., 2001; Yu *et al*., 2002; Shen *et al*., 2017).

The presence of an ankyrin-binding motif sequence loop between domains II and III that is reportedly critical in localizing the mammalian sodium channels to the axon initial segment and nodes of Ranvier (Chahine, 2018).In mammals, the linker sequence between domains III and IV functions as the inactivation gate, the core of which is represented by the well-conserved hydrophobic IFM (Ile-Phe-Met), which binds to the inactivation gate receptor located within or near the intracellular mouth of the sodium channel pore causing

fast inactivation (Dong, 2007).On the contrary, in insects the activation gate is represented by MFM (Met-Phe-Met) where the isoleucine in mammals is replaced with a methionine.

Mammals express sodium channel α-subunits in nine different forms ($Na_v1.1$ to $Na_v1.9$) (Goldin *et al*. 2000; Catterall *et al*. 2003; Dong, 2007). These isoforms display distinct expression patterns. $Na_v1.1$, $Na_v1.2$, $Na_v1.3$, and $Na_v1.6$ are present in the central nervous system, whereas in the peripheral nervous system $Na_v1.7$, $Na_v1.8$, and $Na_v1.9$ are expressed. $Na_v1.4$ and $Na_v1.5$ are expressed in skeletal and cardiac muscles respectively (Yan *et al*., 2017). Insects have only one functional sodium channel gene. However, through extensive alternative splicing and RNA editing of insect sodium channel transcripts, functionally and pharmacologically distinct sodium channel variants are produced (Soderlund, 2017).

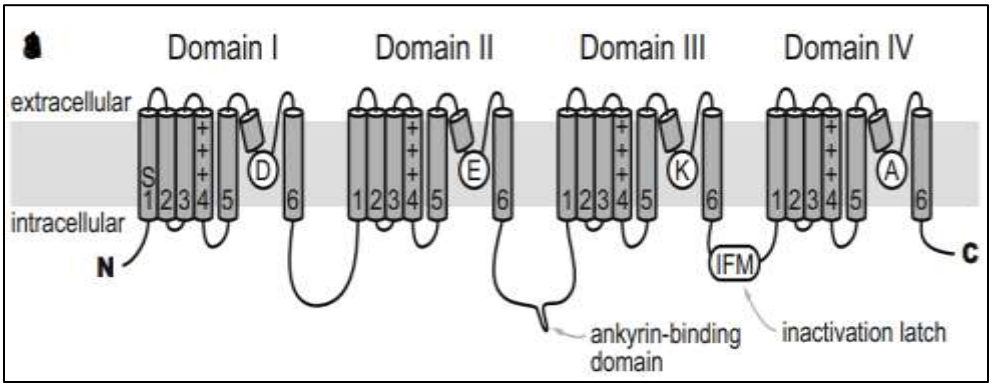


Figure 5: Overall structure of eukaryotic voltage-gated sodium channels (Chahine, 2018)

2.8.2 Functionality of Voltage-gated Sodium Channels

The voltage-gated sodium channels functions by undertaking rapid voltage-dependent transitions between closed and open states, resulting into regenerative wave of electrical signals. They contain “gating charges” which are a set of highly conserved positively charged residues present at every third place along the S4 segment of each domain. Upon

depolarization, as per measurements in potassium channels, approximately 12 gating charges per channel cross across the membrane into the extracellular side (Shen *et al*., 2017) Although the precise mechanism remains to be obscure, it is generally accepted that the S4-S5 linker helices are essential for the voltage-dependent shifts of S4 segments to pore openings with the "canonical" domain swapped arrangement (Long *et al*., 2005; Shen *et al*., 2017). The opening of the activation gate occurs when the S4 segments move outward, initiating the voltage-dependent activation. Fast inactivation takes place a few milliseconds after the channel opening. This process is executed by a cytoplasmic moiety formed by residues (IFM in mammals, MFM in insects) in the linker between repeats III and IV. This inactivation particle blocks the intracellular mouth of the pore thus stopping ion conduction (Rinkevich *et al*., 2013). During prolonged depolarization, when the channel is exposed to one long positive pulse or a series of high-frequency repetitive ones, slow inactivation takes place and /sodium conduction stops (Ulbricht, 2005; Chahine, 2018). The entry and recovery from the slow inactivated state takes longer time ranging from milliseconds to minutes compared to fast inactivation which takes milliseconds. This state is elucidated to be a protective mechanism protecting cells during high stress conditions generating highly repetitive stimuli (Chahine, 2018). Upon repolarization, the activation gate closes as Segment 4 voltage sensors move backwards. Sodium channels deactivate and recover from inactivation state and get back to their resting, excitable state (Silver *et al*., 2014).

2.8.3 Voltage-gated Sodium Channels as a Target Site

Voltage-gated sodium channels play a crucial role in excitation of membranes, thus form the primary target site of a various neurotoxins, such as batrachotoxin, utilized for defense

and predation by plants and animals (Cestele and Catterall, 2000; Wang and Wang, 2003; Dong,2007). Additionally, they are major target sites of synthetic compounds and therapeutic drugs, such as dichlorodiphenyltrichloroethane (DDT) and local anesthetics (Catterall *et al*., 2007; Dong *et al*., 2014). These neurotoxins include Pyrethroids, DDT and Sodium channel blocker insecticides (SCBIs). Pyrethroids are synthetic insecticides structurally derived from pyrethrins that are used to control different insect pests (Zhang *et al*., 2016). Some of these insects include aphids, adult cockroaches, tobacco hornworms, beetles, African armyworms, diamondback moth and earwigs (Von *et al.,* 2013; Du *et al*., 2015). DDT and pyrethroid insecticides are among the initial insecticides identified to act on sodium channels (Narahashi, 2000; Dong *et al*., 2014).

Pyrethroids and DDT bind to the VGSC, modifying the gating transitions thus inhibiting the transition to an inactivated state (Davies *et al*., 2007; Field *et al*., 2017). At the cellular level, pyrethroids interfere with the functioning of nerve system resulting to repetitive discharges, membrane depolarization, and finally synaptic disturbances (Rinkevich *et al*., 2013). This prolonged channel opening leads to persistent depolarization and the insect dies from paralysis, often exhibiting a 'knock-down' response (Field *et al*., 2017).

The sodium channel blocker insecticides, indoxacarb and metaflumizone have high selective toxicity (Silver *et al*., 2014). SCBIs bind preferentially in the slow-inactivated state caused by prolonged or repetitive depolarization. The binding creates a pool of insecticide-bound, non-conducting channels inhibiting activation. The progressive sequestration of channels in this state eventually leads to nerve blocking (Soderlund, 2017).

2.8.4 Knockdown Resistance (*kdr*) at Voltage-gated Sodium Channels

A resistance mechanism to the toxins is referred to as knockdown resistance (kdr), caused by changes within the VGSCs, that renders it less sensitive to the toxin in the compounds (Vais *et al*., 2001: Soderlund and Knipple, 2003) as shown in figure 6. Globally, kdr has been agriculturally and medically documented as significant in arthropod pests (Soderlund, 2005, 2012; Du *et al*., 2015; Rinkevich *et al*., 2013). Not less than 50 VGSCs mutations have been recorded in reference to pyrethroid resistance in various arthropods (Du *et al*., 2013; Li *et al*., 2012; Xu *et al*., 2012; Rinkevich *et al*., 2013). Previous studies document that mutations conferring resistance to these insecticides are mostly common in regional domain II of channel protein (Vais *et al*., 2001; Soderland and Knipple 2003). These are 5 including; Leu^{925}, Thr^{929} and Leu^{932} (IIS5) and Leu1014 located in domain II Segment 6 and Methionine 918 (Met^{918}) located in the linker IIS4-5 (Figure 6). L1014F is the most common mutation, originally in houseflies (Williamson *et al*., 1996; Endersby *et al*., 2011). For instance, a mutation at T929 (the binding site for DDT, deltamethrin, fenfluthrin and permethrin) confers resistance to all the four insecticide compounds, whereas mutations at M918, a distance button from fenfluthrin (pyrethroid) and DDT predicatively bind, confers resistance to deltamethrin and permethrin only (Silver *et al*., 2014). The study shows that, the model prediction for T929I ensues insecticide resistance to all the four compounds, while M918T mutation confers to permethrin resistance and deltamethrin resistance and not DDT nor fenfluthrin (Field *et al*., 2017). Sun *et al*. (2016), has similarly used the same model on live insects' bioassays to support the O'Reilly model above.

Alternatively, other studies proposed a dual-receptor site model that binds with both DDT and pyrethroids (Du *et al.,* 2015, 2016; Zhorov and Dong, 2017). In this models, binding

of two molecules to PyR1 receptor sites and PyR2 simultaneously is necessary for the voltage-gated sodium channel to lock in open state (O'Reilly *et al*., 2006). Proposed location for this interaction is in domain interfaces II/III and I/II, respectively, arranged in a quasi-symmetrical manner. Pyrethroids attach between four helices of adjacent domains that is, S5, linker-helix L45 and two S6 helices (Du *et al*., 2015).

While the original O'Reilly model L1014F affects pyrethroid binding through an indirect allosteric impact, key variances in the Du model indicates the L1014F is located within the PyR2 site. Consequently, L1014F's effect is in slowing opening of VGSCs that is implicated with significantly reducing formation of receptor PyR1, hence limits availability of pyrethroid for binding, conferring the kdr. An additional difference is in the orientation of pyrethroids bound within each pocket that is reversed (which begs the question why M918T would be ineffective against toxic compounds like fenfluthrin), and that the pyrethroids sip deeper into the PyR2 protein domain.

Precise molecular markers through identification of kdr mutations rapidly aids in the assessment of resistance allele frequency in insect populations other than being important in deciphering sodium channels structural features critical in binding and action of the pyrethroids (Silver *et al*., 2014). Studies by Amey *et al*. (2015), in aphid's genome identified unusual properties in VGSCs sequences unique to aphids only. They possess unique heterodimeric channel, having a characteristic ion -selectivity filter, not common in insect and whose insensitive to tetrodotoxin was high. The study implied that it is possible to design selective compounds to act on aphids while sparing other insects.

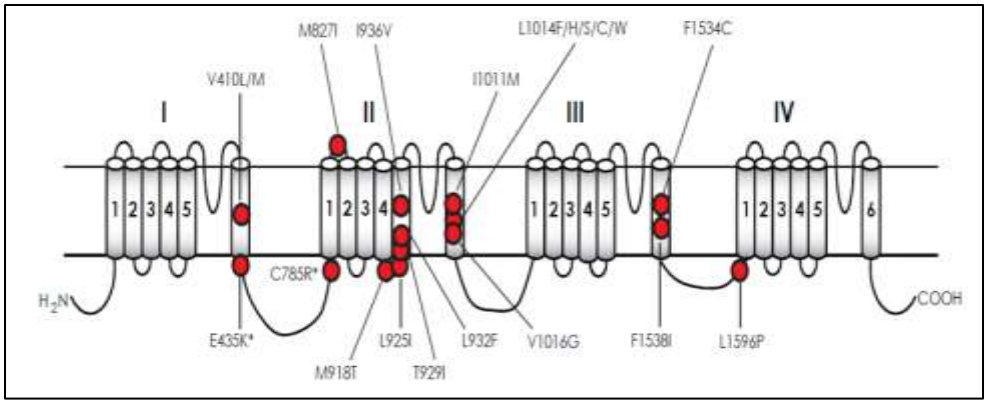


Figure 6: Mutations conferring kdr to pyrethroids in the voltage-gated sodium channel, represented by the red dots (Dong *et al*., 2014).

2.9 Overview of New Chemistries of Management of Insects

2.9.1 The Insecticide Resistance Action Committee (IRAC) Classification

Insecticide resistance in pest insects has been a nuisance for long hence very critical to sustain potency of the old and current insecticides. For this concern, CropLife International came together to form the Insecticide Resistance Action Committee (IRAC) in 1984 (Sparks and Nauen, 2015). It is an international association of companies dealing with protection of crops globally. It entails a technical group of experts from the member companies. Its main mandate is to preserve the efficacy of pest insect control products for a long term, maintain effective resistance management approaches in order to improve public health and sustain agriculture. In addition, IRAC also educates and communicates on insecticide resistance and the traits involved (Nauen *et al*., 2012).

IRAC uses Mode of Action (MOA) Classification Scheme as its key tool to fight against acaricide and insecticide resistance. The scheme provides guidelines on how these control products should be selected in rotational-based or alternation management programs (Sparks *et al*., 2020). The scheme relies on the evidence available about the target sites of

the acaricides and insecticides. This classification lists the major site of action, main group and chemical subgroup, present in the committee's website (http://www.irac-online.org). The groups include insecticides targeting the nerves or muscles, growth, mid-gut, respiration and unknown and non-specific target-sites. Insecticides used in this study both for the baseline susceptibility and *in silico* studies were as follows;

1. Deltamethrin (Sodium channel modulator, Pyrethroid group 3A)
2. Lambda cyhalothrin (Sodium channel modulator, Pyrethroid group 3A)
3. Cartap (nAChRs channel blockers, Nereistoxin analogues group 14)
4. Spinetoram (nAChRs allosteric modulators – site 1, Spinosyns group 5)
5. Spinosad (nAChRs allosteric modulators – site 1, Spinosyns group 5)
6. Fipronil (Gaba-gated chloride channel blockers, Fiproles group 2B)
7. Metaflumizone (VGSC blockers, Semi carbazones group 22)
8. Tebufenozide (Ecdysone receptor agonists, Diacylhydrazines group 18)
9. Indoxacarb (VGSC blockers, Oxadiazines group 22)
10. Chlorfenapyr (Uncouplers of oxidative phosphorylation due to disruption of the proton gradient, Pyrroles group 13)
11. Abamectin (Glutamate-gated chloride channel allosteric modulators, Avermectins group 6)
12. Pyridaben (Mitochondrial complex 1 electron transport inhibitors Meti acaricides and insecticides, group 21A)

13. Imidacloprid (nAChRs competitive modulators, Neonicotinoids, group 4A)

14. Lufenuron (Inhibitors of chitin biosynthesis affecting CHS1, Benzoylureas, group 15)

2.9.2 Pyrethroids

Natural pyrethrum is used in agricultural purposes, for decades now (Yu, 2008). However, over the past two decades synthetic pyrethroids are more used because of their stability in sunlight. Also, they are known to efficiently control most agricultural pests at low rates. Pyrethroids like deltamethrin (Figure 7) and lambda-cyhalothrin have extended residual activity lasting for 10 days under optimum conditions ascribed to its minimal volatility (Ware and Whitacre, 2004). They ensure mammalian safety as systemic absorption via the dermis is very low (Krieger, 2010). Pyrethroids are of two types. Type I have a negative temperature coefficient, lack α-cyano group and causes repetitive discharges upon a single stimulus whereas Type II have a positive temperature coefficient, have an α-cyano group present at the phenoxybenzyl alcohol moiety and causes membrane depolarization suppressing cellular excitability (Ware and Whitacre, 2004; Silver *et al*., 2014). Pyrethroids interfere with insect's peripheral and central nervous system producing repetitive discharges eventually cause paralysis. They work by inhibit channel deactivation and inactivation, block the sodium channels in the open state in neuronal membranes (Silver *et al*., 2014).

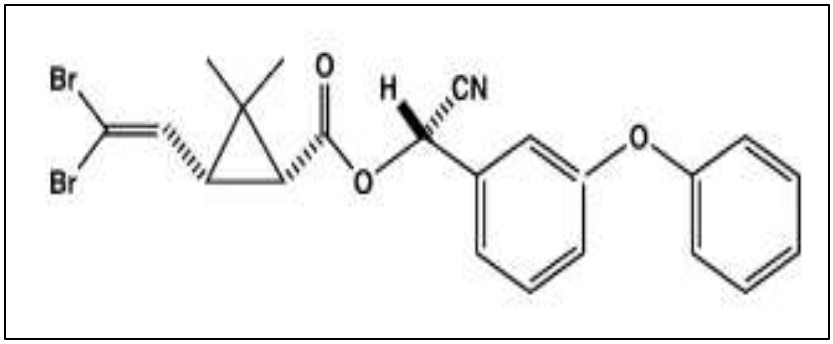


Figure 7 : Structure of Deltamethrin (Soderlund, 2017)

2.9.3 Cartap

Cartap is an insecticide among the analogues of nereistoxin, a broad-spectrum insecticide for controlling coleopterous, lepidopterous and sucking insects (Yu, 2008). It is the analog of a natural toxin *Lumbriconereis heteropoda,* the marine worm, active at cholinergic synapses. Nereistoxin analogues are poisons with stomach, some contact and systemic action. Cartap (Figure 8) is a proinsecticide which degrade into an active component from the manufactured form (Ware and Whitacre, 2004).

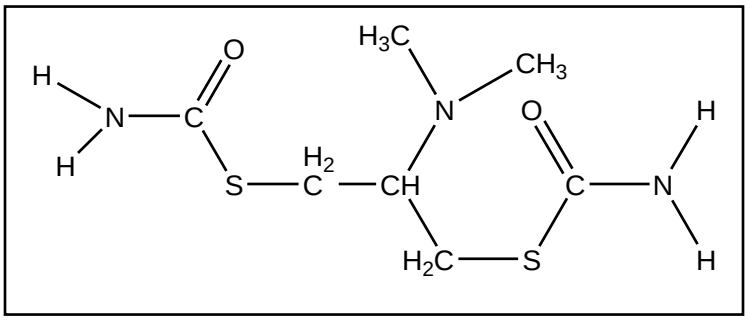


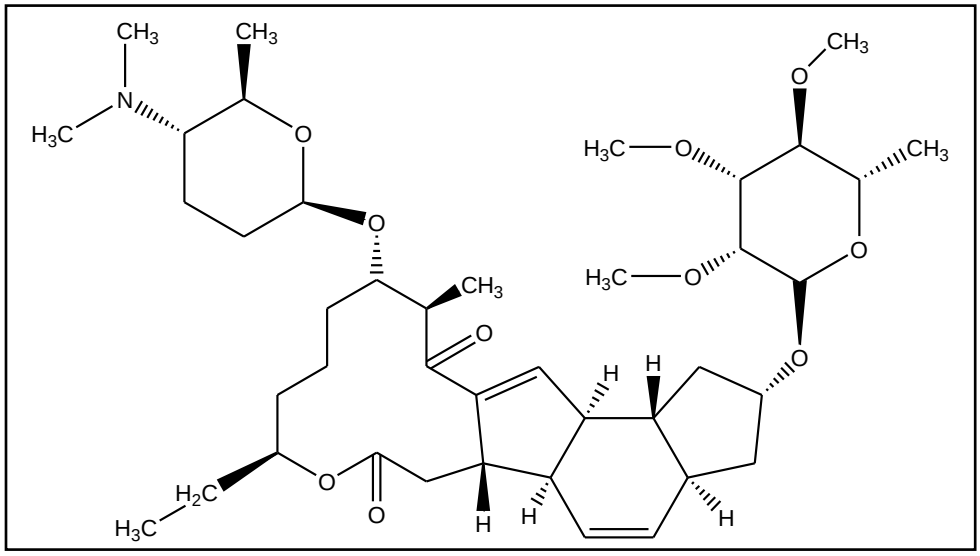
Figure 8: Structure of Cartap (Hazardous Substances Data Bank)

2.9.4 Spinosyns

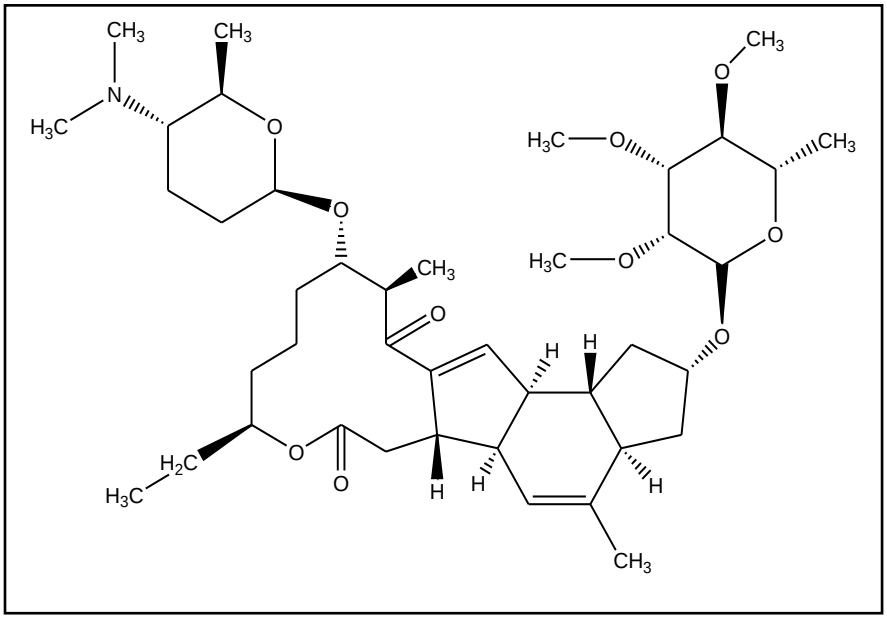
Spinosyn insecticides are crucial in the management of pest as they are less harmful to beneficial insects and have a unique mode of action acting on the nicotinic acetylcholine receptors as allosteric modulators (Lira *et al*., 2020). These insecticides include spinosad,

the first to be introduced into the market and spinetoram, recent in the market. Spinosad is an insecticide effective in controlling lepidopterous insects mostly the noctuid larvae, a group of leaf-feeding pests that are not covered by the neonicotinoid insecticides (Nauen and Bretschneider, 2002; Yu, 2008). Under aerobic fermentation conditions, spinosad is obtained from soil-inhabiting actinomycete, *Saccharopolyspora spinosa*, and contains a mixture of Spinosyn A and D (Figure 9) which are the active ingredients (Ware and Whitacre, 2004). According to Ware and Whitacre, (2004), it has long residual activity, contact toxicity and stomach activity against lepidopterans termites, leaf miners and thrips. Unlike other insecticides like pyrethroids, neonicotinoids, avermectins, carbamates, organophosphates, the spinosyns target the insect nervous system in a distinct manner (Sparks *et al.,* 2012). It causes allosteric activation of insect nicotinic acetylcholine receptors (nAChRs) prolonging the responses of acetylcholine. The binding site on the nAChR of spinosad is different from other nAChR based insecticides (Nauen and Bretschneider, 2002). Sparks *et al*. (2012) demonstrated that strains resistant to spinosad exhibit cross-resistance to spinetoram but not to other classes of insecticides.

Spinetoram, a semisynthetic molecule, has positive toxicological attributes compared to spinosad and high efficacy (Lira *et al*., 2020).



Spinosad A



Spinosad D

Figure 9: Structure of Spinosad (Hazardous Substances Data Bank)

2.9.5 Fipronil

Fipronil is used as a foliar spray with a broad application range and in seed treatment to control foliar and soil insects (Nauen and Bretschneider, 2002; Yu, 2008). In addition, it is applied as bait for cockroaches, and termites and is effective against organophosphate, pyrethroids, and carbamate insecticides resistance strains (Ware and Whitacre, 2004). This insecticide is derived from phenylpyrazole. It is a systemic insecticide with both contact and stomach toxicity. Fipronil antagonizes the 'calming' effect of GABA in neurons, by blocking the chloride channel regulated by gamma-aminobutyric acid (GABA) (Zhang *et al*., 2018). This is achieved by blocking the insect GABA receptor in a closed state hence suppressing GABA-Induced currents without channel activation (Krieger, 2010). Fipronil (Figure 10) has greater affinity for insect GABA receptors than for vertebrates' hence has high selective toxicity towards insects over mammals (Zhang *et al*., 2018).

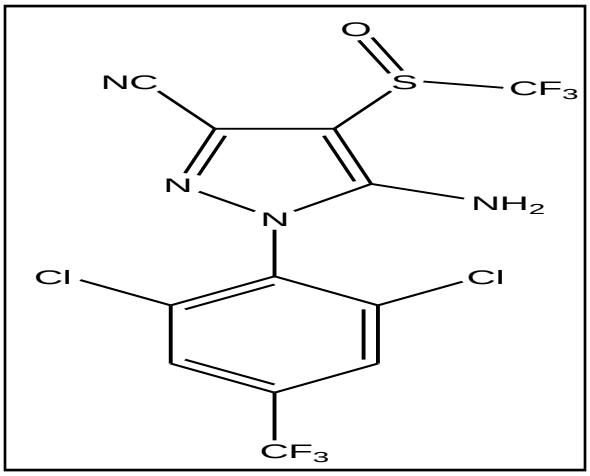


Figure 10 : Structure of Fipronil (Hazardous Substances Data Bank)

2.9.6 Metaflumizone

Metaflumizone is an insecticide that functions against pests by blocking the sodium channels. It is a new semicarbazone insecticide gotten from pyrazole chemistry (Salgado

and Hayashi, 2007). Metaflumizone excellently controls most lepidopterous pests and various pests in the orders Coleoptera, Diptera, Isoptera ,Hemiptera, Hymenoptera and Siphonaptera (Silver *et al*., 2014). Additionally, on a single spot-on application, it offers long-lasting management of fleas on companion animals (Silver *et al*., 2010). Metaflumizone (Figure 11) preferentially interact with the sodium channels when in slow-inactivated state, reducing the number of resting channels available for activation eventually causing a relaxed paralysis via nerve blockage (Rust *et al*., 2007; Soderlund, 2017). It exhibits no cross-resistance to other insecticides.

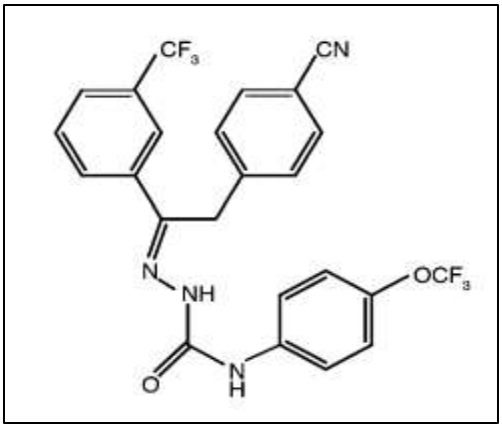


Figure 11: Structure of Metaflumizone (Soderlund, 2017)

2.9.7 Tebufenozide

Tebufenozide has both a stomach and contact action. Tebufenozide (Figure 12) is effective against lepidopterous insects (Yu, 2008), via initiation of premature molting eventually killing insects. It interferes with the molting simply by antagonizing the molting hormone, ecdysone. Insects premature molting is indicated by the slipping forward of the head capsule, occluding the mouthparts and mandibles thus making feeding difficult (Allenza

and Eldridge, 2007).In his study, Qian *et al*. (2008) observed that tebufenozide selected resistant strain exhibit high cross-resistance to abamectin, but selection of a resistant strain with abamectin exhibited no cross-resistance to tebufenozide, a phenomenon explained based on the fact that diverse insecticides tend to select different detoxification enzymes especially MFO which has diverse isoenzymes with a lot of substrates. The selected resistant strain with fufenozide, another novel ecdysone agonist of the same class with tebufenozide, exhibited high resistance to tebufenozide, moderate resistance to abamectin, but no cross-resistance was detected in spinosad, beta-cypermethrin, and chlorfenapyr (Sun *et al.,* 2011).

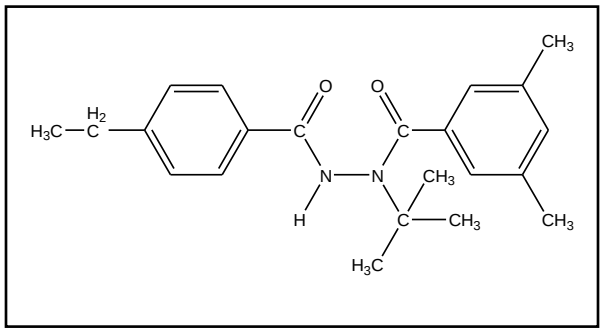


Figure 12: Structure of Tebufenozide (Hazardous Substances Data Bank)

2.9.8 Indoxacarb

Indoxacarb belongs to the same class with Metaflumizone, Sodium channel inhibitors. It is a novel new compound used to control most of lepidopteran insects (Yu, 2008). Indoxacarb (Figure 13) preferentially binds to voltage –dependent sodium channel in its slow-inactivated state which results from prolonged or repetitive depolarization (Salgado and Hayashi, 2007; Khakame *et al.,* 2013). Indoxacarb, in insects, is metabolically converted by cleavage of N-methoxycarbonyl group with esterases it results into NH-derivative which is a highly potent (Lapied *et al.,* 2001; Wing *et al.,* 2010). However, in

mammals, the same insecticide is degraded into nontoxic metabolites and this difference in metabolism ascribes to insect selective toxicity. According to Soderlund, (2017), this selective bioactivation also surmounts the toxicological barriers encountered with the pyrazoline series. Between indoxacarb and pyrethroids, no cross-resistance has been observed as they act on different binding sites (Nauen and Bretschneider, 2002). However, Nehare *et al.* (2010) observed positive cross-resistance between pyrethroid and organophosphate resistant strains to indoxacarb.

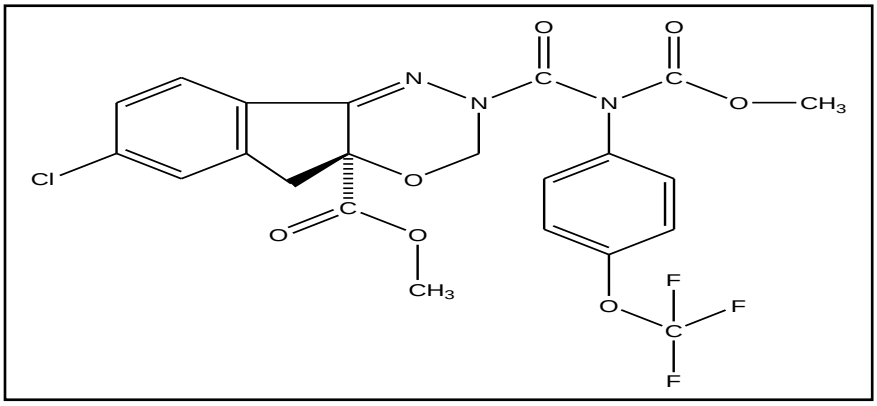


Figure 13: Structure of Indoxacarb (Hazardous Substances Data Bank)

2.9.9 Benzoylphenylurea

Benzoylureas act as growth regulators in insects. They prevent the synthesis of chitin, a key part of the insect exoskeleton (Sun *et al*., 2015). They are used to manage larvae of both caterpillars and beetle more so via ingestion than by contact action. Effects manifested on larvae are death due to starvation and the rupture of malformed cuticle (Ware and Whitacre, 2004). These compounds are derivatives of urea (H_2NCONH_2). Ciba-Geigy discovered lufenuron (Figure 14), a benzoylurea, in the 1980s which later was marketed in

crop protection, animal health and bio protection, in products such as Sentinel™ and Match™ (Poley *et al*., 2018).

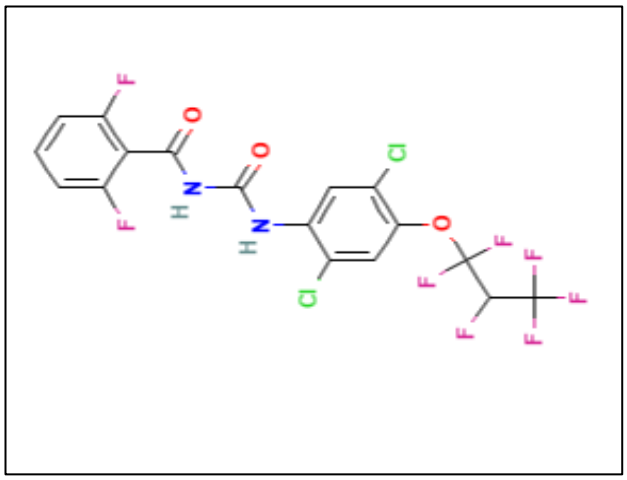


Figure 14 : Structure of Lufenuron (Pubchem)

2.9.10 Chlorfenapyr

Chlorfenapyr belongs to pyrroles class of insecticides and it is the only member belonging to this group. It is synthetically modified from dioxapyrrolomycin which is a natural product derived from *Streptomyces fumanus* (Treacy *et al.*, 1994). Chlorfenapyr (Figure 15) is a contact and stomach insecticide-miticide. Being a pro-insecticide, this insecticide is metabolically activated via N-dealkylation i.e. oxidative removal of the N-ethoxymethyl group (Black *et al.*, 1994; Nauen and Bretschneider, 2002).

In their study, Treacy *et al.* (1994), Nauen and Bretschneider, (2002) observed that in its active form, it disrupts the proton gradient at mitochondrial membrane by uncoupling the oxidative phosphorylation, hence inhibiting conversion of ADP to ATP potentially causing death to the insect.

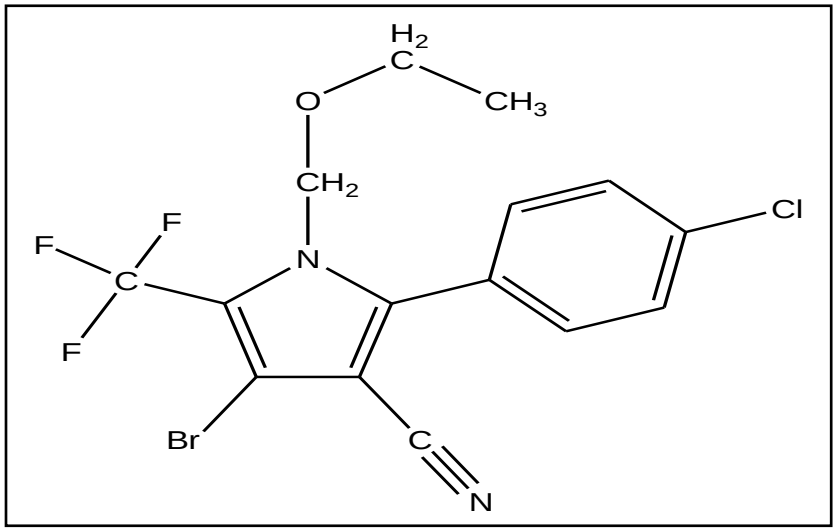
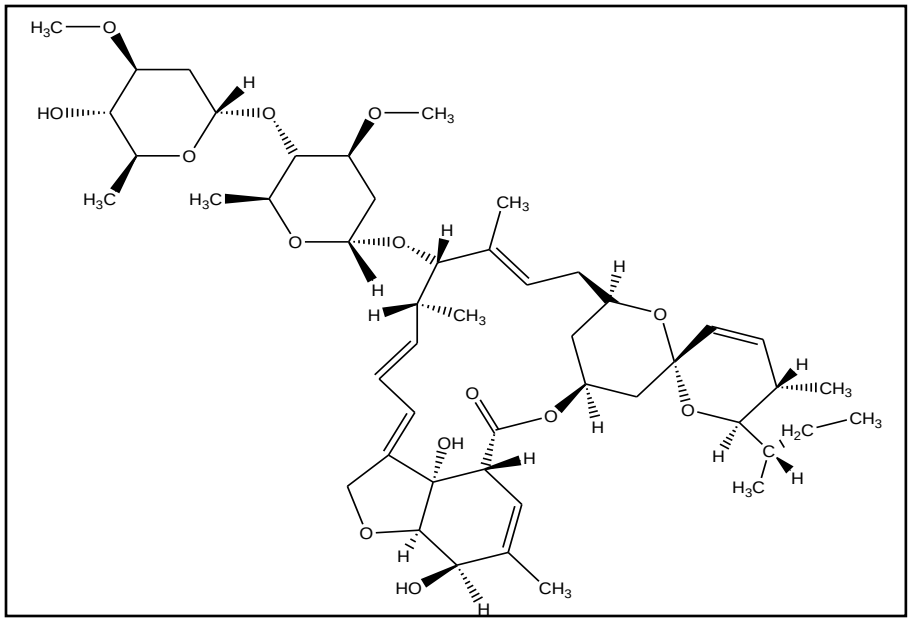


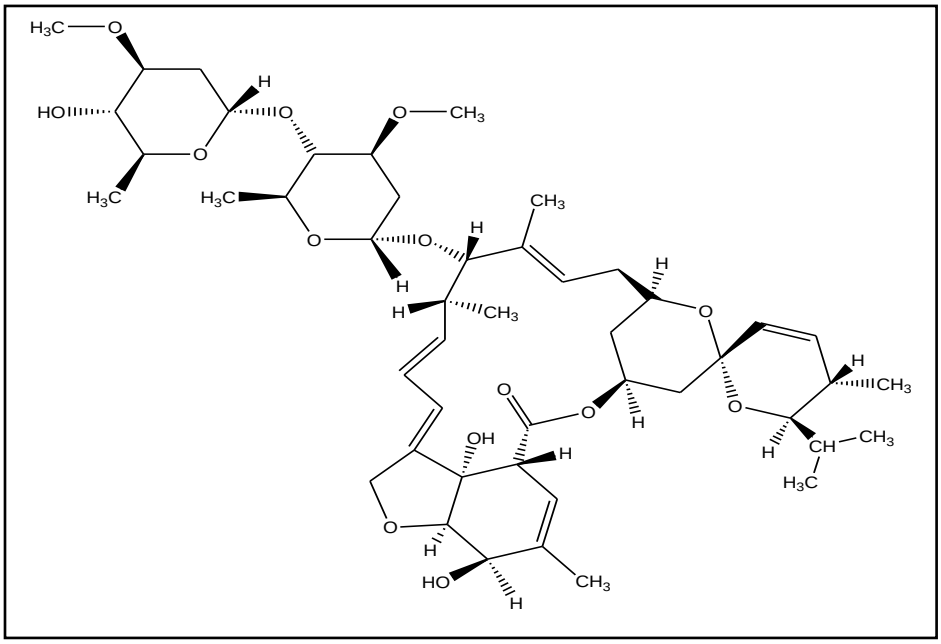
Figure 15 : Structure of Chlorfenapyr (Hazardous Substances Data Bank)

2.9.11 Abamectin

Abamectin is also known as avermectins. Abamectin (Figure 16) entails a mixture of avermectin B_1a (> 80%) and avermectin B_1b (< 20%) as active components with almost equal biological activity (Ware and Whitacre, 2004). It is derived from fermentation products of a soil microorganism, *Streptomyces avermilitis*, actinomycete family (Yu, 2008). Abamectin is used in controlling wide range of insects and mite pests. Avermectins are insecticidal, antihelminthic and acaricidal agents. In their study, Pu *et al*. (2010) observed high level of cross resistance of abamectin selected strains to emamectin benzoate whereas fipronil and spinosad showed low levels. On the contrary, these strains exhibited no cross-resistance to tebufenozide, chlorfenapyr indoxacarb, or chlorfluazuron.



Avermectin B_{1a}



Avermectin B_{1b}

Figure 16 : Structure of Abamectin (Hazardous Substances Data Bank)

2.9.12 Pyridaben

Pyridaben is the only member of pyridazinones class. Pyridaben (Figure 17) provides extended long residual control. Pyridaben is utilized in the management of aphids, whiteflies and leafhoppers. Under diffferent temperatures, Pyridaben provides rapid knockdown. Pyridaben interrupts mitochondrial electron transport (Ware and Whitacre, 2004).

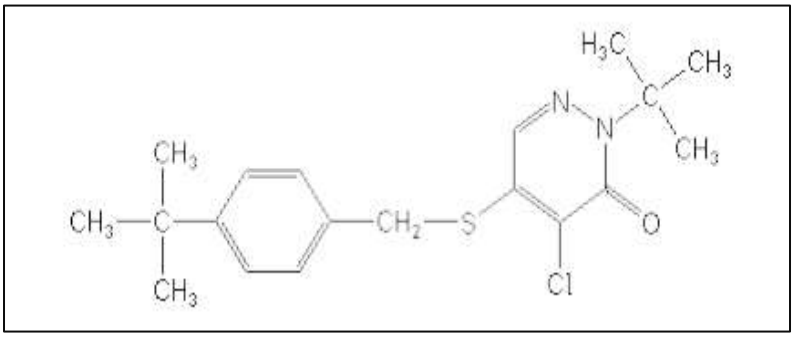


Figure 17: Structure of Pyridaben (Ware and Whitacre, 2004)

2.9.13 Imidacloprid

Imidacloprid (Figure 18) belongs to nicotinoids class of insecticides. The insecticide exhibit long residual control. It is applied as a foliar, soil or seed treatment in cotton, vegetables, rice cereals, peanuts and potatoes. Imidacloprid is effective in controlling sucking insects, soil insects, whiteflies, turf insects and the Colorado potato beetle (Ware and Whitacre, 2004). However, Imidacloprid is not effective against mites and nematodes. It has contact toxicity and stomach action, with excellent root-systemic characteristics. Imidacloprid executes its action on the central nervous system causing an irreversible blockage of postsynaptic nicotinergic acetylcholine receptors.

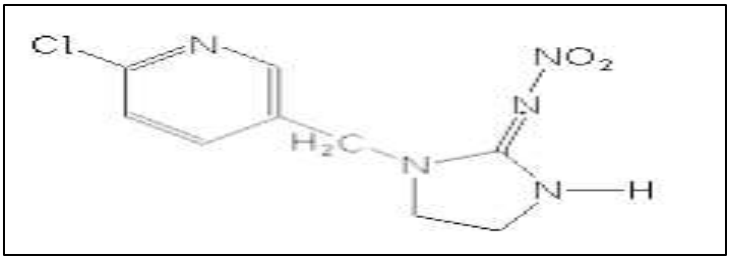


Figure 18 : Structure of Imidacloprid (Ware and Whitacre, 2004)

2.10 Target-Site Mutations Conferring Insecticide Resistance

With global spread of fall armyworm via invasion, there are high chances of spreading resistance mutations to other areas. The 2 most common mechanisms leading to resistance in this pest are target-site mutations and increased insecticides detoxification by various insect's enzymes (Boaventura *et al*., 2020; Guan *et al*., 2021). Target receptor insensitivity and pharmacokinetic processes change the properties of the insecticides and the rate at which they are delivered to the target site (Babithaa *et al*., 2022)**.** Existence of increased proportion of multiple resistance mutations shows strong selective pressure for insecticide resistance. Consequently, most of beneficial mutations in a population are removed because of stochastic fluctuation of allele frequency caused by the genetic bottleneck (Yainna *et al*., 2021). Monitoring the existence and distribution of resistance mutations may avail vital details on FAW control and resistance management by identifying point mutations that confer insensitivity to insecticides.

2.10.1Voltage-gated Sodium Channel Mutations

Mutations in VGSCs makes these channels less insensitive to toxins hence making insects more resistance to insecticides. On these channels, pyrethroids bind in domain III Segment 6 of sodium channels, and domain IIS4-S5 linker, in absence of mutations. VGSCs amino acid substitutions/indels, linked to pyrethroids resistance are T929I, L932F, and L1014F.

The first mutation to be discovered was L1014F mutation in house fly strains resistant to pyrethroid, conferring 10–30-fold resistance (Babithaa *et al*., 2022). L1014F and M918T are point mutations present in domain II of the house fly Vssc1 VGSC alpha subunit that cause knockdown and super knockdown resistance respectively (McComic *et al*., 2020). T929I, a super kdr mutation, was first identified in the pyrethroid-resistant diamondback moth while the L932F mutation is only found in human head lice.

2.10.2AChE Mutations

Carbamates and organophosphates resistance is linked with ace-1 gene mutations hence causing substitutions of amino acids at acetylcholinesterase's active site. Genotyping studies conducted by Carvalho *et al*. (2013) and Boaventura *et al*. (2020) reported existence of G227A, A201S and F290V amino acids substitutions in FAW populations in Brazil. In addition, F290V mutation was found in populations from Indonesia, Kenya and Puerto Rico. In this case, Kenyan populations had high frequency of the F290V mutation in AChE receptors. This high frequency may be due to existence of alleles rendering resistance to these insecticides or continued selection as farmers continue to indiscriminately use carbamate and organophosphates. Other point mutations reported to cause organophosphate resistance are F399V (*Cydia pomonella*), A314S (*Chilo suppressalis*) and G227A, D131G, A201S and A441G (*P. xylostella*) as per studies done by Boaventura *et al*. (2020)**.**

2.10.3Ryanodine Receptor (RyR) Mutations

Ryanodine receptors are homo-tetrameric calcium channels found in the sarco−/endoplasmic reticulum in both nerve and muscle tissues. They have 6 transmembrane domains at the C terminus, with a voltage sensor, and a large N-terminal

cytosolic domain. They are activated by calcium efflux into the cytosol (Gong *et al*.,2021). Diamides, newly introduced insecticides, act selectively on RyRs affecting the neuromuscular functionality. RyR are target-site for diamide insecticides, comprising of the phthalic (flubendiamide) and anthranilic acid diamides (e.g., chlorantraniliprole). Despite of them being two different chemotypes, these insecticides allosterically activate [^{3}H] ryanodine binding which is a common binding site in lepidopteran ryanodine receptors (Boaventura *et al*.,2020). However, the presence of point mutations affects them differently for instance, phthalic insecticides has low efficacy against pests consisting methionine at position 4790 (Nauen and Steinbach, 2016).

G4946E and I4790M mutations were reported to be the most vital resistance mechanism in diamond backmoth, functionally linked to RyR transmembrane domain as a target site. However, the genotyping data from studies done by Boaventura *et al*. (2020). demonstrated low frequency of these two resistance alleles under the field conditions.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Site

The sampling area consisted of different Counties including Kakamega (0.2387^0 N, 34.7515^0 E), Busia (0.4347^0 N, 34.2422^0 E), Kisumu (0.0917^0 S, 34.7680^0 E), Tranz-Nzoia (1.0219^0 N, 35.0015^0 E), Uasin-gishu ($0.5528\ ^0$N, 35.3027^0 E), Siaya (0.0998^0 S, 34.2747^0 E), Vihiga (0.0816^0 N, 34.7229^0 E), Embu (0.6560^0 S,37.7238^0 E), Tharaka-Nithi (0.2965^0 S, 37.7238^0 E), Nandi (0.1036^0 N, 35.1777^0 E), Kiambu (1.748^0 S, 36.8304^0 E), Muranga (0.7237^0 S, $37.1607\ ^0$E) and Bungoma (0.8479^0 N, 34.7020^0 E) Counties as shown in Figure 19. These regions were of great importance to this study as they form most of the Kenyan breadbasket regions where maize is commonly grown on large scale. Most farmers in these regions indiscriminately use the registered chemicals and the Kenyan authorities have no definite program for their use because there is no published data on the use of chemicals in Kenya.

3.2 Study Map

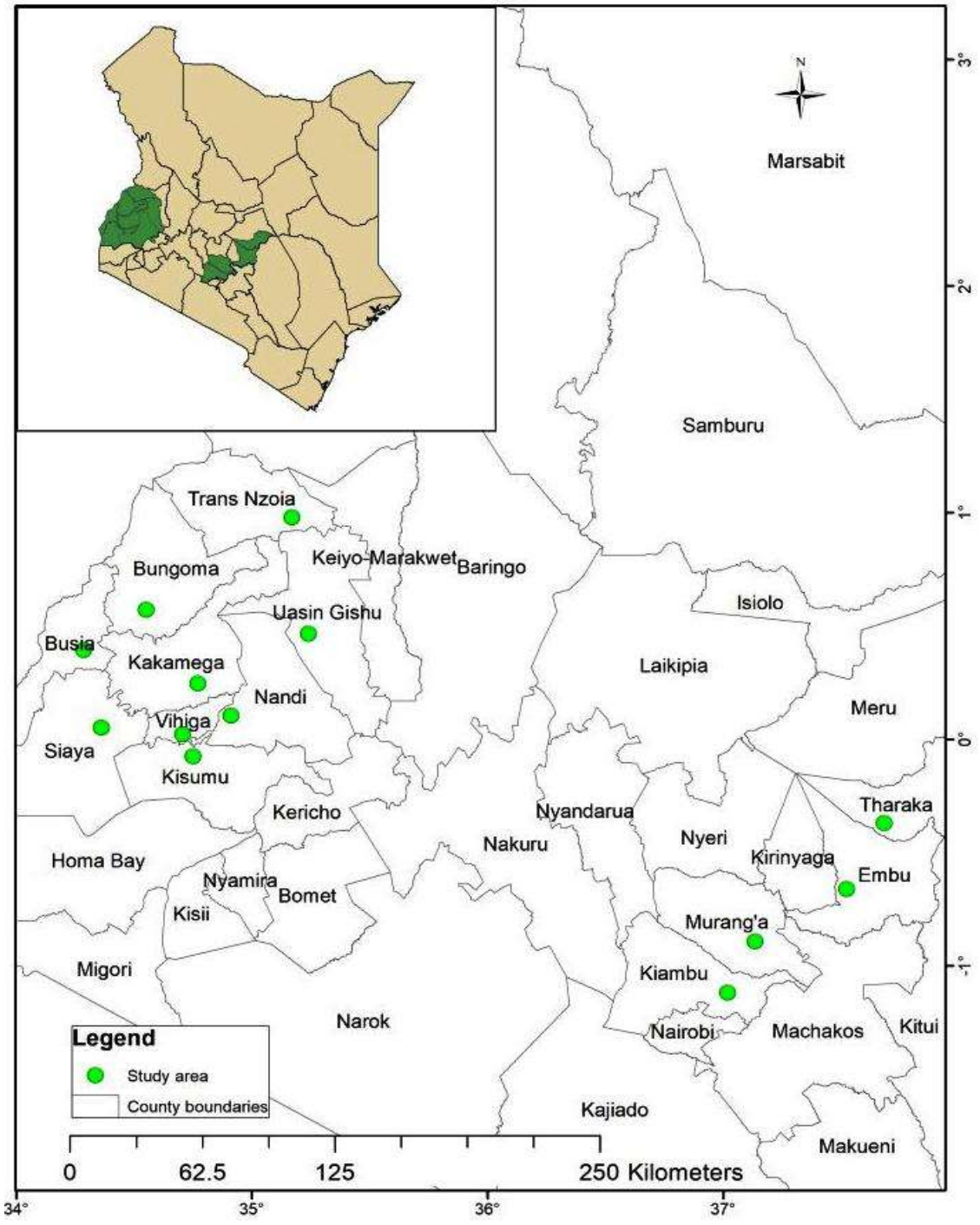


Figure 19: Sampling sites of field populations of *S. frugiperda* from Kenya. Green dots represent sampling locations

3.3 Study Design

This study was conducted in 13 different Counties. Maize growing areas were picked as the key areas of sampling. The number of farms sampled depended on infestation rate and accessibility. For sample collection a modified purposive sampling method was used to collect numerous samples (≥ 100), fourth to sixth instar larvae but only 200 insects reared laboratory populations were used per each insecticide in the bioassays. For the bioassays, complete randomized design was used where the insect samples and the leaf discs used were randomly selected and subjected to different insecticide concentrations and distilled water used as control. Twenty-one (21) treatments were used per each insecticide (5 dosages ×4 replicates + control).

3.4 Sample Collection

A modified purposive sampling method was used in collection of samples. Regular stops were made at predetermined intervals along motorable roads in every area. 4^{th} to 6^{th} instar larvae populations were collected in study fields in a semi-systematic manner via a "W" pattern method used in scouting (Prasanna *et al*., 2018). In the field, about 5 meters away from the farm borders (to avoid the edge effects), zigzags were made in the field, making 5 stops at different locations for collection of insects. The farms selected were over 5 km apart within each county to avoid collection of larvae of the same population. FAW larvae were placed in 10 ml plastic jars filled with soft maize leaves as diet. Upon arrival, larvae were visually confirmed as fall armyworm using morphological features. The moths were mass mated and generations F2 3rd instar larvae were used for the test. The susceptible strain was provided by Kalmer Agricultural consultants and was used as the reference strain. All strains were fed with natural diet, using maize plant leaves.

3.5 Insect Rearing

Insect rearing was conducted as per Gutiérrez-Moreno *et al*. (2019) with some little modifications as show in figure 20. Larvae were held at room temperature, in plastic cans for further larvae development. The plastic cans contained a moisturized kitchen towel and soft maize plant leaves as diet. The lid contained a muslin cloth for aeration. In each day, insect development and fitness was checked, pupated larvae were collected and transferred to different small plastic containers. Upon pupae turning color from orange-red to dark red, they were being placed into cages in groups of 40 for adult emergence and mating, separately for each field population. The cages were covered with grease paper internally as an oviposition substrate. The adults were fed with honey solution (10%) which was replaced every two days. After 3rd day of adult emergence, from each cage the moths were removed and were placed in 1-liter plastic containers with lids covered internally with muslin cloth and 10% honey solution in cotton roll was added as the food source. Egg masses laid were placed in new plastic containers with moistened kitchen towels and soft maize plants and held at 26 ± 1°C until hatching. 3rd instar F2 larvae generation were used in the bioassays.

Figure 20: Insect rearing of field populations of FAW from Kenya

3.6 Insecticides

Commercial formulated insecticides used included; Abamectin 18g/L (Deacarid 1.8 EC, Bio-Medica Laboratories, Nairobi, Kenya), Pyridaben 200g/L (Genomite 200 EC, Geneva Agrochemicals Ltd., Thika, Kenya), Lufenuron 50g/L (Match 050 EC, Syngenta East Africa Ltd., Nairobi, Kenya), Imidacloprid 200 g/L (Concord 20 SL, Agri Scope(Africa) Ltd., Nairobi, Kenya), Deltamethrin 25 g/L (Katrin 25 EC, Twiga Chemicals Industries Ltd., Nairobi, Kenya), Lambda-cyhalothrin 17.5 g/L (Duduthrin 1.75 EC, Twiga Chemicals Industries Ltd., Nairobi, Kenya), Spinosad 480 g/L (Tracer 480 SC, Dow Chemical East Africa Ltd., Nairobi, Kenya), Spinetoram 120 g/L (Radiant 120 SC, Dow Chemical East Africa Ltd., Nairobi), Indoxacarb 150 g/L (Avaunt 150 EC, Elgon Kenya Ltd., Nairobi Kenya).

3.7 Determination of Baseline Susceptibility to Nine Insecticides

Baseline susceptibility was determined using leaf-dip bioassay. 3rd instar larvae from F2 generation laboratory cultures were exposed to different insecticides concentrations via the leaf- dip method as recommended by Insecticide Resistance Action Committee, method no.7 (https://irac-online.org/methods/leaf-eating-lepidoptera-coleotera-larvae/). The insecticides were diluted to generate five serial dilutions with distilled water, for each test insecticide. Since toxicity is related to the logarithm of dose, different dose ranges in a geometric series were preferred for each test insecticide covering 5 to 100% mortality. The concentrations were calculated in milligrams per liter (mg/L). Cleansed maize leaves (the youngest leaf, from 1–3 cm of the leaf apex) were excised into leaf discs of 5 cm in diameter, dipped in the insecticides for 5 seconds with gentle agitation. The leaf discs were allowed to surface-dry on paper toweling for an hour. The control batches were exposed to

the same treatments, except for the inclusion of the insecticide, only treated with distilled water. These leaf discs were placed individually in Petri dishes lined up with water-moistened kitchen towels to avoid leaf desiccation. The kitchen towels were kept moist by adding water daily to preserve leaf turgor. Ten (10) 3rd instar larvae were introduced per Petri dish with 4 replicates for each concentration. The larvae mortality was recorded after 48h for rapidly acting insecticides and 72h for the slow acting insecticides. Larvae was considered dead if unable to move when probed with a brush. The bioassay was performed at an average temperature and relative humidity of 26±1^{o} C and 60-70% respectively.

3.8 Data Analysis

An entire bioassay analysis for one strain was conducted together on the same day, and only leaf-dip bioassays having control mortality <10% were included in the subsequent statistical analyses (inclusion criteria). The Poloplus program (LeOra Software, 2002) was applied for probit analysis to obtain the LC_{50}, fiducial limits (95%), slopes, and Chi-square values. Poloplus automatically tests the normality of data before analyzing it. Overlapping of the 95% fiducial limits was used to judge if there were significant differences in response among the insecticides used in the bioassays. A significant difference between LC_{50} values was indicated by non-overlapping 95% fiducial limits (Zhao *et al*., 2020). The resistance ratios were obtained by dividing the LC_{50} values of the field populations by LC_{50} values of the susceptible strain. Relative potency ratios to estimate the potency of the active ingredients were calculated as the LC_{50} of the least toxic compound divided by the LC_{50} of the most toxic compound (Gutierrez-Moreno *et al*., 2019). Pairwise correlation coefficients were evaluated among log LC_{50} values in field-collected populations and tested chemicals by use of analysis of Pearson's correlation using the IBM SPSS Statistics software package

(Version 22.0) to assess the possible cross-resistance among different chemicals (Zhang *et al*., 2020). A *P*-value of less than 0.05 was regarded as statistically significant (Zhang *et al*., 2020).

3.9 RNA Extraction and Quantitative PCR Protocol to Determine Molecular Tolerance Mechanisms in FAW Against a Range of Insecticides

3.9.1 RNA Extraction Protocol

Total RNA was extracted from *Spodoptera frugiperda* larvae tissues (fourth instar) using TRIzol® reagent (Invitrogen; Carlsbad, CA, United States) following the manufacturer's recommendation (Vatanparast and Park, 2021). RNA was extracted from both insecticides treated and untreated test insect (acted as negative controls) with three replicates for each. Tissues were immediately homogenized in 1 ml TRIzol® reagent (per 100 mg of tissue) and incubated for 10 min at room temperature for dissociation of nucleoprotein complexes. The samples were then centrifuged to remove cell debris and then transfer of supernatant into a new tube followed. 0.2 ml of chloroform was added, vortexed vigorously for 15 seconds before incubation at room temperature for 3 min. The samples were then centrifuged at 12,000× *g* for 15 min at 4 °C. The upper aqueous phase was then transferred to a 1.5 ml tube. RNA was precipitated from the aqueous phase by adding with 0.5 ml of isopropyl alcohol and incubated at room temperature for 15 min. This was followed by centrifugation of the samples at 12,000× *g* for 10 min at 4 °C. Isopropanol was removed carefully, and the pellet was washed twice with 1 ml of 75% ethanol. The sample was vortexed, then centrifuged for 5 minutes at 7500 × g at 4°C. The supernatant was discarded and the RNA pellet was allowed to air dry at room temperature for 10 min. The pellet was suspended in 50 µl of RNase-free water, by pipetting up and down gently. The pellets were

then incubated in a water bath set at 60°C for 10 min. Absorbance at 260 nm with NanoVue (GE Healthcare, Chicago, IL, USA) was used to quantity the RNA. For quality control, the quality of RNA was estimated by determining the 260/280 nm ratio. For inclusion criteria, only samples with this ratio were selected for running the q-PCR process (RNA is considered pure if it has a ratio of ~2).

3.9.2 Quantitative PCR (q-PCR) Protocol

Quantitative PCR was performed to analyze gene expression of VGSC, AChE and RyR genes, as per Vatanparast and Park (2021) and Wekesa *et al*. (2022) with some little modifications. cDNA was synthesized from 1microgram of the previously isolated RNA (only of high quality) using RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Waltham, MA, USA) as per the manufacturer's recommendation. The reaction mixture was as follows: template (10 µg), 2.5 mM dNTPs (2 µL), 10× DreamTaq buffer (2 µL) (Thermo Fisher Scientific, Waltham, MA, USA), 5 U/µL DreamTaq DNA Polymerase (0.25 µL), 20× EvaGreen® Dye (BIOZOL Diagnostica Vertrieb GmbH, Eching, Germany), 10 pM primers (1 µL) for each forward and reverse reactions, and water to top up the total reaction volume to 20 µL. Primers used were designed according to sequences found in the National Center for Biotechnology Information (NCBI) for ace-1 gene (GenBank KC435023), FAW para-type VGSC (GenBank KC435025), and RyR (GenBank MK226188). The list of primers used as by Boaventura *et al*. (2020) are as shown in Table 2. The real-time qPCR was performed on the CFX Connect Real-Time PCR Detection System (Bio-Rad) (Bio-Rad Laboratories GmbH, Feldkirchen, Germany) with the following thermocycling conditions: initial denaturation 95 °C for 3 min, denaturation 95 °C for 10 s, annealing 60 °C for 50 s, extension 72 °C for 1 min, with a total of 40 cycles

(Wekesa *et al*., 2022). Normalization of the expression of the target genes was done using Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene expression level. Evaluation of final products was done via analysis of melting curve. The treatments were each replicated with 3 independent biological sample preparations. Quantitative analysis was done using the comparative CT approach ($2^{-\Delta\Delta CT}$) as follows;

1. Calculate the average of cycle threshold (Ct) values for the Housekeeping Gene (HKG) and Gene of Interest(GOI).
2. Calculate delta Ct (ΔCt)

   Avg. Ct (GOI) – Avg. Ct (HKG)
3. Average (ΔCt) of control group in GOI
4. Calculate delta-delta Ct (ΔΔCt)

   ΔCt of control/treated sample – Avg. ΔCt of control group
5. Calculate $2^{\wedge(-\Delta\Delta Ct)}$
6. Average fold of change for control and treated samples

   Avg. of $2^{\wedge(-\Delta\Delta Ct)}$ of control/treated

The relative fold expression of the control samples is 1 hence if the fold expression of the treated samples is greater than 1 there is upregulation and if less than 1, there is a down regulation.

Table 2: List of Primers Used in q-PCR

Target	Mutation	Primer	Primer Sequence 5' to 3'	Annealing temperature
Ryanodine receptor	G4946E	Sf_G4946_F	GTGATGGGCAACTTCAAC	50
		Sf_G4946_R	TTTTCCGTTATGCGTGAC	
	I4790M	Sf_taq_I4790_F	ACGACGATGCACTAGAAG	60.6
		Sf_taq_I4790_R	CACCTTGAGATGATAGTACC	
Acetylcholinesterase	A201S /G227A	Sf_A201S_G227A_F	TTTGATACCCCTGATGTACC	53
		Sf_A201S_ G227A_R	AATGAAACCGAAACTGCTC	
Voltage-gated sodium channel	L1014F	Sf_L1014_F	TCTTCCTGGCTACAGTCG	50
		Sf_L1014_R	GACAGTAACAGGGCCAAG	
	L932F /T929I	Sf_L932_T929_F	TAATGGGTAGGACAATGG	53
		Sf_L932_T929_R	AATCCACGTAATTTTTCC	

3.10 Modelling the Target-site Mutations in the Voltage-gated Sodium Channels of FAW

3.10.1 Homology Modelling

The crystal structure of the Nav1.4-beta1 complex from electric eel provided the structural template for a homology model of the fall armyworm sodium channel in an activated state. The Nav 1.4 –beta complex from the electric eel represents the first Nav channel to be biochemically purified and cloned. The subunits of the model are represented by the dark-blue colour in figure 5. These subunits correspond to domains I, II, III, and IV in eukaryotic sodium channels that have four domains. The model was produced using the SWISS-MODEL workspace (swissmodel.expasy.org/workspace). The chosen protein had a Global Model Quality Estimation (GMQE) score of 0.37 (the highest among the identified templates). The GMQE score is a quality estimation (quality control check) that combines

properties from the target template alignment and the template structure. The score reflects that expected accuracy of the model built with that of the alignment and template. The study used the ClustalW algorithm to align the amino acid sequences of the fall armyworm sodium channel (XP_035435130.1) with the Nav1.4 channels. The sequence identity of the alignments between the sequences was 33% (Figure 21). The alignments shown below are a portion of a highly identical and repetitive amino acids sequences.

Figure 21: Sequence alignments of the Nav1.4 channel and fall armyworm sodium channel.

3.10.2 Automated Ligand Docking

The crystal structure of the pyrethroids, metaflumizone, indoxacarb, benzophenylurea, cartap, fipronil, spinetoram, chlorfenapyr, and tebufenozide was obtained from the PubChem database (https://pubchem.ncbi.nlm.nih.gov/). The insecticides were downloaded in the sdf (special data file) format and converted to the pdb (protein data bank) format (compatible with Autodock Vina) via Pymol. Automated docking of the insecticides was performed using the AutoDock 4.2.6 software package (Trott and Olson, 2010). The predicted binding affinities between the VGSC and the insecticides were measured in Kcal/mol. The protein molecule (VGSCs) was read into Autodock Vina and water molecules were removed. Water molecules in the binding pocket can interfere with docking. Polar hydrogens and Kollman charges were added to the protein before it was saved in pdbqt format. The protein and ligand (insecticide) were then chosen as

macromolecules in the Autodock Vina. Grid maps with 40×40×40 points were constructed with a grid point spacing of 0.375Å. The grids were centred on positions 138.737, 128.216, and 125.832. A configuration file was then created to indicate the parameters for the docking process. The Iterated Local Search global optimizer algorithm with the parameters of energy range = 4 and exhaustiveness = 8 was used to perform docking simulations. The docking process performed using Autodock Vina was run using the command prompt. Docking predictions with the least binding free energy value (highest negative value) were deemed to be of significance (inclusion criteria). The results of the docking process were visualized using pymol which was also used to identify the specific docking points of individual insecticides.

CHAPTER FOUR

RESULTS

4.1 Introduction

This study sought to determine baseline susceptibility of FAW to different active ingredients of insecticides with varied mode of action, evaluate cross-resistance, determine the FAW molecular mechanisms and model the molecular binding sites of these insecticides on the voltage gated sodium channels of fall armyworm. For baseline susceptibility, leaf-dip bioassays were conducted to determine the LC_{50} insecticide concentrations that killed half of each of the fall armyworm population obtained from 13 different counties used in the bioassays. Additionally, the study also validated the involvement of VGSCs, AChE, RyR of FAW populations via quantification of gene expression. This study also identified amino acid residues that the active compounds could bind to enhance their efficacy against the fall army worm. Insecticides that do not target VGSC also showed interactions with this channel, indicating the possibility of different mode of actions that could be confirmed by experimental studies.

4.2 Baseline Susceptibility of Fall Armyworm Populations in Kenya to Nine Insecticides

4.2.1 Baseline Susceptibility of FAW Populations to Lambda Cyhalothrin

All the 13 fall armyworm populations tested against lambda cyhalothrin exhibited low levels of resistance (3-4 folds) in comparison with the susceptible strain (Figure 22). The LC_{50} values ranged from 139.63 mg/L for the KS (Kisumu strain) to 197.36 mg/L (ppm) for the TZ (Trans- Nzoia strain), showing 1.4-fold difference. LC_{50} values of all fall armyworm populations had overlapping 95% fiducial limits indicating that their response

to lambda cyhalothrin was not significantly different (Table 3). The high baseline LC_{50} values suggest that lambda cyhalothrin may no longer be effective in the management of FAW.

Table 3: Baseline Susceptibility of FAW Field Populations to Lambda Cyhalothrin (Pyrethroid)

Region	Strain	N	Slope+- SE	X^2(df)	LC_{50} (mg/L)	95% FL	RR
Western	KK	200	3.09+-0.77	0.68(3)	143.37	104.41 - 170.45	3
	VH	200	3.53+-0.82	1.02(3)	141.14	107.54 - 164.79	3
	BS	200	2.12+-0.41	0.68(3)	147.45	113.38 - 187.44	3
	BN	200	2.07+-0.56	0.13(3)	143.48	100.12 - 204.60	3
Central	MR	200	3.41+-0.78	0.81 (3)	165.77	133.51- 195.41	3
	KA	200	3.72+-0.84	0.99(3)	160	128.36 - 186.43	3
Eastern	EB	200	4.04+-0.8	1.31(3)	153.73	126.04- 177.29	3
	TN	200	3.92+-0.79	0.74(3)	155.02	127.45- 178.74	3
Nyanza	KS	200	4.45+-0.76	1.53 (3)	139.63	117.32- 158.61	3
	SA	200	3.60+-0.89	1.67(3)	140.62	102.98-165.58	3
Rift valley	UG	200	3.78+-0.79	0.57(3)	179.13	152.25 - 208.14	3
	TZ	200	3.29+-0.82	2.64(3)	197.36	165.03-241.37	4
	NN	200	3.61+-0.72	0.20(3)	162.26	134.88- 188.64	3
	SUS	200	2.06+-0.34	1.55(3)	53.77	34.34- 71.63	1

N = Number of insects used

SE= Standard Error

X^2 (df) = Chi square value (Degrees of freedom)

LC_{50}=Median lethal concentration in milligrams per liter

95% FL=95 % Fiducial limits (Confidence intervals)

RR= Resistance ratios, LC_{50} of field population over LC_{50} of SUS strain

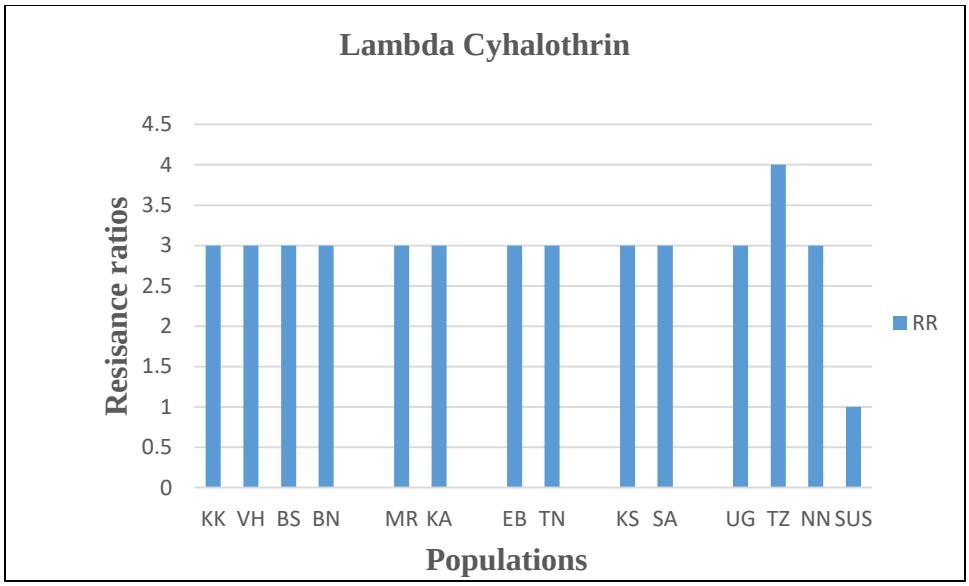


Figure 22: Resistance ratios of different populations of fall armyworm in Kenya to Lambda cyhalothrin

4.2.2 Baseline Susceptibility of FAW Field Populations to Deltamethrin

The LC_{50} values of deltamethrin against fall armyworm were in the range of 130.60 mg/L (82.17 - 248.80) for TZ (Trans-Nzoia) and 61.27 mg/L (40.19 - 87.49) for SA (Siaya). The susceptible strain had LC_{50} value of 29.46 mg/L (Table 4). The fiducial limits gotten at the LC_{50} value overlapped among all the strains, suggesting similar toxicity of deltamethrin across all the populations. Further, a similar scenario was observed for the susceptible population. The resistance ratios ranged from 2 to 4 exhibiting very low levels of resistance to this active ingredient (Figure 23). The populations were less susceptible to lambda cyhalothrin than to deltamethrin despite being in the same group of IRAC classification (Pyrethroids). The low potency was may be due to target-site mutations and metabolic resistance.

Table 4: Baseline Susceptibility of FAW Field Populations to Deltamethrin (Pyrethroid)

Region	Strain	N	Slope+- SE	X^2(df)	LC_{50}	95% FL	RR
Western	KK	200	1.41+-0.27	0.15 (3)	64.71	40.96 - 95.51	2
	VH	200	1.46+-0.27	0.49 (3)	62.84	40.62 - 91.07	2
	BS	200	1.27+-0.26	2.25 (3)	66.31	40.83 - 101.65	2
	BN	200	1.99+-0.28	0.96 (3)	63.25	48.48 - 81.19	2
Central	MR	200	1.12+-0.25	0.34(3)	100.82	62.83 -179.24	3
	KA	200	1.36+-0.27	1.55(3)	97.83	64.08 - 154.5	3
Eastern	EB	200	1.33+-0.26	0.35(3)	73.34	47.39- 110.38	2
	TN	200	1.11+-0.23	1.29(3)	73.17	47.09 - 115.73	2
Nyanza	KS	200	1.50+-0.25	0.19(3)	61.78	43.44 - 85.34	2
	SA	200	1.51+-0.27	1.03 (3)	61.27	40.19 - 87.49	2
Rift Valley	UG	200	1.04+-0.27	0.23 (3)	122.95	69.82 - 264.10	4
	TZ	200	1.16+-0.26	0.30(3)	130.60	82.17 - 248.80	4
	NN	200	1.46+-0.28	0.7(3)	119.21	81.95 - 186.57	4
	SUS	200	1.59+-0.27	0.34(3)	29.46	18.25 - 40.92	1

N = Number of insects used

SE= Standard Error

X^2 (df) = Chi square value (Degrees of freedom)

LC_{50}=Median lethal concentration in milligrams per liter

95% FL=95 % Fiducial limits (Confidence intervals)

RR= Resistance ratios, LC_{50} of field population over LC_{50} of SUS strain

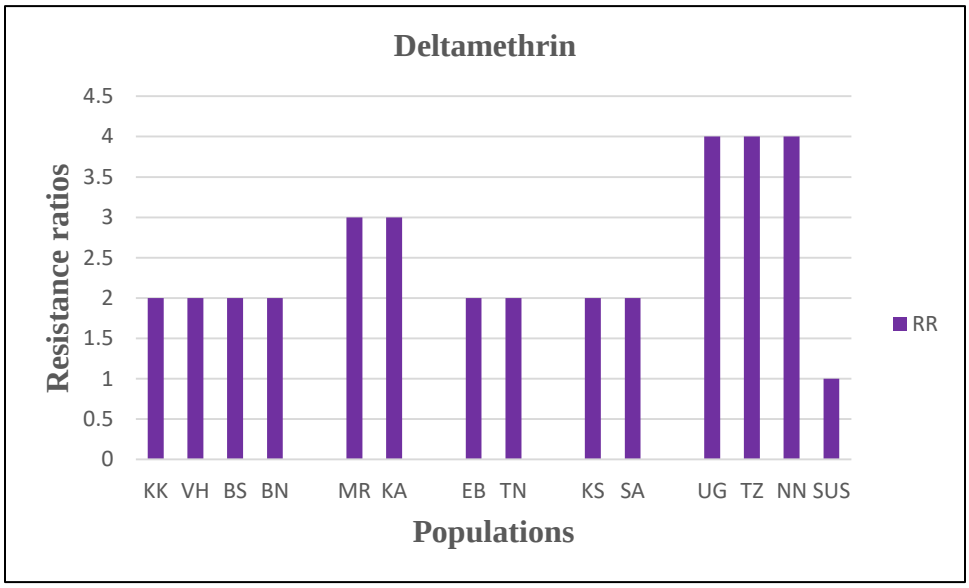


Figure 23: Resistance ratios of different populations of fall armyworm in to Deltamethrin

4.2.3 Baseline Susceptibility of FAW Field Populations to Indoxacarb

The fall armyworm population from TZ had higher LC_{50} value, 26.28 mg/L, for indoxacarb compared to other populations (Table 5). Even though the populations exhibited very low resistance (1 to 2-fold at LC_{50}) as shown in figure 24 the LC_{50} values of indoxacarb were high hence low potency against the pest. The overlapping fiducial limits showed that the FAW field populations exhibited no statistical difference in their response to this active ingredient.

Table 5: Baseline Susceptibility of FAW Field Populations to Indoxacarb (Oxadiazine)

Region	Strain	N	Slope+-SE	X^2(df)	LC_{50} (mg/L)	95% FL	RR
Western	KK	200	1.91+-0.37	2.06(3)	16.03	10.72 - 21.05	1
	VH	200	1.86+-0.33	2.72(3)	15.49	10.9 - 20.02	1
	BS	200	2.24+-0.45	1.58(3)	16.47	10.66 - 21.41	2
	BN	200	1.81+-0.33	1.47 (3)	16.30	11.55 - 21.15	2
Central	MR	200	1.95+-0.37	0.51 (3)	21.92	16.31 - 28.21	2
	KA	200	1.98+-0.4	0.23(3)	20.24	14.24-26.25	2
Eastern	EB	200	2.44+-0.52	0.73(2)	18.66	12.33 - 23.74	2
	TN	200	1.92+-0.4	2.54 (3)	18.81	12.59 - 24.72	2
Nyanza	KS	200	1.62+-0.32	0.21 (3)	14.96	9.56 - 20.31	1
	SA	200	1.47+-0.32	1.05(3)	14.21	8.47 - 19.84	1
Rift Valley	UG	200	1.78+-0.4	0.13(3)	25.94	18.54 - 35.57	2
	TZ	200	1.77+-0.39	2.53(3)	26.28	19.04 -35.81	2
	NN	200	1.71+-0.35	1.97 (3)	22.33	16.06 - 29.93	2
	SUS	200	1.94+-0.35	1.74(3)	10.85	6.66 - 14.68	1

N = Number of insects used

SE= Standard Error

X^2 (df) = Chi square value (Degrees of freedom)

LC_{50}=Median lethal concentration in milligrams per liter

95% FL=95 % Fiducial limits (Confidence intervals)

RR= Resistance ratios, LC_{50} of field population over LC_{50} of SUS strain

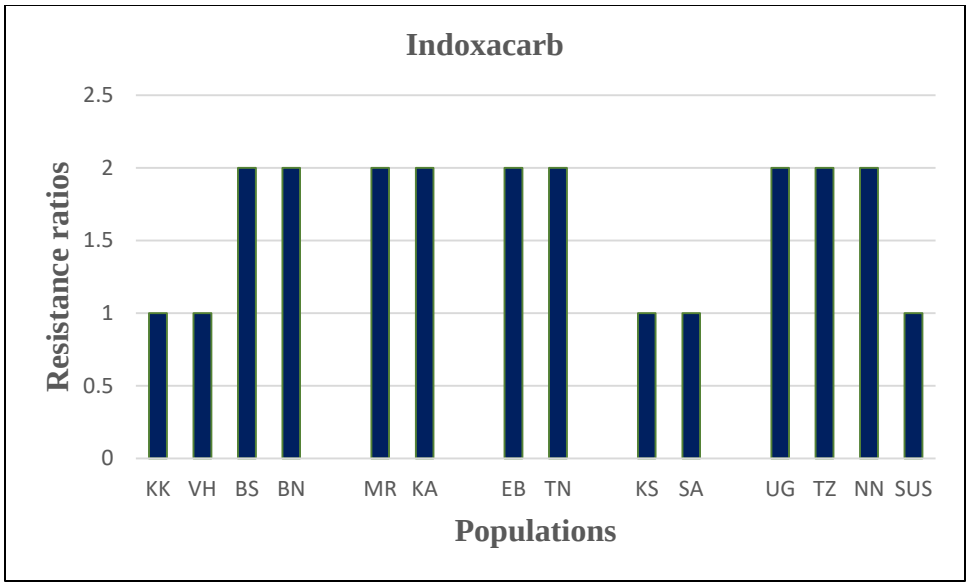


Figure 24: Resistance ratios of different populations of fall armyworm in Kenya to Indoxacarb

4.2.4 Baseline Susceptibility of FAW Field Populations to Spinosad

The LC_{50} values for the fall armyworm varied between 0.76 and 0.36 (Table 6), a 2.09-fold variability between the least and most sensitive populations. This confirmed that the field populations were more susceptible to spinosad than the pyrethroids. The TZ strain had a higher resistance ratio (3) compared to other populations as shown in figure 25. The log dose-probit regression slopes for spinosad among the populations were similar. This observation suggest that this compound had similar toxicity levels against the populations.

Table 6: Baseline Susceptibility of FAW Field Populations to Spinosad (Spinosyn)

Region	Strain	N	Slope+- SE	X^2(df)	LC_{50}	95% FL	RR
Western	KK	200	1.47+-0.26	0.48(3)	0.4	0.263 - 0.56	2
	VH	200	1.22+-0.24	1.8(3)	0.39	0.234 - 0.58	1
	BS	200	1.53+-0.25	2.41(3)	0.39	0.274 - 0.53	1
	BN	200	1.37+-0.25	2.47(3)	0.39	0.237 - 0.56	1
Central	MR	200	1.47+-0.253	2.47(3)	0.45	0.31 - 0.63	2
	KA	200	1.65+-0.27	0.25(3)	0.43	0.29 -0.58	2
Eastern	EB	200	1.22+-0.25	1.45 (3)	0.44	0.26 - 0.67	2
	TN	200	1.56+-0.26	0.76 (3)	0.44	0.30 - 0.62	2
Nyanza	KS	200	1.66+-0.27	2.99(3)	0.37	0.25 - 0.51	1
	SA	200	1.50+-0.26	1.27 (3)	0.36	0.24 - 0.50	1
Rift Valley	UG	200	1.51+-0.26	1.84(3)	0.63	0.442 - 0.90	2
	TZ	200	1.28+-0.25	0.7(3)	0.76	0.52 - 1.19	3
	NN	200	1.25+-0.24	0.75(3)	0.61	0.41 - 0.93	2
	SUS	200	1.93+-0.28	0.1(3)	0.26	0.19 - 0.34	1

N = Number of insects used

SE= Standard Error

X^2 (df) = Chi square value (Degrees of freedom)

LC_{50}=Median lethal concentration in milligrams per liter

95% FL=95 % Fiducial limits (Confidence intervals)

RR= Resistance ratios, LC_{50} of field population over LC_{50} of SUS strain

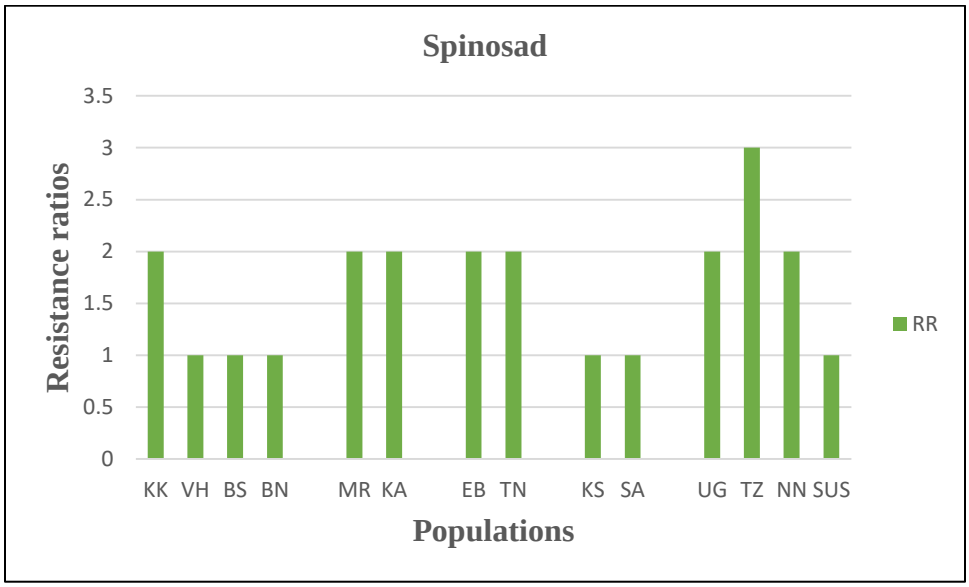


Figure 25: Resistance ratios of different populations of fall armyworm in Kenya to Spinosad

4.2.5 Baseline Susceptibility of FAW Field Populations to Spinetoram

The baseline LC_{50} values of spinetoram were in the range of 0.48 mg/L for the TZ strain and 0.16mg/L for KS strain as shown in Table 7. In this study, spinetoram came out to be the most toxic active ingredient amongst the tested insecticides as it exhibited the lowest values of LC_{50} values compared to other active ingredients despite of the TZ strain exhibiting a resistance ratio of 3 (low resistance levels). This implies that this insecticide may be cost effective for the famers due to their high potency and broad activity showed

against many problematic pests. All the populations displayed very low levels of resistance (1 to 3 fold) to spinetoram as shown in figure 26.

Table 7: Baseline Susceptibility of FAW Field Populations to Spinetoram (Spinosyn)

Region	Strain	N	Slope+- SE	X^2(df)	LC_{50}	95% FL	RR
Western	KK	200	1.64+-0.26	1.2 (3)	0.18	0.12 - 0.24	1
	VH	200	1.64+-0.27	1.86 (3)	0.16	0.11 - 0.22	1
	BS	200	1.69+-0.26	0.95(3)	0.19	0.14 - 0.25	1
	BN	200	1.63+-0.27	2.13 (3)	0.16	0.11 - 0.23	1
Central	MR	200	0.92+-0.23	1.33 (3)	0.27	0.16 -0.48	2
	KA	200	1.19+-0.24	1.93(3)	0.28	0.18 -0.44	2
Eastern	EB	200	1.42+-0.27	0.13 (3)	0.25	0.16- 0.37	2
	TN	200	1.41+-0.26	2.08 (3)	0.22	0.14 -0.32	2
Nyanza	KS	200	1.85+-0.27	1.35 (3)	0.16	0.11 - 0.21	1
	SA	200	1.82+-0.29	0.72(3)	0.16	0.11 - 0.22	1
Rift Valley	UG	200	1.35+-0.28	0.07 (3)	0.35	0.22 - 0.55	2
	TZ	200	1.3+-0.25	2.09 (3)	0.48	0.33 - 0.8	3
	NN	200	2.03+-0.30	0.97(3)	0.30	0.23 - 0.4	2
	SUS	200	1.86+-0.28	0.85(3)	0.14	0.10- 0.19	1

N = Number of insects used

SE= Standard Error

X^2 (df) = Chi square value (Degrees of freedom)

LC_{50}=Median lethal concentration in milligrams per liter

95% FL=95 % Fiducial limits (Confidence intervals)

RR= Resistance ratios, LC_{50} of field population over LC_{50} of SUS strain

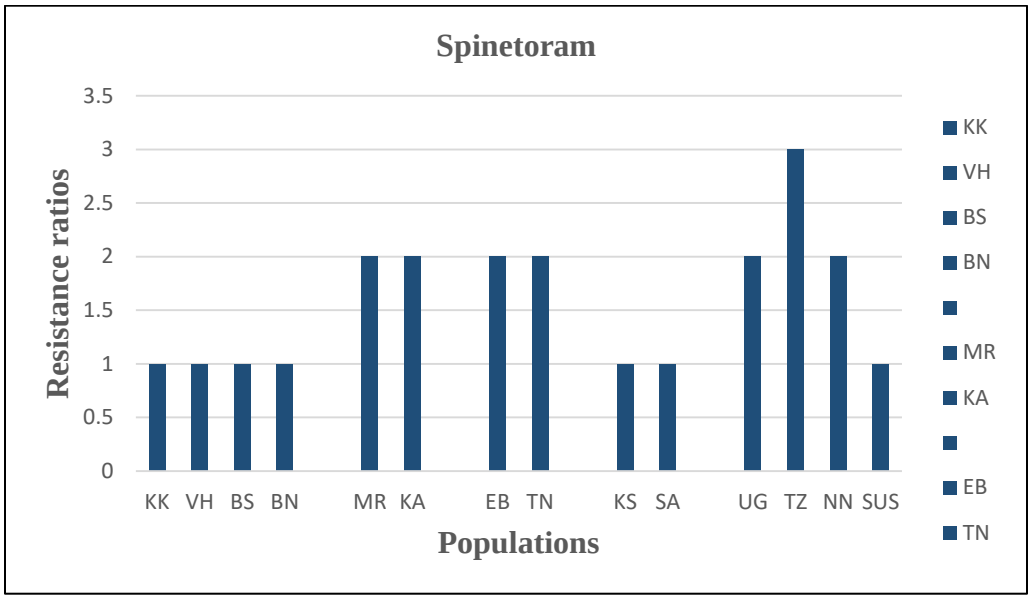


Figure 26: Resistance ratios of different populations of fall armyworm in Kenya to Spinetoram

4.2.6 Baseline Susceptibility of FAW Field Populations to Lufenuron

LC_{50} values of lufenuron against fall armyworm were in the range of 6.13 mg/L for UG strain and 4.23 mg/L for SA population, showing less than 1.5-fold difference (Table 8). All strains had a resistance ratio of 2 except SA population which had a resistance ratio of 1 (Figure 27). Strains from the Rift valley region displayed high baseline LC_{50} values compared to strains in other regions suggesting that these 3 strains were less susceptible to lufenuron. However, the fiducial limits at LC_{50} level overlapped indicating that the

response for these field populations were not statistically different to lufenuron. The low values of LC_{50} implies that the insecticide is effective in the field in controlling FAW.

Table 8: Baseline Susceptibility of FAW Field Populations to Lufenuron (Benzoylurea)

Region	Strain	N	Slope+- SE	X2(df)	LC_{50}(mg/L)	95% FL	RR
Western	KK	200	3.0+-0.58	1.83 (3)	4.40	3.22 - 5.36	2
	VH	200	2.64+-0.47	0.64 (3)	4.32	3.29 - 5.25	2
	BS	200	2.34+-0.45	0.21(3)	4.42	3.30 - 5.46	2
	BN	200	2.68+-0.46	0.83 (3)	4.45	3.46- 5.36	2
Central	MR	200	2.55+-0.5	1.45 (3)	4.91	3.72 - 6.02	2
	KA	200	2.93+-0.58	1.01(3)	4.88	3.65 - 5.92	2
Eastern	EB	200	3.21+-0.61	1.03 (3)	4.62	3.46 - 5.56	2
	TN	200	3.15+-0.49	2.49 (3)	4.62	3.77 - 5.41	2
Nyanza	KS	200	3.24+-0.56	0.69(3)	4.25	3.26 - 5.09	2
	SA	200	2.78+-0.45	1.91(3)	4.23	3.34 - 5.04	1
Rift Valley	UG	200	2.51+-0.51	0.40(3)	6.13	4.86 - 7.62	2
	TZ	200	2.37+-0.47	1.55(3)	5.76	4.58 - 7.13	2
	NN	200	2.51+-0.51	2.67 (3)	5.05	3.80 - 6.22	2
	SUS	200	2.30+-0.43	0.50(3)	2.82	1.87 - 3.59	1

N = Number of insects used

SE= Standard Error

X^2 (df) = Chi square value (Degrees of freedom)

LC_{50}=Median lethal concentration in milligrams per liter

95% FL=95 % Fiducial limits (Confidence intervals)

RR= Resistance ratios, LC_{50} of field population over LC_{50} of SUS strain

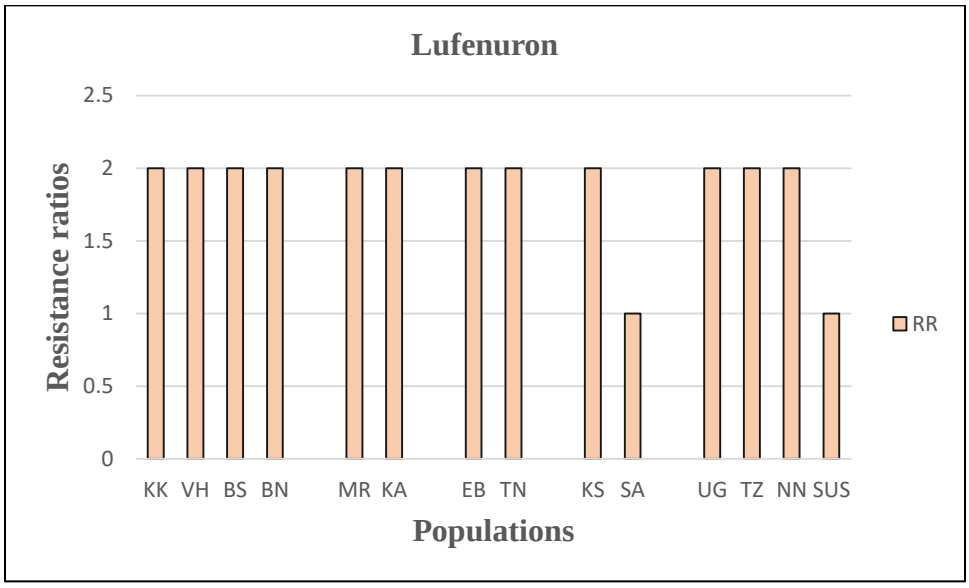


Figure 27: Resistance ratios of different populations of fall armyworm in Kenya to Lufenuron

4.2.7 Baseline Susceptibility of FAW Field Populations to Abamectin

The LC_{50} values of abamectin were higher (Table 9) compared to other active ingredients used in this study. This results suggest that this compound is less effective in controlling fall armyworm from the sampled regions as this mean that large quantities of the insecticide is needed in the management of the pest which may not be economical. Nevertheless, the resistance ratios ranged from 1 to 2 hence no resistance of the field populations to abamectin as shown in figure 28.

Table 9: Baseline Susceptibility of FAW Field Populations to Abamectin (Avermectin)

Region	Strain	N	Slope+-SE	X^2(df)	LC_{50}	95% FL	RR
Western	KK	200	2.76+-0.51	1.43(3)	4246.42	3134.31 - 5198.31	1
	VH	200	2.96+-0.46	0.09(3)	4168.13	3356.92 - 4916.31	1
	BS	200	2.64+-0.47	0.64 (3)	4319.99	3292.52 - 5244.81	1
	BN	200	2.69+-0.44	1.08 (3)	4325.42	3444.55 - 5155.33	1
Central	MR	200	2.75+-0.46	0.08 (3)	4767.97	3850.98 - 5667.42	2
	KA	200	2.44+-0.45	0.68(3)	4581.29	3493.61 - 5612.33	1
Eastern	EB	200	2.91+-0.51	0.20 (3)	4396.50	3352.09 - 5310.22	1
	TN	200	2.93+-0.51	1.52(3)	4350.45	3340.59 -5229.24	1
Nyanza	KS	200	2.88+-0.45	0.24(3)	4201.76	3372.33 - 4969.76	1
	SA	200	2.75+-0.45	2.43(3)	4100.59	3230.84 - 4894.78	1
Rift Valley	UG	200	2.49+-0.49	0.73(3)	5105.51	3910.34 - 6270.82	2
	TZ	200	2.58+-0.46	0.17(3)	5359.01	4346.40 - 6453.85	2
	NN	200	2.99+-0.55	0.86 (3)	5040.48	3930.81 -6043.45	2
	SUS	200	2.94+-0.48	0.74(3)	3089.31	2269.0 - 3782.95	1

N = Number of insects used

SE= Standard Error

X^2 (df) = Chi square value (Degrees of freedom)

LC_{50}=Median lethal concentration in milligrams per liter

95% FL=95 % Fiducial limits (Confidence intervals)

RR= Resistance ratios, LC_{50} of field population over LC_{50} of SUS strain

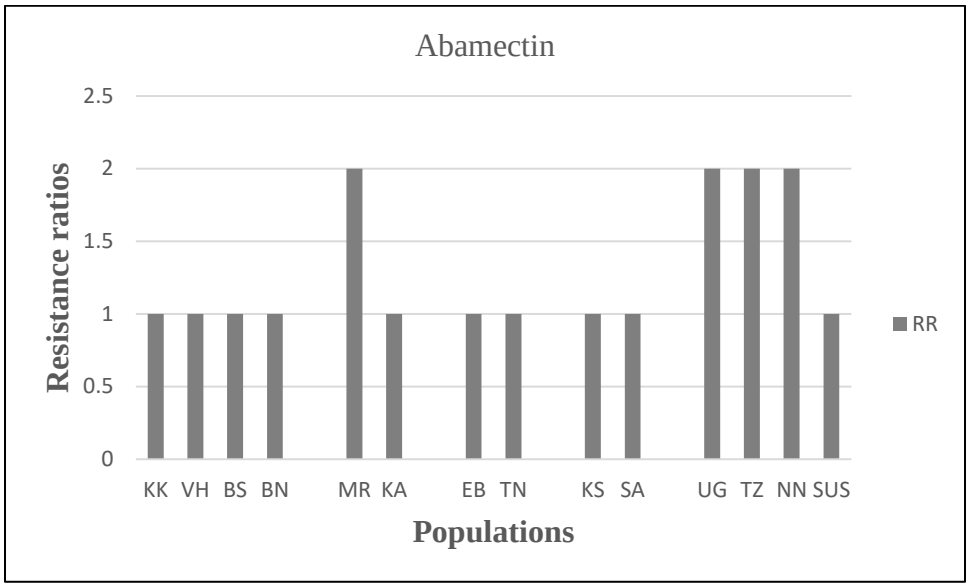


Figure 28: Resistance ratios of different populations of fall armyworm in Kenya to Abamectin

4.2.8 Baseline Susceptibility of FAW Field Populations to Pyridaben

The LC_{50} values to pyridaben varied from 6.96 mg/L (TZ) to 5.33 mg/L (VH), showing 1.3-fold difference (Table 10). The low LC_{50} values indicate that pyridaben is a highly potent active ingredient against fall armyworm. The populations had same resistance ratio of 1 as displayed in figure 29. This suggests that fall armyworm has not developed resistance to pyridaben in Kenya. The slopes values obtained were similar indicating that FAW are heterogeneous in their response to pyridaben.

Table 10: Baseline Susceptibility of FAW Field Populations to Pyridaben (Mitochondrial Complex I Electron Transport Inhibitor)

Region	Strain	N	Slope+- SE	X2(df)	LC_{50}	95% FL	RR
Western	KK	200	4.25+-0.67	0.69(3)	5.60	4.68 - 6.35	1
	VH	200	3.56+-0.63	2.29 (3)	5.33	4.23 -6.18	1
	BS	200	4.01+-0.63	0.25(3)	5.95	5.05 - 6.72	1
	BN	200	4.02+-0.65	2.09(3)	5.71	4.74 - 6.49	1
Central	MR	200	3.66+-0.62	0.12(3)	6.47	5.48 - 7.36	1
	KA	200	4.10+-0.64	0.71(3)	6.44	5.55 - 7.24	1
Eastern	EB	200	4.21+-0.69	1.37 (3)	6.16	5.19 - 6.98	1
	TN	200	4.49+-0.72	0.97 (3)	6.17	5.22 - 6.95	1
Nyanza	KS	200	4.71+-0.72	2.49 (3)	5.57	4.69 - 6.28	1
	SA	200	4.20+-0.69	1.81 (3)	5.33	4.35 - 6.11	1
Rift Valley	UG	200	3.76+-0.63	0.49(3)	6.82	5.86 - 7.72	1
	TZ	200	4.06+-0.73	0.04 (3)	6.96	5.81 - 7.95	1
	NN	200	4.13+-0.65	0.58(3)	6.75	5.85 - 7.57	1
	SUS	200	4.16+-0.68	0.93(3)	4.96	4.02 - 5.70	1

N = Number of insects used

SE= Standard Error

X^2 (df) = Chi square value (Degrees of freedom)

LC_{50}=Median lethal concentration in milligrams per liter

95% FL=95 % Fiducial limits (Confidence intervals)

RR= Resistance ratios, LC_{50} of field population over LC_{50} of SUS strain

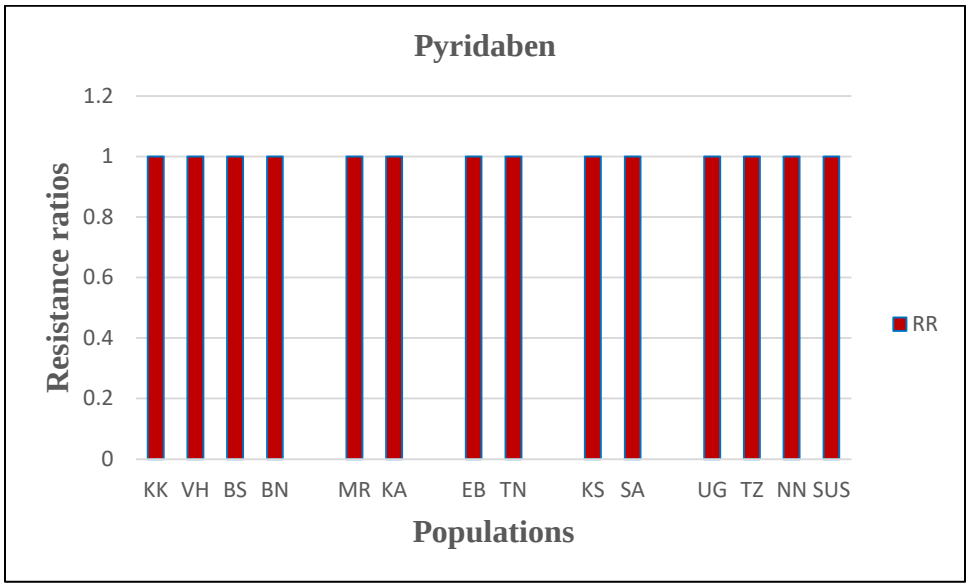


Figure 29: Resistance ratios of different populations of fall armyworm in Kenya to Pyridaben

4.2.9 Baseline Susceptibility of FAW Field Populations to Imidacloprid

Evidenced by similar values of the slopes and the overlapping 95% fiducial limits, all the populations had a similar response to imidacloprid toxicity. LC_{50} values of imidacloprid against the field populations ranged from 1168.39 mg/L for SA strain to 1748.02 mg/L for TZ population (Table 11). The high LC_{50} values suggest that the pest populations are less susceptible to imidacloprid thus less potent in its management. The results displayed very low levels of resistance to this active ingredient as the resistance ratios ranged from 1-2 fold as shown in figure 30.

Table 11: Baseline Susceptibility of FAW Field Populations to Imidacloprid (Neonicotinoid)

Region	Strain	N	Slope+-SE	X2(df)	LC_{50}	95% FL	RR
Western	KK	200	3.94+-0.68	0.36(3)	1397.14	1188.75- 1587.29	1
	VH	200	3.49+-0.61	1.94(3)	1288.41	1082.51 - 1470.58	1
	BS	200	3.60+-0.68	0.37(3)	1476.64	1241.16 - 1701.55	2
	BN	200	3.0+-0.60	0.27(3)	1427.66	1187.70 - 1667.36	1
Central	MR	200	3.91+-0.81	0.51(3)	1654.45	1388.68 - 1916.66	2
	KA	200	4.13+-0.79	0.61(3)	1684.33	1447.96 - 1933.26	2
Eastern	EB	200	3.91+-0.77	1.65(3)	1544.96	1296.75 - 1772.16	2
	TN	200	3.36+-0.72	0.63(3)	1565.02	1276.63 - 1847.47	2
Nyanza	KS	200	4.16+-0.67	0.28(3)	1230.25	1038.01 - 1392.80	1
	SA	200	4.14+-0.70	0.10(3)	1168.40	960.90 - 1336.15	1
Rift valley	UG	200	3.55+-0.70	1.60(3)	1726.10	1481.32- 2026.78	2
	TZ	200	4.04+-0.86	0.21(3)	1748.02	1480.99 - 2027.77	2
	NN	200	3.64+-0.79	0.80(3)	1696.73	1411.02 - 1996.02	2
	SUS	200	4.11+-0.69	0.1(3)	955.68	761.82 - 1103.0	1

N = Number of insects used

SE= Standard Error

X^2 (df) = Chi square value (Degrees of freedom)

LC_{50}=Median lethal concentration in milligrams per liter

95% FL=95 % Fiducial limits (Confidence intervals)

RR= Resistance ratios, LC_{50} of field population over LC_{50} of SUS strain

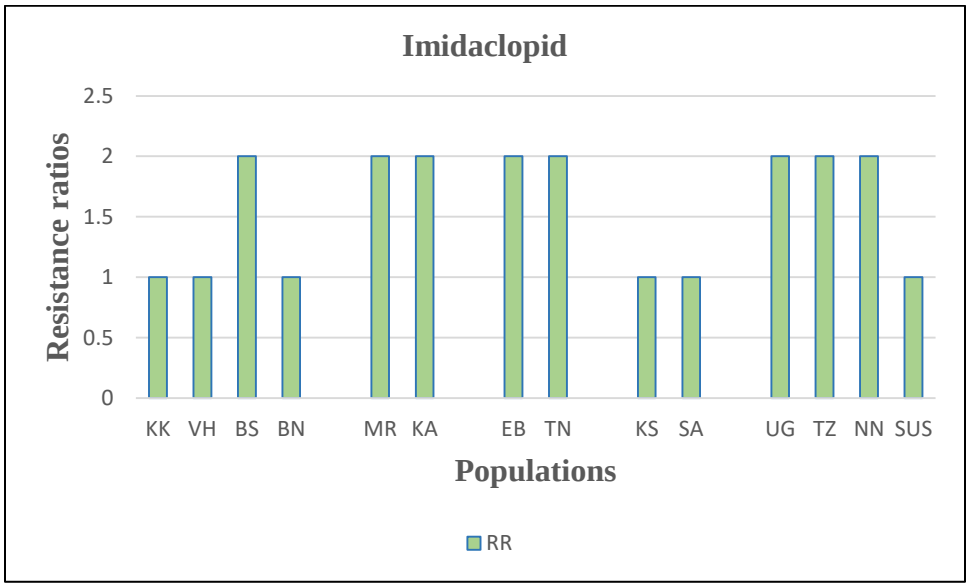


Figure 30: Resistance ratios of different populations of fall armyworm in Kenya to Imidacloprid

4.2.10 Relative Potency of the Nine Active Ingredients

As per the findings of this study, Spinosyns were the most potent active ingredients against all the populations. Spinetoram was more potent than spinosad with relative potency ratio of 11188 and 7079 respectively as shown in figure 31. This indicates that the fall armyworms are highly susceptible to these 2 newer chemistries. All insecticides had a relative potency ratio above 20 except for abamectin (1) and imidacloprid (3). Abamectin having the lowest relative potency ratio was used as the index insecticide to in the calculation of relative potency ratios. The findings show that abamectin is the least efficacious compound hence the field populations are less susceptible to this insecticide. Pyridaben and lufenuron had relatively high ratios hence also relatively effective against

the field populations. This is also evidenced by the lower LC_{50} values from the susceptibility bioassays results.

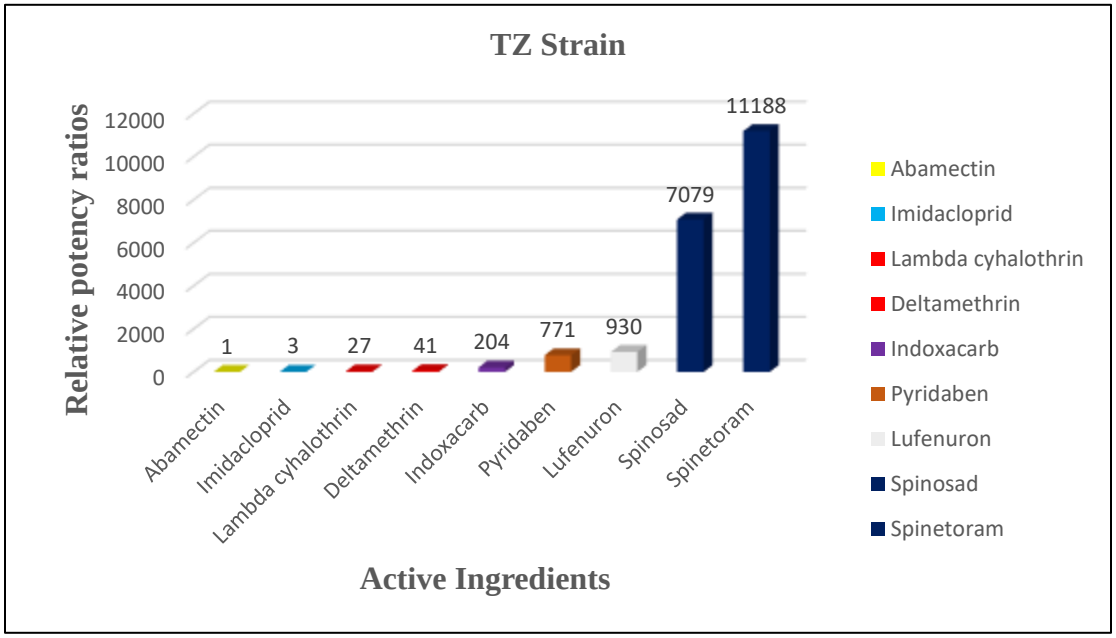


Figure 31: Relative potency ratios of different insecticides against fall armyworm in Kenya, showing the most to the least efficacious insecticide.

4.3 Cross- Resistance Evaluation

The assessment of pairwise correlation coefficients was done between the log LC_{50} values of the tested chemicals for FAW field-collected populations (Table 12). This analysis is a continuation of the first objective results which gives us more insight on how test insecticides correlate with each, to understand the nature, strength and significance of their relationship. The generated knowledge can be used in development of insecticides rotational management strategies without affecting their efficacy due to cross-resistance.

Resistance to lufenuron had a significant correlation with pyridaben resistance (r=0.878, p<0.01), abamectin (r=0.976, p<0.01), imidacloprid (r=0.907, p<0.01), deltamethrin (r=0.959, p<0.01), indoxacarb (r=0.944, p<0.01), spinosad (r=0.912, p<0.01 and spinetoram (r=0.818, p<0.01). Significant correlation was also observed in spinetoram with pyridaben resistance (r=0.943, p<0.01), abamectin (r=0.840, p<0.01), imidacloprid (r=0.848, p<0.01), deltamethrin (r=0.890, p<0.01), indoxacarb (r=0.939, p<0.01) and spinosad (r=0.922, p<0.01). This means that despite of spinetoram having the highest potency, its rotational use with these insecticides may low its efficacy due to cross resistance. Similarly, spinosad had positive significant correlations with pyridaben (r=0.907, p<0.01), abamectin (r=0.936, p<0.01), imidacloprid (r= 0.854, p<0.01), deltamethrin (r=0.935, and indoxacarb (r=0.947, 0.01). Indoxacarb had significant correlation with pyridaben (r=0.973, p< 0.01), abamectin (r=0.952, p<0.01), imidacloprid (r=0.949, p<0.01) and deltamethrin (r=0.973, p<0.01). Significant correlation was also observed in deltamethrin with pyridaben (r=0.940, p<0.01), abamectin r=0.982, p<0.01), and imidacloprid (r=0.931, < p<0.01). Imidacloprid exhibited a strong correlation with pyridaben (r=0.951, p<0.01) and abamectin (r=0.923, p<0.01). Abamectin exhibited significant correlation with pyridaben (r=0.907, p<0.01). However, there was no significant correlation of lambda cyhalothrin with other eight tested chemicals in the collected FAW field populations. This implies that this insecticide can be used in rotational program of FAW management. All the insecticide which had a significant correlation with another insecticide may cause cross-resistance to the second insecticide if used consecutively in rotational program of FAW management.

Table 12: Pairwise Correlation Analysis of the LC_{50} Values for Nine Insecticides in the 13 Field Populations of FAW

	Pyridaben	Abamectin	Imidacloprid	Lambda-cyhalothrin	Deltamethrin	Indoxacarb	Spinosad	Spinetoram
Abamectin	.907**							
Imidacloprid	.951**	.923**						
Lambda-cyhalothrin	0.46	0.432	0.384					
Deltamethrin	.940**	.982**	.931**	0.445				
Indoxacarb	.973**	.952**	.949**	0.441	.973**			
Spinosad	.907**	.936**	.854**	0.389	.935**	.947**		
Spinetoram	.943**	.840**	.848**	0.4	.890**	.939**	.922**	
Lufenuron	.878**	.976**	.907**	0.419	.959**	.944**	.912**	.818**

** Correlation is significant at the 0.01 level (2-tailed)

4.4 FAW Molecular Tolerance Mechanisms Against a Range of Insecticides (q-PCR Results)

After performing comparative CT approach [2^(-ΔΔCT)], the relative fold expression for VGSC Control was 1.04 and for VGSC Treated sample, fold expression was 13.59 as shown in appendix 2. For AChE Control and Treated sample relative fold expression was 1.28 and 34.93 respectively. Insecticide treated samples had 4.90-fold high expression of RyR genes compared to the control samples whose fold expression was 1.14. The positive fold change values which were also greater than 1 indicates that the genes in these target sites were up-regulated in samples exposed to insecticides (treated) than in samples which had no prior exposure to insecticides(control).

The overexpression of these genes at these target sites may be as a result of stress and defence mechanisms for insects against the toxins. The toxins bind to these target regions leading to neurotoxin effects which cause stress, nervous system malfunctioning, paralysis or even death of the insect. Consequently, the insect may overexpress the genes present at these sites leading to more expression of transcripts coding for these target proteins(receptors) so that the neurotransmitters can find enough receptors to bind to so as to sustain the normal functioning of the nervous system during the stress period. This explains why in the current study, transcripts for expression of all the 3 proteins were elevated in the insecticides treated samples than in the untreated samples. The upregulation of the present genes may lead to mutations in coding regions that causes structural changes in these 3 study proteins hence insecticide resistance.

4.5 Molecular Modelling the Target Site Mutations in the Voltage-gated Sodium Channels of FAW Populations

4.5.1 Homology Modelling

The homology model of the fall armyworm VGSCs is largely based on the X-ray structure of Nav1.4-beta1 complex from electric eel (PDB accession number (5XSY). The model of the VGSC used is shown in figure 32.

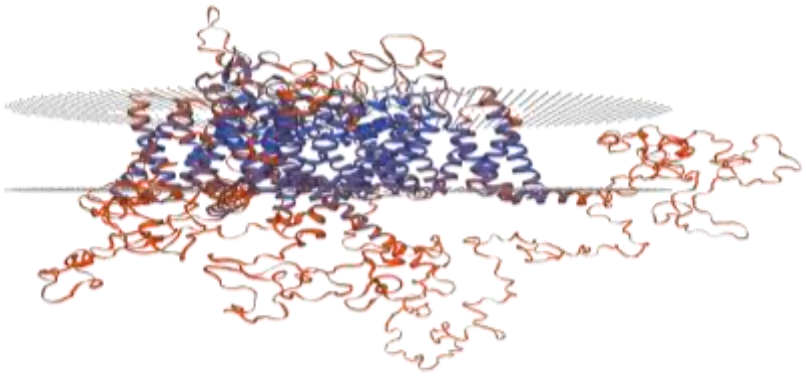
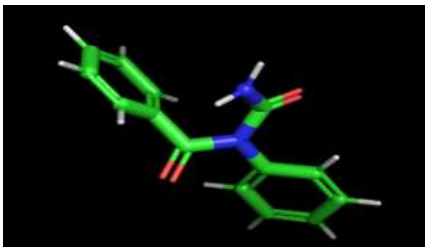


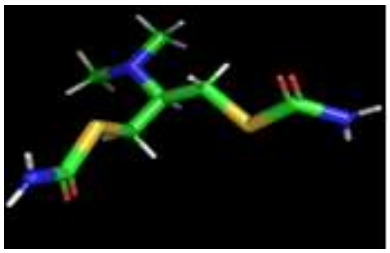
Figure 32: Model of the voltage-gated sodium channel

4.5.2 Automated Docking Predictions

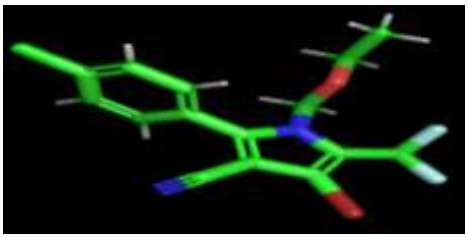
Insecticides have a distinct structure-activity relationship that relates to its physical properties and 3-D configuration of the entire molecule. Figure 33 illustrates the different chemical structures of the nine insecticides that were retrieved from the PubChem database. (https://pubchem.ncbi.nlm.nih.gov/).



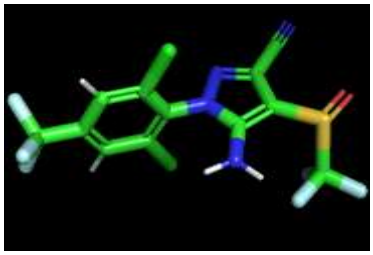
Benzoylphenylurea



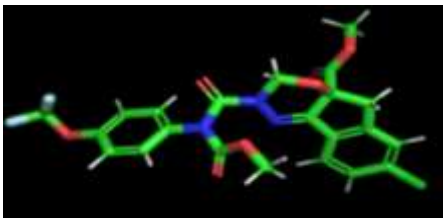
Cartap



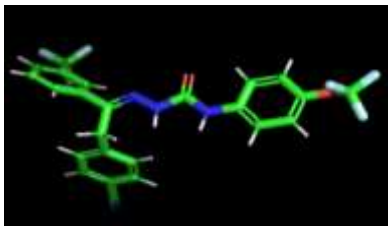
Fipronil



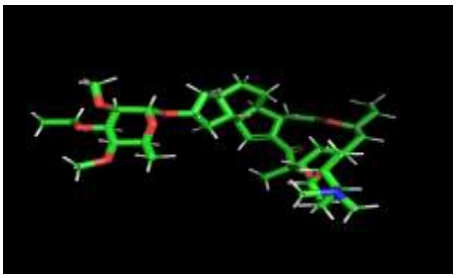
Chlorfenapyr



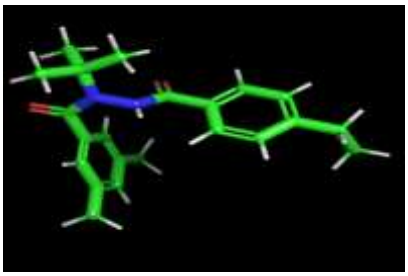
Indoxacarb



Metaflumizone



Spinetoram



Tebufenozide

Figure 33: Chemical structures of the insecticides that were used in the docking.

The program Autodock was utilized in generating docking predictions for the insecticides and the modelled VGSC. The study analyzed the energetically favorable docking predictions (i.e. those with negative values for binding free-energy) to determine the interactions between the insecticides and the residues in the protein model (Table 13).

Table 13: Binding Sites Identified Through the Molecular Docking Process

Insecticide	Amino Acid	Binding Position
Benzoylphenylurea	Tyrosine	476
Cartap	Glutamine	1580
	Tyrosine	433
	Phenylalanine	1579
	Threonine	430
	Threonine	1578
Chlorfenapyr	Serine	1873
Fipronil	Serine	1873
	Tyrosine	1927
Metaflumizone	Alanine	1577
Spinetoram	Glutamine	1580
	Serine	1873
Tebufenozide	Serine	1873
Indoxacarb	Serine	1873
	Tyrosine	1927
	Asparagine	1045
Pyrethroid	Serine	1873

For each insecticide, the Autodock vina software identified 9 potential binding sites. The ranking of the binding sites was based on their affinity (kcal/mol). Visualizations on the pymol software helped us identify the specific amino acids that interacted with the VGSC and the binding site where the interaction was identified. The residue Ser^{1873} stood out with six of the nine insecticides indicating interactions at this position (Table 13). Indoxacarb and pyrethroids, were among the insecticides that indicated interactions with the Ser^{1873} residues. The other identified interacting residues were specific for each insecticide (Figure 34-41).

Binding site between Benzoylphenylurea and the VGSC at position Tyrosine 476 represented by a dotted yellow line (Figure 34). The amino acid was located at the third binding site.

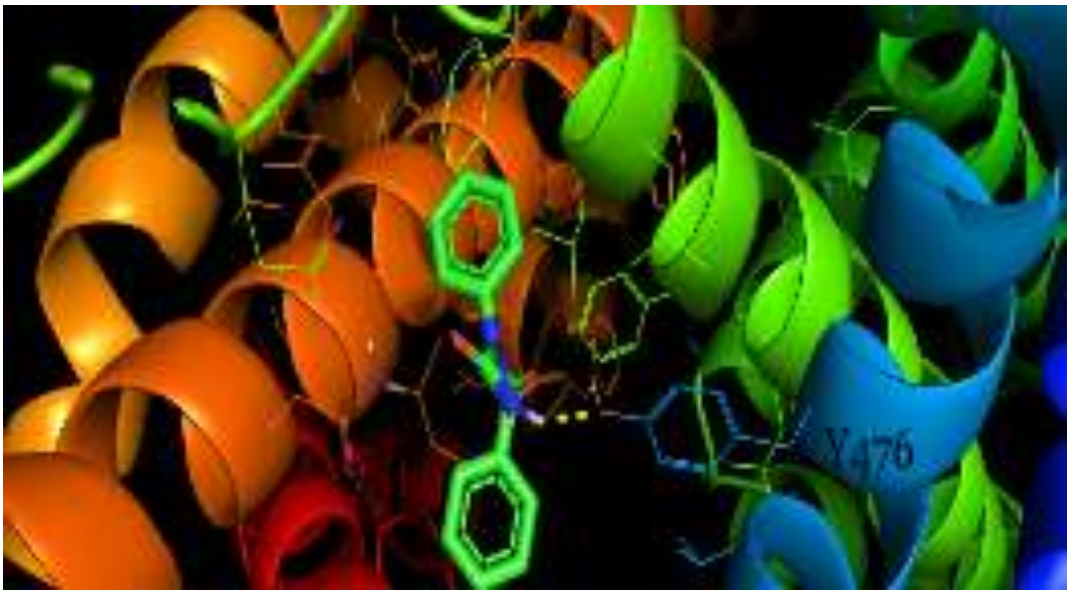


Figure 34: Binding sites for Benzoylphenylurea

Binding site between cartap and the VGSC at positions Tyrosine 433, Glutamine 1580, Tyrosine 433, Phenylalanine 1579, Threonine 430, and Threonine 1578 represented by a

dotted yellow line (Figure 35). These amino acids were identified on the 1st, 2nd, and 3rd binding sites.

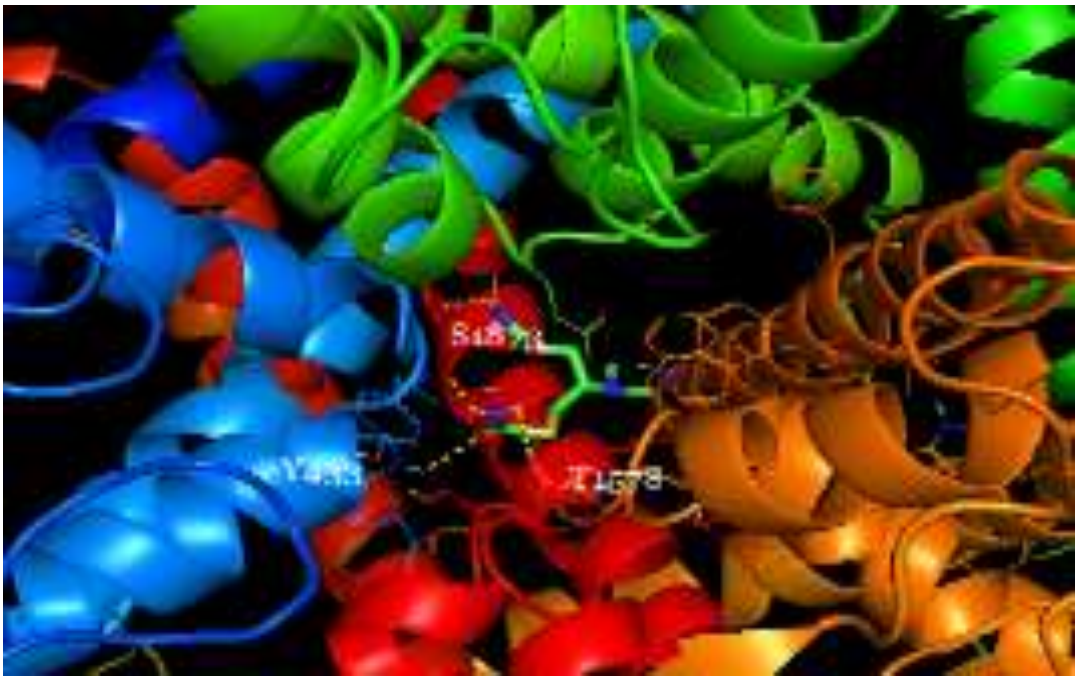


Figure 35: Binding sites for Cartap

Binding site between chlorfenapyr and the VGSC at position Serine 1873 represented by a dotted yellow line as shown in figure 36. The amino acid was identified on the 2nd binding site.

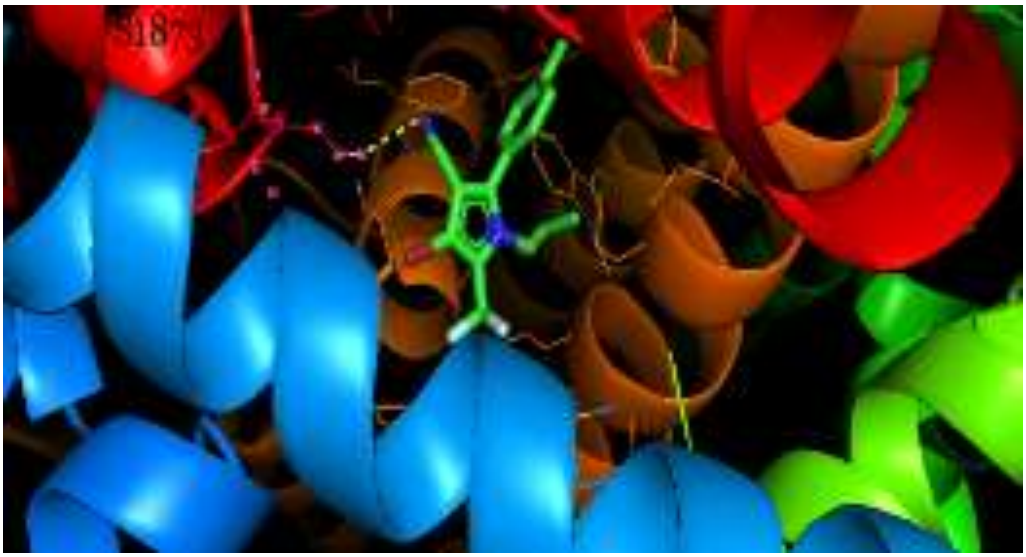


Figure 36: Binding sites for Chlorfenapyr

Binding site between fipronil and the VGSC at positions Serine 1873 and Tyrosine 1927 represented by a dotted yellow line (Figure 37). The protein was identified on the 1st binding site.

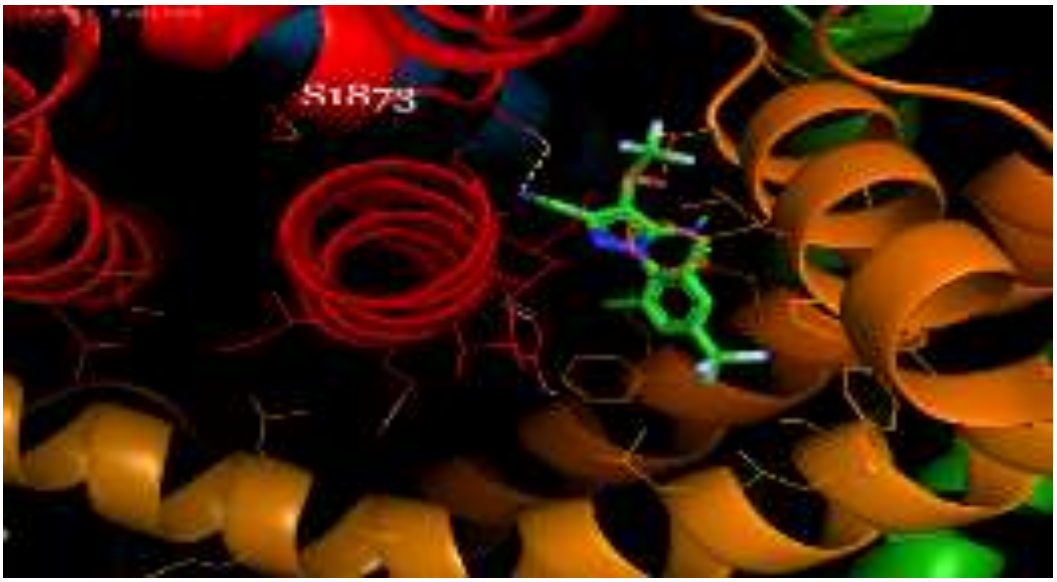


Figure 37: Binding sites for Fipronil

Binding site between indoxacarb and the VGSC at positions Serine 1873, Tyrosine 1927, and Asparagine 1045 represented by a dotted yellow line (Figure 38). The amino acids were identified on the 7th binding sites.

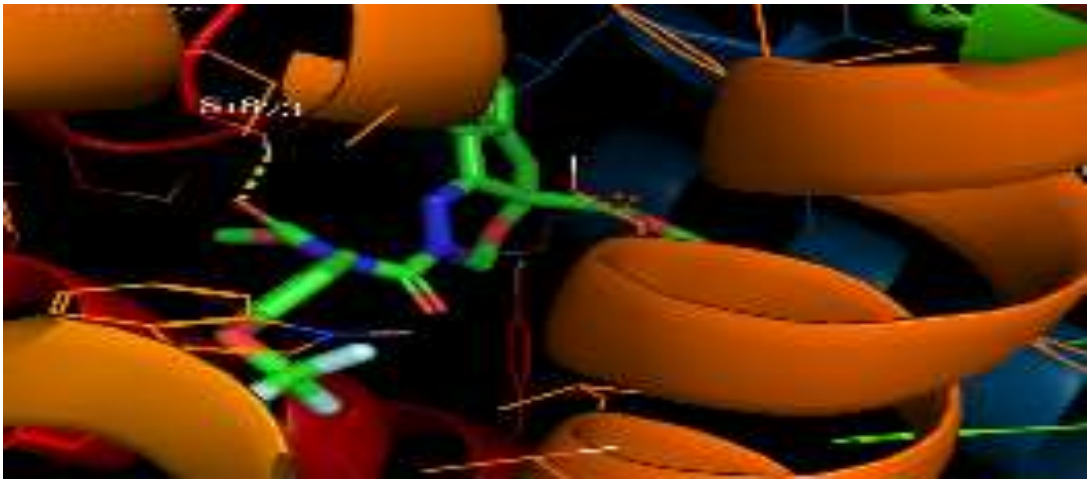


Figure 38: Binding sites for Indoxacarb

Binding site between metaflumizone and the VGSC at positions Alanine 1577 represented by a dotted yellow line (Figure 39). The amino acid was identified on the 8th binding site.

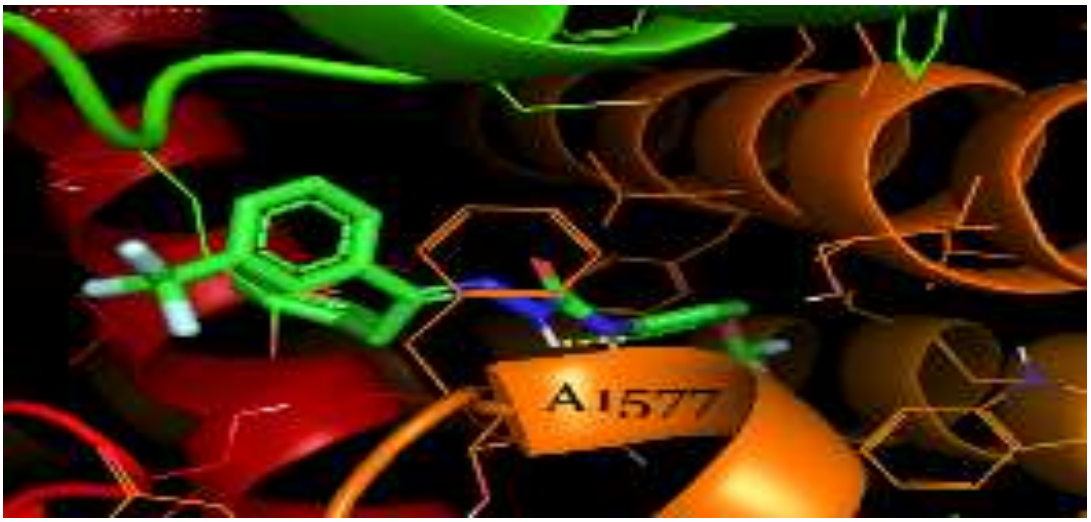


Figure 39: Binding sites for Metaflumizone

Binding site between spinetoram and the VGSC at positions Serine 1873 and Glutamine 1580 represented by a dotted yellow line (Figure 40). The amino acid was identified on the 1st pose.

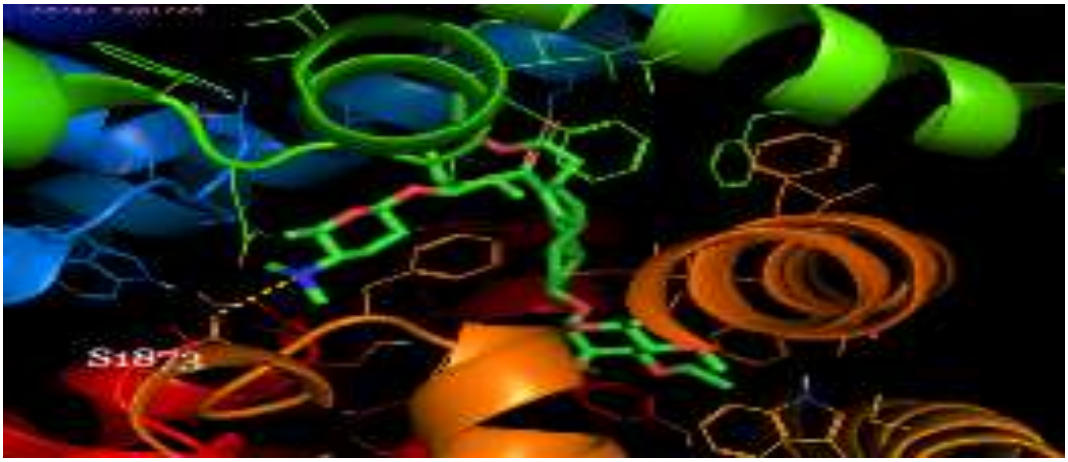


Figure 40: Binding sites for Spinetoram

Binding site between tebufenozide and the VGSC at positions Serine 1873 represented by a dotted yellow line (Figure 41). The amino acid was identified on the 7th binding site.

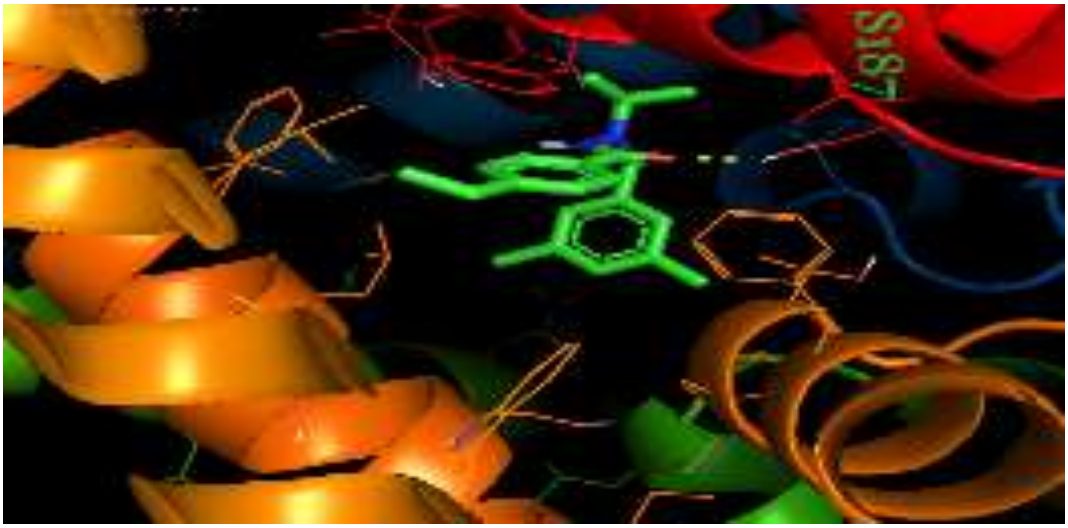


Figure 41: Binding sites for Tebufenozide

Binding site between pyrethroids and the VGSC at position serine 1873(Figure 42) identified on the 1st pose

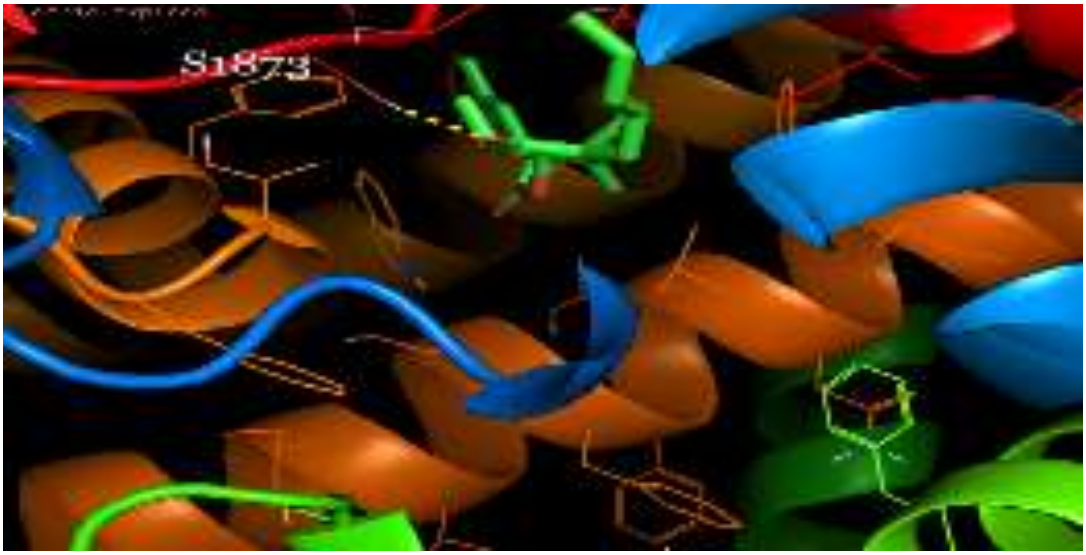


Figure 42: Binding sites for Pyrethroids.

CHAPTER FIVE

DISCUSSION

5.1 Baseline Susceptibility of FAW Populations in Kenya to Nine Insecticides

In the current study we report baseline susceptibility in fall armyworm from Kenya to nine different insecticides having different mode-of action. These chemicals were deltamethrin, lambda cyhalothrin, abamectin, spinosad, spinetoram, lufenuron, pyridaben, imidacloprid and indoxacarb. These insecticides are readily available in the Kenyan market and farmers have been using them to control different pests including the fall armyworm. The findings from this study represent initial efforts to develop baseline data for insecticides with reference data for several commercial insecticides currently used against this pest, in Kenya. In addition, the results obtained suggest that the pattern of response of fall armyworm for each of the nine insecticides used was similar across all the sampled locations. From the results, spinetoram, spinosad, lufenuron and pyridaben exhibited high toxicity to fall armyworm while indoxacarb, deltamethrin, lambda cyhalothrin, imidacloprid and abamectin had low toxicity hence fall armyworm populations were less susceptible to them (Table 3-11).

Spinosyns are allosteric modulators of nicotinic acetylcholine receptors and consist of two active ingredients, spinetoram and spinosad (Okuma *et al*., 2018). For their special mode of action and low toxicity to non-target insect, this group of insecticides has been a vital component in management of fall armyworm (Lira *et al*., 2020). Spinetoram is effective and have been adopted to control fall armyworm in the field (Lira *et al*., 2020). The current study showed that spinetoram has high toxicity (Table 7; Figure 26; Figure 31) against fall armyworm which is consistent with results by Zhao *et al*. (2020) despite of the bioassay

method used being different. Despite of the TZ strain showing a resistance ratio of 3, the populations were still susceptible to spinetoram following the criterion used by Shao *et al*. (2013). In addition, experiments by Hardke *et al*. (2011) also indicated that spinetoram was the most toxic of the insecticides tested against this pest with lower LC_{50} values than the values from findings of this study. Spinosad is also an important tool of controlling fall armyworm in Puerto Rico. In the current study, spinosad was second in terms of efficacy evidenced by the low baseline LC_{50} values (Table 6). The populations showed highly susceptible to spinosyns (1 to 3-fold) as shown in figure 25 and 26 respectively. Similarly, these very low resistance levels were also reported in all Pakistani populations of *Spodoptera litura* tested during 1997–2013 (Ahmad and Gull, 2017). In contrast, a recent study by Gutiérrez *et al*. (2019), the PR (Puerto Rico) population showed a higher resistance ratio (8-fold) for spinosad suggesting that the fall armyworms from Kenya are more susceptible to this active ingredient. This may be because of the intensive application of insecticides in Puerto Rico. A study by Lira *et al*. (2020) reported existence of cross-resistance between spinosad and spinetoram which can jeopardize their excellent efficacy against fall armyworm.

Lufenuron is a Benzoylurea which bind chitin synthase 1 in terrestrial arthropods resulting into inhibition of chitin biosynthesis (Douris *et al*., 2016). In spite of the bioassay method used by Zhao *et al*. (2020) being different from the assay used in this study, the studies presented similar levels of toxicity for lufenuron (Table 8). As shown in figure 26, this compound also exhibited very low resistance levels (1 to 2-fold) thus can also be recommended to be used against fall armyworm. In this study, pyridaben also showed high toxicity (Table 10) to fall armyworm but lower than that of lufenuron hence can be applied

as alternative insecticides to control this pest. Interestingly, resistance ratio for this insecticide was 1 (Figure 29) suggesting that this pest has not developed resistance of this pest to this active ingredient.

Indoxacarb bind and block sodium channels in a slow inactivated states leading to pseudoparalysis (Zhang *et al*., 2016). Present study reveals that the LC_{50} values of indoxacarb were high (Table 5) hence low potency against the pest. Nevertheless, the resistance of pest populations to this compound was found to be very low (1 to 2- fold) as shown in figure 24. A previous study by Deshmukh *et al*. (2020) using the same bioassay method with our study also reported low potency (2-fold) of indoxacarb against fall armyworm collected from unsprayed maize farms in India. Ahmad *et al*. (2018) documented very low resistance of *Spodoptera exigua* to indoxacarb during 1998-2009. Contrarily, a moderate to very high level of resistance of *Spodoptera exigua* from Pakistan and diamondback moth, Plutella xylostella (L.), from China to indoxacarb have been documented ((Ishtiaq *et al*., 2012; Zhang *et al*., 2016).

Pyrethroids target the voltage-gated sodium channels. They act by inhibiting channel deactivation and inactivation thus stabilize their open state, leading to prolonged channel opening (Soderlund, 2017). Pyrethroids are a vital group used in control of fall armyworm in Mexico (IRAC, 2016). In our, study, fall armyworms were less susceptible to lambda cyhalothrin than to deltamethrin. For lambda cyhalothrin, all the field populations had a significantly different response from the SUS population based on the non- overlapping 95% fiducial limits (Table 3). However, in the case of deltamethrin, only VH, BS and SA had comparable susceptibility to SUS population (Table 4). Even though the resistance ratios for the two insecticides was very low (Figure 22,23) the high baseline LC_{50} values

obtained for these two pyrethroids suggest that they may no longer be effective in the management of fall armyworm in the long run the observation also reported by Zhao *et al*. (2020). Previous studies by Carvalho *et al*. (2013) recorded that fall armyworm insensitivity to pyrethroids and organophosphates is brought by target-site mutations. Genes that code for glutathione S-transferases, carboxylesterases and cytochrome P450s enzymes were upregulated in the pyrethroid and organophosphate-resistance strains, hence implicated in metabolic resistance (Carvalho *et al*.,2013).

Imidacloprid acts on insects as an agonist of insect nicotinic acetylcholine receptors (Singh, 2014). From our findings, imidacloprid was the second least potent active ingredient from abamectin, with high baseline LC_{50} values as shown in Table 11. These results suggest that large quantities of imidacloprid are needed to kill half of fall armyworm population. However, the resistance ratio was 1 to 2-fold (Figure 30). Previous studies have documented that various species have developed resistance to imidacloprid, including tobacco whitefly (116-fold), small brown planthopper (18-fold), western flower thrips (14-fold), Colorado potato beetle (110.8-fold), peach aphid (7-fold) and 10-fold resistance ratio in tobacco aphid (Abbas *et al*., 2012)

In this study, abamectin was the least potent insecticide with a potency ratio of 1 as displayed in figure 28. The baseline LC_{50} values were the highest compared to all insecticides used in this study (Table 9) suggesting that the fall armyworm from Kenya are highly less susceptible to this compound hence may not be recommended for use in its management. Fall armyworm populations from Muranga (MR), Uasin-gishu (UG), Trans-Nzoia (TZ) and Nandi (NN) counties had significantly different response with the susceptible population based on non-overlapping 95% fiducial limits. Nonetheless, the

populations exhibited very low resistance ratios (1 to 2-fold) to abamectin (Figure 28). Ahmad *et al*. (2018) also reported moderate resistance of *Spodoptera exigua* to abamectin. In *Plutella xylostella*, a point mutation associated with glutamate-gated chloride channel was implicated with abamectin resistance (Ahmad *et al*., 2018). Increased detoxification by mixed function oxidases and carboxylesterases underlie its resistance in *Plutella xylostella* from China (Ahmad *et al*. 2018; Wang *et al*., 2016).

5.2 Cross Resistance Evaluation

Cross-resistance is a phenomenon in insects that renders the selecting insecticide, with or without a similar mode of action, ineffective (Zhang *et al*., 2017). It can be caused by target-site mutations, over-expression and increased activities of esterase, GSTs and cytochrome P450 monooxygenase (Zhang *et al*., 2017). Knowhow on cross-resistance is critical in adoption of resistance management strategies, which can assist in delaying occurrence of resistance and preserve insecticide potency (Afzal *et al*., 2018). A negative correlation was reported by Zhao *et al*. (2020) between spinetoram and lambda cyhalothrin (R=-0.559). The current study revealed a weak correlation between the two chemicals (Table 10). Lambda cyhalothrin and indoxacarb did not show any correlation (Zhang *et al*., 2020). We report no correlation between the two chemicals an indication of a lack of cross-resistance. Muraro *et al.* (2021) observed low levels of cross-resistance of abamectin to lambda cyhalothrin, indoxacarb, and spinetoram. Our study revealed a strong correlation of abamectin to indoxacarb and spinetoram, but a weak correlation to lambda cyhalothrin (Table 12). Lira *et al*. (2020) reported the existence of cross-resistance between spinosad and spinetoram which can jeopardize their excellent efficacy against *Spodoptera frugiperda* in the field. A recent study by Stacke *et al*. (2020) reported that Lambda-

resistant strain showed low cross-resistance to deltamethrin (6.2-fold) in soybean looper, *Chrysodeixis includens* (Lepidoptera: Noctuidae). Our study revealed a weak correlation (R=0.445) between deltamethrin and lambda cyhalothrin.

In our current research analysis of pairwise correlation of log LC_{50} values found levels of cross-resistance among the eight insecticides tested. However, lambda cyhalothrin exhibited weak correlations to the eight insecticides tested implying a lack of cross-resistance to them.

5.3 Molecular Resistance Mechanisms of FAW

In the current study, relative quantification of VGSC, AChE and RyR genes both for the treated and untreated samples was done to give more details on the existence of resistance at target-sites and monitor the frequency of mutated sites in fall armyworm. The VGSC acts as a target site mostly for pyrethroids while acetylcholinesterase is targeted by carbamate and organophosphate insecticides. Ryanodine receptor is a molecular target site for diamide insecticides which bind and affect the neuromuscular functioning hence killing the pest. Mutations in the amino acids present at these sites affects the binding of these insecticides hence conferring resistance by rendering the insecticides less active (Boaventura *et al.,* 2020). It remains unclear whether its resistance or poor application of insecticides that leads to low efficacy of various insecticides in Africa. Therefore, understanding the genetic resistance mechanisms for FAW is vital for development of successful resistance management strategies.

From the current study results, the fold changes obtained for the treated samples were greater than 1, that is, 13.59, 34.93 and 4.90 for VGSC, AChE and RyR respectively as shown in appendix 2. This means that the expression of genes in the VGSC, AChE and

RyR proteins were all up-regulated. Gene amplification and overexpression may lead to structural changes in proteins, changing the receptors needed for toxins to attach and kill pests hence linked to target insensitivity (Vontas *et al*., 2005). These mutations are known to cause insecticide resistance in insects. The disruption/ interactions of resistance causing genes at the level of regulation, hints on how there may be increased levels of resistance in insects (Liu, 2015).

Studies have reported the occurrence of point mutations in these regions, that have a possibility of conferring insecticide resistance. Study done by Boaventura *et al*. (2020) reported the existence of F290V mutation in AChE with a relatively high frequency, from Kenyan FAW populations. This explains the low efficacy of the older active chemical compound (e.g. carbamates and organophosphates). Yainna *et al*. (2021) reported that fall armyworm populations collected from India exhibited the presence of F290V mutations in the acetylcholinesterase and A201S mutation in less prevalence. Genotyping studies carried out by Boaventura *et al*. (2020) presented the presence of A201S, G227A and F290V mutations targeting AChE in FAW from Brazil.

I4790M mutation in the ryanodine receptor has been implicated with causing cross-resistance among diamide insecticides used against FAW(Chlorant-R) populations in Brazil (Kulye *et al*., 2021). Mutation I4790M is reported to render resistance in most lepidopteran pests (Richardson *et al*., 2020). G4946E mutation has been shown to increase levels of diamide resistance in *T. absoluta* and *P. xylostella* pests (Roditakis *et al*., 2017). These mutations make ryanodine receptor to be less sensitive to diamide insecticides hence the pests develop resistance. Carvalho *et al*. (2013) detected the presence of VGSC mutations (T929I, L932F and L1014F) in pyrethroid-resistant *S. frugiperda* from Brazil.

Therefore, VGSC, RyR, and AChE target sites play a crucial role in potency of the active compounds used to control pests. Mutations at these sites lower the potency of theses insecticides hence existence of insecticide resistance insects.

5.4 Molecular Modelling of Target-site Mutations in the Voltage-gated Sodium Channels

Voltage-gated sodium channels are essential integral transmembrane proteins, crucial for electrical signaling in excitable cells. Their critical role in excitability has made them a target site of multiple neurotoxins. In addition, they are also the primary target of modern sodium channel binding inhibitors. The intensive insecticides application has, however, lead to resistance development against common insecticides. Knockdown resistance caused by multiple mutations in the insecticide binding sites of VGSCs is a major mechanism of insecticide resistance among different insects. Insects that exhibit kdr show reduced target-site sensitivity to insecticides targeting sodium channels rising from one or more point mutations. Understanding common insecticide binding sites for different classes of insecticides is an important step in finding a lasting solution to the growing menace of insecticide resistance. The model of the VGSC used is shown in figure 32. Different chemical structures of the nine insecticides that were retrieved from the PubChem database are shown in figure 33. Ten different amino acid residues that showed interactions with the insecticides under study were identified from analyses of the current study (Table 13, Figure 34-42). Residue Ser^{1873} indicated the most frequent interactions, with 6 of the 9 insecticides used indicating interactions. The residue formed close (<4 Å) binding contacts with the analyzed insecticides. Super kdr resistance has previously been attributed to Met918, Thr929, Leu925, and Leu932 (O'Reilly *et al.,* 2006). None of these residues

indicated any interactions with the two SCBIs and pyrethroid used in this study. Results from the current study could indicate that the pyrethroid, metaflumizone, and indoxacarb have different target sites that allow them to interact with the fall armyworm's VGSC. The results also confirm that the mutations previously reported have influenced the insecticides binding affinity, resulting to insecticide resistance. This could be attributed to a lack of interactions in the previously identified binding residues. Residues Val^{410} and Leu^{1014} known for kdr-type resistance to DDT and pyrethroids were also not picked up by this study (O'Reilly *et al.*, 2006).

Of the 9 insecticides analyzed in the present study, cartap indicated the highest number of binding sites in the VGSCs. The insecticide successfully interacted with five different amino acids, including Gln 1580, Tyr 433, Phe 1579, Thr 430, and Thr 1578. These interactions were identified in pose 4 of the docking results which had a binding affinity of -4.2kcal/mol and an rmsd value of 4.712. This insecticide is known to cause neurotoxicity among insects (Liao *et al*., 2003). Interactions with the VGSCs have, however, not been reported by previous studies. Residue Phe^{1579} has been previously been described as an essential determinant of SCBIs binding and mode of action (von Stein *et al.,* 2013). Mutations of this residue usually interferes with the binding ability of the sodium channel inhibitors (SCIs) drugs to its receptors (Mike and Lukacs, 2010). Such results indicate the possibility of multiple mode of actions for this class of insecticides. We suggest that further experimental studies be performed to look at the possibility of cartap actually binding to VGSCs and effecting its toxicity through this mode of action. These studies could also be extended to the other classes of insecticides which indicate a different mode-of action to that of the SCBIs. Indoxocarb, a different class of insecticides, indicated binding in three

different amino acids, including Ser 1873, Ty 1927, and Asp 1045. Resistance to this insecticide has previously been attributed to Ser^{989} and Val^{1016}, both of which did not indicate any interactions from analyses of the current study (Leticia *et al.,* 2017). The different interacting residues in our context could probably indicate alternative binding sites of the insecticides to the VGSCs. Studies on the mutant insects could help to confirm the efficaciousness of these binding sites. Serine 1873 was a highly targeted binding site with 6 of the 9 insecticides indicating binding interactions with the amino acid. This binding position has, however, not been implicated in mutations that are known to cause kdr among fall armyworm insects.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The major challenge affecting agricultural production is the development of insecticides that will concur resistance in the world today. This study reports the initial efforts to develop baseline susceptibility data of field FAW populations from different counties in Kenya to various synthetic insecticides found readily on the Kenyan market. Some of results from baseline susceptibility and cross-resistance study may not correlate with the recommended concentration use in the field by respective manufacturers, as FAW may have developed insecticide resistance for instance abamectin. The current status of FAW susceptibility to various insecticides applied during the study revealed that the farmers could still use the existing chemicals and maintain effective control of the fall armyworm. The study generated new knowledge and concluded as follows;

1. Spynosyns, lufenuron and pyridaben exhibited high toxicity to FAW while pyrethroids, imidacloprid and abamectin were the least potent. Lambda cyhalothrin can be used in rotational programs to as it exhibited weak correlation with other insecticides.
2. Lower quantities of relative transcripts and the positive fold changes in expression validates mutations in VGSCs, AChE and RyR conferring FAW resistance.
3. Residue Ser^{1873} indicated the most frequent interactions. Ser 1873, Ty 1927, and Asp 1045, were the new mutations attributed to resistance to indoxacarb. Cartap had the highest number of binding sites at VGSCs which has not been reported before.

We suggest;

1. Further studies on FAW baseline susceptibility against other insecticides in market.
2. Further studies on other resistance mechanisms to various insecticides by FAW and whole transcriptome analysis of VGSCs, RyR, and AChE to fully understand resistance.
3. Further studies on modelling of AChE and RyR regions to identify mutations conferring resistance to the insecticides used against FAW.

6.2 Recommendations

The following recommendations may be adopted to give an inclusive picture of the baseline susceptibility, resistance of FAW in Kenya to synthetic insecticides used against this pest and molecular mutations conferring resistance at a target-site. This will help in controlling the pest and monitoring resistance. We recommend;

1. Application of spinosyns and lufenuron insecticides in control of FAW as they had high potency. This will help reducing purchase cost and crop damage on staple crops (SDGs N0. 2, Zero hunger). We recommend that lambda cyhalothrin may be rotated with other insecticides in the management program of the FAW.
2. Researchers to determine more target sites which new compounds may target to cope up with the evolvement of resistance at VGSCs, AChE, and RyR target sites.
3. The manufacturing companies to consider making new chemicals targeting residue Ser 1873 as it indicated the most frequent interactions with VGSC. This may increase the efficiency of less potent insecticides.

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APPENDICES

Appendix 1: Fall Armyworm Strains Used in the Bioassays

Region	Population	Strain Code
Western	Kakamega	KK
	Vihiga	VH
	Busia	BS
	Bungoma	BN
Central	Muranga	MR
	Kiambu	KA
Eastern	Embu	EB
	Tharaka-Nithi	TN
Nyanza	Kisumu	KS
	Siaya	SA
Rift Valley	Uasin-Gishu	UG
	Trans Nzoia	TZ
	Nandi	NN
	Susceptible	SUS

Appendix 2: q-PCR Comparative CT Approach ($2^{-\Delta\Delta CT}$) Analysis Output

HOUSEKEPPING GENE			**GENE OF INTEREST**								**FOLD EXPRESSION**
SAMPLE	CT	Avg CT		SAMPLE	Avg CT	dct	ddct	2^-ddct			
Untreated	20.18		VGSC	untreated	31.55	11.25	-0.06	1.04			
Untreated	20.63			untreated	32.17	11.87	0.56	0.68			
Untreated	20.09	20.3		untreated	31.11	10.81	-0.50	1.41		Control	1.04
Treated	20.34			Treated	27.15	6.58	-4.73	26.54			
Treated	20.81			Treated	28.57	8	-3.31	9.92			
Treated	20.56	20.57		Treated	29.77	9.2	-2.11	4.32		Treated	13.59
			ACHE	Untreated	33.48	13.18	11.85	0.00			
				Untreated	32.4	12.1	0.25	0.84			
				Untreated	30.56	10.26	-1.59	3.00		Control	1.28
				Treated	28.74	8.17	-3.68	12.79			
				Treated	29.26	8.69	-3.16	8.92			
				Treated	26.04	5.47	-6.38	83.09		Treated	34.93
			RYR	Untreated	31.93	11.63	0.91	0.53			
				Untreated	30.1	9.8	-0.92	1.90		Control	1.14
				Untreated	31.04	10.74	0.02	0.99			
				Treated	29.33	8.76	-1.96	3.90			
				Treated	28.23	7.66	-3.06	8.36			
				Treated	30	9.43	-1.29	2.45		Treated	4.90
				VGSC	AVG DCT CONTROL	11.31					
				ACHE	AVG DCT CONTROL	11.8467					
				RYR	AVG DCT CONTROL	10.7233					

Appendix 3: Approval by Institutional Ethical Review committee

MASINDE MULIRO UNIVERSITY OF SCIENCE AND TECHNOLOGY
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Institutional Ethics Review Committee (IERC)

Ref: MMU/COR: 403012 Vol 3 (01)

Date: 24th May, 2021

Savinda Gichere,
Masinde Muliro University of Science and Technology,
P.O. Box 190-50100,
Kakamega.

Dear Ms Gichere,

RE: Target Site Mutations, Baseline Susceptibility and Gross Resistance Evaluation of Fall Armyworm Infecting Maize in the Lake Basin Region. - MMUST/IERC/194/2021

Thank you for submitting your proposal entitled as above for initial review. This is to inform you that the committee conducted the initial review and approved (with no further revisions) the above Referenced application for one year.

This approval is valid from **24th May, 2021** through to **24th May, 2022**. Please note that authorization to conduct this study will automatically expire on by **24th May, 2022.** If you plan to continue with data collection or analysis beyond this date please submit an application for continuing approval to the MMUST IERC by **24th April, 2022.**

Approval for continuation of the study will be subject to submission and review of an annual report that must reach the MMUST IERC Secretariat by **24th April, 2022.** You are required to submit any amendments to this protocol and any other information pertinent to human participation in this study to MMUST IERC prior to implementation.

Please note that any unanticipated problems or adverse effects/event resulting from the conduct of this study must be reported to MMUST IERC. Also note that you are required to seek for research permit from NACOSTI prior to the initiation of the study.

Yours faithfully,

Dr. Gordon Nguka (PhD)
Chairman, Institutional Ethics Review Committee

Copy to:
- The Secretary, National Bio-Ethics Committee
- Vice Chancellor
- DVC (PR&I)