DIVERSITY AND PERFORMANCE OF EGG PARASITOIDS OCCURING ON FALL ARMYWORM (*SPODOPTERA FRUGIPERDA* J.E SMITH) ON MAIZE IN KENYA

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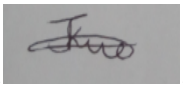
Signature ……  ……Date …01/07/2021.....

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DEDICATION

I dedicate this thesis to my dear parents, Mr. John Kibii Rutto and Mrs. Rose Tarkok Kibii; my son, Emmanuel Kibet; my uncle, Mr. Willian Chelimo and his family; my loving sister, Mrs. Valens Bett, my brothers and sisters, brothers and sisters' in-laws, friends and classmates for their continued support, encouragement and prayers during my study. I lack words that can express my gratitude to you, God bless you all.

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ACRONYMNS AND ABBREVIATIONS

NCBI	National Center for Biotechnology Information
FAW	Fall armyworm
BLAST	Basic Local Alignment Search Tool
PCR	Polymerase chain reaction
DNA	Deoxyribonucleic acid
FAO	Food and Agricultural Organization
CO1	Cytochrome oxidase 1
KALRO	Kenya Agricultural and Livestock Research Organization
MoALF	Ministry of Agriculture, Livestock and Fisheries
MLND	Maize Lethal Necrosis Disease
MSV	Maize Streak Virus
CABI	Center for Agriculture and Biosciences International
ICIPE	International Center for Insect Physiology and Ecology
IPM	Integrated Pest Management
US$	United State Dollar
KOH	Potassium Hydroxide
SPSS	Statistics For Social Sciences
ANOVA	Analysis of variance
NCPB	National Cereals and Produce Board
ML	Maximum likelihood
GLM	Generalized Linear Model
FAOSTAT	Food and Agriculture Organization of the United Nations
UK	United Kingdom
RH	Relative Humidity

GENERAL ABSTRACT

Fall armyworm, *Spodoptera frugiperda* (J.E Smith) (Lepidoptera: Noctuidae) is an insect of economic importance, indigenous to tropical and sub-tropical regions of America. This pest mainly attacks maize crop and it also has a wide host range. Characteristic damage symptoms on maize crop include ragged feeding, windowing and short holes on maize leaves and presence of moist saw dust-like frass found on top surface of maize leaves and whorl of the crop. *Trichogramma* and *Telenomus* species are egg parasitoids which are commonly used worldwide to keep lepidopteran pest under check. This study aimed at carrying out field survey across selected agro-ecological zones in Kenya, collect egg parasitoids of FAW and determine the exact native parasitoid species using morphological and molecular techniques and determine their effectiveness under laboratory condition. A field survey of egg parasitoids of FAW was conducted in eighty farms in five counties across different agro ecological zones namely, Kilifi, Kwale and Taita Taveta (coastal lowlands), Makueni (midland) and Kirinyaga (highland). About fifty maize plants (from young maize crop to before flowering) per field were randomly searched for FAW egg masses adopting a "W" pattern of sampling. Fall armyworm egg masses collected were put in glass vials and taken to the International Centre for Insect Physiology and Ecology (*icipe*). Egg masses were incubated under room temperature, 25 ± 1°C, 12:12 h (L: D) photoperiod and 60–70% relative humidity (RH) in the laboratory to observe for emergence of parasitoids or FAW larvae and percent parasitism determined. The egg parasitoids recovered from the field were identified using both morphological and molecular techniques to determine the native parasitoid species of FAW present in Kenya. Morphological characterization was done based on antennal type, while molecular characterization involved the use of LCO/HCO primer targeting mitochondrial cytochrome oxidase 1

(COI) barcode gene region. Colonies of FAW, factitious host, *Corcyra cephalonica* (Stainton) and the egg parasitoids were established in the laboratory. The egg parasitoid, *Telenomus remus* Nixon (Platygastridae: Hymenoptera) was raised using FAW eggs, while *Trichogramma chilonis* Ishii (Trichogrammatidae: Hymenoptera) was raised using factitious eggs, *C. cephalonica* and FAW eggs. Subsequently, performance of egg parasitoid species on FAW eggs was assessed under controlled conditions in the laboratory. Fall armyworm egg densities (20, 40 and 60) were used as treatments and exposed to an individual mated female parasitoid (*T. remus* or *T. chilonis*) for 24 hours. Data on percent parasitism, FAW emergence, egg mortality and sex ratio were recorded. Sex ratio was determined after emergence; whereby adult parasitoids were killed by freezing them at 0˚C for 5 minutes. They were sexed by observing individual adults using a dissecting microscope (Leica Laz EZ), at magnification of 10x, and this was based on the antennal characteristics. Field data collected was analyzed using GLM with binomial distribution while data collected from the assessment of egg parasitoids under laboratory conditions was analyzed using one-way Analysis of Variance (ANOVA) and the mean separation was done using Tukey multiple comparison test. The two native parasitoids species to Kenya, namely: *T. remus* and *T. chilonis* were key egg parasitoid species recovered from the FAW eggs. Diversity of egg parasitoids and percent parasitism was higher in midland and highland than lowland regions. Field percent parasitism significantly varied with altitudes for both parasitoid species where it ranged from 4.4 to 13.2% for *T. chilonis* and 0 to 21.4% for *T. remus*, with the latter being predominant. Percent parasitism, FAW emergence, egg mortality and sex ratio varied significantly per female parasitoid species and with FAW egg densities offered. More female parasitoids emerged from the FAW eggs which is beneficial in relation to biological control. However, the male parasitoids were

few but could still mate with many females. Under controlled conditions, 57 to 96% and 53 to 90% of FAW eggs were either killed or parasitized by *T. remu*s and *T. chilonis* respectively. Egg parasitoid species kill the host eggs by attacking it more than once (super parasitism). Sex ratio (% female progeny) varied significantly among the parasitoid species with *T. remus* recording the highest sex ratio (83.3%). The parasitoids *T. remus* and *T. chilonis* could be adopted as biocontrol agents in the management of FAW through conservation or augmentative release.

Key words: *Telenomus, Trichogramma,* Parasitism, Factitious, Egg density

CHAPTER ONE

INTRODUCTION

1.1 Background information

Maize, *Zea mays* L. is the major staple food crop cultivated mainly by small holder farmers in diverse agro ecological zones of Sub-Saharan Africa (SSA) (FAO, 2017a). Majority of Kenyan farmers mainly cultivate maize under rain-fed conditions, while it is also grown under irrigation to a much lesser extent (FAO, 2017a). Maize cultivation is challenged by both biotic and abiotic constraints. Examples of abiotic constraints include: (i) frequent famine and poor distribution of rainfall due to change of climate which result into total or partial crop failures (Cairns *et al.*, 2013), (ii) decline of soil quality and fertility due to unsustainable farming practices, (iii) low fertilizer use (soil amendments) and (iv) slow rates of implementation of better varieties, recent technologies and appropriate agronomic practices; all these leading to poor yields (MoALF, 2017).

Biotic factors include disease causing pathogens and parasitic weeds. Diseases of maize crop caused by fungus includes; Anthracnose caused by *Colletotrichum graminicola* (Ces) G.W. Wilson; Cercospora leaf spot, *Cercospora zeae-maydis* Tehon & E.Y. Daniels; Common rust, *Puccinia sorghi* Schwein; downy mildews, *Peronosclerospora sorghi* (W. Weston & Uppal) C.G. Shaw; Common smut, *Ustilago zeae* (DC.) Corda and Giberrella stalk and ear rot, *Gibberella zeae* (Schwein.) Petch. Bacterial diseases of maize include; Bacterial leaf blight, *Acidovorax avenae subsp. Avenae* (Manns); Bacterial stalk rot, *Erwinia chrysanthemi*; Goss's bacterial blight, *Clavibacter michiganensis* (Vidaver & Mandel) and Stewart's wilt (*Erwinia stewartia syn Pantoea stewartia*) (E.F. Smith) Dye.

Viral diseases of maize crop are; *Maize streak virus* (MSV), *Maize dwarf mosaic* (*Maize dwarf mosaic virus*) (MDMV**)** and recently *Maize chlorotic mottle virus* (MCMV) was reported in Kenya affecting maize crop. In synergy with maize infecting Potyviruses, they caused Maize Lethal Necrosis Disease (MLND) which causes chlorotic mottle on the leaves. The weeds affecting maize crop is mainly purple witch weed (*Striga hermonthica*), an invasive parasitic weed which inhabit maize roots and suck nutrients causing yield losses.

Maize crop and its plant parts are attacked by various pests. The roots are attacked by root knot nematode, *Meloidogyne incognita* (Kofoid & White) Chitwood; white grubs, *Phyllophaga* spp. and rootworms, *Diabrotica* sp. Maize seeds are attacked by seed-corn maggots, *Delia platura* (Meigen). The maize leaves are attacked by corn leaf aphids, *Rhopalosiphum maidis* (Fitch), grasshoppers, various thrips species, various spider mite species and flea beetles, *Chaetocnema pulicaria* Melsheimer. The maize stems are attacked by cut worms, *Agrotis ipsilon* (Hufnagel); maize stalk borer, *Busseola fusca* Fuller; the spotted stalk borer, *Chilo partellus* (Swinhoe) and termites, *Coptotermes* spp. The maize ears and tassels are attacked by *A. ipsilon* and Corn earworm, *Helicoverpa zea* (Hübner).

Apart from the losses which result before harvest, significant losses after harvest (up to 80%) have also been recorded. In the tropics due to larger grain borer, *Prostephanus truncatus* (Horn); the grain weevil, *Sitophilus zeamais* Motschulsky; Angoumois grain moth, *Sitotroga cereallela* (Olivier) and the lesser grain weevil, *Sitophilus oryzae* (Linnaeus) result to approximate loss of 20-30% and this impacts food safety and income generation negatively (Shiferaw *et al.,* 2011). Despite several of the above

destructive pests, the most recent biotic threat to food safety of many people is the Fall armyworm, *Spodoptera frugiperda* (J.E. Smith), (FAO, 2019). It occurs in many areas growing maize and causes damage to plants from the vegetative to reproductive stages (Thereza *et al.,* 2013) which result to decreased grain yield amounting to 55% and up to US $ 400 million annual yield loss.

In Kenya, this pest was first reported in early 2017 in Trans Nzoia county in an off-season maize crop (MoALF, 2017). The initial counties affected were Trans Nzoia, Busia, Bungoma, Nandi and Uasin Gishu (FAO, 2017a). The current reports show that FAW has been reported in 43 out of 47 counties (FAO, 2019). Fall armyworm larvae is the most destructive stage attacking the growing points of young maize (whorl) and burrowing in the cobs in older crop, hence affecting negatively the yield, quality and quantity (Burkhardt, 1952; FAO, 2018; Tambo *et al.*, 2019).

Egg parasitoids are used during inundative biocontrol programs all over the world (Pomari *et al.*, 2012). *Telenomus* and *Trichogramma* sp. have been found attacking various lepidopteran pests of economic importance. These egg parasitoids have been revealed to parasitize eggs of FAW on maize crop in many parts of the Americas, for example, *T. remus* in Brazil and Venezuela (Cave, 2000). *Telenomus* spp. possess characteristics of high reproductive rate which makes it a suitable agent for biocontrol of lepidopteran pests, especially those which belong to *genus Spodoptera* (Pomari *et al.,* 2012). On the other hand, *Trichogramma* spp. are inexpensive to use since they can be mass reared using factitious host like rice meal moth, *C. cephalonica*. Different *Trichogramma* spp. has been reported to attack FAW eggs on maize crop. For example, *T. demoraesi* (Zucchi) in Chile, *T. minutum* Riley in Nicaragua and *T. pretiosum* (Riley)

in Brazil (Molina-Ochoa *et al.,* 2009; De Sa and Parra, 1994).

In the native region of Fall armyworm, natural enemies which include predators, parasitoids and entomopathogens are known to keep its populations under check (Molina-Ochoa *et al.,* 2009). For example, in the Americas and Caribbean, 150 parasitoid species were shown to attack FAW (Molina-Ochoa *et al.,* 2009). Different egg-larval parasitoids species like *Chelonus insularis* Cresson were found attacking eggs of FAW on maize crop in Argentina (Virla *et al.,* 1999) and *C. cautus* Cresson on maize and sugarcane in Mexico (Molina-Ochoa *et al.,* 2001).

Fall armyworm being invasive to Africa, a few parasitoid species parasitizing eggs and larval stages have been documented in East, South and West Africa (Kenis *et al.,* 2019; Sisay *et al.,* 2018, 2019). This indicates the potential of biological control of FAW in Africa hence extensive survey remains urgently required to be carried out to explore for more natural enemies particularly the egg parasitoids. It is important to consider employment of indigenous natural enemies specifically the egg parasitoids to combat the FAW. The main aim of this work was, to collect indigenous FAW egg parasitoids, identify then assess their effectiveness under controlled conditions in the laboratory.

1.2 Problem statement

Fall armyworm is an invasive insect indigenous to the Americas and infested sub Saharan Africa in the year 2016 (Goergen et al., 2016b). This insect is a voracious feeder and it attacks mainly maize crop and over 353 other crop species (Montezano *et al.,* 2018). This threatens maize production which is a main staple food crop and a raw material for livestock feed and income generation (Nagoshi *et al.,* 2018). The pest has

high reproductive potential with one female laying between 1500 and 2000 eggs in her lifetime and it can have up to six generations annually (Luginbill, 1928). Currently, chemical pesticide use seems to be the only method used to manage FAW. African and Asian countries have faced high maize production costs due to losses incurred because of spraying chemical insecticides. However chemical control has deleterious effects to human beings, the environment and the non-target organisms like honeybees. Also, the usage of insecticides has caused FAW to develop resistance against it (Carvalho *et al.*, 2018; Yu, 1991; Yu *et al.*, 2003). This emphasizes the need to look for alternative control methods which are reliable, sustainable and cost effective for small scale farmers in Kenya.

Biological control offers the best alternative solution to chemical control against fall armyworm because it is pest specific, self-perpetuating, density dependent, cost effective and safe to humans and the environment. In this regard, egg parasitoids remain the most useful agents of biocontrol as they kill pest eggs before further damage to crops from larval feeding. However, in Africa many studies have highlighted the association of the natural enemies with FAW regarding the field surveys done in a few regions in Kenya, for example, Western and North Rift regions (Sisay *et al.*, 2019). However, scarce knowledge on the presence of native parasitoids of FAW in other counties in Kenya especially in different agro ecological zones, like Kirinyaga, Makueni, Taita Taveta, Kilifi and Kwale. The current study aims to identify the indigenous egg parasitoids of FAW across the selected agro ecological zones of Kenya and determine their effectiveness under laboratory condition.

1.3 Justification of the study

Fall armyworm is nocturnal in nature and the time for application of chemical products is limited to early morning or late evening and this makes it not reliable for effective control. Biological control through the use of natural enemies for pest control is an important aspect of integrated pest management (IPM). Egg parasitoids can act as biocontrol agents since they are highly pest specific, sustainable, effective at little or no cost and they impose no harm to the public health or the environment. Parasitoid species have shown excellent results when it comes to decreasing pest population numbers in the crop field (Mills *et al.,* 2000). Local species of parasitoids are most preferred since it is much well adapted to ecological conditions of the region when compared to exotic species (Smith, 1996). *Trichogramma* and *Telenomus* species are egg parasitoids which are commonly used worldwide to keep lepidopteran pest under check.

Since FAW invaded Africa in the year 2016, recent evidences have highlighted new association of parasitoids infesting egg and larval stages in Africa (Sisay *et al.,* 2018). During the field survey, the districts surveyed were in the Western and North Rift regions of Kenya (Sisay *et al.,* 2018). Furthermore (Kenis *et al.,* 2019) carried out a field survey in coastal regions of Kenya, Kilifi County. However, this study aims to carry out field survey in other counties of different agro ecological zones in Kenya which include; Kilifi, Kwale, Taita Taveta, Makueni and Kirinyaga.

1.4 Objectives of the study

1.4.1 General objective

To carry out field survey across selected agro ecological zones in Kenya, collect egg parasitoids of FAW and determine the exact native parasitoid species using

morphological and molecular techniques and determine their effectiveness under laboratory condition.

1.4.2 Specific objectives

1. To determine the diversity of indigenous egg parasitoids of Fall armyworm (FAW) in Kenya and identify using morphological and molecular techniques
2. To assess the performance of egg parasitoids on Fall armyworm egg densities in the laboratory

1.5 Hypotheses

1) Diversity of indigenous egg parasitoids of FAW does not exist in different agro ecological zones in Kenya
2) There is no variation in the morphological and molecular characteristics of egg parasitoids collected
3) Indigenous egg parasitoid species have similar abilities to parasitize Fall armyworm eggs

CHAPTER TWO

LITERATURE REVIEW

2.1 Importance of maize

Maize being a major staple food crop in Africa, small scale farmers cultivate it mainly for food and an estimated 50% population depend on them as income source (FAO, 2019). Maize can be cultivated in a wide array of climatic conditions. Maize is rich in starch, protein, iron, vitamin B, and minerals. In Africa, maize is eaten in different ways, for example, green fresh maize with cobs can be roasted or boiled then eaten. Githeri is a traditional Kenyan meal of boiled maize and legumes (usually beans).

Dried maize grain is normally grinded into flour, which can be used to make different meals such as Ugali, which is very popular in Kenya. The oil from the maize embryo can be extracted and utilized in manufacturing cooking oils, butter and salad dressings. Maize remains the world's unique fodder and a major source of calories in livestock feeds. In 2017, Africa cultivated 7.4% of the 1,135 million tonnes cultivated worldwide in 40 million hectares of land (FAO, 2017b).

2.1.1 Constraints of maize production

Maize is cultivated worldwide, with widely varying yields that depend on the area where it is being cultivated and the season of the year. The average maize yield per hectare in various African countries is very low. This is because of many biotic and abiotic stresses affecting maize cultivation in Africa. Biotic stresses result from harm caused by pests and diseases such as, cut worms, aphids, stem borers, nematodes, bacteria, viruses, fungi and weeds. Abiotic factors that adversely affect maize crop include drought, extreme temperatures and poor soil fertility. Cultivation is also

affected due to inaccessibility to quality seeds and fertilizers, decreased mechanization and poor management of produce after harvesting. All these factors contribute to low maize yields in Africa. For example, fall armyworm is estimated to impact on up to US$13 billion worth crops (rice, corn, sugarcane, and sorghum) annually in Africa (Day *et al.,* 2017).

2.1.2 Other host plants attacked by Fall armyworm

Besides maize crop, which is widely preferred by FAW, this insect has an extensive host range that exceeds 80 crop species which includes; beans (*Phaseolus vulgaris*), sorghum (*Sorghum bicolor* (L.) Moench), cotton (*Gossypium hirsutum*), sugarcane (*Saccharum officinarum* L.), kales (*Brassica oleracea var. sabellica* L.), cabbages (*Brassica oleracea var. capitata*), tomatoes (*Solanum lycopersicum* L.), pepper (*Capsicum annuum* L.) and forage grasses like Napier grass (*Pennisetum purpureum* Schumach) (Day et al., 2017; Prasanna, Huesing, Eddy, & Peschke, 2018). Recently, a review from its native regions, shows that FAW can attack over 353 host crop species (40% of them are economically important) which belong to seventy six families with the biggest plant host taxa found in the family Poaceae, followed by Fabaceae then Asteraceae (Montezano *et al.,* 2018).

2.1.3 Fall armyworm in Africa

Fall armyworm is a migratory insect, prevalent to North and South America. In the year 2016, FAW invaded Africa and it is the first invasion of this pest into the Eastern Hemisphere (Goergen *et al.,* 2016). It was first reported in Africa on the Island nation of Sao Tome and Principe in April 2016, which later spread widely in the West African countries such as Ghana (Cock *et al.,* 2017) and Nigeria, Benin and Togo (Goergen *et*

al., 2016). By October 2017, FAW was present in 44 countries (Rwomushana *et al.,* 2018) and in all of the sub-Saharan African countries except Lesotho and Djibouti (FAO, 2018; 2019; Rwomushana *et al.,* 2018, Nagoshi *et al.,* 2018).

Moreover, this pest is likely to spread further in many other regions and cause significant yield losses to crops dependent on by smallholder farmers (FAO, 2017) due to climate suitability and host plants distribution. Rapid FAW population is fostered by the prevailing environmental and climatic conditions in Africa. In West and Central Africa, *S. frugiperda* larvae significantly damaged corn than other crops (Prasanna *et al.,* 2018). The FAW damage to maize production in African could total up to US$ 6.1 billion per annum over the following years (Nagoshi *et al.,* 2018) with the significant losses still to be determined in other crops.

2.1.4 Fall armyworm in Kenya

Fall armyworm was reported for the first time in Kenya in March 2017 in an off-season maize crop in Trans Nzoia and Bungoma counties (FAO, 2017a). The pest has rapidly spread to 43 out of the 47 counties and the pest is infesting mainly maize crop (FAO, 2019). The Semi-Arid counties like Garissa, Marsabit, Lamu, Mandera and Wajir have not reported any occurrence of FAW to date due to not growing maize crop. Characteristic damage symptoms on maize include ragged feeding and presence of wet frass which looks like sawdust on the upper leaves and whorl of the crop (Goergen *et al.,* 2016; Nagoshi *et al.,* 2018). Fall armyworm infestation and rapid spread predominate small-scale maize farming regions and thus threatens maize production as the main staple food crop, income security besides a key component of cattle feedstuff (Nagoshi *et al.,* 2018).

2.1.5 Fall armyworm host plants in Kenya

Fall armyworm is polyphagous insect which attack nearly 100 plants which belong to 27 families (FAO and CABI, 2019). The preference of this pest is found in the family (graminaceous) which include maize (Zea mays), millet, sugarcane (*Saccharum officinarum* L.), wheat (*Triticum aestivum*), sorghum (*Sorghum bicolor*) and rice (*Oryza sativa*). It also attacks crops of economic importance, such as cotton (*Gossypium hirsutum*), potato (*Solanum tubersum*), groundnut (*Arachis hypogaea*), soybean (*Glycine max*) and cowpea (*Vigna unguiculata*) (FAO and CABI, 2019). Grasses which are fed on by FAW include Bermuda grass (*Cynodon dactylon*), sudan grass (*Sorghum* × *drummondii*), ryegrass (*Lolium perenne*), barley (*Hordeum vulgare*), oat (*Avena sativa*) and clover (*Trifolium* spp). Lastly FAW attack weeds which include nut sedge (*Cyperus* spp.), pigweed (*Amaranthus* spp.), crabgrass (*Digitaria* spp.), johnson grass (*Sorghum halepense*), morning glory (*Ipomoea* spp.), and sandspur (*Cenchrus* sp.) and fruit crops like apple (*Pyrus malus*), grape (Vitis spp.), orange (*Citrus aurantium*), papaya (*Carica papaya*) peach (*Prunus persica*), strawberry (*Fragaria* × *ananassa*) and many flowers (FAO and CABI, 2019).

2.1.6 Biology of Fall armyworm

The FAW belongs to order Lepidoptera and family Noctuidae. It is a moth which is nocturnal in nature and endemic to the tropical and subtropical regions of the Americas (Sparks, 1979). The larvae or caterpillar is a stage which causes damage to crops while the adult is the moth which can fly up to 100km per night and it can have up to six or more generations annually (Luginbill, 1928). They usually mate in the evening and female moths deposits its eggs during night hours in layers of (two or more) of between 100-200 eggs (Luginbill, 1928). They prefer either, the lower or upper side of the maize

crop when the crop is still young (V4-V6 stages). The eggs hatch after 2-3 days into larvae and immediately start dispersing into different directions while feeding.

Fall armyworm distribution is very wide in North, South, Eastern and Central America but they became invasive into Africa in 2016 (Goergen *et al.*, 2016). The larvae undergo up to six different instars. The caterpillars are voracious feeders and attack all parts of the maize crops (stems, leaves, cobs and tassels) at all stages. Larval stages take 14 to 21 days and this depend on the temperatures. Each larval stage varies from each other slightly in physical appearance and the pattern. The larvae of armyworms normally display marching behavior when moving to feeding sites, hence the name. They spread rapidly and cause yield loss in crops like, maize and sorghum with potential to damage rice, pasture, millet, cotton and some vegetable crops (Day *et al.,* 2017; FAO, 2017b; Prasanna *et al.,* 2018; Rwomushana, *et al.,* 2018). Fall armyworm breeds continuously throughout the year especially where host crops like maize is available. Reports have shown existence of two strains of FAW in Africa, the corn strain (C-strain) and the rice strain (R-strain) (Day *et al.,* 2017; Gichuhi *et al.,* 2020; Nagoshi *et al.,* 2018). Rice strain is alleged to prefer feeding on rice and different pastures e.g. grasses but the corn strain feeds mainly on maize, sorghum and cotton.

2.1.7 Migratory behavior of Fall armyworm

Fall armyworm is a migratory insect endemic to tropical and sub-tropical regions of North, Central and South America (Sparks, 1979). This pest was observed first in West Africa in the late 2016 (Goergen *et al.,* 2016), by the year 2017, the pest was reported in the Sub-Saharan Africa and Asian continent (Deole & Paul, 2018; Sisodiya *et al.,* 2018) such as, India (Shylesha *et al.,* 2018), China (Wu *et al.,* 2019) then the most

recent is Japan. In Sub-Saharan Africa, reports showed that FAW has spread to 44 countries except Lesotho and Djibouti (FAO, 2019; Rwomushana *et al.*, 2018) and this indicate that this notorious pest is capable of spreading faster therefore a risk to national food safety, livelihood as well as economy of millions of people (FAO, 2018). It is very difficult to eradicate and stop FAW from spreading to other unaffected areas due to environmental suitability in Africa (FAO, 2017b) and the moth's ability to soar up to 100 km per night.

Fall armyworm migrations is reliant on the prevailing winds as well as the availability of host plants (Luginbill, 1928). The pest is unable to diapause in cold temperatures (Foster, 1987). Adult moths are migratory and have localized dispersal habit, a characteristic that is common with genus *Spodoptera*. The moths can migrate beyond 300 miles assisted by wind before stopping and laying eggs (Johnson, 1987). However, the damaging populations usually depend on factors like planting date, cropping practices, migration patterns of insects, weather conditions, and presence or absence of natural enemies. For example, late planting of maize highly attracts the moths, leading to considerable damage of the crop.

2.1.8 Life history of Fall armyworm

More than 200 years ago, FAW was known as a destructive pest of various cultivated crops (Luginbill, 1928). This pest has posed serious threat in agriculture in the Western Hemisphere where it is indigenous. As typical of most noctuids, FAW adult females are relatively short lived but highly reproductive (Johnson, 1987). For mating purposes, females release sex pheromone which attract high populations of males to trace them at the top of the host crop for mating. After mating, the females deposit their egg masses

when they arrive in new field with favorable host plants.

After 2-3 days, eggs hatch, and the larvae starts moving while feeding on plants close to the ground surface or in whorl of maize crop while hiding itself as they feed. This pest undergoes complete metamorphosis: eggs, six to seven larval instars, pupae and adult (Hardke *et al.,* 2015; Nagoshi *et al.,* 2018). For 14-21 days, the larvae grow through six instars, then burrows into the ground to pupate. Depending on the underground temperatures, the moth emerges from pupa within 1-5 weeks and in most cases migrate to new areas for infestation. The complete life cycle occurs in 30, 60 and 90 days with varying number of generations in summer, spring/autumn, and winter, respectively.

2.1.9 Description of life stages of Fall armyworm

2.1.9.1 Egg stage

Oblate-spheroidal is the shape of FAW egg and it measures (0.39mm long x 0.47mm round) (Luginbill, 1928). The eggs are green/brown in color at the time of oviposition and turns grey to black when it is about to hatch (Hardke *et al.,*2015). Oviposition is initiated during evening hours. Eggs takes 2-3 days before it matures and hatch at optimal temperature (20 to 30°c). The FAW eggs are normally deposited in masses, and the number per mass considerably vary between 100 to 200 which are laid in 2-4 layers. The moth normally covers its eggs with scales which are grey in color from the female' abdomen. The total egg number per mass produced by an individual female ranges between 1,500 and 2,000 (Capinera, 1999).

2.1.9.2 Larval stage

This stage can take up to 14 days when warm (28°c) and around 30 days during cold

weather conditions. The FAW consists of 7th instars (Goergen *et al.,* 2016). The first larval stage (L1) which eclose from eggs appear creamish yellow in color with a dark head, it also has small black spots where primary setae protrude. The larvae change from dark to greenish as they continue to feed (Sparks, 1979). The 2nd and 3rd instars (L2-L3) look similar in color, but they darken as they proceed to molt into other instars (Hardke *et al.,* 2015).

The last three instars (L4, L5 and L6) are dark in color, but their color differs from each other depending on the food they eat. In the last three instars, the larvae display a distinguishing inverted "Y" shape feature yellow in color on the head (Hardke *et al.,* 2015). Fourth to sixth instar are generally smooth due to lack of primary setae. Older larvae have non-continuous marking lines which appear on mid dorsal of the body which are white in color, and the abdomen have "flecking". On the eighth abdominal segment, there is presence of "dots" which are arranged in square form. The length of first instar larva measures 1.68mm, while last instar measures 34.15mm. For first instar larva, the head capsule width is 0.314mm, while for last instar it is 2.78mm.

2.1.9.3 Pupal stage

As typical of most noctuid, the appearance of pupal case is orange brown in color, but with age they turn black. The pupation depth depend on factors like the soil texture, moisture and temperature (Sparks, 1979). In the soil, pupation normally occurs at the depth ranging from 2 - 8 cm. With silk and soil particles, the larvae form a cocoon which is loose and oval shaped and measures 20- 30 mm lengthwise. In case the topsoil material is very tough, larvae can decide to mesh together fragments from leaves which form a cocoon. Measurement of the pupa is 14 to 18mm in length and 4 to 5mm in

width. Pupal period during summer ranges between 8 to 9 days while in winter time it may extend up to 30 days (Sparks, 1979). Immediately the moth emerges from the soil, they can copulate with males and fly about 300 miles before oviposition (Ashley *et al.*, 1989).

2.1.9.4 Adult stage

The male moth of FAW has filiform (threadlike) antennae which is common in Noctuid. The male moth has a wingspan of 32mm to 40mm. The length of a male body measures 1.6cm and wings' length is 3.7cm. The front wings are mottled (light brown, grey, straw) with white dots at the tip and near the middle of the wing which are triangular.

The length of a female body measures 1.7cm and wing length is 3.8cm. The forewings are mottled (dark brown, grey). In both sexes, the rear wing is lustrous grey-white with a black edge which is narrow. Adults moths are nocturnal and more active in the evening when conditions are warm and humid. After copulation, the female undergoes 3 to 4 days' period of pre-oviposition before depositing most eggs in the first 4 -5 days of her lifetime, though some oviposition takes place for a period of up to three weeks. The average moth's lifespan is about 10 days, particularly ranging between 7 and 21 days (Luginbill, 1928; Sparks, 1979).

2.2 Damage of Fall armyworm on maize plant

The adult female moth lays between100 and 200 eggs covered by scales on underneath of maize leaves which hatch after 2-3 days. The first instars feed on the bottom of the leaves which result into semitransparent patches on the surface. Young larvae can disperse in different directions and attack other plants with the help of silken threads

which catch the wind and transport them (Curry, 2017).

The 2nd and 3rd instars prefer feeding on the leaf whorl of the young maize crop whereas in older plants, the leaves around the cob silks are attractive to them. Attacking the whorl of young maize crop can destroy the budding region which result to no leaves growing or no cobs developing (Curry, 2017). The larvae can also feed on silk and emerging tassels, thus preventing pollination of the ear. Where the maize cobs have already developed, the larvae will eat its way over the protecting leaf bracts and starts to feed on developing kernels which may result in fungal infection or aflatoxins, and poor grain quality.

Feeding inside the whorl of maize plant is normally characterized by perforations and ragged feeding, and presence of large quantities of frass which look like sawdust around the whorl and top leaves of the plant (Goergen *et al.*, 2016). By this time FAW will have reached 4th to 6th instar stage. Due to cannibalistic behavior, number of larvae are normally lowered to one or two per every plant when the feeding larva is near one another to avoid competition for food (Curry, 2017). Extensive damage through defoliation is caused by older larvae on the maize crop, normally leaving a torn appearance on both ribs and stalks of maize plants. After about 14 days, the sixth larval stage will fall on the soil and start burrowing 2- 8cm and pupate inside.

Fall armyworm attacks maize crop in all its growing stages, for example, from the vegetative to the reproductive stages. Leaf damage of the maize crop does not necessarily result to a yield loss due to the crop's ability to compensate for the loss of leaf area affected (FAO, 2018). Foliar damage should be assessed and rated on a 1-9

scale whereby the crops which are not affected (highly resistant) to FAW are rated as 1 (no visible leave attack) while the ones which are mostly affected (highly susceptible) are rated as 9 (completely damaged leaves) (Prasanna *et al.,* 2018). Ear damage takes place once the larvae eat through the emerging ears and the entries are scored during the harvest period. When there is no ear damage, it means that the crop is highly resistant, but when the ear is almost 100% damaged, it shows that the crop is highly susceptible (Prasanna *et al.,* 2018).

2.3 Host range and Fall armyworm distribution

2.3.1 Host range

Fall armyworm have a wide range of hosts which exceed 80 species but prefers grass family (Poaceae) (CABI, 2017). The larval stage mainly prefer maize (*Zea mays* L.), but it feeds on additional crops like sorghum (*Sorghum bicolor* (L.) Moench), rice (*Oryza sativa* L.), millet (*Panicum miliaceum* L.), sugarcane (*Saccharum officinarum* L.), cotton (*Gossypium hirsutum*) (Day *et al.,* 2017; FAO, 2018; Prasanna *et al.,* 2018) and vegetable crops like; cabbage (*Brassica oleracea var. capitata*), kales, (*Brassica oleracea var. sabellica* L.), pulses or legumes, like pigeon pea (*Cajanus cajan* (L.) Millsp.), pepper (*Capsicum annuum* L.), tomatoes (*Solanum lycopersicum* L.), spinach (*Spinacia oleracea* L.), ginger (*Zingiber officinale* Roscoe), onions (*Allium cepa* L.), common amaranth, cucumber (*Cucumis sativus* L.) and sunflower (*Helianthus annuus* L.). In addition, recent review and surveys in Brazil shows that FAW larvae can feed on 353 crops that belong to seventy six families with the biggest host taxa (106) found in the family Poaceae, followed by Asteraceae and Fabaceae each consisting of 31 taxa (Montezano *et al.,* 2018).

2.3.2 Fall armyworm distribution

Fall armyworm spreads faster across wide geographic areas particularly in warm climatic conditions. According to research 28°C is the optimum temperature for larval development, however, not favorable for oviposition and pupation (CABI, 2017). In Northern areas of America, FAW have only single or two generations unlike the tropics which may have up to four or six generations annually (Luginbill, 1928). At low temperatures, rate of activity and development slow down and stop, which usually leads to death of all the stages of development (Garcia *et al.,* 2018).

Fall armyworm is a migratory insect endemic to tropical and sub-tropical areas in America (Sparks, 1979). In the African continent , first report on FAW occurrence was in January 2016 in Nigeria, Sao Tome, Benin and Togo respectively (Goergen *et al.,* 2016). Early 2017, the pest infested Sub-Saharan Africa and by February 2018, FAW occurrence was confirmed in 44 African countries (Rwomushana *et al.,* 2018). Fall armyworm has been able to reach Madagascar and other Islands through Indian Ocean (Chinwada, 2018). The major reason of Madagascar getting invaded by FAW is due to its favorable climatic conditions and continuous maize growing activities (Chinwada, 2018). In Angola and Nigeria, it is expected to have more FAW widespread outbreaks than earlier reported, due to favorable climatic conditions and maize distribution (Day *et al.,* 2017).

In August 2018, fall armyworm was reported from the India in maize fields of Karnataka and Andhra Pradesh (Deole & Paul, 2018; Shylesha *et al.,* 2018) and Maharashtra, Tamil Nadu and Telangana (Saravanan *et al.,* 2019). In July 2019, FAW larvae were detected in the southwestern part of Japan in a corn field in Minamikyushu

City, Kagoshima Prefecture on Kyushu Island and China (Wu *et al.,* 2019). Afterwards, it has spread rapidly through 65 countries across Africa and Asia. More recently, in February 2020, FAW was officially reported in Australia. This indicates the fast spread of the pest, hence a threat to economy, livelihood, and food security of many people in the world. Fall armyworm has the potential to spread further due to trade and the moth's durable soaring capability (Johnson, 1987). Potential ways of FAW spread involved wind aided flying and the contaminants of goods being exported to other countries (Cock *et al.,* 2017).

2.4 Diversity of Fall armyworm natural enemies

Many studies in the America have shown multiplicity of antagonists related with FAW which include both parasitoids of egg, larval, pupal and adult (Molina-Ochoa *et al.,* 2009). Approximately one hundred and fifty parasitoid species belonging to 13 families have been reported in the Americas (Molina-Ochoa *et al.,* 2009). Out of 150 species, 22 have been reported in Mexico. Parasitoids associated with FAW in Northern States of Mexico are completely hymenopterans, while in Southern States like; Tabasco, Quintana Roo and Chiapas, they comprise both wasps and Tachinid flies (Molina-Ochoa *et al.,* 2001).

In Argentina, three and eight parasitoid species belonging to order Hymenoptera and Diptera respectively, parasitize on FAW (Murúa *et al.,* 2009). Egg-larval parasitoid such as *C. insularis* occur as parasitoids of FAW throughout North America and are reported to cause 63% parasitism in Southern Florida (Murúa *et al.,* 2009). However, lower parasitism level of 5% by *C. insularis* was observed in Puerto Rico and Mexico, respectively (Molina-Ochoa *et al.,* 2001). In South and Latin America, *C. insularis* is

broadly distributed and it is considered a successful candidate for augmentative release due to its overwintering behavior. Therefore, it is capable of colonizing the region early and assist in pest management when released on specific crops (Lewis & Nordlund, 1984). In Vipos, the parasitism level caused by Ichneumonid wasps was low and it ranged between 0.8 and 1.3% during a four-year study (Murúa *et al.,* 2009) (Murúa *et al.,* 2009). In Southern Georgia, parasitism rate of the three late instars (4^{th}, 5^{th} and 6^{th}) by *Ophion flavidus* was successful by 19.5% (Gross & Pair, 1986).

In South Asia, most of the parasitoid species found attacking fall armyworm, *S. frugiperda* have been reported from the survey carried out in different locations of Karnataka. For example, egg parasitoids, *Telenomus* and *Trichogramma* sp., and larval parasitoids, *Glyptapanteles creatonoti* (Viereck), *Campoletis chlorideae* Uchida and *Forficula* sp (Shylesha *et al.,* 2018).

Similarly, in Africa, parasitoid species attacking *S. frugiperda* have been documented. Field surveys conducted in East Africa, for instance, Ethiopia, Kenya and Tanzania reported parasitoids species from FAW eggs and larvae with over 50% parasitism rates (Sisay *et al.,* 2019). *Telenomus remus* and *Trichogramma* sp were found attacking egg masses of FAW with 69.3 and 20.9% egg parasitism, respectively (Sisay *et al.,* 2019). Kenya's main, larval parasitoid was Tachinid fly, *P. zonata,* recording a parasitism rate of 12.5% , while *Cotesia icipe* (Triana and Fiaboe) was the predominant species in Ethiopia, with a range of 33.8 to 45.3% parasitism (Sisay *et al.,* 2018). However, in Kenya and Tanzania, *Charops ater* and *Coccygidium luteum* were reported as the major parasitoid species, with parasitism ranging between 6 and 12% and 4 and 8.3%, respectively (Sisay *et al.,* 2018). *Telenomus remus*, were observed parasitizing eggs of

S. frugiperda in 5 countries in East, West, and South Africa (Kenis *et al.*, 2019).

Recently in West Africa; Ghana and Benin, FAW egg, larvae and pupa parasitoid species were reported; (Agboyi *et al.*, 2020). For example, egg-larval parasitoid species, *Chelonus bifoveolatus and Coccygidium. luteum* were the major species of parasitoids causing up to 75% parasitism while egg parasitoid, *T. remus* caused parasitism ranging between 14.5% and 25.9%, respectively (Agboyi *et al.*, 2020). *Trichogramma* sp was among the egg parasitoids collected. (Koffi *et al.*, 2020) reported three predators and seven parasitoid species of FAW in Ghana whereby *C. bifoveolatus and C. luteum* were the most abundant with parasitism percent of 1.04% and 0.85% respectively.

2.5 Egg parasitoids of Fall armyworm

2.5.1 Biology of *Trichogramma* species

Trichogramma sp. belong to order Hymenoptera and family Trichogrammatidae. They are the egg parasitoids which has been extensively studied because of its ease of mass rearing (Smith, 1996). As a biological control agent, *Trichogramma* sp. has been widely employed in augmentative release against many crop pests of economic importance (Smith, 1996). Most species of *Trichogramma* reproduce by arrhenotokous parthenogenesis whereby, unfertilized eggs develop into males while females develop from fertilized eggs.

In Brazil, the release of *Trichogramma* sp. as a biocontrol of lepidopteran crop pests like *S. frugiperda,* and *D. saccharalis* Fabricius has been considered as the best method of controlling these pests in maize crop (Parra & Zucchi, 2004). In this regard, parasitoids have shown excellent results when it comes to decreasing pest population

numbers in the crop field (Mills *et al.,* 2000). In Brazil, genus *Trichogramma* mostly parasitizes *S. frugiperda* eggs in corn crops (Beserra *et al.,* 2005a). Egg parasitoid, *Trichogramma pretiosum* Riley is commonly found in different agroecosystems attacking many crop pests. However, the presence of scales and deposition of eggs in layers reduces the efficacy and prolongs time spent by *Trichogramma* females to parasitize *S. frugiperda* eggs (Beserra *et al.,* 2005b).

2.5.2 Biology of *Telenomus* species

Telenomus remus Nixon is a suitable agent for biocontrol of many pests belonging to the genus *Spodoptera* (Pomari *et al.,* 2013). Adult female parasitoid produces up to 270 eggs before it dies which are normally laid on host eggs. Furthermore, *T. remus* is effective in parasitizing *Spodoptera* spp. eggs as with a long ovipositor it is capable of attacking eggs which are located in the innermost regions of egg mass layers (Pomari *et al.,* 2013). This feature is not often found with other species of egg parasitoids. *Telenomus remus* disperses widely since it has high host search capabilities making it suitable for use in augmentative release (Cave, 2000). Studies worldwide have been conducted to determine its efficacy on *S. frugiperda* eggs (Bueno *et al.,* 2010; Cave, 2000). Parasitism rate of *T. remus* in Barbados on different populations of *Spodoptera* sp. varied from 50-70% under laboratory conditions (Cave, 2000). In Venezuela, *T. remus* has been used in IPM programs for FAW through large scale inoculative release in corn fields causing between 78-100% parasitism levels (Cave, 2000).

In Brazil, this egg parasitoid was introduced in the mid 1980's with the aim of evaluating its effectiveness to mitigate FAW in a classical control program. It has also been used in soybean IPM (Carmo *et al.,* 2010) to control pests like *S. cosmioides*

(Walker) and *S. eridania* (Cramer) which normally attack crops at reproductive stage whereby they feed on foliage and affect the pods. According to Pomari *et al.,* (2013), *T. remus* is effective against *S. frugiperda* (Smith, 1996) and *S. albula* (Walker). In Mexico, *T. remus* is stated to be effective on *S. frugiperda* egg masses with parasitism rate ranging between 78% -100% when 5000 to 8000 parasitoids are released per hectare of land (Bueno *et al.,* 2010).

In Latin America, *T. remus, T. pretiosum* and *T. atopovirilia* Oatman & Platner are the most reported egg parasitoids of FAW (Beserra *et al.,* 2005a; Parra & Zucchi, 2004). Among the three species, *T. remus* is the most appropriate candidate for biological control of crop pests due to its robustness, larger size than *Trichogramma* sp. and its ability to attack eggs located in the inner layers of the FAW egg masses (Cave, 2000). Experiments conducted in the laboratory have revealed that *T. remus* is capable of parasitizing *S. frugiperda* egg masses quickly than *T. pretiosum* (Carmo *et al.,* 2010). When the two parasitoid species are placed at the same time in an area with FAW eggs, more *T. remus* adult emerge than *T. pretiosum* (Carmo *et al.,* 2010).

CHAPTER THREE

GENERAL MATERIALS AND METHODS

3.1 Field collection of Fall armyworm egg masses and egg parasitoids

Fall armyworm egg masses were collected during a field survey carried out in the year 2018 in five counties of different agro ecological zones, which include Kilifi, Kwale, Taita Taveta, Kirinyaga and Makueni. The eggs were monitored daily for FAW egg hatchability or parasitoid emergence. After the field survey, colonies of FAW, egg parasitoids and the factitious host *C. cephalonica* was established at the International Centre of Insect Physiology and Ecology (*icipe*) laboratory to multiply their numbers for further bioassays. Fall armyworm was reared on both natural (maize leaves) and artificial diet prepared following the procedure developed by the (*icipe*). The factitious host, *Corcyra cephalonica* was collected from the National Cereals and Produce Board (NCPB) and reared on maize bran. *Telenomus remus* was reared on FAW eggs while *Trichogramma chilonis* was reared on FAW and factitious eggs.

3.2 Rearing of Fall armyworm colony

3.2.1 Preparation of Fall armyworm artificial diet

Fraction A: Using a plastic spoon, all the powdered ingredients which included bean powder, wheat germ, maize leaf powder, milk powder, torula yeast, ascorbic acid, methyl paraben and sorbic acid were mixed and put in sterile container inside a fume hood except methyl-hydroxybenzoate. Sterile water was boiled and cooled to 60°C then mixed with ingredients (mixture) above and blended for one minute. Methyl-p-hydroxybenzoate (dissolved in 20ml of 100% ethanol) was added to the combination and blended for another two minutes.

Fraction B: Agar powder was measured using weighing balance and put in a different container, then 150ml distilled water added in a different saucepan. It was then boiled while being stirred continuously and then allowed to cool to 60°c. Ingredients of A were added to ingredients of B and blended for 3 minutes.

Fraction C: Formaldehyde (40%) and vitamin mix were finally added to both fractions A and B and blended together and mixed for 3 minutes under room temperature, (25±1°C); 12:12 light: dark photoperiod; and 75±5%, RH. The required volume, 10ml of the diet was dispensed into plastic/glass vials.

3.2.2 Diet infestation with Fall armyworm 2nd instar

After preparation of the diet, it was dispensed into sterilized glass/plastic vials and plastic jars then placed inside the fumehood to cool and make the chemicals to evaporate. In each glass/plastic vial, shallow holes were made on the surface of the diet using a sterilized laboratory plastic rod to make it easy for larvae to feed through the diet. Also, black FAW egg masses that are ready to hatch were put inside the plastic jars containing the diet. When the eggs hatch and larvae reach 2nd instar, two larvae were transferred from plastic jar into the glass/plastic vials to avoid cannibalism which could arise when they reach the third instar. Vials were then closed tightly using cotton wool plugs.

3.2.3 Management of Fall armyworm larvae and pupae

Development of larvae was monitored on daily basis to record any contamination with fungi/virus. Close monitoring of pupal harvest was done between 14 to 21 days after diet inoculation to avoid adult emergence on the glass vials. Pupae were harvested when

50% of the larvae had pupated. This was done by carefully removing the diet from each vial onto a clean tray using a spatula to avoid crushing the pupae. The pupae were put in petri dishes lined with paper towel. The pre-pupae which had not pupated were kept in plastic jar lined with moist paper towel until they pupated completely. Pupae were then cleaned by gently spraying distilled water and excess moisture drained using paper towel. Pupae were then transferred onto a clean petri dish lined with a paper towel then placed in an oviposition cage (45cm×60cm×45cm) ventilated with a fine net on the sides. The cages were incubated under room temperature (25±1°C); 12:12 light: dark photoperiod; and 75±5%, RH until the moths emerged.

3.2.4 Management of Fall armyworm moths

A piece of wax paper was spread/lined inside the oviposition cage to provide oviposition site where the adult moths could lay their eggs. Moths were nourished with 10% honey solution saturated in a piece of cotton wool and put in a small plastic petri dish inside the cage. The oviposition cage was checked daily to collect the eggs which were deposited on the wax paper. Occasionally, the oviposition cage was cleaned by removing the dead moths.

3.2.5 Management of Fall armyworm eggs

Fall armyworm egg masses which was laid on waxed paper were cut using a pair of scissors. They were then surface disinfected by plunging in 10% formaldehyde for 15 minutes and thereafter dipped carefully in distilled water and dried using filter then transferred into clean plastic jars.

3.2.6 Mass rearing of *Telenomus remus* Nixon on Fall armyworm *eggs*

Colony of *T. remus* was initiated from a cohort of fifteen wasps (1:2 - males: females) obtained from parasitized eggs of FAW collected during the field survey. They were maintained in glass vials in the laboratory at *icipe*, at room temperature under conditions, 25 ± 1°C, 12 L:12 D photoperiod, and 60-70% relative humility (RH). The parasitoid was reared on freshly laid FAW egg masses which were pasted onto rectangular manila cards (1cm by 5cm) using white glue and exposed to wasps to parasitize. Date of exposure was recorded at the back of the card to calculate the expected adult parasitoid emergence. A plain paper (2cm by 1cm) was thinly layered with honey and placed inside a glass as a source of food. Glass vials containing parasitoids were placed on the shelves in the laboratory.

After 3 to 4 days of exposing the card, the eggs turned black and this showed that it has been parasitized. The parasitized egg cards were removed and placed on a separate clean glass vial and incubated at room temperature to observe for parasitoid emergence. The parasitoids took a period of 6-13 days before emerging. The above process was repeated for colony maintenance. The colony was maintained for 16 generations before starting the bioassays.

3.2.7 Colony of rice meal moth, *Corcyra cephalonica* (Stainton)

Rice meal moth, *C. cephalonica* served as the factitious host for laboratory mass rearing of *T. chilonis.* A starter colony of twenty-three moths (fifteen females and eight males) was obtained from the National Cereals and Produce Board, Nairobi. The moths were kept in a transparent plastic bucket (5-liter capacity) with a lid whose center has been cut and protected with nylon mesh for air circulation. Besides the rearing buckets, a

plastic jar (1liter) was used for adult oviposition. The lid of the oviposition jar has a round hole in the center with mesh nylon for ventilation.

A nylon mesh strip was suspended in the oviposition container, with the upper part fitted with the lid acting as oviposition substrate, walking and resting for adult moths. Collection of eggs was done by shaking thoroughly the nylon mesh to enable eggs to drop on the bottom of the jar and collected onto a plain paper. Freshly laid eggs were cleaned free from impurities such as scales of moths and their broken appendages by tilting a plain piece of paper to allow eggs to roll slowly into a petri dish. Eggs used for parasitoid rearing were sterilized by exposing them to ultraviolet rays of 30-watt UV tube for 10 minutes to avoid hatching. While eggs used for maintaining the colony of rice meal moth were left to hatch and fed with maize bran.

3.2.8 Mass rearing of *Trichogramma chilonis* Ishii on factitious host

Colony of *T. chilonis* was initiated from a cohort of fifteen wasps (five males and ten females) obtained from field collected FAW parasitized eggs. The *T. chilonis* wasps were kept in the laboratory under the same conditions described above for *T. remus.* This parasitoid was mass reared using factitious host, rice meal moth, *C. cephalonica*. For colony maintenance, Trichocards were prepared manually with white rectangular manila card (1cm by 5cm) which can accommodate around 200 eggs. Using a Camel-hair brush, white glue was applied on the card and spread evenly. Host eggs were sprinkled slowly over the Trichocards using a paper, tilted slightly downward as dust particles and other debris remain on the top side of the paper. The card was gently shaken to remove excess eggs on the Trichocard if there were any. The card with eggs was allowed to dry for five minutes before being exposed to *T. chilonis*. The

Trichocards were then exposed to the 15 parasitoid wasps (1: 2 - male: female), and plain paper (2cm by 1cm) was thinly layered with honey, put inside the glass vial as a food source.

Once the eggs turned black in color (mostly after 3 to 4 days), indicating that they have been parasitized, Trichocards were removed and replaced with new ones. Trichocards were incubated under room temperature for two weeks till all parasitoid emerged. The parasitoids emerged after a period of 6-12 days. The above process was repeated for colony maintenance. The colony was maintained for 16 generations before starting the bioassays.

CHAPTER FOUR

TO DETERMINE THE DIVERSITY OF INDIGENOUS EGG PARASITOIDS OF FALL ARMYWORM IN KENYA AND IDENTIFY USING MORPHOLOGICAL AND MOLECULAR TECHNIQUES

4.1 Abstract

Fall armyworm, *S. frugiperda* (J.E Smith) is a devastating indigenous insect to the tropical and sub-tropical regions of America and has invaded Africa. This pest is a polyphagous in nature and it has a wide host range, attacking mainly maize crop besides other crop species. A survey was carried out focusing on diverse agro ecological regions of Kenya to explore for parasitoids attacking FAW eggs in maize crop. The survey was conducted in the year 2018 in five counties in Kenya and in each county, two wards were selected. In Kilifi (Kakuyuni and Gongoni), Kwale (Matuga and Kinango), Taita Taveta (Werugha and Mboghoni), Makueni (Kwakjai and Kiboko) and Kirinyaga (Nyangati and Murinduko). These counties were selected because they practice subsistence farming of maize crop, regarding this they minimize the use of pesticide in FAW control. Therefore, the probability of getting parasitoids in these regions was high and this justified for our field survey in these regions. Furthermore, these counties grow maize crop throughout the year under irrigation schemes. During field survey which was done in one season, FAW egg masses were randomly searched from young maize crop (2 weeks) to before flowering adopting a "W" pattern of sampling (FAO, 2018) with the aim to collect as many egg masses as possible. Fifty maize plants per field were randomly chosen to create uniformity between the small and large farms sampled. Egg masses were collected and taken to (*icipe*) and incubated under room temperature, 25 ± 1°C, 12:12 h (L: D) photoperiod, and 60 – 70% RH in the laboratory to observe for emergence of parasitoids and percent parasitism determined. Collection of data was

done in the month of November and December, analyzed using GLM with binomial distribution and means separated using Tukey multiple comparison test. The egg parasitoids collected from the field survey were identified using morphological and molecular techniques. The egg parasitoids of lepidopteran insects, *Telenomus* and *Trichogramma* sp. are minute in size (<1 mm) and challenging to identify morphologically and requires qualified taxonomist to carry out the identification. Molecular identification provides efficient tools for the study of natural population genetics. Therefore, species identification with molecular techniques is fast, accurate and widely applicable. Morphological identification of adults (male and female) parasitoids of both species was done based on the nature of the antennae as the main morphological character that distinguishes one species from another using dissecting microscope (Leica Laz EZ) using magnification (10x). Molecular identification of the field collected samples was done by extracting genomic DNA using DNA extraction kit (Bioline, London, UK) following the manufacturer's instructions. Polymerase chain reaction was performed using forward primer; LCO 1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and reverse primer; HCO 2198 (5'-TATTTTTTGGTCACCCTGAAGTTTA-3'), respectively, targeting the mitochondrial cytochrome oxidase 1 (COI) barcode gene region. Amplified PCR products were purified and shipped to Macrogen Europe for bi-directional sequencing. Sequence identities were determined using, Basic Local Alignment Search Tool (BLAST) lodged in the NCBI GenBank.

Field percent parasitism significantly varied with different altitudes, it ranged from 4.4 to 13.2% for *T. chilonis* and 0 to 21.4% for *T. remus.* Parasitoids was absent in Taita Taveta and present in other counties. Midland (Makueni) and highland (Kirinyaga)

regions had both parasitoid species (*T. chilonis* and *T. remus*), but the latter had higher percent parasitism; 19.24% and 21.43%, respectively. In the same altitude, percent parasitism for *T. chilonis* was higher than parasitism observed in the coastal lowlands (Kilifi and Kwale).

Two egg parasitoids species were recovered from FAW egg masses collected from the field. Morphological and molecular characterization confirmed them to be *Telenomus remus* and *Trichogramma chilonis. T. remus* and *T. chilonis* differ morphologically based on the type of antennae they possess, for example, *T. remus* males have 12-segmented moniliform antennae in which flagella segments are of almost equivalent diameter, while females have 11-segmented clavate antennae, where the last 4 or 5 segments are slightly larger than the previous ones. In *T. chilonis* male antennae was long, distinct, and more plumose; while female antennae were short, not distinct, clubbed and less plumose. Molecular identification confirmed that the egg parasitoids were *T. remus* and *T. chilonis*.

4.2 Introduction

Maize is the main staple food crop to a large population of the world. It is the main income and livelihood source to various smallholder farmers in the developing countries. Maize production is limited by pests and diseases. The diseases include, downy mildews (*Peronosclerospora sorghi*) (W. Weston & Uppal) C.G. Shaw and *Maize chlorotic mottle virus* (MCMV) which are reported to affect maize in Kenya. In synergy with Potyviruses infecting maize, MCMV recently caused the Maize Lethal Necrosis Disease (MLND). The pests attacking maize crop are stem borers, *Chilo partellus* (Swinhoe) and *Busseola fusca* (Fuller); cut worms, *Agrotis ipsilon* (Hufnagel)

and Fall armyworm, *Spodoptera frugiperda* (J.E. Smith). Numerous approaches have been applied over several years to manage maize pests and diseases. These mainly include the use of resistant varieties, often use of highly hazardous classes of insecticides either as seed treatment or foliar application. In sub-Saharan Africa biological control options and habitat management have proved effective for the management of stemborers and striga weeds. However, with the recent invasion of FAW there is need to look for effective biological control solutions.

Several egg parasitoids species, *Trichogramma and Telenomus* are available and used as lepidopteran pests biological control agents. Use of egg parasitoids, *T. remus* and *T. chilonis* as a biological control is a sustainable and cost-effective management option for FAW. *Telenomus remus* is an efficient egg parasitoid of insect pests of genus *Spodoptera* (Pomari *et al.,* 2012). Furthermore, *T. remus* is significant for its productive attack on *Spodoptera spp.* eggs which are deposited in two or more layers (Pomari *et al.,* 2013). This feature is less often found with other egg parasitoids.

Trichogramma chilonis are egg parasitoids that are extensively studied and utilized because of their ease to mass rear (Smith, 1996). In Brazil, the release of *Trichogramma* sp. as a biological control of lepidopteran pests like *S. frugiperda,* and *D. saccharalis* has been considered as the best method of controlling these pests in maize crop (Parra & Zucchi, 2004).

The successful assessment of the role of natural enemies to combat pests must comprise a field survey in order to define their species composition and how their population dynamics differ with the pest insect (Sivasubramaniam *et al.,*1997). Field surveys of

parasitoids and other antagonists in diverse areas of the pest origin have been accomplished (Molina-Ochoa *et al.,* 2001). These parasitoids can potentially prevent an outbreak of fall armyworm. The collection of parasitoid species suited to specific pests and to a particular environmental conditions is crucial for their success (Herz & Hassan, 2006). Indigenous species widely prevalent in similar ecosystem as the invasive pest at any given time are expected to possess high potential for biological control, unlike those from different zones and cropping systems (Glenn *et al.,* 1997). This is because they are well adapted to their natural environment. Indigenous species are the most preferred than exotic ones because the latter one can eliminate indigenous species by competition (Orr *et al.,* 2000). Identification of these egg parasitoids using both morphological and molecular techniques is essential for successful use in biocontrol programs. The main aim of this objective is to determine the diversity of indigenous egg parasitoids of fall armyworm in Kenya and identify using morphological and molecular techniques.

4.3 Materials and Methods

4.3.1 Description of the study area

Since the presence of parasitoids in the field is not dependent on the season, a field survey was done in one season. The survey was conducted in the year 2018 in five counties in Kenya and in each county, two wards were selected. In Kilifi (Kakuyuni and Gongoni), Kwale (Matuga and Kinango), Taita Taveta (Werugha and Mboghoni), Makueni (Kwakjai and Kiboko) and Kirinyaga (Nyangati and Murinduko). The agro ecological characteristics of these regions are illustrated in (Table 4.1). These counties were chosen because they practice subsistence farming of maize crop hence, they minimize the use of pesticide in FAW control and the probability of getting parasitoids

was high. Again, these counties grow maize crop throughout the year under irrigation schemes.

Table 4.1. Characteristics of the agro ecological zones in five counties

County	Study site	Latitude	Longitude	Elevation (m)	Average temp. range (°C)	Annual rainfall range (mm)
Kilifi	Kakuyuni and Gongoni	02°56′11.0″S	040°07′35.4″E	30-310	21-30	300-1300
Kwale	Matuga and Kinango	03°22′11.1″S	038°19′30.7″E	402	19.4-31.7	500-1000
Taita Taveta	Werugha and Mboghoni	03°22′11.1″S	037°40′31.4″E	790-980	21-23.3	480-680
Makueni	Kiboko and Kibwezi	02°23′12.8″S	038°00′12.2″E	1400-1770	13.9-30	300-600
Kirinyaga	Nyangati and Murinduko	00° 37′14.7″S	037°21′05.3″E	1158-5199	12-26	1100-1250

4.3.2 Collection of Fall armyworm egg masses

The FAW egg masses were collected in October and December 2018 in different altitudes as described in Table 4.1. The sample size used during field sampling was 80 farms. The sample size was calculated using the formula.

$$\text{Sample size (n)} = \frac{\frac{Z^2 x\, p(1-p)}{e^2}}{1+\frac{Z^2 x\, p(1-p)}{e^2 x\, N}}$$

Where;

Z=z-score (1.96)

P=probability of success

e= margin of error (95%)

N=population size (106)

Fifty maize plants from young maize crop (2 weeks) to before flowering per field were randomly searched for FAW egg masses adopting a “W” pattern of sampling (FAO, 2018) with the aim to collect as many egg masses as possible. Fifty maize crops were

used to ensure uniformity between the small and large farms sampled. Upon finding the egg mass, it was carefully cut with a pair of scissors along with part of maize plant infected, counted and put in a glass vial and plugged with cotton wool. The glass vial containing the eggs was labelled with the date, site of collection and GPS coordinates of the collection point. Glass vials were put in cool box and taken to the laboratory at the *icipe*, where they were incubated under room temperature, 25 ± 1°C, 12:12 h (L: D) photoperiod and 60–70% RH.

The eggs were monitored daily for FAW egg hatchability or parasitoid emergence. This observation was carried out for a period of four weeks in the laboratory. The emerged parasitoids were counted, recorded and plain paper (2cm by 1cm) thinly layered with honey placed inside the glass vial as a source of food. Eggs which failed to hatch was ignored, because the study aimed to observe for parasitoid emergence from the parasitized FAW eggs only. The adult parasitoids which emerged and died were preserved in 70% ethanol.

Also, the hatched FAW larvae were counted, recorded and removed daily to prevent cannibalism to the parasitized eggs. These larvae were put in plastic jars (4 liters) and given fresh leaves of maize as food while waiting to reach sixth instar to observe for egg-larval parasitoids. At sixth instar, they either formed FAW pupae or cocoon if egg-larval parasitoid were present. The emerged egg parasitoids from field survey were used to initiate a colony in the laboratory to multiply their numbers for further bioassays. For each site, the percent parasitism by each parasitoid species was evaluated by the use of the formula:

$$\text{Percent parasitism} = \frac{\text{Total No.of emerged parasitoids}}{\text{Total No.of FAW eggs collected}} \times 100$$

4.3.3 Morphological and molecular identification of the parasitoids

The study was carried out at the Arthropod Pathology and Biosystematics Unit, International Centre for Insect Physiology and Ecology *(icipe)*.

4.3.3.1 Morphological identification of the parasitoids

4.3.3.1.1 Point mounting of Telenomus remus and Trichogramma chilonis

The parasitoid species recovered during the field sampling were identified using morphological techniques. At least two individuals of each sex (male and female) were used for point mounting. Parasitoids were killed by freezing at 0°C for 5 minutes in the refrigerator before point mounting. Parasitoids were identified at the *icipe* biosystematics unit and through further consultations with Dr. Andrew Polaszek, Natural History Museum, UK. The antennae of the parasitoids were observed using dissecting microscope (Leica Laz EZ) using magnification (10x).

4.3.3.2 Molecular characterization of the parasitoids

The egg parasitoids used in molecular characterization were collected from the field survey carried out in 2018 in five counties of different agro ecological zones. Field collected egg parasitoids were put in 1.5ml micro centrifuge tubes separately. Genomic DNA was extracted using the Isolate II Genomic DNA Kit (Bioline, London, UK) following the manufacturer's instructions. This was done by crushing individual parasitoid using a sterilized pestle before adding 180 µl lysis buffer (GL) and 25 µl proteinase K solution to break the disulphide bonds linking the proteins. The mixture was vortexed with advanced vorter mixer (Velp Scientifica), to mix well the solution and incubated overnight at 56° C in a water bath.

On the following day, the sample solution was vortexed briefly and 200 µl lysis buffer (G3) was added and vortexed vigorously then the mixture incubated at 70°C for 10 minutes. If DNA is present, the solution appears cloudy. After vortexing, 210 µl ethanol was added into the mixture. The solution was placed in isolate II genomic DNA spin column and placed in a 2ml collection tube and centrifuged at 11,000×g. After the flow through was discarded the spin column was blotted using paper towel and re-used as a collection tube. To wash out the silica membrane, 500 µl wash buffer GW1 and 600 µl wash buffer GW were added and centrifuged for 1minute at 11,000×g. To remove residual ethanol and dry the silica membrane, the isolate II genomic DNA spin column was centrifuged for 1 minute at 11,000×g. The extracted DNA solution placed in a 1.5ml micro centrifuge tube. The DNA was eluted twice by adding 15 µl preheated Elution Buffer G (70°C) onto the center of the silica membrane and incubated at room temperature for 5 minutes before being centrifuged at 12,000×g for 7 minutes. Purity and concentration of the extracted DNA was determined using a Nanodrop 2000/2000c spectrophotometer (Thermo Fischer Scientific, Wilmington, USA).

Polymerase chain reaction was performed using forward primer; LCO 1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and reverse primer HCO 2198 (5'-TATTTTTTGGTCACCCTGAAGTTTA-3'), respectively, targeting the mitochondrial cytochrome oxidase 1 (COI) barcode gene region in a total reaction volume of 20 µl containing; PCR H_2O, 5× My Taq Buffer, 10 Pmoles LCO/HCO (forward and reverse) primers, 25Mm $MgCl_2$, My Taq Polymerase and DNA template. The PCR conditions were as follows; initial denaturation at 95°C for 2 min, followed by 40 cycles for 30 seconds at 95°C, annealing at 50.6°C for 45 seconds, extension at 72°C for 1 min, final

extension at 72°C for 10 min after the last cycle.

After DNA amplification, PCR products were resolved through a 1.2% agarose gel (1.5g of agarose dissolved in 150ml TAE buffer after which 3 µl ethidium bromide added as stain) in TAE buffer. Three micro liters of each PCR products were also mixed with a drop of loading dye prior to loading into the gel for electrophoresis. A well was loaded with the control which included 3 µl of distilled water with a drop of loading dye. Mixer were separated by electrophoresis on a 1.2% agarose gel in TAE. A 700-bp DNA ladder was used as a size marker. Electrophoresis was run for 40 min at 80V. DNA bands on the gel were analyzed and documented using KETA GL Imaging System Trans-Illuminator (Wealtec Corp, Meadowvale Way Sparks, USA).

The successfully amplified PCR products were excised and purified using Isolate II PCR and Gel Kit (Bioline) following manufacturer's instructions. Purified samples were shipped to Macrogen Europe BV (Meibergdreef, Amsterdam, the Netherlands) for bi-directional sequencing.

4.3.4 Data analysis

4.3.4.1 Data analysis of the field collected data

The data on presence (1) and absence (0) of FAW egg masses, eggs and the prevalence in different altitudes were analyzed using generalized linear model (GLM) with a binomial distribution. Where significant difference was observed, pairwise comparison between means was done using Tukey multiple comparison test ($\alpha = 0.05$). All statistical analysis was done in R statistical software version 3.5.3 (R Development Core Team, 2016).

4.3.4.2 Molecular Data analysis

Sequences obtained were assembled and edited using BioEdit software (Version 7.2). The primer sequences were identified and removed from the consensus sequences generated from both the forward and reverse reads. Sequences identities were determined using the Basic Local Alignment Search Tool (BLAST) lodged in the NCBI GenBank.

4.4 Results

4.4.1 Prevalence of Fall armyworm in different altitudes in Kenya

From the survey, it was found that FAW has spread widely in different agro-ecological zones. The number of egg masses significantly varied with altitudes (LR $\chi 2 = 5114.5$, df = 4, $p < 0.001$), being higher (50.0 ± 15.07) at (Kirinyaga County) i.e., mid highland (Nyangati ward) and highland (Murinduko ward) while it was lowest (18.52 ± 7.62) at coastal lowland (Kinango and Matuga wards) in Kilifi County. Similarly, total number of FAW eggs was also influenced by altitude (LR $\chi 2 = 5114.5$, df = 4, $p < 0.001$) with highland (Kirinyaga County) recording the highest number (384.58 ± 196.46), while it was lowest (64.48 ± 28.87) at coastal lowland (Kilifi County) (Table 4.2).

Table 4.2. Mean number of Fall armyworm eggs and egg masses per area

County	Number of egg masses (Mean ±SE)	Number of eggs (Mean ±SE)
Kilifi	18.52 ± 7.62a	64.48 ± 28.87a
Kirinyaga	50.0 ± 15.07e	384.58 ± 196.46e
Kwale	28.57 ± 18.44b	134.28 ± 97.95b
Makueni	23.53 ± 10.60d	232.41 ± 122.94d
Taita Taveta	46.67 ± 13.33c	158.53 ± 63.53c

Means followed by different letters in the same column for the FAW egg number and egg masses were significantly different using adjusted Tukey multiple comparison procedure ($\alpha = 0.05$).

4.4.2 Emergence of egg parasitoids from Fall armyworm eggs

Two egg parasitoids species emerged from the FAW egg masses (Figure 4.2) collected from field, namely: *T. remus* and *T. chilonis*. The identity of these parasitoids was confirmed using both morphological and molecular techniques. Fall armyworm unparasitized egg masses hatched after 2 to 3 days while the parasitized eggs were dark in colour. It was observed that parasitoids emerged from these dark egg masses after 6 to 14 days after FAW larvae had emerged. For example, *T. remus* emerged after 6 to13 day and *T. chilonis* emerged after 6 to 12 days.

Figure 4.1. Fall armyworm egg masses

Percent parasitism varied with altitudes ($F_{2,16}$ =8.25; P<0.01) for both parasitoid species. For *T. remus,* the highest percent parasitism (21.43 ± 0.03%) was recorded at midland (Makueni) and at highland (Kirinyaga) (19.24 ± 0.03%). The percent parasitism for *T. chilonis* followed a similar trend, being highest (13.14% ± 0.02%) at highland (Kirinyaga) and lowest (4.44 ± 0.001 %) at coastal lowland (Kilifi) (Figure 4.3). In the midland (Makueni) and highland (Kirinyaga), percent parasitism was higher

for *T. remus* compared to *T. chilonis*. No parasitoid species was recorded in highland (Werugha ward) and mid lowland (Mboghoni ward) in Taita Taveta county (Figure 4.2).

4.4.3 Morphological identity of the egg parasitoids

Results from morphological identification revealed that the two parasitoid species, *T. remus* and *T. chilonis* as the key egg parasitoid species collected from the maize agro-ecologies in the sampled fields. *Telenomus remus* and *Trichogramma chilonis* differ morphologically based on the type of antennae they possess, for example, *T. remus* males have 12-segmented moniliform antennae in which flagella segments are of almost equivalent diameter, while females have 11-segmented clavate antennae, where the last 4 or 5 segments are slightly larger than the previous ones. *T. chilonis* male antennae was long, distinct, and more plumose; while female antennae were short, not distinct, clubbed and less plumose (Figure 4.4).

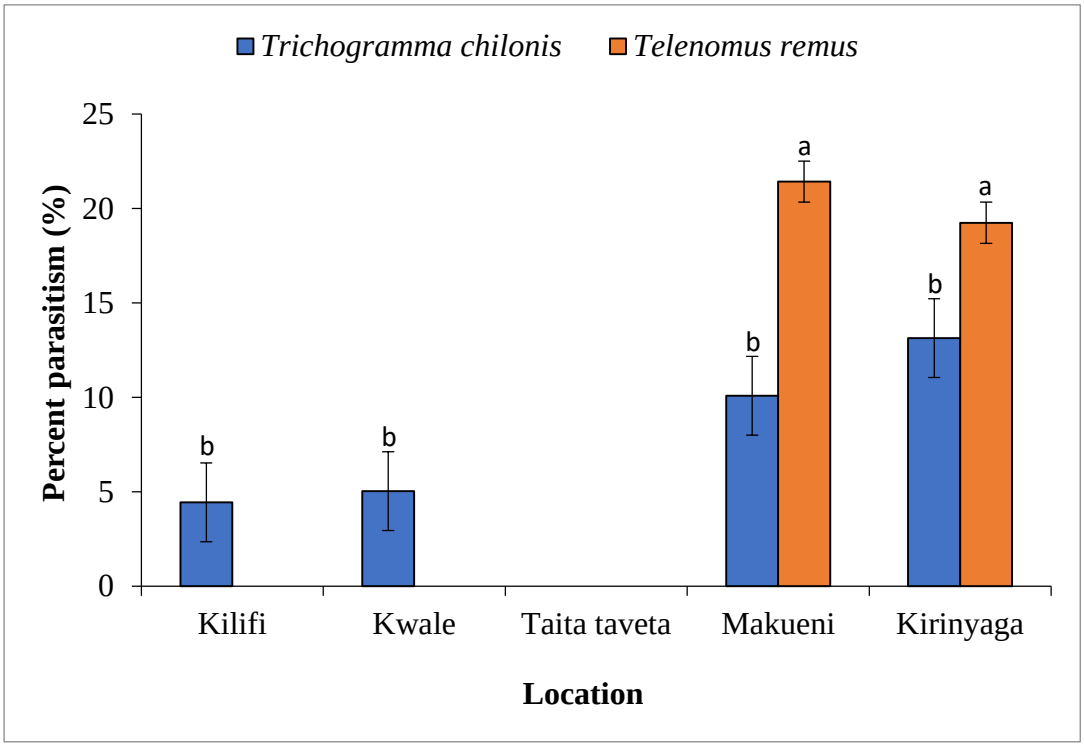

Figure 4.4.2. Field percent parasitism of eggs parasitoids per area

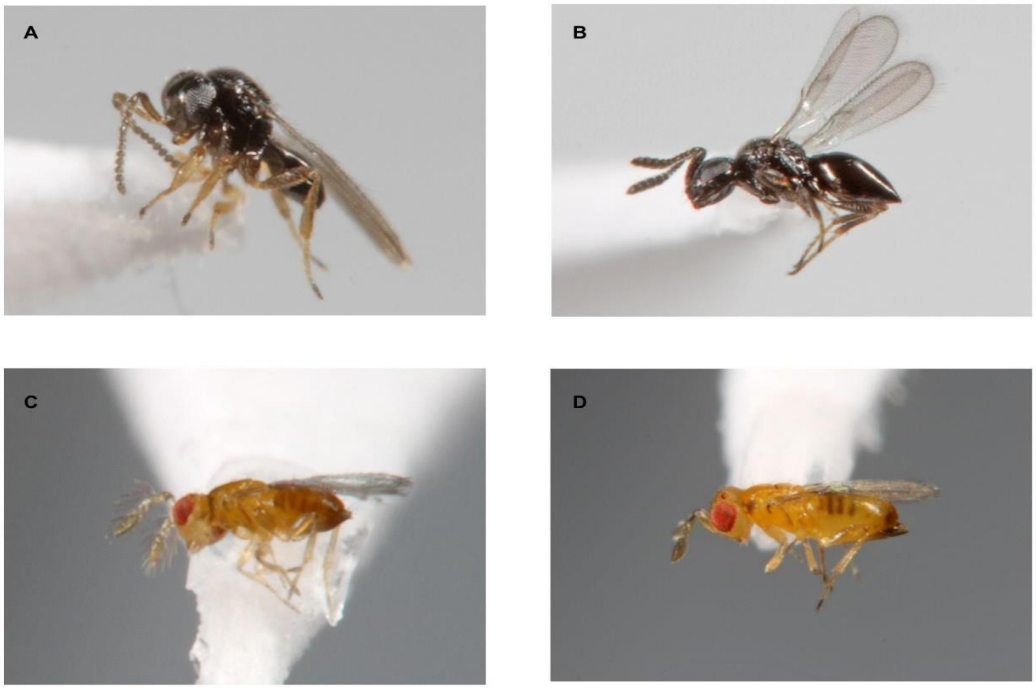

Figure 4.3. Morphological identification of egg parasitoids using antennal characteristics: A) Male *Telenomus remus*, B) Female *Telenomus remus*, C) Male *Trichogramma chilonis* and D) Female *Trichogramma* chilonis (Courtesy: Dr. Robert Copeland, icipe)

4.4.4 Molecular identity of the egg parasitoids

All analyzed sample sequences (Table 4.3) when blasted, they gave hits to *T. remus* and *T. chilonis* samples from GenBank with percent similarity ranging between 96-98% and 99-100%, respectively for the two egg parasitoid species (Table 4.4).

Table 4.3. Study sample cleaned sequences using BioEdit software (Version 7.2)	
Sample	**Cleaned sequences**
TR 1	GGATCAAAAAAAGAAGTATTTAAATTTCGATCAAACAATAATATTGTAATTG CTCCAGCTAAACTGGTAAAGATAATAATAATAAAATTGCTGTTAATAATATA GCCCAAGAAAATAAAGAATTAATTCAATTTTATAAATTTTTATATTTAAAAT TGTTGTAATAAAATTAATTAGAACCTATAATTGAAGAAACCCCAGCAATATG TAAAGAAAAAATTGATAAATCAACTGAAGGTCCCCTATGAGATAAATTAGA AGATAAAGGTGGATAAACAGTTCACCCTGTACCTGTACCAGTTCCAATAAAT ATTCTTGAAGTAATAATATTAATCTTGGAGGTAATAATCAAAATCTTATATT ATTTATTCGTGGAAAAGCTATATCAGGTCTTCCTAATATTAATGGAATTAAA TAATTTCCAAAACCTCCCATTATAACAGGTATCACAAAAAAAAAAAATTATTA AAAAAGCATGACTTGTAACAATAGAATTATAAATTTGATCATTTCCAATTAA AGAACCAGGATTTCCTAATTCTAAACGAATAATTATTCTTATTGATAAACCT ACAATTCCTGCTCACATACCAGAAATAAAATACAAAAT ACCAATATCTTT
TR 2	GGATCAAAAAAAGAAGTATTTAAATTTCGATCAAACAATAATATTGTAATTG CTCCAGCTAAAACTGGTAAAGATAATAATAATAAAATTGCTGTTAATAATAT AGCCCAAGAAAATAAAGAAATTAATTCAATTTTATAAATTTTTATATTTAAA ATTGTTGTAATAAAATTAATTGAACCTATAATTGAAGAAACCCCAGCAATAT GTAAAGAAAAAATTGATAAATCAACTGAAGGTCCCCTATGAGATAAATTAG AAGATAAAGGTGGATAAACAGTTCACCCTGTACCTGTACCAGTTCCAATAAA TATTCTTGATAGTAATAATATTAATCTTGGAGGTAATAATCAAAATCTTATAT TATTTATTCGTGGAAAAGCTATATCAGGTCTTCCTAATATTAATGGAATTAA ATAATTTCCAAAACCTCCCATTATAACAGGTATCACAAAAAAAAAAATTATT AAAAAAGCATGACTTGTAACAATAGAATTATAAATTTGATCATTTCCAATTA AAGAACCAGGATTTCCTAATTCTAAACGAATAATTATTCTTATTGATAAACC TACAATTCCTGCTCACATACCAAAAA
TR 3	AAATATAATAATAATAAAATTGCTGTTAATAATATAGCCCAAGAAAATAAA GAAATTAATTCAATTTTATAAATTTTTATATTTAAAATTGTTGTAATAAAATT AATTGAACCTATAATTGAAGAAACCCCAGCAATATGTAAAGAAAAAATTGA TAAATCAACTGAAGGTCCCCTATGAGATAAATTAGAAGATAAAGGTGGATA AACAGTTCACCCTGTACCTGTACCAGTTCCAATAAATATTCTTGATAGTAAT AATATTAATCTTGGAGGTAATAATCAAAATCTTATATTATTTATTCGTGGAA AAGCTATATCAGGTCTTCCTAATATTAATGGAATTAAATAATTTCCAAAACC TCCCATTATAACAGGTATCACAAAAAAAAAAATTATTAAAAAAGCATGACTT GTAACAATAGAATTATAAATTTGATCATTTCCAATTAAAGAACCAGGATTTC CTAATTCTAAACGAATAATTATTC TTATTGATAAACCT
TR 4	CCTCCTGAAGGATCAAAAAAAGAAGTATTTAAATTTCGATCAAACAATAATA TTGTAATTGCTCCAGCTAAAACTGGTAAAGATAATAATAATAAAATTGCTGT TAATAATATAGCCCAAGAAAATAAAGAAATTAATTCAATTTTATAAATTTTT ATATTTAAAATTGTTGTAATAAAATTAATTGAACCTATAATTGAAGAAACCC CAGCAATATGTAAAGAAAAAATTGATAAATCAACTGAAGGTCCCCTATGAG ATAAATTAGAAGATAAAGGTGGATAAACAGTTCACCCTGTACCTGTACCAGT TCCAATAAATATTCTTGATAGTAATAATATTAATCTTGGAGGTAATAATCAA AATCTTATATTATTTATTCGTGGAAAAGCTATATCAGGTCTTCCTAATATTAA TGGAATTAAATAATTTCCAAAACCCCCATTATAACAGGTATCACAAAAAAAA AAATTATTAAAAAAGCATGACTTGTAACAAATTATAAATTTGATCATTTCCA ATTAAAGAACCAGGATTTCCTAATTCTAACGAAATAATTATTCTTATTGATA AACCTACAATTCCTGCTCACATACCAAAAATAAAAT ACAAAATACCAATATCTTT

Table 4.3. Study sample cleaned sequences using BioEdit software (Version 7.2)	
Sample	**Cleaned sequences**
TR 5	TATTTTGTATTTTATTTTTGGTATGTGAGCAGGAATTGTAGGTTTATCAATAA GAATAATTATCGTTTAGAATTAGGAAATCCTGGTATCTTTAATTGGAAATGA TCAAATTTATAATCTCTATTGTTACAAGGTCATGCTTTTTTAATAATTTTTTTT TTTGTGATACCTGTTATAATGGGAGGTTTGGAAATTATTTAATTCCATTAATA TTAGGAAGACCTGATATAGCTTTTCCACGAATAAATAATATAAGATTTTGAT TATTACCTCCAAGATTAATATTATTACTATCAAGAATATTTATTGGAACTGGT ACAGGTACAGGGTGAACTGTTTATCCACCTTTATCTTCTAATTTATCTCATAG GGGACCTTCAGTTGATTTATCAATTTTTTCTTTACATATTGCTGGGGTTTCTTC AATTATAGGTTCAATTAATTTTATTACAACAATTTTAAATATAAAAATTTATA AAATTGAATTAATTTCTTTATTTTCTTGGGCTATATTATTAACAGCAATTTTA TTATTATTATCTTTACCAGTTTTAGCTGGAGCAATTACAATATTATTGTTTGA TCGAAATTTAAATACTTCTTTTTTTG
TR 6	TCCAGCTAAAACTGGTAAAGATAATAATAATAAAATTGCTGTTAATAATATA GCCCAAGAAAATAAAGAAATTAATTCAATTTTATAAATTTTTATATTTAAAA TTGTTGTAATAAAATTAATTGAACCTATAATTGAAGAAACCCCAGCAATATG TAAAGAAAAAATTGATAAATCAACTGAAGGTCCCCTATGAGATAAATTAGA AGATAAAGGTGGATAAACAGTTCACCCTGTACCTGTACCAGTTCCAATAAAT ATTCTTGATAGTAATAATATTAATCTTGGAGGTAATAATCAAAATCTTATATT ATTTATTCGTGGAAAAGCTATATCAGGTCTTCCTAATATTAATGGAATTAAA TAATTTCCAAAACCTCCCATTATAACAGGTATCACAAAAAAAAAAATTATTA AAAAAGCATGACTTGTAACAATAGAATTATAAATTTGATCATTTCCAATTAA AGAACCAGGATTTCCTAATTCTAAACGAATAATTATTCTTATTGATAAACCT ACAATTCCTGCTCACATACCAAAAA
TR 7	TCAAAAAAAGAAGTATTTAAATTTCGATCAAACAATAATATTGTAATTGCTC CAGCTAAAACTGGTAAAGATAATAATAATAAAATTGCTGTTAATAATATAGC CCAAGAAAATAAAGAAATTAATTCAATTTTATAAATTTTTATATTTAAAATT GTTGTACCATAAAATTAATTGAACCTATAATTGAAGAAACCCCAGCAATATG TAAAGAAAAAATTGATAAATCAACTGAAGGTCCCCTATGAGATAAATTAGA AGATAAAGGTGGATAAACAGTTCACCCTGTACCTGTACCAGTTCCAATAAAT ATTCTTGATAGTAATAATATTAATCTTGGAGGTAATAATCAAAATCTTATATT ATTTATTCGTGGAAAAGCTATATCAGGTCTTCCTAATATTAATGGAATTAAA TAATTTCCAAAACCTCCCATTATAACAGGTATCACAAAAAAAAAAATTATT AAAAAAGCATGACTTGTAACAATAGAATTATAAATTTGATCATTTCCAATTA AAGAACCAGGATTTCCTAATTCTAAACGAATAATTATTCTTATTGATAAACC TACAATTCCTGCTCACATACCAAAAA
TR 8	TCCTCCTGAAGGATCAAAAAAAGAAGTATTTAAATTTCGATCAAACAATAAT ATTGTAATTGCTCCAGCTAAAACTGGTAAAGATAATAATAATAAAATTGCTG TTAATAATATAGCCCAAGAAAATAAAGAAATTAATTCAATTTTATAAATTTT TATATTTAAAATTGTTGTAATAAAATTAATTGAACCTATAATTGAAGAAACC CCAGCAATATGTAAAGAAAAAATTGATAAATCAACTGCAAGGTCCCCTATG AGATAAATTAGAAGATAAAGGTGGATAAACAGTTCACCCTGTACCTGTACC AGTTCCAATAAATATTCTTGATAGTAATAATATTAATCTTGGAGGTAATAAT CAAAATCTTATATTATTTATTCGTGGAAAAGCTATATCAGGTCTTCCTAATAT TAATGGAATTAAATAATTTCCAAAACCTCCCATTATAACAGGTATCACAAAA AAAAAAATTATTAAAAAAGCATGATAAACGAATAATTATTCTTATTGATAAA CCTACAATTCCTGCTCACATACCAAAAA

Table 4.3. Study sample cleaned sequences using BioEdit software (Version 7.2)	
Sample	**Cleaned sequences**
TR 9	CTCCAGCTAAAACTGGTAAAGATAATAATAATAAAATTGCTGTTAATAATAT AGCCCAAGAAAATAAAGAAATTAATTCAATTTTATAAATTTTTATATTTAAA ATTGTTGTAATAAAATTAATTGAACCTATAATTGAAGAAACCCCAGCAATAT GTAAAGAAAAAATTGATAAATCAACTGAAGGTCCCCTATGAGATAAATTAG AAGATAAAGGTGGATAAACAGTTCACCCTGTACCTGTACCAGTTCCAATAAA TATTCTTGATAGTAATAATATTAATCTTGGAGGTAATAATCAAAATCTTATAT TATTTATTCGTGGAAAAGCTATATCAGGTCTTCCTAATATTAATGGAATTAA ATAATTTCCAAAACCTCCCATTATAACAGGTATCACAAAAAAAAAAATTATT AAAAAAGCATGACTGTAACAATAGAATTATAAATTTGATCATTTCCAATTAA AGAACCAGGATTTCCTAATTCTAAACGAATAATTATTCTTATTGATAAACCT ACAATTCCTGCTCACATACCAAAAATAAAATA CAAAATACCAATATCTTTA
TEL 10	AACATTATATTTTTATTTTGGTGTTTGAGCAGGAATATTAGGTTCAGCAATAA GAGCTTTAATTCGAATAGAACTTAGAGTTCCAGGAATACTTATTGGAAATGA CCAAATTTATAATTCAATTGTAACTTCACACGCATTTATTATAATTTTTTTTAT AGTTATACCAATTATACTTGGAGGATTTGGAAATTGACTTGTACCATTAATA ATTAATGCTCCAGATATAGCATTTCCACGATTAAATAATATAAGTTTTTGATT ATTAATTCCATCTTTAATTTTATTAATTTATAGAAATATTTTTGGATCAGGAA CAGGAACAGGATGAACTGTATACCCACCTTTATCTTCACAATTAAATCCATC AATTGATTTAACTATTTTTTCACTTCATATTGCAGGAATTTCATCTATTCTTAG ATCAATTAATTTTTTATGTACAATTATTAATATATCAAATACTTCAATAAATA ATTGAACCTTATTTACTTGATCGGTATTAATTACAACAATTTTATTATTATTA TCACTTCCAGTACTAGCAGGAGCAATTAAAATAATTTTAACTGATCGAAACT TAAATACAGCATTTTTTGGTCCACCAGGAGGAGGAGA
TEL 11	AACATTATATTTTTATTTTGGTGTTTGAGCAGGAATATTAGGTTCAGCAATAA GAGCTTTAATTCGAATAGAACTTAGAGTTCCAGGAATACTTATTGGAAATGA CCAAATTTATAATTCAATTGTAACTTCACACGCATTTATTATAATTTTTTTTAT AGTTATACCAATTATACTTGGAGGATTTGGAAATTGACTTGTACCATTAATA ATTAATGCTCCAGATATAGCATTTCCACGATTAAATAATATAAGTTTTTGATT ATTAATTCCATCTTTAATTTTATTAATTTATAGAAATATTTTTGGATCAGGAA CAGGAACAGGATGAACTGTATACCCACCTTTATCTTCACAATTAAATCCATC AATTGATTTAACTATTTTTTCACTTCATATTGCAGGAATTTCATCTATTCTTAG ATCAATTAATTTTTTATGTACAATTATTAATATATCAAATACTTCAATAAATA ATTGAACATTATTTACTTGATCTGTATTAATTACTACAATTTTATTATTATTAT CACTTCCAGCACTATCAGGAGCAATTACC
TEL 12	CAATTGTAACTTCACACGCATTTATTATAATTTTTTTTATAGTTATACCAATT ATACTTGGAGGATTTGGAAATTGACTTGTACCATTAATAATTAATGCTCCAG ATATAGCATTTCCACGATTAAATAATATAAGTTTTTGATTATTAATTCCATCT TTAATTTTATTAATTTATAAAAATATTTTTGGATCAGGAACAGGAACAGGAT GAACTGTATTCCCACCTTTATCTTCACAATTAAATCCATCAATTGATTTAACT ATTTTTTCACTTCATATTGCAGGAATTTCATCTAGTCTTTGATCAATTAATTTT TTCTGTACACTTATTAATATATCAAAGACTTCCCCAATAATGGATTGTACCTT ATTTAATTGATATGCATTACTAACAGAGTTTTTATTATTTTTATTAGGGTTAC CAGAGGAGAATTCACAAAAATTTAACTGGTCCAAATTAAAACCCTTAATTTT T

Table 4.4. Molecular identification of egg parasitoids *Telenomus remus* and *Trichogramma chilonis* parasitizing Fall armyworm in Kenya

Study sample Name	**ID from GenBank**	**Sample identification**	**% Identical similarity**	**Query cover %**	**E value**	**Sample GenBank accessions**
TR 1	*Trichogramma chilonis* cytochrome oxidase subunit 1 gene, partial cds; mitochondrial	KJ911023	99.7	96.3	0	MT465117
TR 2	*Trichogramma chilonis* isolate Tc1 cytochrome oxidase subunit I (CO1) gene, partial cds; mitochondrial	KM501046	100	92.28	0	MT465118
TR3	*Trichogramma chilonis* isolate Pak3835 cytochrome c oxidase subunit I gene, partial cds; mitochondrial	JQ598687	99.8	96.92	0	MT465119
TR4	*Trichogramma chilonis* cytochrome oxidase subunit 1 gene, partial cds; mitochondrial	KJ911023	99.8	94.06	0	MT465120
TR5	*Trichogramma chilonis* cytochrome oxidase subunit 1 gene, partial cds; mitochondrial	KJ911023	99.5	100	0	MT465121
TR 6	*Trichogramma chilonis* isolate Tc1 cytochrome oxidase subunit I (CO1) gene, partial cds; mitochondrial	KM501046	100	91.91	0	MT465121
TR 7	*Trichogramma chilonis* isolate Tc1 cytochrome oxidase subunit I (CO1) gene, partial cds; mitochondrial	KM501046	99.5	92.28	0	MT465123
TR 8	*Trichogramma chilonis* isolate Tc1 cytochrome oxidase subunit I (CO1) gene, partial cds; mitochondrial	KM501046	99.8	92.55	0	MT465124
TR 9	*Trichogramma chilonis* strain Anakapalle cytochrome c oxidase subunit 1 (COX1) gene, partial cds; mitochondrial	MN116707	100	100	0	MT465125
TEL 11	*Telenomus remus* voucher BIOUG15396-B12 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochodrial	KY835081	98.6	89.55	0	MT465126
TEL 12	*Telenomus remus* voucher BIOUG15396-B12 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	KY835081	96.1	97.04	0	MT465127
TEL 13	*Telenomus remus* voucher BIOUG15396-B12 cytochrome oxidase subunit 1 (COI) gene, partial cds; mitochondrial	KY835081	98.9	95.01	0	MT465128

4.4.5 Diversity of the parasitoids characterized in the study

Phylogenetic analysis was done using MEGA X (Kumar *et al.*, 2018) and the evolutionary history was inferred using the Maximum Likelihood method and Kimura 2-parameter model (Kimura, 1980). The analysis involved 12 nucleotide sequences generated from the study and the codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). The tree was rooted using *Trichogramma brassicae* (JX442923.1) and *Megaphragma amalphitanum* (KT373787.1) from GenBank.

4.4.5.1 Phylogenetic analyses of Trichogramma chilonis

The analysis showed that all the *T. chilonis* from this study clustered together to *T. chilonis* (Figure 4.5 and 4.6) of GenBank accession number KJ911023.1 and forming a sister clade with *T. brassicae*. The *M. amalphitanum* as expected, branched separately from the two *Trichogramma* species.

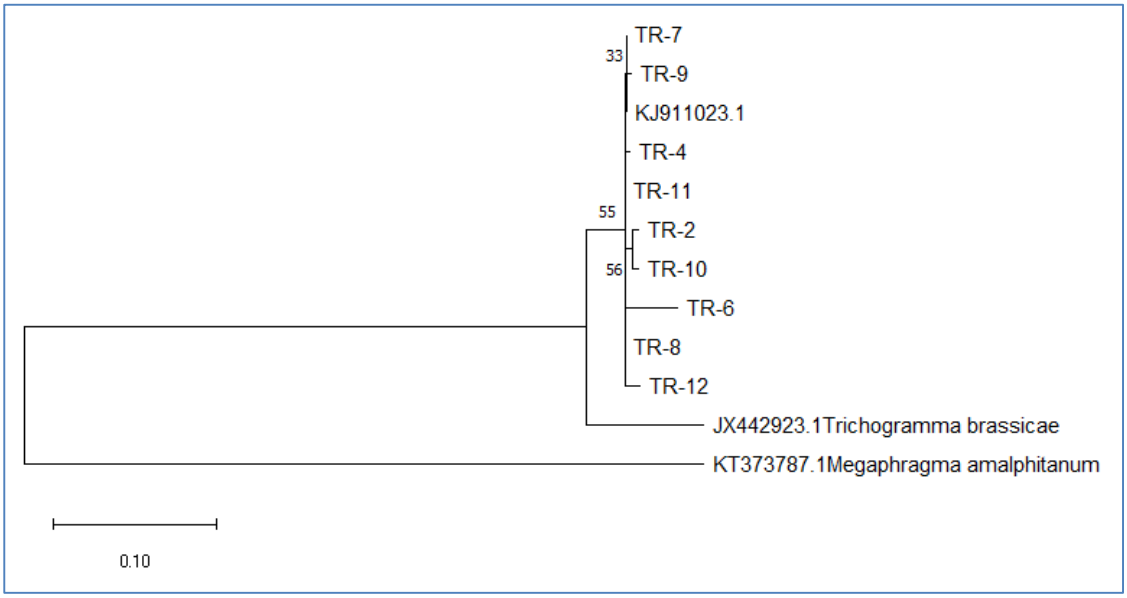

Figure 4.4. Phylogenetic tree of *Trichogramma chilonis*

Figure 4.5. Trichogramma chilonis

4.4.5.2 ***Telenomus remus***

The BLAST results linked all the T. remus from this study to *T. remus* (Figure 4.7) from GenBank of accession number KY835081. The T. remus phylogenetic tree was not generated because, only three samples were cleaned successfully using Bioedit software from the sequences obtained after bi directional sequencing.

Figure 4.4.6. Telenomus remus

4.5 Discussion

The FAW egg masses hatched after 2 to 3 days while the parasitized eggs were dark in color and emerged parasitoids after a period of 6 to 14 days. For instance, *T. chilonis* emerged from the parasitized eggs after 6 to 12 days and *T. remus* emerged after 6 to 13 days. *Telenomus remus* and *Trichogramma chilonis* were the key egg parasitoids recovered from FAW eggs. This was revealed from morphological identification and further confirmed by molecular characterization. The field percent parasitism significantly varied with altitudes for both parasitoid species and it ranged from 4.44 to 13.15% for *T. chilonis* and 0 to 21.43% for *T. remus* with the latter being predominant. Outcomes of the study are consistent with those recently stated by Sisay *et al.* (2019) that *T. remus* is the dominant egg parasitoid species in Kenya and Tanzania with percent parasitism of 69.3% and 58.5%, respectively while *T. chilonis* contributed to only 20.9% of FAW egg parasitism. In addition, Kenis *et al.* (2019) stated the occurrence of *T. remus* in five countries in East, South and West Africa parasitizing eggs of *S. frugiperda*.

Percent parasitism was lower for *T. chilonis* especially in lowland regions. Factors that might have contributed to the lower parasitism rate could be; difference in population size of the parasitoids, ability of parasitoids to search for their host eggs and parasitize, climatic conditions, plant morphology and crop architecture. Romeis *et al.* (1997) reported that plant volatiles influence Trichogrammatidae behavior with certain plant species being attractive while others being repellant.

These findings put more emphasis on further surveys on antagonists of FAW in diverse locales of spread of FAW (Molina-Ochoa *et al.,* 2001). Our investigation showed that

the two egg parasitoid species (*T. remus* and *T. chilonis*) were recovered from the midland and highland altitudes (Makueni and Kirinyaga) while only one egg parasitoid species (*T. chilonis*) was got from the coastal lowland regions (Kilifi and Kwale). The *T. chilonis* was the most widely distributed parasitoid being found in four counties, Kilifi, Kwale, Makueni and Kirinyaga, although with very low percent parasitism. On the other hand, *T. remus* was found only in two counties, Makueni and Kirinyaga but with higher percent parasitism when compared to *T. chilonis*. The occurrence of egg parasitoids of FAW, *T. remus* and *T. chilonis* in the sampled farms indicates a great potential for FAW biological control which needs to be enhanced and conserved.

This study confirms the identity of the two collected egg parasitoid species of FAW as *T. remus* and *T. chilonis* through morphological and molecular identification. These results recommend for testing the two egg parasitoid species to determine their potential as biocontrol agents of FAW and be incorporated within an IPM context for its management in Kenya. Molecular analysis of the mitochondrial COI barcode gene region of *Trichogramma chilonis* samples in this study linked them to *T. chilonis* (KJ911023, MN116707 and KM501046) from India and *T. chilonis* (JQ598687) from France at 99 to 100% similarity. On the other hand, *Telenomus remus* samples from this study linked to *T. remus* (KY835081) from Canada at 96 to 98% similarity. In Africa, *T. remus* has been reported to attack eggs of FAW (Kenis *et al.*, 2019). Field surveys conducted in Ethiopia, Kenya and Tanzania by Sisay *et al.* (2019) reported *T. remus* and *Trichogramma* sp attacking egg masses of FAW. This study further confirms this result on *T. chilonis* with both morphological and molecular techniques. Morphological identity of *Trichogramma* species attacking FAW eggs in Africa was also confirmed as *T. chilonis* by Dr. Andrew Polaszek of Natural History Museum,

United Kingdom.

In Ghana and Benin, *T. remus* and *Trichogramma sp.* have been reported to attack FAW eggs with 25.9% and 14.5% parasitism, respectively (Agboyi *et al.,* 2020). In West Africa, the new associations of indigenous parasitoids with FAW may be related to the occurrence of several other *Spodoptera* species, for example; black armyworm (*S. exempta*), beet armyworm (*S. exigua*) and cotton leaf worm (*S. littoralis*) (Agboyi *et al.,* 2020). In Pakistan, *T. chilonis* has been used excellently to control *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) attacking tomato crops (Abbas *et al.,* 2020). Cave (2000) reported that *T. remus* is effective in augmentative biological control of FAW infestation in the field. In Brazil, the release of *Trichogramma* sp. as a biological control of lepidopteran pests like *S. frugiperda* and *Diatraea saccharalis* Fabricius has been considered as the best method of controlling these pests in maize cropping systems (Parra & Zucchi, 2004). Results from this study show that field percent parasitism of *T. remus* ranged between 0 to 21.4% and 4.4 to 13.2% for *T. chilonis* which needs to be conserved and enhanced for the FAW management in Africa.

In North and South America, where FAW originated from, many parasitoids species attacking FAW have been documented. For instance, a total of one hundred and fifty species of parasitoids of FAW have been recorded (Molina-Ochoa *et al.,* 2003). This indicates that emphasis should be given to designing a successful biological control program for FAW through conservatory and augmentative field releases of these indigenous parasitoid species.

4.6 Conclusion

The recovery of *T. remus* and *T. chilonis* in different altitudes surveyed in 2018 confirms the associations of indigenous egg parasitoids with FAW in Kenya. The findings from this study justify more extensive surveys to collect for more natural enemies of FAW even in other counties which can contribute to the management of FAW through integrated pest management. Percent parasitism differed significantly with altitudes. Up to 21.43% percent parasitism of FAW eggs by *T. remus* and 13.15% by *T. chilonis* were observed in the field, which needs to be conserved and enhanced through minimizing indiscriminate usage of insecticides. Therefore, we recommend both the egg parasitoids to being the most efficient candidates to be incorporated in IPM strategy for Fall armyworm.

Morphological and molecular identification confirms that the field collected egg parasitoids were T. remus and T. chilonis. These egg parasitoids were found attacking the FAW egg masses in the selected agro ecological zones surveyed in five counties. This finding has important implications for designing biological control program within an Integrated Pest Management (IPM) strategy for fall armyworm in Africa. Egg parasitoids are amenable for mass rearing and augmentative releases and they kill FAW eggs before hatching into the destructive larval stage.

CHAPTER FIVE

PERFORMANCE OF EGG PARASITOIDS OF FALL ARMYWORM UNDER LABORATORY CONDITIONS

5.1 Abstract

Fall armyworm is a destructive insect constraining maize production in Africa. It is naturally attacked by parasitoids which can be used for managing outbreaks. This study was done to assess the performance of egg parasitoids of FAW eggs at different densities under laboratory conditions. Colony of egg parasitoids were maintained in the laboratory at the (*icipe*) at 25 ± 1°C, 12:12h (L: D) photoperiod and 60 ±10% relative humidity (RH). Parasitoids were permitted to mate for 1 to 2 days after emerging and individual female picked randomly then exposed to 20, 40 and 60 fresh FAW eggs for 24 hours while being fed on honey. Data on percent parasitism, FAW emergence and egg mortality was recorded. Sex ratio (percent female progeny) was determined after emergence. Parasitoids were killed by freezing them at 0°C after emergence and sexed by observing individual adults using dissecting microscope (Leica Laz EZ) using magnification 10x based on the antennal characteristics. Percent parasitism, FAW emergence, egg mortality and sex ratio varied significantly per female parasitoid species with FAW egg densities offered. Under controlled conditions, 57 to 96% FAW eggs were either killed or parasitized by *T. remus* and 53 to 90% FAW eggs were either killed or parasitized by *T. chilonis*. Sex ratio (percent female progeny) varied significantly among the parasitoid species with *T. remus* recording the highest percent (83.3%).

5.2 Introduction

The selection of a suitable natural competitor (egg parasitoid) to be introduced into the field is the main concern in establishment of biocontrol approaches (Ballal & Singh,

2003). *Telenomus remus* and *Trichogramma chilonis* are tiny parasitoids which parasitize the eggs of its host and prevent the crop damage reaching above economic threshold levels. The use of parasitoids is the most efficient, inexpensive, and suitable for smallholder farmers who do not have enough money to buy insecticides. However, determining which parasitoid species is the most effective at parasitizing a pest in a specific ecosystem might assist in enhancing biocontrol (Jourdie *et al.,* 2008). Testing the efficacy of natural enemies in the field can consume a lot of time particularly when numerous parasitoids need to be evaluated. Therefore, laboratory and screen house testing are important for selecting an appropriate candidate for a biological control programme (Ballal & Singh, 2003). The present study assesses the performance of *T. remus* and *T. chilonis* under controlled conditions in the laboratory.

5.3 Materials and Methods

5.3.1 Description of the study area

Before the commencement of the bioassay, the parasitoids were maintained in the laboratory for at least 16 generations. Freshly laid FAW eggs were obtained from the colony and separated using soft camel hair brush. Small pieces of papers were prepared by measuring (0.5cm by 0.5 cm) and cut using a pair of scissors. Fall armyworm egg densities (20, 40 or 60) were randomly counted carefully to minimize the eggs getting cleaned from its natural state (scaly eggs) and pasted on small pieces of paper using white glue. The papers containing the glued eggs were placed individually inside a clean glass vial. One-day old mated female wasps of either *T. chilonis* or *T. remus* were introduced in the glass vial containing FAW egg (one wasp per vial per egg density) and the date of exposure was recorded. After exposure for 24hours, the piece of paper containing FAW eggs were removed, placed separately in a new glass then incubated

in the laboratory at the same conditions as described above.

Eggs were monitored daily to observe and record parasitoid emergence, FAW emergence and egg mortality. The total number of emerged parasitoid and FAW larvae were counted daily, removed from the vials, and recorded. After a period of two weeks the number of eggs which neither hatched as FAW larvae nor resulted emerged parasitoid, were also counted, and recorded as egg mortality. To get percent parasitism, the number of emerged parasitoids was divided by the total number of exposed eggs and multiplied by 100.

To determine sex ratio, the emerged wasps were first killed by keeping them under refrigerator at $0^{\circ}C$ for 5 minutes. Adult parasitoids were sexed by observing the morphological characteristics of antennae under a dissecting microscope (Leica Laz EZ) with magnification (10x) using the same characteristics described under section (5.3.2.1) for the differential type of antennae. The experiment was laid in a completely randomized design and for each egg density, the experiment was replicated fourteen times for each parasitoid species.

$$\text{Percent (parasitism, FAW emergence and egg mortality)} = \frac{\text{No.of parasitoids,faw emergence \& egg mortality}}{\text{Total no.of eggs exposed}} \times 100.$$

5.3.2 Data analysis

Laboratory data recorded on the performance of egg parasitoids of FAW in the laboratory was tested for normality with the Shapiro-Wilk test and were arcsine transformed to ensure normal distribution. Thereafter, one–way ANOVA was conducted to determine the significance difference between transformed data of percent

parasitism, FAW emergence, egg mortality and sex ratio (% female progeny) on FAW egg densities. Where significant difference was observed, pairwise comparison between means was done using the Tukey multiple comparison test ($\alpha = 0.05$). Furthermore, the parameters were compared between the parasitoid species, *T. remus* and *T. chilonis* per each FAW egg density using t test. All statistical analysis was carried out in R statistical software version 3.5.3 (R Development Core Team, 2016).

5.4 Results

5.4.1 Egg parasitoids parasitism rate

Fall armyworm egg density significantly influenced percent parasitism ($F_{2,77}$= 31.41; P<0.001) (Figure 5.1 and 5.2). Percent parasitism significantly varied with FAW egg density for both parasitoid species. *T. remus* had significantly ($F_{2,39}$=13.82; P<0.001) higher percent parasitism (62.7±4.17%) at low (20) FAW egg density and decreased with increase in egg density. Likewise, percent parasitism (67.4±6.78%) by *T. chilonis* was highest ($F_{2,38}$=19.59; P<0.001) at low egg density (20) and it also decreased as the FAW egg density was increased. There was an interaction effect between FAW egg density and parasitoid species ($F_{2,77}$ =4.20; P<0.05) on percent parasitism.

Comparing the performance of the two parasitoid species on the same FAW egg density, percent parasitism was significantly higher ($F_{1,26}$=16.58; P<0.001) for *T. remus* than *T. chilonis* at FAW egg density of 60. Conversely, there was no effect on the performance of the two parasitoid species at the low FAW egg densities ($F_{1,25}$=0.43; P=0.52 and $F_{1,26}$=1.50; P=0.23) of 20 and 40, respectively).

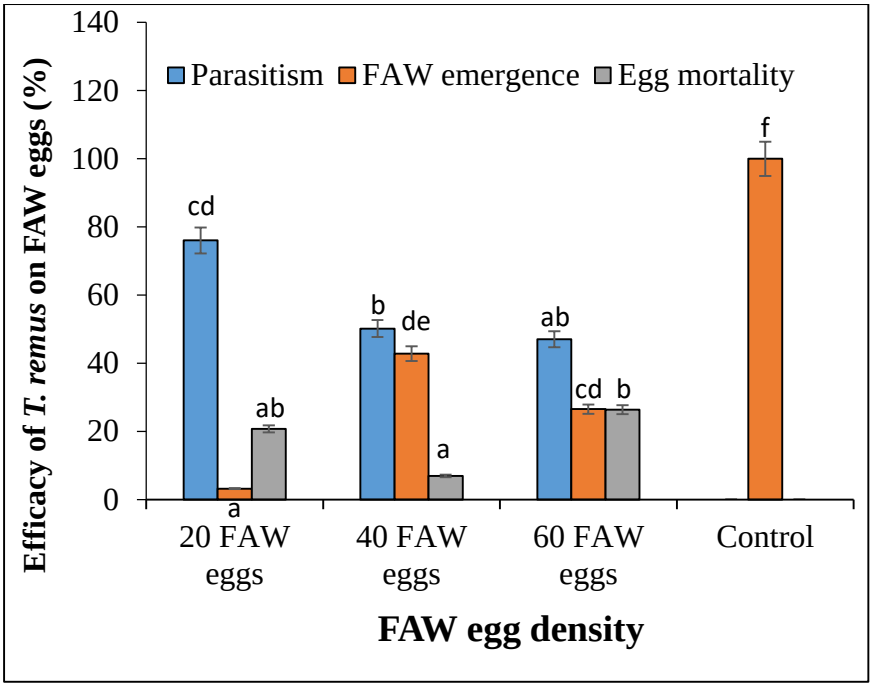

Figure 5.1. Performance of *Telenomus remus* on Fall armyworm egg density

Bars caped with the same letter are not significantly different using Tukey's multiple comparison test (α = 0.05).

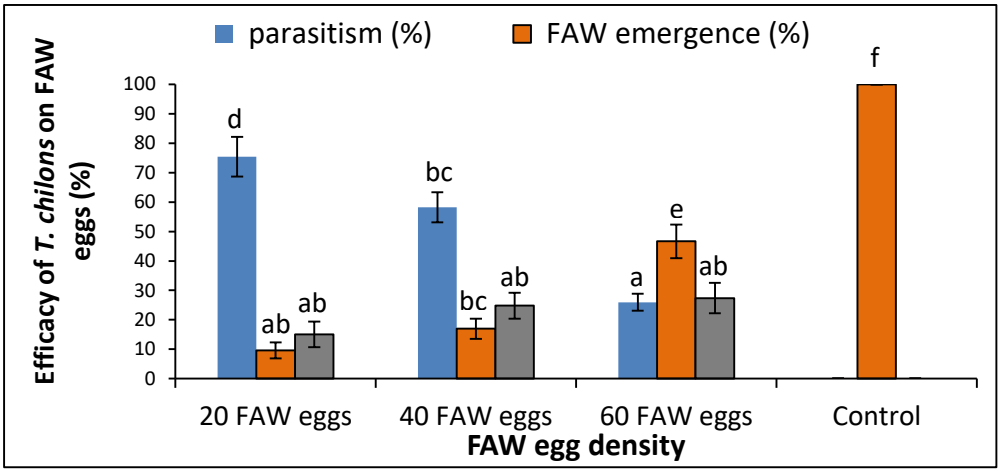

Figure 5.2. Performance of *Trichogramma chilonis* on Fall armyworm egg density

Bars caped with the same letter are not significantly different using Tukey's multiple comparison test (α = 0.05).

5.4.2 Fall armyworm emergence

Similar to percent parasitism, FAW emergence varied with the FAW egg density and parasitoid species. The percentage FAW emergence was significantly ($F_{2,38}$ =34.45; P<0.001) lowest (6.64±1.20%) at egg density of 20 when subjected to parasitism by *T.*

remus. Also, when subjected to parasitism by *T. chilonis*, percent FAW emergence was significantly ($F_{2,38}$=19.3, P<0.001) lower (13.16±2.75%) at the same egg density and it increased at higher FAW egg density. FAW emergence for same FAW egg density varied between the two parasitoid species, except for FAW egg density of 20. At FAW egg density of 40, FAW emergence was significantly ($F_{1,26}$=19.89; P<0.001) lowest (22.45±3.43%) when the eggs were offered to *T. chilonis*, and it was significantly ($F_{1,26}$=6.68; P<0.05) lower (29.55±5.19%) for *T. remus* at FAW egg density of 60.

5.4.3 Fall armyworm egg mortality

The FAW egg mortality was significantly influenced by FAW egg density ($F_{2,77}$=3.30; P<0.05) (Table 5.1). Percent egg mortality varied significantly ($F_{1,39}$=9.63; P<0.001) between FAW egg density treatments, when subjected to parasitism by *T. remus.* Lowest (13.4 ±1.66%) egg mortality was observed when 40 eggs were exposed. On the other hand, percent mortality of FAW egg was not affected ($F_{2,38}$=2.22; P=0.12) by egg density when the eggs were subjected to *T. chilonis*. Mortality of FAW eggs for the same density was not affected by parasitoid species except at the egg density of 40, whereby egg mortality was significantly ($F_{1,26}$=13.43; P<0.01) higher (28.1 ±4.40%) when the eggs were subjected to parasitism by *T. chilonis*.

Table 5.1. Performance of *Telenomus remus* and *Trichogramma chilonis* per each Fall armyworm egg density using t test

FAW egg density	Parasitoid species					
	T. remus	***T. chilonis***	***T. remus***	***T. chilonis***	***T. remus***	***T. chilonis***
	Parasitism (%)		**Hatched FAW egg (%)**		**FAW egg mortality (%)**	
20	62.7±4.17cdA	67.4±6.78dA	6.64±1.20aA	13.16±2.75abB	25.0±3.71abA	16.7±4.35abB
40	45.1±4.19bA	50.3±5.08bcA	40.63±4.39deA	22.45±3.43bcB	13.4 ±1.66aA	28.1±4.40abB
60	43.2±4.08abA	30.2±2.90aB	29.55±5.19cdA	42.93±5.71eB	29.8 ±3.33bA	28.1±5.14abA

Means in the same column followed with the same lowercase letters are not significantly different using Tukey's multiple comparison test ($\alpha = 0.05$); Means in the same row followed by the same uppercase letters for the same parameter are not significantly different using t test.

5.4.4 Parasitoid sex ratio (% female progeny)

Percentage of female progeny varied significantly across FAW egg density ($F_{2,39}$=18.52; P<0.001) for *T. remus*. Conversely, there was no effect on the same FAW egg density ($F_{2,39}$=2.61; P=0.09) for *T. chilonis*. *T. remus* yielded more female progeny (66.2±1.72%) at FAW egg density of 20, while for *T. chilonis*, the highest female progeny (58.5±5.60%) was recorded at egg density of 40. There was an effect on egg density of 20 ($F_{1,26}$=28.12; P<0.001), when percent female progeny was compared among the parasitoid species on FAW egg density. On the other hand, there was no effect on FAW egg density of 40 ($F_{1,26}$=0.45; P=0.51) and 60 ($F_{1,26}$=3.50; P=0.07), respectively.

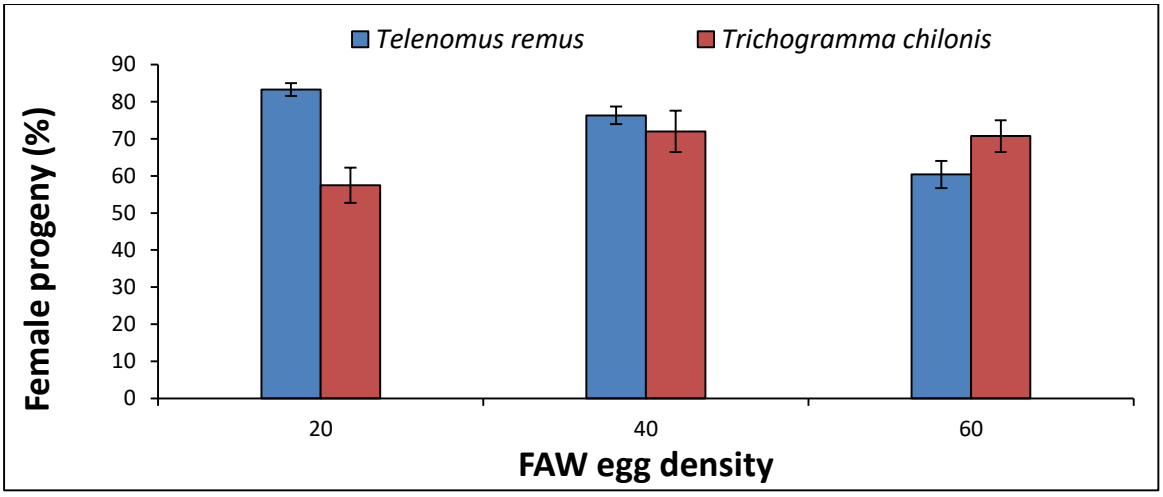

Figure 5.1. Percent female progeny of *Telenomus remus* and *Trichogramma chilonis* on Fall armyworm egg density

5.5 Discussion

For the successful deployment of egg parasitoids in the field, it is necessary to identify the essential quantity of egg parasitoids in regard to the estimated host eggs existing in the field with the aim to manage it and prevent crop loss from its damage. To achieve this, parasitoids collected from the field survey was assessed under laboratory to determine their performance with the FAW egg densities. This will be relevant regarding enforcement of biological control programs using egg parasitoids. Parasitoid species that showed a high potential of parasitism when assessed under laboratory conditions can also perform well in the field.

Percent parasitism rate, FAW emergence and egg mortality were among the parameters used to assess the efficacy of the egg parasitoids (Vásquez *et al.*,1997) in the laboratory. The laboratory output showed significant difference in percent parasitism, FAW emergence and egg mortality when different densities of FAW eggs (20,40 and 60) were subjected to parasitism by *T. remus* and *T. chilonis*. For instance, 53 to 90% FAW eggs were either killed or parasitized by *T. chilonis,* while 57 to 96% eggs were either killed or parasitized by *T. remus*. The differences in parasitism rates among the *T. remus*

and *T. chilonis* could be attributed the differences in searching behavior of the parasitoids. However, parasitism of egg parasitoids in the laboratory conditions is considered as being unnatural (Kalyebi *et al.,* 2005) due to its difference in dimension of the region parasitoids need to look and find their hosts.

The egg mortality was significantly affected by density of eggs exposed. Exposure to *T. remus* resulted in significantly higher egg mortality than *T. chilonis*. Significant effect on egg mortality was detected when 40 eggs of both *T. remus* and *T. chilonis* were exposed, while no effect was detected with 20 and 60 eggs were exposed. However, in some circumstances, parasitoids and the FAW larva fail to emerge and it was recorded as egg mortality. There is a likelihood of the parasitism on the host eggs, although, the parasitoids probably died before attaining the third larval stage hence making the host eggs darken. (Hansen & Jensen, 2002). Observation of parasitoid efficiency based on parasitoid emergence only may undervalue the potential effect of parasitoids on pest population (Vásquez *et al.,* 1997).

The *T. remus* registered the highest parasitism than *T. chilonis* under laboratory conditions, hence *T. remus* could be a suitable candidate for management of FAW outbreaks when compared to *T. chilonis*. These outcomes are consistent to those of Pomari *et al.* (2012), who indicated that *T. remus* is an aggressive parasitoid that can penetrate the layered and scaly FAW eggs and cause over 80% parasitism. In addition, *T. remus* is the best candidate to be used as the biological control of FAW through augmentative release due to its wide prevalence (Kenis *et al.,* 2019).

Sex ratio (percent female progeny) varied significantly among the parasitoid species

with *T. remus* recording the highest percent (83.3%). More female parasitoids emerged from the FAW eggs and this is beneficial in relation to biological control. On the other hand, male parasitoids were few because they can still mate with many females. This can be related to suitability of FAW eggs to these wasps. When the female ratio is higher it can be beneficial in biological control because female parasitoids can lay their eggs on host eggs and prevent larval hatch which are the destructive stage. This can effectively eliminate the potential for the pest to inflict damage on host crops.

5.6 Conclusion

These results showed that the percent parasitism, FAW emergence, egg mortality and sex ratio was significantly different among parasitoid species on FAW egg densities. Under laboratory conditions, 53 to 90% FAW eggs were either killed or parasitized by *T. chilonis,* while 57 to 90% eggs were either killed or parasitized by *T. remus*. Percent parasitism under laboratory conditions were more effective than the field parasitism and the average parasitism was >44% for both parasitoid species. These results recommend for testing the two egg parasitoid species in the field to determine their potential as biocontrol agents of FAW and be incorporated within an IPM context for its management in Kenya. Surprisingly, with increasing eggs densities, egg mortalities and FAW larva emergence increased, while percent parasitism of eggs decreased, this needs to be researched further. The knowledge obtained in this study on the total parasitism and egg mortality caused by egg parasitoids on FAW egg densities can help in choosing the most efficient parasitoid species for FAW biological control. The *T. remus* recorded the highest percent parasitism and egg mortality than *T. chilonis,* similarly to field parasitism. These results propose the two parasitoid species, *T. remus* and *T. chilonis* to be incorporated in the Integrated pest management development strategies for FAW.

CHAPTER SIX

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

6.1 General discussion

Wide distribution of fall armyworm was observed in all the maize fields surveyed on different agro ecological zones of Kenya in the five counties. Environmental parameters such as favorable climatic conditions and presence of host plants and further the adult moth's strong flying ability whereby it can fly up to 100km in one night could have contributed to the FAW dispersion (Johnson, 1987). Prevalence of FAW varied significantly among the different altitudes surveyed, with Kirinyaga registering the highest prevalence. The number of FAW eggs recorded in highland (Kirinyaga) was significantly higher in comparison with other altitudes. *Telenomus remus* and *T. chilonis* were the key egg parasitoids recovered from FAW eggs. This was revealed from morphological and molecular identification conducted.

The field parasitism rate significantly varied among the different altitudes and it ranged from 4.44 to 13.15% and 0 to 21.43% for *T. chilonis* and *T. remus*, respectively. In Ghana and Benin, field parasitism by egg parasitoids *T. remus* and *Trichogramma sp.* was low, with 25.9% and 14.5%, respectively. Sisay *et al.* (2019) stated egg parasitism rates by *T. remus* to be over 50% in Kenya and Tanzania. Variations in diversity of indigenous parasitoid species and the parasitism rate may result from the differences in agronomic practices, crop type, crop stage, and geographical locations, (Sisay *et al.,* 2019). No parasitoid was recorded in Taita Taveta. Percent parasitism of *T. chilonis* was low in Kilifi and Kwale counties. Plant volatiles influence Trichogrammatidae behavior with specific plant species being attractive and others repellant (Romeis *et al.,* 1997). Other factors which might have contributed to the lower parasitism rate, include

difference in population size of the parasitoids, ability of parasitoids to search for their host eggs and parasitize and climatic conditions. Furthermore, (Romeis *et al.,* 2005) revealed that, plant color, plant structure and chemistry are also believed to cause fluctuation of parasitism level by the parasitoids.

Midland (Makueni) and highland (Kirinyaga) regions had both parasitoids (*T. chilonis* and *T. remus*) but the latter had higher parasitism; 19.24% and 21.43%, respectively. In the same altitudes, percent parasitism for *T. chilonis* was higher compared to coastal lowlands (Kilifi and Kwale). Findings from this study are similar with those reported by Sisay *et al.* (2019) that *T. remus* is the dominant egg parasitoid in Kenya and Tanzania with percent parasitism (69.3%) and (58.5%), respectively, while *T. chilonis* only inflicted 20.9% parasitism. In addition, Kenis *et al.* (2019) reported the parasitism of eggs of *S. frugiperda* by *T. remus* in five East, South and West African countries.

These results highlight the necessity for more detailed surveys for natural enemies in various agroecosystems where FAW has spread (Molina-Ochoa *et al.*, 2001). Our investigation showed that there was higher diversity of egg parasitoids in the midland and highland altitude (Makueni and Kirinyaga) than low altitude regions (Kilifi and Kwale) counties. The occurrence of egg parasitoids of FAW (*T. remus* and *T. chilonis*) in the sampled farms indicates a great opportunity for biological control of FAW and hence there is a need to reduce the indiscriminate use of broad-spectrum insecticide.

To effectively release egg parasitoids in the field for management, it is necessary to understand the optimal density needed in relation to the level of pest infestation to manage it and prevent pest damage which result to crop loss. To achieve this,

parasitoids collected from the field survey was assessed under laboratory to determine their performance with the FAW egg densities. This will be relevant in the implementation of biocontrol programs using egg parasitoids. The species of parasitoids that showed a high potential of parasitism when assessed under laboratory conditions can also perform well in the field. Percent parasitism rate, FAW emergence and egg mortality were among the parameters used to assess the performance of the egg parasitoids in the laboratory (Vásquez *et al.,* 1997).

Under laboratory conditions, there was a high significant difference in parasitism rate, FAW emergence and egg mortality of FAW eggs at different densities (20,40 & 60 eggs) by the *T. remus* and *T. chilonis*. Laboratory output shows that, 57 to 96% FAW eggs were either killed or parasitized by *T. remu*s and 53 to 90% FAW eggs were either killed or parasitized by *T. chilonis*. Egg parasitoid species kill the host eggs by attacking it more than once (superparasitism). Furthermore, it can kill the host by providing their young one with food through laying an egg in or on a host. The egg hatch and the larvae of the parasitoid feed on the host ending up killing it preceding the parasitoid's pupation.

There was higher diversity of egg parasitoids in the midland and highland altitude (Makueni and Kirinyaga) than coastal lowlands region (Kilifi and Kwale) counties. The existence of differences in parasitism rates among the *T. remus* and *T. chilonis*, suggests their differences in abilities to parasitize host eggs. This may result from the effects on the ability of the parasitoids to search for the host. However, parasitism by egg parasitoids in the laboratory conditions could be considered as being unnatural (Kalyebi *et al.,* 2005) due to its difference in dimension of the zone parasitoids must search and

find their hosts.

The FAW egg densities significantly affected egg mortality. The *T. remus* showed highly significant difference on egg mortality than *T. chilonis*. Significant difference on egg mortality was detected between *T. remus* and *T. chilonis* when 40 eggs were exposed, while no difference was detected with 20 and 60 eggs of FAW. However, in some circumstances, and both parasitoids the host larva may fail to emerge resulting in egg mortality. There is a likelihood of the parasitism on the host eggs, although, the parasitoids probably died before attaining the third larval stage hence making the host eggs darken. (Hansen & Jensen, 2002). Hence both the parasitism percent and the egg mortality has to be considered while assessing the efficiency of egg parasitoids (Vásquez *et al.*, 1997). The knowledge obtained in this study on the percent parasitism and egg mortality caused by parasitoids on FAW egg densities could be used to choose the most effective parasitoid species for FAW management.

Telenomus remus registered the highest percent parasitism than *T. chilonis* in the field and under laboratory conditions, therefore more evidence need to be built and determined to allow T. *remus* to be incorporated in the integrated management of FAW. These results are similar with those reported by Pomari *et al.* (2012), who showed that *T. remus* is an aggressive egg parasitoid that can penetrate the layered and scaly FAW eggs and cause over 80% parasitism. In addition, Kenis *et al.* (2019) reported that *T. remus* is the best candidate to be used in the biological control of FAW through augmentative release. Sex ratio (% female progeny) varied significantly among the parasitoid species with *T. remus* recording the highest (83.3%). This can be related to suitability of FAW eggs to these wasps. When the female ratio is higher it can be

beneficial in biological control because female parasitoids can lay their eggs on host eggs and destroy hence prevent subsequent damage to crops from larval feeding (Smith, 1996). This can effectively eliminate the potential for the pest to inflict damage on host crops. The findings from this study justify more extensive surveys to collect for more natural enemies of FAW even in other counties which can contribute to the management of FAW through integrated pest management.

6.2 Conclusions

In conclusion, findings from this study have shown that two egg parasitoid species, *T. remus* and *T. chilonis* were recovered from the FAW egg masses collected from the field survey carried out in five counties of different agro-ecological zones. These parasitoids are effective and this study recommend for their further testing in the field to determine their potential as biocontrol agents of FAW and be incorporated within an IPM context for its management in Kenya. It was also noted that the occurrence of indigenous egg parasitoids of FAW (*T. remus* and *T. chilonis*) is significant in designing biological control of FAW either through conservation or augmentative release. Thus, emphasis should be put to conserve these egg parasitoids by avoiding the use of broad-spectrum insecticides and adopt Integrated Pest Management, which is cost effective to smallholder farmers, is sustainable and environmentally friendly. Also, *Telenomus remus* and *T. chilonis* recorded up to 21.43 and 13.15% percent parasitism of FAW eggs in the field, which needs to be conserved through minimizing chemical control and encouraging high plant diversity in the farms. The percent parasitism, FAW emergence, egg mortality and sex ratio varied significantly among the two parasitoid species. The *T. remus* displayed the highest percent parasitism and egg mortality than *T. chilonis,* similarly to field parasitism rates. These results propose both parasitoids to

be incorporated in the IPM strategy for FAW. Under controlled conditions, 53 to 90% FAW eggs were either killed or parasitized by *T. chilonis,* while 57 to 96% eggs were either killed or parasitized by *Telenomus remus*. Surprisingly, with increasing FAW eggs densities, FAW larvae emergence and egg mortality increased, while percent parasitism decreased, this needs to be researched further.

6.3 Recommendations

1) There is need to carry out further field investigations to explore for more egg parasitoids of Fall armyworm.
2) Incorporate the two species of egg parasitoids from this study (*T. remus* and *T. chilonis*) in the development of FAW Integrated pest management (IPM) programmes since it is cost-effective, sustainable, readily available and environmentally friendly.
3) To identify the missing link to help in decision making on aspects of utilization of the egg parasitoids

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