BIOLOGY, DISTRIBUTION AND MANAGEMENT OF FALL ARMYWORM, *Spodoptera frugiperda* J.E. SMITH (LEPIDOPTERA: NOCTUIDAE), ON MAIZE (*Zea mays* L.), IN ETHIOPIA

PhD DISSERTATION

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Biology, Distribution and Management of Fall Armyworm, *Spodoptera frugiperda* J.E. Smith **(Lepidoptera: Noctuidae), on Maize** (*Zea mays* L.) **in Ethiopia**

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DOCTOR OF PHILOSOPHY IN AGRICULTURAL ENTOMOLOGY

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APPROVAL SHEET

HARAMAYA UNIVERSITY SCHOOL OF GRADUATE STUDIES

We hereby certify that we have read and evaluated this Dissertation entitled "**Biology, Distribution and Management of Fall Armyworm,** *Spodoptera frugiperda* J.E. Smith **(Lepidoptera: Noctuidae), on Maize** (*Zea mays* L.) **in Ethiopia**" prepared under our guidance by Tesfaye Hailu Terefe. We recommend that it be submitted as fulfilling the dissertation requirements.

Prof. Emana Getu ____________________ ____________________
Chairman, Advisory committee Signature Date

Dr. Mulatu Wakgari ____________________ ____________________
Member, Advisory committee Signature Date

Dr. Muluken Goftishu ____________________ ____________________
Member, Advisory committee Signature Date

As members of the Board of Examiners of the PhD Dissertation Open Defense Examination, we certify that we have read, and evaluated the dissertation prepared by **Tesfaye Hailu Terefe** and examined the candidate. We recommend that the dissertation be accepted as fulfilling the dissertation requirements for the Degree of Doctor of of Philosophy in Agricultural Entomology.

Professor________________ ____________________ ____________________
Chairperson Signature Date

Dr.______________________ ____________________ ____________________
Internal Examiner Signature Date

Dr.______________________ ____________________ ____________________
External Examiner Signature Date

DEDICATION

This thesis is dedicated to my late father, Hailu Terefe, and my mother Elfenesh Merga for nursing me with love and for their great dedication in educating me. Besides, it is dedicated to my wife Meseret Sisay; my childrens Yanaten Tesfaye, Robenus Tesfaye, Alesen Tesfaye, Yadan Tesfaye, and Bontu Chimdi for their dedicated support for the success of my study.

STATEMENT OF THE AUTHOR

First, I declare that this dissertation is my own work and all sources of materials used for this dissertation have been duly acknowledged. The dissertation has been submitted in partial fulfillment of the requirements of a PhD degree from Haramaya University and will be deposited at the University Library to be made available to borrowers under the rules of the Library. I solemnly declare that this dissertation is not submitted to any other institution anywhere for the award of any academic degree, diploma, or certificate.

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BIOGRAPHICAL SKETCH

Tesfaye Hailu Terefe was borne from his father Mr Hailu Terefe, and his mother Mrs Elfenesh Merga in Ambo town, West Shoa Zone, Ethiopia, on April 16, 1978 G.C. He attended his primary and secondary schools at Ambo Addis Ketema Primary School and Ambo Senior Secondary School, respectively, from 1985 to 1997 G.C. He joined Ambo University in 1998 and graduated with a Diploma in General Agriculture in 2000. The author was then employed by the Ethiopian Institute of Agricultural Research (EIAR) and was assigned to the Ambo Agricultural Research Center (AmARC), formerly knowen as Ambo Plant Protection Research Center (APPRC) and surved for 3 years. He joined Ambo University for his first degree in 2003 G.C and graduated in Crop Production in 2007 G.C. At the end of 2009/10 G.C., he re-joined Ambo University for postgraduate studies, specializing in Crop Protection. Having completed his MSc study in July 2011, he resumed his duty as a Researcher of Agricultural Entomology at Ambo Agricultural Research Center and surved for 5 years and then in October 2017, he joined Haramaya University, School of Plant Sciences, for his Doctor of Philosophy in Agricultural Entomology.

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ACRONYMS AND ABBREVIATIONS

AKLDP	Agriculture Knowledge, Learning Documentation and Policy
AmARC	Ambo Agricultural Research Center
ANOVA	Analysis Of Variance
CABI	Centre for Agriculture and Biosciences International
CM	Control Mortality
CSA	Central Statistics Agency
CV	Coefficient of Variation
DAT	Days After Treatment
EARO	Ethiopian Agricultural Research Organization
EPF	Entomo-Pathogenic Fungi
EPNs	Entomo-Pathogenic Nematodes
FAO	Food and Agriculture Organization (of the United Nations)
GAIN	Global Agricultural Information Network
GMC	Genetically Modified Crops
GPS	Geographical Positioning System
HSD	Tukey's Honestly Significant Difference Test
IITA	International Institute of Tropical Agriculture
IJs	Infective Juveniles
IPM	Integrated Pest Management
IRAC	Insecticide Resistance Action Committee
km	kilometer(s)
LC_{50}	Lethal Concentration kill 50% of treated organism
LSD	Least Significant Difference
LT_{50}	Lethal Time to kill 50% of treated organisms
m.a.s.l.	meter above sea level
NaOCl	Sodium Hypochlorite
NPV	Nuclear Polyhedrosis Virus
OBs	Occlusion Bodies
SDAY	Sabouraud Dextrose Agar with Yeast Extract
SDW	Sterile Distilled Water

SSA	Sub-Saharan Africa
Std Dev	Standard Deviation
Std Err.	Standard Error
TAF	Triethanomine 2 mL, Formalin (40% Formaldehyde)

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II. *Chapter 4:* Seasonal distribution of *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae) in Ethiopia [Published in Advances in Life Science and Technology, 98:8-17(2023)]

III. *Chapter 5*: Biology and host preference of *Spodoptera frugiperda* Smith (Lepidoptera: Noctuidae) in Ethiopia [Published in SINET: Ethiop. J. Sci., 46(3): 326-339, 2023]

IV. *Chapter 6*: Occurrence and pathogenic potential of entomopathogenic nematodes isolates from maize growing areas of Ethiopia [Published in Pest Mgt. J. Eth. 22: 1–22 (2021)]

V. *Chapter 7*: Evaluation of Entomopathogenic Fungi for the Biological Control of *Spodoptera frugiperda* Smith *(Lepidoptera: Nocutidae)* Under Laboratory and Wire-house Condition (under review on Journal of Pest management)

GENERAL ABSTRACT

Fall armyworm (FAW) Spodoptera frugiperda *J.E. Smith (*Lepidoptera*: Noctuidae) is a polyphagous insect pest and has long been known as a serious economic pest in the tropical and subtropical regions of America. However, outside of the native range in North and South America, FAW was recently invaded different African countries and caused significant damage to maize. The invasive FAW first invaded Ethiopia in February 2017 and has since become established in many areas across the country and threatened food security of smallholder maize growing farmers. Maize,* Zea mays *L. is one of a strategic and staple crop that is prmarlly grown as a source of food in several Sub-Saharan Africa (SSA) countries including Ethiopia. In SSA, maize is essential for economic and food security for over 208 million people. In Ethiopia, maize occupies about 2.1 million hectares of land, of which, smallholder farms accounted for more than 95% of the total area and production. However, maize has a very low average grain yield in SSA due to a variety of abiotic and biotic constraints. Among the biotic constraint the damage caused by the recently introduced FAW is the most significant biotic component. Therefore, the present study aimed at determining the biological characters of FAW, their current distribution, damage level and its associated natural enemies which may provide further insights for future effective integrated management options. Recently, the damage caused by FAW has increased year after year. The current assessment of FAW distribution and infestation in different parts of Ethiopia confirmed that during the main season, the overall mean average infestation of FAW for the 2018/19 and 2019/20 crop seasons was 16.38 and 21.41%, respectively. Moreover, the dry season assessment recorded an average infestation of 42.5%.*

The results of natural enemy assessment revealed that six different types of indigenous natural enemies namely Entomopathogenic fungi (EPF), Entomopathogenic nematodes (EPN), Ladybird beetle, Earwig, Trichogramma sp., and Cotesia sp. that cause natural mortality on FAW were recorded and identified. The identified EPF and EPN were further screened against FAW to develop a biocontrol program against FAW. Among the EPN isolates, Steinernema sp. (Aso-Tes-287) and Heterorhabditis sp. (Am-Ger-Tes-74, Am-Adm-Tes-369, Z9) caused larval mortality within ten days under laboratory and lath house pot experiments. Particularly, based on the pot experiment, 74.7 (Aso-Tes-287), and 78.3% (Am-Ger-Tes-74) larval mortality were obtained at 600 IJ/ml. Most of the isolated EPF from different maize growing areas are virulent *against the 3rd instar larvae of FAW larvae and caused 50% mortality within 5.6 days. The four most virulent EPF isolates were further tested in a wire-house pot experiment for dose response at four different conidial concentration levels. Accordingly, two of the EPF isolates (ABe28 and SMe162) caused significant larval mortality at the higher conidial concentration (i.e., 1x10*^{9} *conidia ml*$^{-1}$*). Therefore, two EPN (Aso-Tes-287 and Am-Ger-Tes-74) and two EPF (ABe28 and SMe162) isolates, which caused higher mortalities within shorter periods, were promising bio-agents for the management of FAW. Future work would therefore be focused on field validation of these isolates against different stages of FAW in different agro-ecologies. The biology of FAW was studied in the greenhouse (usingr no-choice test) on different crops namely, maize, sorghum, chickpeas, barley, and wheat. Thus, the life cycle of FAW ranged between 31 to 38 days at rearing room temperature of 25.5 to 37.4°C and relative humidity of 35.33 to 45.46%. The highest number of days from egg to adult was recorded on sorghum and maize, while the lowest duration of on chickpea, barley, and wheat. The host preference study was also conducted using both choice and nonchoice tests on the major crops grown in Ethiopia. The host preference study result discovered that maize, sorghum, Swiss chard, teff, elephant grass, and cabbage were the most preferred hosts for egg-laying and larval development.*

Keywords: Biological control, Natural enemies, *Heterorhabditis, Steinernema*, *Beauveria* species, *Metarhizium* species, Pathogenicity, Ethiopia.

CHAPTER I

I. GENERAL INTRODUCTION

1.1. BACKGROUND AND JUSTIFICATION

Maize (*Zea mays* L.) is a staple food crop in Africa, where over 200 million people depend on it for food and nutritional security. It accounts for almost half of the calories and protein consumed in eastern and southern Africa, and one-fifth in West Africa (Macauley, 2015). With the high demand for maize in the developing world, where population growth is projected to double by the year 2050 (Rosegrant *et al*., 2009), maize yield losses due to Fall armyworm (FAW) will exacerbate challenges in meeting the demand. For example, FAW is estimated to cause up to 25 to 100% grain yield losses in maize cultivation in Central America (Womack *et al*., 2018), whereas in Zimbabwe, Uganda, Kenya, and Ethiopia, the yield losses on maize plants were 32, 87.66, 34, and 60%, respectively (Baudron *et al*., 2019; De Groote *et al*., 2020; Sharon *et al*., 2020; Wondimu *et al*., 2021). This is a major shock to food supply and it affects the already fragile economic situation of many households across SSA.

Fall armyworm (FAW), *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae), is native to tropical and subtropical regions of the Americas. In 2016, it was first reported from central and western African regions and rapidly spread through most African countries and later to Asian countries (Goergen *et al*., 2016). By 2017, it had spread to all of Sub-Saharan Africa (SSA) (Day *et al*., 2017), where it became the most important crop pest of maize (Kumela *et al*., 2019). Fall armyworm invaded India in 2018 (Ganiger *et al*., 2018) and by early 2019 expanded to most of Asia, Australia, Oceania, and the United Arab Emirates (FAO, 2021; Everington, 2019). It is a polyphagous insect pest and an economically important pest of maize (*Zea mays* L.) and recorded from 353 host plant species in 76 families (Montezano *et al*., 2018; Kumela *et al*., 2019; De Groote *et al*., 2020). Fall armyworm has genetically two distinct strains: the rice strain (R-strain) and the corn strain (C-strain). The R-strain is more frequently detected in rice and grasses, while the C-strain is more frequently found in corn, cotton, and sorghum (Dumas *et a*l., 2015; Nagoshi *et al*., 2021).

Since FAW is a serious invasive and destructive insect pest, and maize is a food security crop in many African countries, particularly in Ethiopia, the establishment of effective management strategies is decisively important. After the occurrence of FAW in African countries, synthetic chemical insecticides have been widely used as an emergency response to reduce the distribution of this pest and minimize damage in maize fields. Although chemical insecticides play an important role in FAW management, given confirmed reports of insecticide resistance development in the FAW population (Chen *et al*., 2023), their unavailability, high cost as well as other adverse effects on humans and the environment, sole dependence on insecticide is not feasible.

A key component of crop protection in modern agriculture is the use of biological control agents (bioagents), such as entomopathogenic fungi (EPF) (Ramanujam *et al*., 2020; Idree *et al*., 2023) and entomopathogenic nematodes (EPNs) (Tesfaye *et al*., 2019; Fallet *et al*., 2022; Wang *et al*., 2022), parasitoids and predators (Birhanu *et al*., 2019) to manage insect pests. The use of entomopathogenic fungi and nematodes can be an important alternative to reduce the impact of synthetic chemical insecticides on the environment, while boosting maize production.

1.2. STATEMENT OF THE PROBLEM

Fall armyworm (FAW) has a high reproduction rate, a relatively short generation time, and wide distribution. The spread of FAW has a substantial impact on both the economic and food security. To prevent this enormous invasion, basic knowledge of FAW distribution, seasonal distribution, and its biological aspect is important. However, obtaining these pieces of information seems to be complicated and still limited due to its new occurrence in Ethiopia. Therefore, distribution, biology, and assessment of natural enemies are used to indicate the damage level by the insect pest and naturally co-existing local natural enemies. Having all of the information will be useful in deciding the appropriate management strategies to manage FAW. Since the occurrence of FAW in Ethiopia, maize-producing farmers have sprayed different pesticides (insecticides) to reduce the maize damage level. However, evenif chemical insecticides play an important role in FAW management, continuous applications of synthetic insecticides by farmers to manage FAW are not only uneconomical but also harmful to the environment and non-target organisms and, consequently, lead to the development of insecticide resistance. Use of safe alternative methods, especially naturally occurring microbial bio-control agents, such as entomopathogenic fungi and nematodes, are the most preferred and sustainable management options of FAW while reducing impacts of senthetic insecticides on health and the environment. However, empirical information on the availability and efficacy of EPF and EPN against FAW in Ethiopia is inadequate.

1.3. RESEARCH QUESTIONS

1) How much of FAW distributed and caused damage in maize production in Ethiopia?
2) What are the major natural enemies of FAW existing in Ethiopia?
3) Do locally isolated entomopathogenic nematodes and fungi (indigenous/native bioagents) have the potential efficacies against FAW?
4) Does the FAW have different feeding preference for the major crops grown in Ethiopia?
5) Does the life cycle of FAW vary on the different host crops grown in Ethiopia?

1.4. OBJECTIVES OF THE STUDY

1.4.1. General Objective

To enhance basic knowledge of FAW host preference along with its biology and develope environmental friendly and sustaunable management options to reduce FAW damage and losses on maize.

1.4.2. Specific Objectives

The research project was carried out with the specific objectives to:

1) To assess the distribution and infestation level due to FAW and its natural enemies in maize fields at different agro-ecological zones of Ethiopia;
2) To study the seasonal distribution of FAW in maize fields in the major maize-growing regions of Ethiopia;
3) To study the biology, feeding, and oviposition preference of FAW on selected major crops grown in Ethiopia;
4) To isolate, identify, and evaluate the efficacies of local entomopathogenic nematodes against FAW on maize; and
5) To isolate and evaluate the efficacy of local entomopathogenic fungi against FAW on maize.

1.5. ORGANIZATIONAL STRUCTURE OF THE DISSERTATION

The dissertation has been organized into eight chapters:

Chapter I: is mainly focused the general introduction including the problem, rationale of the study, Research questions, Objectives and dissertation structure,

Chapter II: Review and summarizes avalable literatures on maize, FAW and its control options,

Chapter III: Status, distribution and infestation level of FAW and its associated natural enemies in Ethiopia,

Chapter IV: Seasonal distrinution and infestation level of FAW,

Chapter V: Locally available botanicals that were selected tested for their effectiveness and the results obtained discussed under this chapter,

Chapter VI: Occurance and pathogenicity of EPN on FAW,

Chapter VII: Occurrence and efficacy of EPF on FAW,

Chapter VIII: This section summarizes the overall research findings, followed by general conclusions and recommendations.

CHAPTER II

II. LITERATURE REVIEW

2.1. DESCRIPTION OF MAIZE

2.1.1. Maize Production and Its Importance in Ethiopia

Maize (*Zea mays* L.) holds a prominent position as one of the top three crops (Rice, Wheat, and Maize) worldwide (FAO, 2021). It is a multipurpose crop utilized for various purposes, including food, feed, and as a raw material for the synthesis of biofuel and beer. In Sub-Saharan Africa (SSA), maize plays a vital role as the primary source of calories (466.5 kcal per capita per day) and it is the second most significant source of protein (12 g per capita per day) after wheat (Dagne *et al*., 2019). In Ethiopia specifically, maize is an essential staple crop, ranking first (30.88%) among cereals in total grain production and second (19.46%) in area coverage (CSA, 2021). Its versatile uses and importance highlight the significance of maize as a crop in sustaining agricultural and economic development.

Maize has become increasingly popular in Ethiopia due to its high value as a food crop, as well as the growing demand for its stover as animal fodder and a source of fuel for rural families, according to research conducted by Abate *et al*. (2015). Approximately 88% of maize produced in Ethiopia is consumed as food, both in its green and dry grain forms. Maize is processed and prepared in various ways for consumption in Ethiopia. For example, green maize is often boiled/roasted and eaten as a snack. Additionally, maize is used in the production of local food (*Gonfo, Injera, Kolo, Nifro, Asharo*) and drinks (*Tela, Borde, and Arekie,* and similar others). The versatile nature of maize as a food crop and its ability to serve multiple purposes make it an important staple in the diets of many Ethiopians and a highly important crop to the national economy of the country by also exporting it to neighboring countries, including Kenya, Djibouti and the Sudan (Birhan, 2021).

The oil found in maize, particularly in the embryo, is widely utilized in both cooking and the production of soaps (Rajendran *et al*., 2012). Additionally, the sticky gum extracted from maize contains dextrin, which is commonly employed for sealing envelopes and labels. Maize starch is well-regarded in the cosmetics and pharmaceutical industries for its role as a diluent. Maize seeds are utilized in the production of alcohol, while maize stem fibers are utilized in the manufacture of paper. Overall, maize plays a crucial role in various industries due to the diverse array of valuable components it contains (Kumar and Jhariya, 2013).

Maize is an essential source of major phytochemicals, like carotenoids, phenolic compounds, and phytosterols. These phytochemicals play a crucial role in preventing chronic diseases and promoting overall health. Maize oil, derived from the crop, is a beneficial component that can fulfill the essential fatty acid requirements for both children and adults. Just a tablespoon of maize oil can contribute significantly to a healthy diet (Kumar and Jhariya, 2013). Furthermore, various parts of the maize plant, including the silk, roots, leaves, and cob, have been utilized in traditional medicine for treating bladder problems, nausea, vomiting, and stomach complaints. Decoctions made from these parts of the maize plant are known for their therapeutic properties and have been used effectively for maize is not just a staple food, but also a valuable source of phytochemicals and health benefits that can aid in maintaining optimal and preventing disease (Rouf-Shah *et al*., 2016). Zein, an alcohol-soluble prolamine found in maize endosperm, exhibits unique and promising potential applications in the pharmaceutical and nutraceutical industries (Luo *et al*., 2011; Kumar *et al*., 2023).

2.1.2. Major Constraints of Maize Production

Several key constraints impact maize production, and these constraints are primarily within three main categories: pests, poor agronomic practices, and postharvest losses (Akowuah *et al*., 2012; Zhou *et al*., 2019). These factors present significant challenges for maize farmers and must be addressed to improve overall crop yield and quality (Liliane and Charles, 2020). From pests, insects, diseases and weeds can cause extensive damage to crops (Mosisa *et al*., 2011; Abate *et al*., 2017; Tolera *et al*., 2018). Implementing effective pest management measures is essential to minimize crop losses and protect crop health. Poor agronomic practices highly constrained maize production. Poor soil management, improper planting techniques and cropping system, and insufficient fertilization can all hinder crop growth and reduce grain yields (Liliane and Charles, 2020). By implementing recommended agronomic practices, farmers can improve the overall health and productivity of their maize crops. Postharvest losses are other major constraints in maize production. Improper storage and handling techniques can result in significant losses of maize after harvest, reducing overall profitability and quality for farmers (Temesgen and Getu, 2023). Insect pests, plant diseases, and competitive weeds are pest problems that could hinder the production of maize (Mosisa *et al*., 2011; Abate *et al*., 2017; Tolera *et al*., 2018).

Generally, the Lepidopterous stem and cob borers are among the most injurious insect pests of maize, sorghum, millet, rice, and sugarcane in SSA (Benjamin *et al*., 2024). In Ethiopia, economically important insect pests include maize stem borer, leaf aphids, thrips, leafhoppers, grasshopper and termites (Tolera *et al*., 2018; Tufa *et al*., 2023). More recently, however, the invasive and polyphagous insect pest, named fall armyworm (FAW), *Spodoptera frugiperda*, is becoming a major insect pest causing substantial yield losses on maize in different areas of Africa, including Ethiopia (Birhanu *et al.,* 2019; Kumela *et al*., 2019).

2.2. DESCRIPTION OF FALL ARMYWORM

2.2.1. Origin and Distribution of Fall Armyworm

Fall Armyworm, FAW (*Spodoptera frugiperda*J.E. Smith) (Lepidoptera: Noctuidae), is a polyphagous insect pest native to tropical and subtropical regions of the Americas (FAO, 2017; Cock *et al*., 2017), which has recently appeared in African countries (Goergen *et al*., 2016; FAO, 2017). It is regularly intercepted in intercontinental trade (CABI, 2016), but has not previously become established outside of the Americas (except a report of its presence in Israel (Wiltshire, 1977) was based on a misidentification) (Goergen *et al.*, 2016; Cock *et al*., 2017). However, FAW was reported from Africa for the first time in Central and Western Africa in early 2016 (Benin, Nigeria, Sao Tome and Principe, and Togo) and in the whole of mainland southern Africa, in Burundi, Cameroon, Cape Verde, Ethiopia, Ghana, Kenya, Niger Rwanda, South Sudan and Uganda (Goergen *et al*., 2016; Nagoshi *et al*., 2018). According to Abrahams *et al*. (2017), up until September 2017, 54 African countries were surveyed, and 28 countries were invaded by this insect, even so, the precise means of introduction to Africa is angibily tunknown.

Fall armyworm is a dangerous transboundary insect pest with a high potential of continuing to spread due to its natural distribution capacity and trade (FAO, 2017). It was classified as a sporadic pest due to its migratory behavior. This species does not enter diapauses, so it migrates from warmer climates. Fall armyworm movement each year generally creates sporadic problems across multiple crops, including cotton. FAW outbreaks and subsequent damage can be unpredictable. When outbreaks do occur, the severity of the problem is compounded by the ability of FAW to damage a range of vegetative to reproductive plant structures, creating the opportunity to cause devastating crop losses (Hardke *et al*., 2015; Makgoba *et al*, 2021).

Fall armyworm has several generations per year and the moth can fly up to 100 km per night (FAO, 2017; Devi, 2018). The FAW is one of the most serious pests of maize and grasses throughout the Americas and Africa (Assefa *et al*., 2019; Nagoshi *et al*., 2021). The caterpillars of the FAW larvae feed on leaves, stems, and reproductive parts of more than 350 plant species (FAO, 2017), causing major damage to economically important cultivated grasses, such as maize, rice, sorghum and sugarcane, as well as other crops, including alfalfa, beet, cabbage, cotton, millet, onion, pasture grasses, peanut, potato, soybean, and tomato if not well managed (CABI, 2016). Although widely agreed to be one of the most damaging crop pests in the Americas (Cock *et al*., 2017), economic assessments of crop losses and the costs of management are not comprehensive studied. However, in Brazil and Kenya, for example, FAW is considered the major insect pest for maize, causing up to 33-34% reduction in grain yield (Lima *et al*., 2010; Cock *et al*., 2017; De Groote *et al*., 2020). Farmers considerably need great support to sustainably manage their cropping systems through Integrated Pest Management, IPM (FAO, 2017).

2.3. BIOLOGY AND AND LIFE CYCLE OF FALL ARMYWORM

Fall armyworm is nocturnal and capable of flying long distances. Mating of adults takes place during calls initiated by virgin females when calling the female site near the top of the host canopy, extending her ovipositor and emitting the sex pheromone to indicate that the female is available to mate (Meagher *et al*., 2013; Van *et al*., 2017). Generally, virgin females mate early in the night; females mated once a night, and also mating activities peak before midnight, depending on the temperature and time of season strong maternal effects could be shown in the time of male and female calling, copulation timing was influenced by a mix of maternal effects and corn strain dominant autosomal components, and oviposition timing was inherited in a maize strain dominant manner (Hänniger, 2015; Zweerus *et al*., 2022). Oviposition by mated females follows closely and may overlap with the early evening feeding period; the female normally oviposits on the lower side of leaves in cluster form and is protected by a dense covering of scales. Masses contain from a few to hundreds of eggs, which hatch in 2-4 days if the mean temperature is 21,1-26.7 °C (Harrison *et al*., 2019; FAO and CABI, 2019).

The FAW has compelete metamorphosis and has six generations per year, which completes its life cycle within 30 days during the summer 60 days prolonged in the spring and autumn, and 80 to 90 days during the winter (Capinera., 2020; Kona *et al*., 2021). The newly hatched larvae are often observed feeding on the leaves of host plants until they reach about the third in star or 2-3 weeks of age, at which time they are large enough to feed the shoot and leaves of the host plants and they move downward, sometimes through several internodes (Ball *et al*., 2006; Storer *et al*., 2010). At this stage, the suitability of the host plant is probably a limiting factor to the feeding of the larvae (Silva *et al*., 2017). The 6th instar drops to the ground and pupates 2-8 cm deep in the soil, depending on soil texture, moisture, and temperature. According to Du Plessis *et al*. (2020) and Kalyan *et al*. (2020), length of the pupal period varies from 8.96 and 30 days, again depending on soil mean temperature ranging from 18-32 °C.

In general, the life cycle of the FAW is sequential in four cycles as described below (Figure 1).

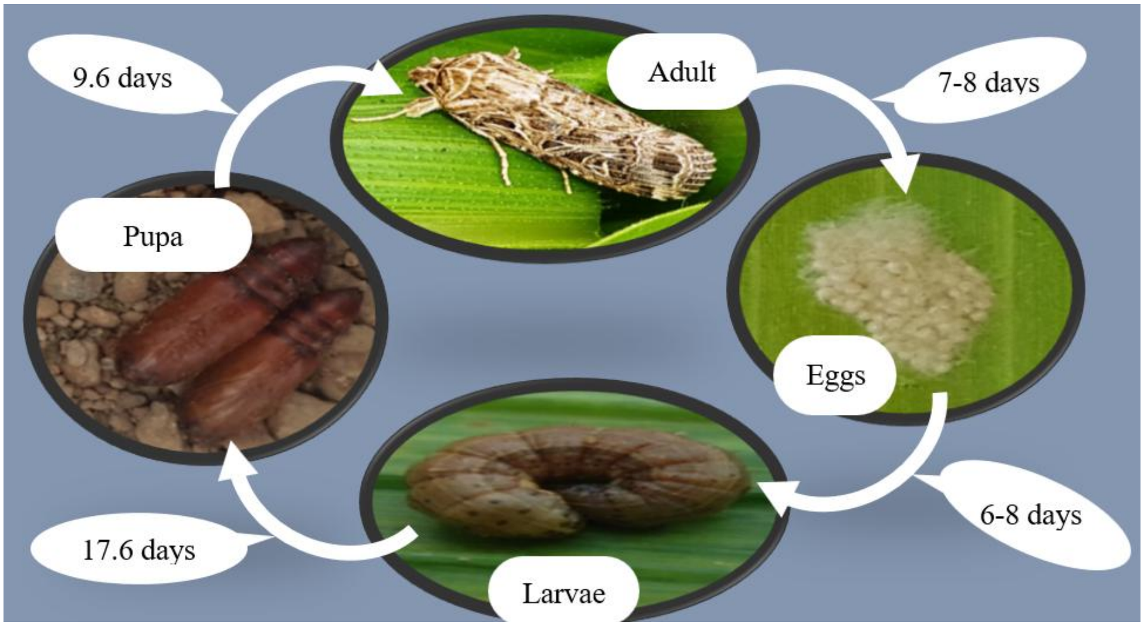

Figure 1. The life cycle of FAW on maize (Source: Tesfaye *et al*., 2023)

2.3.1. FAW Egg Stage

The egg is dome–shaped; the base is flattened and the egg curves upward to a broadly rounded point at the apex (Kannan *et al*., 2021). The egg measures about 0.4 mm in diameter and 0.3 mm in height and the female lays 1500-2000 eggs and takes 2-3 days for hatching. The eggs are sometimes deposited in layers, but most eggs are spread over a single layer attached to foliage. The female also deposits a layer of grayish scales between the eggs and over the egg mass, imparting a furry or moldy appearance. The duration of the egg stage is only two to three days during the summer months (Capinera, 2020; Kannan *et al*., 2021; Russianzi *et al*., 2021).

2.3.2. FAW Larval Stage

Fall armyworm has six instars and the instars have different (0.35, 0.45, 0.75, 1.30, 2.00, and 2.60 mm, respectively) head capsule widths for 1-6 instars and larvae attain lengths of about 1.7, 3.5, 6.4, 10.0, 17.2, and 34.2 mm, respectively. During these instars (Capinera, 2017), young larvae are greenish with a black head, the head-turning orangish in the second instars. In the second, but particularly the third instars, the dorsal surface of the body becomes brownish, and lateral white lines begin to form. In the fourth to the sixth instars, the head is reddish brown, mottled with white, and the brownish body bears white sub-dorsal and lateral lines. Elevated spots occur dorsally on the body; they are usually dark, and bear spines. The face of the mature larva is also marked with a white inverted "Y" and the epidermis of the larva is rough or granular in texture when examined closely (Assefa *et al*., 2019; FAO and PPD, 2020). However, this larva does not feel rough to the touch, as does the corn earworm, *Helicoverpa zea* Boddie, because it lacks the microspines found in the similar-appearing corn earworm. In addition to the typical brownish form of the FAW larva, the larva may be mostly green dorsally. In the green form, the dorsal elevated spots are pale rather than dark. Larvae tend to conceal themselves during the brightest time of the day. The duration of the larval stage tends to be about 14 days during the summer and 30 days during cool weather. Mean development time was determined to be 3.3, 1.7, 1.5, 1.5, 2.0, and 3.7 days for instars 1 to 6, respectively, when larvae were reared at 25 °C (Schlemmer., 2018).

2.3.3. FAW Pupal Stage

According to Schlemmer, (2018), Capinera (2020) and Prasanna *et al*. (2018), the pupal development stage is 8 to 9 days during the summer but reaches 20 to 30 days during cooler weather. Pupation takes place inside the soil, leaf sheath, and in crop residue. The newly formed pupa is reddish-brown and immobile but shows wiggling movement of the abdomen when touched. It can roll on its side as well. The cocoon is oval and 20 to 30 mm in length (Prasanna *et al*., 2018). If the soil is too hard, larvae may web together leaf debris and other material to form a cocoon on the soil surface. The pupa measures 14 to 18 mm in length and about 4.5 mm in width. The pupal stage of FAW cannot withstand protracted periods of cold weather. Schlemmer, (2018) studied winter survival of the pupal stage in Florida and found 51% survival in southern Florida, but only 27.5% survival in central Florida and 11.6% survival in northern Florida. Maize and other host plants do not affect the pupal development stage.

2.3.4. FAW Adult Stage

The moths have a wingspan of 32 to 40 mm. In the male moth, the forewing generally is shaded gray and brown, with triangular white spots at the tip and near the center of the wing. The forewings of females are less distinctly marked, ranging from a uniform grayish brown to a fine mottling of gray and brown. The hind wing is iridescent silver-white with a narrow dark border in both sexes. Adults are nocturnal and are most active during warm, humid evenings. After a pre-oviposition period of three to four days, the female normally deposits most of its eggs during the first four to five days of life, but some oviposition occurs for up to three to four weeks (Silva *et al*., 2017). Several investigators reported that larval diet had a significant effect on adult longevity and is, therefore, dependent on larval food quality and quantity that determines adult longevity, gamete production, fat reserves, and muscle tissue (Silva *et al*., 2017; Gopalakrishnan and Kalia, 2022).

2.4. HOST RANGE AND STRAIN OF FAW

Fall armyworm consists of two subpopulations that are morphologically indistinguishable but differ in their host plant distribution and certain physiological features with overlapping spatial and temporal distribution occurring in different parts of the world, including Africa and Ethiopia inclusive (Nagoshi *et al*., 2018). The rice-strain (R-strain) is most consistently found in millet and grass species associated with pasture habitats, while the maize-strain (C-strain) prefers maize, rice and sorghum (EFSA *et al*., 2015). FAW is by far the most economically important insect pest in maize and sorghum. According to Goergen *et al*. (2016), the FAW is a prime nocturnal insect pest of maize on the American continents where it has remained confined (endemic) despite occasional interceptions by European quarantine services in recent years. The pest has currently become a new invasive species in West and Central Africa where outbreaks were recorded for the first time in early 2016. The presence of at least two distinct haplotypes within samples collected on maize in Nigeria and São Tome suggests multiple introductions into the African continent. It has not previously been established outside the Americas but its two strains have now appeared in Africa and are rapidly spreading throughout the tropical and subtropical regions of the continent. It is widely agreed to be one of the most damaging crop pests in the Americas, feeding on over 350 different crops. Its impact on maize yields in Africa has been and is likely to continue to be significant. Research to date suggests that both strains entered Africa as stowaways on commercial aircraft, either in cargo containers or airplane hold, before subsequent widespread dispersal by the wind (Abrahams *et al*., 2017).

Due to their suitable climate, reports of FAW presence and impact are to be expected in further West African countries, such as Sierra Leone, Mali, Senegal, Liberia, and Cote D'Ivoire, which so far have not officially reported FAW, plus the Central African Republic and the Sudan. Angola and Nigeria appear at risk of suffering increased pest outbreaks given their environmental suitability for FAW, or the relative proportion of maize grown in suitable areas. Madagascar, which has not as yet reported FAW, is also at risk. Of particular note is the high environmental suitability on the Mediterranean coast in Algeria, Libya, Morocco andTunisia, increasing the possible spread of this insect to Europe, and the high suitability areas in Ethiopia that could enable the pest to progress towards the Middle East and Asia (Abrahams *et al*., 2017; Day *et al*., 2017).

2.5. ECONOMIC IMPORTANCE OF FAW

Several authors, Blanco *et al*. (2016); Fatoretto *et al*. (2017); Goergen *et al*. (2016); Abrahams *et al*. (2017), and Tambo *et al*. (2023) have indicated that FAW is the most serious economically important insect pest of maize and other graminae plants native to the American regions.

Crop damage results mainly from larvae consuming leaf tissue, but larvae will also burrow into the growing point (bud, whorl, and other parts), destroying potential future plant growth. Yield loss can reach 34-60% but the severe infestation due to the insect may cause 100% yield loss. The life cycle of FAW is highly temperature dependent and lasts about 30 to 60 days in summer and 80 to 90 days in winter, resulting in multiple generations per year. There is no diapause in this species. FAW has a high reproductive rate. Adults are nocturnal and are most active during warm, humid evenings. The occurrence of multiple generations, the ability to migrate, and the ability to feed on a wide range of host plants make fall armyworm one of the most severe economic pests in the Western Hemisphere (Hardke *et al*., 2015; IRAC., 2016).

Fall armyworm is a polyphagous insect pest that shows a definite preference for the Poaceae (Gramineae). It is most commonly recorded from grasses and maize, rice, sorghum, and sugarcane. Also recorded on Brassicaceae, cotton, Cucurbitaceae, groundnuts, lucerne, onions, *Phaseolus*, sweet potatoes, tomatoes, and other Solanaceae (aubergines, *Capsicum*, tobacco) and various ornamental plants (chrysanthemums, carnations, and *Pelargonium*). Most larvae are conditioned to the host on which they first feed, usually the plant on which the eggs were laid (Praveen and Mallapur, 2019; Sotelo-Cardona *et al*., 2021).

Some studies showed the considerable impacts of FAW on maize yield and economics: national, continental, household, and trade perspectives. Infestations during the mid-to-late maize stage may result in yield losses of 15-73% when 55-100% of the plants are infested (Cock *et al*., 2017; De Groote *et al*., 2020). The FAW caterpillars appear to be much more damaging to maize in West and Central Africa than most other African *Spodoptera* species (Georgen *et al*., 2016). Outbreaks of FAW have been reported in several countries in Africa. Around 330,000 hectares of staple crops, especially maize, have been affected.

2.6. FALL ARMYWORM MANAGEMENT METHODS

2.6.1. Preventive Measures

Different preventive management methods for FAW, such as avoid planting susceptible varieties, dig a deep trench around the corn field and filling it with water, plough the soil deeply to expose larvae and pupae to the upper surface of the soil, sow early to avoid peak immigration of adults, balanced fertilization, clear the grass weeds surrounding the plot which provide shelter and food to the pest, conserve shelters for beneficial insects, remove and destroy all crop residues and by minimum- and zero-tillage. (Patrick, 2016; Plantwise, 2016; Baudron *et al*., 2019).

2.6.2. Monitoring and Scouting

The presence of FAW was typically related to fields planted after June 1, thus maize growers should pay particular attention to late-planted areas or fields with a history of FAW problems. As a result, early infestation detection will enable more efficient for this pest management. The FAW is widely distributed in the field in harmful numbers, so it needs to be managed, while the larvae are still young. It is a major and frequent issue to discover an infestation when it is too late to apply effective management. Late-planted fields should be inspected carefully since FAW damages the whole stage of the plants (Ric, 2011; Plantwise, 2016; Jaramillo-Barrios *et al*., 2020).

2.6.3. Cultural Management Methods

Cultural management of FAW implies the manipulation of the environment in such a way as to render it unfavorable to the pest. Cultural management is considered the first line of defense against pests and includes technologies, such as the destruction of crop residues, tillage, mulching, intercropping, manipulation of planting dates, and fertilizer application. Many cultural management practices are labor intensive, but they have little adverse effects on the environment and are readily available without extra investment in equipment (Murray *et al*., 2019; FAO. 2021).

The FAW feeds and multiplies on a wide range of alternate host plants. Many are weeds and removal of some of them, like prickly caterpillars (*Amaranthus* spp.), from the fields will reduce the breeding grounds for the pest. Thorough plowing and cultivation of the field following the final harvest of the crop will also destroy large numbers of FAW pupae in the soil and help to lessen pest infestation of surrounding forage crops (Grzywacz *et al*., 2014; Matova *et al*., 2020). Some smallholder farmers in the Americas report using ash, sand, sawdust, or dirt in whorls to suppress FAW larvae. Ash, sand, and sawdust may desiccate young larvae (FAO, 2018; Attaluri *et al*., 2022).

2.6.4. Host Plant Resistance

2.6.4.1. Conventional resistant maize

Plant resistance has long been recognized as an important component of pest management of the FAW in Africa (Prasanna *et al*., 2021). Resistance to FAW appears to be under polygenic suppression (Matova *et al*., 2020). Many morphological, anatomical, physiological, and biochemical factors have been reported to be associated with resistance, each controlled by different sets of genes (de Bastos *et al.,* 2022). Resistant cultivars, when available to farmers for planting, express their resistance as complex contributions from several mechanisms. According to Omuut *et al*. (2023), the genetic identity and diversity of FAW were partially responsible for the resistance of mitochondrial cytochrome oxidase to the FAW. Mookiah *et al*. (2021) and Sharma, (2007) found a significant negative correlation between the rind hardness of the internode first accessible to attack and mean percent internodes subsequently FAW larvae. Nuambote-Yobila *et al*. (2023) reported that tolerance to larval feeding also could be an important mechanism of resistance against FAW.

On the other hand, several researchers reported that the FAW has no ovipositional preference among different crop varieties (Silva *et al*., 2017; Gopalakrishnan and Kalia, 2022). However, the host selection for oviposition by FAW among cotton, maize, millet, oat, soybean and wheat and their relationship with the biological characteristics were investigated under laboratory conditions revealed that similar oviposition preference among the host plants but duration of pre-pupal, pupal, and larva-adult period, pupal weight, sex ratio, survival, larva feeding preferences, oviposition preferences, and nutritional quality of different hosts were evaluated. Insects fed on wheat showed the shortest larva-adult period. The insects fed on cotton and soybean had longer larval development cycles and pupae of lower weight. Feeding preference was evident for third-instar larvae and did not differ between maize, oat, soybean and wheat,which were the preferred hosts. Moths oviposited to a greater extent on the upper canopy of wheat than on other plants. According to Prasanna *et al*. (2022), host plant resistance as an approach to pest management in gramineous crops confers many advantages. Resistant crop varieties provide inherent control that involves no environmental problems, and they are generally compatible with other insect management methods. Hence, several efforts are underway worldwide to develop resistant varieties against FAW (Kasoma *et al*., 2022; Prasanna *et al*., 2022).

A study conducted by Signoretti (2012) aimed to assess the olfactory response of FAW-mated female moths toward odors released by mechanically and herbivore-induced corn at different time intervals. Results showed that female moths strongly responded to corn volatiles although fresh-damaged corn odors (0–1 h) are not recognized by moths. Moreover, females preferred volatiles released by undamaged plants over herbivore-induced plants at 5–6 h. This preference for undamaged plants may reflect an adaptive strategy of moths to avoid competitors and natural enemies for their offspring (Signoretti *et al*., 2012).

In the maize varieties screening trials conducted in Kenya (Navasha) and Ethiopia, against FAW, showed promising results in the development of resistant varieties of maize (Personal communication) Maize resistance to FAW is categorized as a combination of physical characteristics that hinder feeding (i.e., rind hardness, leaf-sheath appression), variety specific tolerance to feeding, and antibiosis mechanisms that contribute to differences in survival in larvae that have damage into the plant. The extent of this resistance also is influenced by the severity of infestations.

2.6.4.2. Genetically Modified Resistant Maize

Maize genetically engineered is important to produce insecticidal protiens from the *Bacillus thuringiensis* (Bt) is a potentially useful tool for controlling FAW. Abrahams *et al,* (2017) reported about the BT maize varieties, that suppress or control FAW and other lepidopteran insect pests. Natural strains of *Bacillus thuringiensis* tend not to be very potent, and genetically modified strains improve performance of maize varieties resistance to FAW and other insect pests (Ni *et al*., 2011; Abrahams, 2017: FAO, 2017). In the Americas pesticides and genetically modified (GM) crops are the main methods of management,although FAW has developed some resistance to both. Most countries in Africa do not yet plant GM crops. (Bio) Pesticides, including Bt, are an option in Africa though these are not always affordable to many small-scale farmers.

2.6.5. Mechanical Control Methods

A very important management option for smallholder farmers in Africa, based on the experience of smallholders in the Americas, is to visit their fields regularly and crush egg masses and young larvae ("use your fingers, not pesticides"). Farmers should visit fields twice a week during the vegetative stage, especially in periods of heavy oviposition by FAW, and once a week or every 15 days in later stages (Abrahams, 2017; FAO. 2018).

2.6.6. Biological Control

2.6.6.1. Parasitoids and Predators

Fall armyworm has many natural enemies; few act effectively enough to prevent crop injury. A study on the classification and distribution of natural enemies of FAW by Abang *et al*. (2021) showed that 2 species of parasitoids representing *Telenomus remus* and *Cotesia* icipe attack FAW in Cameroon. In Burkina Faso, Ahissou *et al*. (2021) recorded 3 species of Hymenopterans Coccygidium luteum, Chelonus bifoveolatus and Dipterans that cause average parasitism of 17.9% and Predators families: Carabidae (1.5%), Coccinellidae (13%), Forficulidae (51%), Forficula senegalensis (24.3%), Formicidae (15%) and Mantidae 4.1%, Pentatomidae (2.3%) and Reduviidae (4.9%). The most abundant predators were members of the Forficulidae (51%), Formicidae (15%) and Coccinellidae (13%) and, also, more than 44% of natural parasitism has been recorded in the insecticide not sprayed fields of America (FAO, 2017). Similarly, Koffi *et al*. (2020) surveyed parasitoids of FAW from 106 maize fields in Ghana and recorded an overall parasitism rate of 3.58%. Chelonus bifoveolatus, *Coccygidium luteum*, *Cotesia icipe*, *Meteoridea testacea*, and Bracon sp., *Anatrichus erinaceus* and Diptera sp. The parasitoids *C. bifoveolatus* and *C. luteum* relative abundance 29.0% and 23.7% of the total with parasitism rates of 1.04% and 0.85% respectively. However, *C. bifoveolatus* was the most widely distributed parasitoid,

being present in 6.6% of the sites surveyed across all agroecological zones in Ghana. This species is a strong contender for serving as a biological control method for FAW in Africa. The most common and widespread predator on the farms was *P. megacephala*, making up 46.0% of the total population and found on 3.8% of the farms (Koffi *et al*., 2020).
Among parasitoid species that affect FAW, *Cotesia marginiventris* Cresson and *Chelonus texanus* Cresson (both Hymenoptera: Braconidae), are the most commonly reared wasp parasitoids from larvae of FAW in the United States. Among fly parasitoids, *Archytas marmoratus* Townsend (Diptera: Tachinidae) and *Megaselia scalaris* (Diptera: Phoridae) is the most abundant (Murúa *et al*., 2009; Deshmukh *et al*., 2021). One important problem that affects the efficiency of parasitoids is competition among them as the same individual host may be attacked by different species of parasitoids (McLean *et al*., 2017). In this case, if the less efficient species survives, its attack can serve to reduce the number of more efficient parasitoid species. Hence, a large number of parasitoids attacking the same host may result in less rather than more suppression of that host. Otim *et al*. (2021) reported 13 species of parasitoids, which caused an average of 9.2% larval mortality of FAW.

The predators of FAW are general predators that attack larvae of other lepidopterans. The most important predators of FAW include various ground beetles (Coleoptera: Carabidae); the striped earwig, *Labidura riparia* Pallas (Dermaptera: Labiduridae); the spined soldier bug, *Podisus maculiventris* Say (Hemiptera: Pentatomidae); and the insidious flower bug, *Orius insidiosus* Say (Hemiptera: Anthocoridae) (Baudron *et al*., 2019). Among the vertebrate predators, birds, skunks, and rodents are important ones that feed on larvae and pupae of FAW. De Pedro *et al*., (2022) reported a 100 percent loss of FAW to predators in Georgia, indicating the importance of the predator (*Pseudoophonus rufipes*) and the parasitoid (*Aganaspis daci*) in the biological control of FAW.

De Lourdes *et al*. (2015) found that a potential solution for organic maize is to apply the biological control agent *Trichogramma pretiosum* to reduce FAW populations. Here, it was tested the application of one, two, or three releases of *T. pretiosum*. Then it was measured plant damage ratings, egg masses were parasitized., In general, consequently, biological control with egg and larval parasitoids is a promising alternative to control FAW.

2.6.6.2. Entomopathogens

Fall armyworm is also attacked by several entomopathogens, including viruses, fungi, nematodes and a bacterium, that cause significant levels of mortality in the FAW population and help to reduce leaf damage in maize plants (Williams *et al*., 2022; Bateman *et al*., 2023; Cokola *et al*., 2023; Ratnakala *et al*., 2023).

2.6.6.2.1. Virus

The efficacy of baculoviruses against FAW and other lepidopterans has been reported in several studies (Harrison and Hoover, 2012; Paiva *et al*., 2021). When the FAW consumes occlusion bodies (OBs) on the treated leaf surface, the baculovirus infection begins. The larvae's midgut is alkaline, which dissolves the OBs, releasing virions that bind to and infect the midgut epithelial cells after passing the peritrophic membrane (PM). Infected midgut cells generate a second virus phenotype known as the budded virus (BV), which induces systemic infection (Behle *et al*., 2012).

Plant allelochemicals, like maysin, a luteolin-C- glycoside, when used with a nuclear polyhedrosis virus (NPV), reduced damage by FAW by about 27%, and doubled the mortality of FAW compared to the susceptible check (Cuartas-Otálora *et al*., 2019). A nuclear polyhedrosis virus (NPV), obtained from caterpillars of *Spodoptra littiralis* Boisduval (Lepidoptera: Noctuidae), has been effective against different insects (Khattab, 2013; Ayyub *et al*., 2019; Sarwar *et el*., 2021; Williams *et al*., 2022).

2.6.6.2.2. Bacteria

Crystal proteins of the Gram-positive bacterium *Bacillus thuringiensis* (Bt) Berliner are the most commonly used biopesticides against lepidopteran pests worldwide. Two sub-species (Bt aizawai and Bt kurstaki) are effective against FAW (Guo *et al*., 2020; Bateman *et al*., 2021), but toxicity may vary largely between strains and proteins (Liu *et al*., 2019; Wang *et al*., 2022). Some Bt strains only have sublethal effects or growth inhibition, such as reduced pupal weight and fecundity of adult females (Horikoshi *et al*., 2021; Liu *et al*., 2022). However, the efficacy of Bt biopesticides may also be influenced by the adjuvants used in its formulation (Rogers, 2012; dos Santos *et al*., 2021). *Bacillus thuringiensis* (Bt) biopesticide is the most effective against early larval instars because it works via swallowing, and the chance of reaching older larvae is less (Prasanna *et al*., 2021). *Bacillus thuringiensis* (Bt) biopesticide has been registered against FAW and is commercially accessible in many recently invaded countries, either alone or in combination with other biopesticides or insecticides (Bateman *et al*., 2021). In countries, such as Ghana, Kenya, Rwanda, South Africa, Uganda, Zambia, and Zimbabwe, Bt-based biopesticides are among the most commonly used products against FAW (Bateman *et al*., 2023).

2.6.6.2.3. Fungi

Entomopathogenic fungi (EPF) are ubiquitous and contribute to both the natural suppression of insect pests and the development of commercial biopesticides. The natural infection of EPFs has been widely reported on FAW in different areas (Gichuhi *et al*., 2020; Cokola *et al*., 2023). Most EPF isolates have been isolated from the soil and infected arthropods (Tuininga *et al*., 2014; Liu *et al*., 2021). Application of *Metarhizium* and *Beauveria* species on FAW reduced the FAW infestation by 85.5% (Hussain *et al*., 2023). Glasshouse and field efficacy tests with *Metarhizium* and *Beauveria* species against FAW has been demonstrated and high efficacy has been reported on different stages of FAW (Grijalba *et al*., 2018; Mallapur *et al*., 2018; Akutse *et al*., 2020). On the other hand, the compatibility of EPFs with FAW pheromones and that they affect egg viability and fertility indicate the possibility of creating lure and infection application techniques have also been demonstrated (Rivero-Borja *et al*., 2018; Akutse *et al*., 2020). Furthermore, endophytic colonization in maize plants by *Metarhizium* and *Beauveria* species affects FAW survival, growth, reproduction, and food preference offering a promising potential for incorporating EPFs into FAW management practices (Russo *et al*., 2021; Gustianingtyas *et al*., 2021; Altaf *et al*., 2023).

2.6.6.2.4. Nematodes

Entomopathogenic nematodes (EPNs) are generalist lethal parasites of ground-dwelling insects that are present in soils worldwide (Alfy and Malak, 2023; Ashenafi *et al*., 2019) and it has symbiotic bacterial association responsible for their control action against insect pests within a short period (Yuksel and Canhilal, 2019; Mallikajun *et al*., 2022). In a survey Comparative Screening of Mexican and Rwandan Commercial EPN, Fallet *et al*. (2022) recorded a 75 to 100% FAW larval mortality in Switzerland due to entomopathogenic nematodes. Their potential and effectiveness evaluated in the laboratory and field against FAW on maize and they are highly pathogenic, 25-100% mortality has been recorded (Acharya *et al*., 2020; Ratnakala *et al*., 2023).

However, the efficacy of EPNs to subdue FAW larvae feeding on maize plants is adversely affected by unfavorable abiotic conditions (Lacey and Georgis, 2012). Application of EPN with water only reduces FAW infestation, but not consistently across fields (Fallet *et al*., 2022). Negrisoli *et al*. (2010) found that EPN sprayed with water reduced FAW larva mortality by 25% but had synergistic effects when combined with pesticides.

Developing protective formulations is key to EPN effectiveness against FAW. Negrisoli *et al*. (2010) and Fallet *et al*. (2022) recently reported the EPNs formulated by a carboxymethyl cellulose gel have been effective as the cypermethrin insecticide in killing FAW in laboratory and field experiments.

2.6.7. Pheromonal Control/Mass Trapping/Mating Disruption

Biological insecticides are routinely employed as biocontrol agents against this insect pest in Mexico although recently some alternatives to biological control have begun to be explored, particularly the use of entomopathogenic agents (Irsad *et al*., 2023). However, complementary strategies of pest management remain to be tested, including the use of pheromones. Lepidopteran pheromones have been successfully used for insect monitoring, mass trapping, and mating disruption of a diversity of insect pests (Tarekegn *et al*., 2020).

The pheromone composition also differs in the two host strains. Corn strain females produced significantly more of the second most abundant pheromone compound, (Z)-11-hexadecen-1-yl acetate, and significantly less of most other compounds than rice strain females (Muthukumar and John, 2021).

Commercially available FAW sex pheromones have been used in the USA, and are a useful tool for monitoring FAW males (Cruz *et al*., 2012). In the study of inhibition of the responses to sex pheromone of the fall armyworm, they suggest that Z9-14: TFMK is a mating disruptant of FAW and it may be a good candidate to consider in future strategies to manage the pest. (Malo *et al*., 2013).

Many studies have addressed the effect of female pheromones on the ability of males to find calling females but, so far, fewer have addressed the effect of pheromones on the mating behavior of females. Kuhns *et al*. (2012) hypothesized that mating of female moth species may be adversely affected following sex pheromone auto-exposure due to abnormal behavioral activity and/or antennal sensitivity and they found that, for *Grapholita molesta* and *Pandemis pyrusana* females, copulation, but not calling, was reduced following pre-exposure to sex pheromone. In contrast, for *Cydia pomonella* and *Choristoneura rosaceana*, sex pheromone pre-exposure did not affect either calling or copulation propensity. Therefore, in some species, mating disruption may include a secondary mechanism that affects the mating behavior of female moths, in addition to that of males (Kuhns *et al*., 2012).

2.6.8. Botanical Control

Bioinsecticides are used as major components of IPM to manage the pest because their ability to migrate long distances and feed on a broad host range makes other control options less efficient. Although chemical insecticides can provide effective control of crop pests, including FAW (Ahmad *et al*., 2023), control of FAW has been fully dependent on insecticides and, as a result, the pest has developed resistance to major classes of insecticides in several locations (Chen *et al*., 2023). However, the use of botanicals, like neem, *Azadirachta indica* A. Juss. (Meliaceae) has been used as fertilizer as well as in the management of pests in maize crops, and emerging as a viable alternative for smallholder farmers (Ngegba *et al*., 2022). Indeed, this fertilizer in many regions is residue from the pressing of the neem seed for the extraction of the oil (Abbasi *et al*., 2005; Schmutterer, 2009), which is used as a botanical insecticide and provides toxicity in maize and other crops to caterpillars (Lima *et al*., 2010; Silva *et al*., 2015).

Most studies with pest control utilizing neem are reported using oil from the seed, which has been a commercial product (Lokanadhan *et al*., 2012; Ikeura *et al*., 2013; Stanley *et al*., 2014), or leaf extracts (aqueous or organic) as the crop protector. However, the application of extracts of neem seedcake and azadirachtin (a compound toxic to caterpillars) utilizing root system (in the soil), caused a systemic effect in the suppression of sucking pests (Lynn *et al*., 2010; Hikal *et al*., 2017). On the other hand, the insecticides with contact effect, most commonly used to subdue/contain FAW, often fail to reach the insect, particularly the late-larvae instars, that are located between the young leaves inside the stalk of the plant as described by Phambala *et al*., (2020), in which the chemical insecticide activity depends on the plant architecture. For this and other reasons, the application of products with systemic action in crops has a large advantage due to the translocation of the active compound to all parts of the plant, besides being selective to natural enemies.

2.6.9. Chemical Control

Chemical treatment of maize is hampered by several factors, including high plant biomass, prolonged insect activity in a benign climate, and the cryptic lifestyle of the pest, where larvae tunnel within stalks and pack the entrances with frass. The larval habit of tightly packing its tunnels with frass prevents physical access by biocontrol agents (Dequech *et al*., 2013). Chemical control, despite its limitations, is an important tool for any pest management. However, based on the knowledge regarding the ecology and larval behavior of FAW, which feed in plant whorls, insecticides can be used with great success in containing this pest. The identification of the most susceptible stage in its life cycle is, however, necessary to ensure timely and effective chemical control. (Hardke *et al*., 2015; Varella, 2015; IRAC., 2016) found that a large percentage of FAW larvae feed behind leaf sheath and main shoot of maize, where they are not reached by insecticide applications. Late infestation of FAW feed in maize ears, where they are protected from insecticide application by husk leaves (Goergen *et al*., 2016; Abrahams *et al*., 2017; Kumela *et al*., 2019).

Consideration of specific insect habitats, peak periods of activity, and vulnerable stages of the pest is essential for efficient and successful management (IRAC., 2016). Knowledge of economic thresholds for FAW is also important for cost-effective application and minimum impact on the environment (Prasanna *et al*., 2018). Abrahams *et al*. (2017) and Patrick (2016) evaluated thresholds of 20 and 50% of maize whorls damaged and concluded that the 20% threshold generally performed better. This depended on the subsequent rate of increase in the number of injured whorls: as the infestation is difficult to forecast, the 20% level was deemed most appropriate for small-scale farmers in Nicaragua. This threshold may be applicable up to 30-40 days after planting, while for plants between 40 and 60 days after planting the threshold can be reduced to 10% (Jaramillo-Barrios *et al*., 2020; Lowry *et al.*, 2022).

Several insecticides have been used for subduing of maize FAW in different regions in Latin America and Africa. Insecticides that have been found effective as spray or dust treatments include carbofuran, carbaryl, deltamethrin, endosulfan, and synthetic pyrethroids (Ribeiro *et al*., 2014; Abrahams *et al*., 2017; Kumela *et al*., 2019).

2.6.10. Integrated Management Methods

In considering the strategies for the management of FAW, it is essential to develop integrated pest management (IPM) strategies that are sustainable, effective, environmentally safe, economically feasible, and acceptable to resource-limited/poor farmers. Fall armyworm management using only one method has been proven to be difficult by many scientists working in the area (FAO., 2018; Prasanna *et al*., 2018). In addition, relying on a single control method, like chemicals, may also result in undesirable consequences, such as pesticide resistance development, secondary pest outbreaks, adverse effects on non-target species, hazards of pesticide residues, and direct hazards from pesticides and environmental pollution (IRAC, 2016; Abrahams *et al*., 2017).

To address one or more problems arising from the use of a single control method and have sustainable management on FAW integrating different containing methods in a compatible manner would be the best solution (CABI, 2018; Birhanu *et al*., 2019; Mooventhan *et al*., 2019). Karlsson *et al*., (2020) suggested having directed efforts toward an integrated approach, particularly one, which incorporates biological control to improve crop production and insect pest management.

The FAW has been controlled by an integrated pest management program (IPM) comprised of cultural measures, host plant resistance, biological control, botanical control and pesticide application (Abrahams *et al*., 2017; FAO., 2018; Kumela *et al*., 2019). Matova *et al*. (2020) estimated that cultural practices contribute approximately 10% of season-long control; plant resistance and biological control each contribute 25%, and chemical control the remaining 40%. This pest management program has provided effective and stable management of the FAW.

CHAPTER III

III. DISTRIBUTION AND INFESTATION STATUS OF FAW AND ITS ASSOCIATED NATURAL ENEMIES IN MAIZE GROWING AREAS OF ETHIOPIA

ABSTRACT

Currently, FAW has become the most important threat to maize production in the maize belt areas of Ethiopia causing significant yield losses, which were not properly quantified. Therefore, the current study was conducted to know the distribution, and status of FAW and the associated natural enemies in major maize-growing areas of Ethiopia. The field assessment was conducted during the 2018/2019 and 2019/2020 main cropping seasons. An aggregate of 434 farmers' fields, 97 districts of 31 zones, and six regional states (Amhara, Benishangul-Gumuz, Gambella, Oromia, SNNPR, and Tigray) were surveyed to assess the invasion level of FAW in 2018/19. Similar surveys were conducted in 2019/2020 by adding a few locations. The survey results revealed that FAW infested almost all surveyed farmers' fields and locations. Moreover, 249 farmers' fields from a total of 434 farmers' fields surveyed in 2018/19 were infested by FAW. The overall mean average infestation by FAW for that year was 16.38%. In the 2019/20 crop season, 313 farmers' fields out of 384 farmers' fields surveyed were infested by FAW with an average infestation of 21.41%. In the first season, the highest (48.84%) infestation level was recorded in Benishangul-Gumuz, followed by Gambella Region, which was 35.72. In the same season, the lowest (6.06) infestation was recorded in the Amhara Region. In 2019/20, the highest (46.67) average infestation was recorded from the Afar Region, which was followed by the Somali region with 41.18 infestation. The lowest (11.25) infestation was from the Tigray Region. In the present study, six different types of indigenous/native natural enemies of FAW were recorded and identified from the host pest. Entomopathogenic Fungi and Entomopathogenic Nematodes ranged from 80 to 100% pathogenic for the third to sixth larval instars. The ladybird beetle was 60% predaceous for the first to third instar larvae; earwig was 30% predaceous for the first to the third instars; Trichogramma *spp 40% parasitism on eggs of FAW;* Cotesia *sp. parasitized 36 to 38% parasitism on second to sixth larval instars of FAW. These native natural enemies are promising for the management of FAW on maize. The insect pest was more severe and more rapidly spread throughout all surveyed areas than ever before. The current study revealed that FAW was devastating maize farms in Ethiopia and implied the need for designing effective, efficient, inexpensive, and sustainable management of the FAW for sustainable maize production and productivity.*

Keywords*:* Distribution, Entomopathogenic Fungi, Fall armyworm, Nematodes, Parasitoids, Predators

3.1. INTRODUCTION

3.1.1. Background

Maize (*Zea mays* L.) is the most important staple food crop grown predominantly by smallholder farmers in Sub-Saharan Africa (SSA) countries (Midega *et al.*, 2015). In SSA, maize occupies more than 36 million hectares of land each year. More than 208 million people in SSA depend on maize for food security and economic well-being (Abate *et al.*, 2017). Similarly, maize is the major cereal crop in Ethiopia, ranking first and second in yield per hectare and area coverage, respectively (CSA, 2017). In Ethiopia, maize occupies about 2 million hectares of land, and smallholder farms account for more than 95% of the total area and production in the country (CSA, 2017).

Despite the large production area and importance of maize, the average grain yield of this crop in SSA is very low, with yields being generally <1.0 $tonha^{-1}$, representing some of the lowest in the world (Kiboi *et al.*, 2019). This may be due to several abiotic and biotic constraints. Among the biotic factors, the damage caused by insect pests is the major one (Groote, 2002; Kfir *et al.*, 2002; Emana *et al.*, 2008). In Ethiopia, more than 40 species of insect pests have been recorded on maize in the field (Abraham *et al*., 1998). Among these, the maize stem borer *Busseola fusca*, *Chilo partellus*, and various termite species (*Macrotermes* and *Microtermes* spp.) are recognized to be the key pests (Emana *et al.*, 2008). More recently, however, an invasive insect pest *Spodoptera frugiperda*, fall armyworm (FAW) is becoming a major insect pest causing substantial yield losses on maize in different areas of African countries, including Ethiopia (Birhanu *et al*., 2018; Birhanu *et al.*, 2019; Kumela *et al*., 2019).

Fall armyworm (FAW) is a polyphagous insect pest native to tropical and subtropical regions of the Americas (Cock *et al.*, 2017; FAO, 2017; FAO, 2018), which has recently appeared in Africa (Goergen *et al*., 2016; FAO, 2017). It is regularly intercepted in intercontinental trade (CABI, 2016), but it has not previously become established outside of the Americas (Cock *et al*., 2017). However, FAW was reported in early 2016 for the first time in Africa (Goergen *et al.*, 2016; IITA, 2016). Currently, the occurrence of this pest is a threat to maize production in 44 African countries (Prasanna *et al*., 2018; Rwomushana *et al*., 2018; Birhanu *et al*., 2019).

In Ethiopia, FAW was reported for the first time in February 2017 on irrigated maize fields in the Bench Maji Zone of southern Ethiopia. Since arriving in Ethiopia, the pest has spread to six administrative regions: Southern Nations, Nationalities, and People's Region (SNNPR); Amhara; Benishangul; Gambella; Oromia and Tigray (AKLDP, 2017), where it has infested almost 685,000 hectares or about 23% of the total area planted with maize in the country (GAIN, 2017).

The FAW causes devastating damage to almost 100 plant species, including cotton, maize, rice, sorghum, soybean, sugarcane and wheat. However, recent studies revealed that a total of 353 plant species were hosts for FAW (Montezano *et al.*, 2018). Due to its ability to rapidly spread and inflict on widespread damage across multiple crops, FAW poses a serious threat to the food and nutrition security and livelihoods of millions of farming households in Sub-Saharan Africa (SSA).

In maize, FAW attacks all crop stages from seedling emergence through to ear development. It defoliates and kills younger plants, whorl damage, and ear feeding can result in grain quality and yield reductions. Detecting FAW infestations before they cause economic damage is the key to design for its management strategies. If infestations are detected too late, the impacts of damage may be irreversible. Recent estimates by CABI in 12 maize-producing countries showed that without control, FAW can result in maize yield losses ranging from 4.1 to 17.7 million tonnes per year, which is equivalent to a loss ranging from 1088 to US$4661 million per year. Recently, Baudron *et al*. (2019) reported a yield loss of 11.57% due to FAW damage in smallholder maize fields in Zimbabwe, which is relatively lower than the perceived losses reported by smallholder farmers in different countries, including Ghana and Zambia.

Synthetic chemical pesticides have been widely utilized as an emergency response to reduce the spread of FAW and minimize damage to maize crops in African countries since its introduction. Although chemical insecticides play an important role in FAW management, given confirmed reports of insecticide resistance development in the FAW population (Matova *et al*., 2020; Chen *et al*., 2023), their unavailability, high cost, and other negative effects on humans and the environment, sole dependence on insecticide is not feasible.

A key component of crop protection in modern agriculture is the use of biological control agents (bioagents), such as entomopathogenic fungi (EPF) to suppress insect pests (Meyling and

Eilenberg, 2006). The use of entomopathogenic fungi, entomopathogenic nematodes, parasitoids, and predators can be important alternatives to reduce the impact of chemical insecticides on the environment while boosting maize production (Fallet *et al*., 2022; Meyling and Eilenberg, 2006; Birhanu *et al.*, 2019).

3.1.2. Statement of the Problem

The newly occurring and menace insect pest FAW affects maize production throughout the year and, thus, poses a challenge to the production of maize in Ethiopia and serious threat to the country's food safety and security and has direct implications for the economic outlook of farmers. However, FAW infestation, and its damage levels on maize due to this new pest are not very well known in different maize-production areas of the country. In addition, natural enemies associated with FAW have not been adequately assessed, characterized and documented. Several efforts have been made by farmers and the government to mitigate these losses and protect the country's modest advances in guaranteeing food and nutrition security. The purpose of this study was to collect information from maize-producing farmers and inspect farms to establish the general status of the FAW and its impact on maize output in Ethiopia. Proper assessment of distribution, importance, and associated natural enemies is crucial in developing environmentally sustainable integrated pest management (IPM) strategies and boosting maize production and productivity.

3.1.3. Scope of the Study

The distribution and impact of FAW on maize crops in different localities of Ethiopia is poorly understood. The aim of this study was to collect information, understand and docummented the distribution and impact level of FAW. Knowing the availabilty and identification of FAW natural enemies are also very importan for further considereing the option of biocontrol strategies. The field assessment was conducted during 2018/19 and 2019/20 cropping seasons both in dry and wet easons.

3.1.4. Significance of the Study

Since the FAW recenty occurring insect pest, there is a lack of information on its distribution and the availabilty of its natural enemies under Ethiopian conditions. Thus, the results of this study will give a clue for local farmers, policy makers, students, researchers, extensonists and development agents to design appropriate control mechanisms and in looking for better solutions and options to overcom the problem cuased by FAW.. The main aim of this survey was to study the distribution of FAW and its natural enemies in different maize growing areas of Ethiopia.

3.1.5. Objectives of the Study

General Objective:

- To make the situation of FAW and its natural enemies more clear and understandable in the study areas for future development of control strategies

Specific Objectives

- To assess the distribution and infestation level of FAW on maize and identify their natural enemies

3.2. MATERIALS AND METHODS

3.2.1. Assessment of Distribution and Infestation Level of FAW in Different Agro-ecological Zones of Ethiopia

Surveys of FAW were conducted in major maize-growing regions (Amhara, Benishangul Gumuz, Gambella, Oromia, SNNPR, Somali and Tigray Reions) of Ethiopia for two consecutive years in 2018 to 2019 and 2019 to 2020 during the main cropping seasons (Table 1). The study sites were purposively selected based on representations of the different agro-ecologies and production statistics of maize. The agro-ecological zones are categorized as highlands (above 2000 m.a.s.l), mid-highlands (between 1500-2000 m.a.s.l.), and lowlands (below 1500 m.a.s.l.) (EARO, 1998). Three to ten districts recognized for maize production and those with accessible maize fields were selected from each major maize-growing agro-ecology. Two to three villages within each district were selected at the distance of 5 to 10 km intervals. Three to 5 farmers' maize fields within each village/PA and four maize plants sampling points within each farmers' field were randomly selected. From each selected sampling point, maize plants within the 1 × 1 m^2 quadrant were inspected against FAW. From each quadrant, the total plant density and the number of plants infested by FAW were counted to estimate the proportion of infested plants (FAW infestation in the crop field) with total plants in the quadrant (Koffi *et al*. 2020).

The number of larvae and egg mass were counted per ten sampled plants and the average FAW density was calculated by dividing the total number of larvae to total inspected plants and multiplied by 100.

Coordinates of the sampling points (localities) were recorded using GPS and study localities were mapped using ArcGIS software to determine the distribution of FAW.

The percentage of infested fields per district, zone, and region (FI) were determined by the formula:

$$\mathrm{FI} = \frac{\mathrm{Fi}}{\mathrm{Ft}} * 100$$

Where FI is the percentage of infested fields, Fi is the number of fields insect infestation reordered, and Ft is the total number of fields surveyed (Albasini *et al.*, 2020).

Table 1. Descriptions of surveyed regions, zones, and districts by altitude in the cropping seasons of 2028/19 and 2019/2020.

	2018 to 2019 cropping season			**2019 to 2020 cropping season**		
Region	**Zone**	**District**	**Altituddinal rang (m.a.s.l.)**	**Zones**	**District**	**Altitude range (m.a.s.l.)**
Gambella	Anywaa, Nuer, Majang	Habobo; Lare, Itang; Makuey	451-510 415-437 410-423	Anywaa, Nuer, Gambella	Habobo, Abol; Itang, Lare; Gambella	430-540 414-447 455-1686
B.Gumuz	Asosa Zuria, Kamashi, Metekel	Bambasi, Homosha; Kamashi; Dangur, Pawe	1240-1531 1120-1326 1117-1203	Asosa	Banbasi, Homosha, Asosa,	1253-1720
Amhara	West Gojam, Awi, South Gojam, North Gondar	Mankusa, JabiTehenan, Wonberma, Bure, South Achafer, Mecha, Bahidar Zuria; Ankesha, Dangila, Ayew Gagugsa; Dera; Chilga, Dambia	1747-2336 1897-2063 1279-1880 1283-2244	East Gojam, West Gojam, Awi Zone, Bahidar Zuriya, South Gonder, North Gonder	Awobele, Andid, Gozamen; Dembecha, Jabi Tehinan; Banja; Mecha; Fogera, Libo Kemkem Adi Arkay	2170-2461 1697-1832 1955-2502 1222-1994 1783-1876 1216
Tigray	Northwest, South Tigray	Lealai Adaw, Tahtay Kararo, Medabai Zana; Mehoni	1788-2013 1535-1625	Northwester, Southwest, Central zone, South zone	Tselemti, Laelay Adiabo, Tahtay Adiabo, Wukiro, Hintale Wojerat, Adeigudem; Tahtay Koraro, Laelay Koraro; May Zegiray, Laelay Maichew; Wukiro, Hintale	1160-1914 1937-1972 2040-2136 1460-1758

Oromia	Guji, Bedele, Buno Bedele, Jima, East Showa, East Arisi, West Hararghe, East Hararghe, North Showa, H/G/Wolleg, West Wollega, East Wollega, West shoa, Southwest shoa	Bore, Adola, Shakiso & Sora; Kerka, Bedele, Buno; Gachi, Didesa; Goma,Mana, Seka Chekorsa, Shebe Sombo; Adama, Boset, Fentale; Jeju; Gumbi Bordede, Miheso, Chiro, Tulo, Doba; Goro Gutu,Kersa,Meta, Haromaya, Kurfa Chele,Gurawa, Dire Tiyara, Kombolcha; Minjar Shenkora;Horo, Abay Choman, Guduru, Choman Guduru, Jima Rare; Gimbi, Lalo Asabi;Gida, Kiremu, Gida Ayana, Guto Gida, Diga, Wayu Tuka,Sibu Sire, Gobu Seyo; Bako, I/Gelan, Dano,Cheliya, L/Jawi, T/Kutaye, Ambo, D/Inchini, Shenen Jibat, Nono; Welliso, Becho, Dawo & Dendi.	1686-2765 1419-1909 1590-2064 1383-2010 967- 1772 1194-1313 1060-2400 1755-2546 1316-1348 2229-2622 1267-1911 1247-2237 1525-2592 2159-2342	East Showa, East Arsi, West Showa, East Wollega, West Wollega, Buno Bedele, Illubabore, Jima, Borena, West Guji	Fentale, Boset, Adama, Merti, Batu, Dugda Bora; Jeju; Bako, Illu Gelan, Dano, Gudeya Bila, Gobu Seyo, Sibu Sire, Diga, Wayu Tuka, Nekemte, Leka Dulecha, Gutu Gida, Belo Gigafo; Gimbi, Nejo, Laloasabi, Boji Dirmji, Leta sibu, Kiltu kara, Mene Sibu; Bedele, Chora, Gehi, Didesa; Ale, Didu, Halu, Hurumu, Yayo; Goma, Seka Chekorsa, Shebe Sobo, Kersa, Gumay, Mana; Dire, Dubuluk, Mio, Wlwaye, Gomole, Moyale, Teltele, Yabelo; Dugda Dawa	959-1644 1238 1642-1947 1187-2224 1552-1959 1519-2194 1557-1923 1366-2005 880-1619 1618-1716
Afar		Not done in this season		Afar	Asaita, Dubti	349-371
Somali		Not done in this season		Fafen	Gursum, Dendema, Babile, Jigjiga, Kebiribeya, Ararso, Haruris & Wuchale	1331-1813

3.2.1. Assessment of FAW Natural Enemies and Their Parasitism Level

Similar assessment procedures were used as that of FAW assessments. Eggs, larvae, and pupae of FAW found on maize plants were collected and taken to the Entomology laboratory, Ambo Agricultural Reseach Centetr (AmARC). Predators were collected when they were preying on eggs and/or larvae of FAW. The cocoons or pupae of parasitoids and any unknown insects associated with any life stages of FAW were collected and taken to the laboratory and maintained in plastic vials for the emergence of any natural enemy of FAW. The dead cadavers of FAW were collected for entomopathogens. The larvae were reared in glass jars on fresh stem cuttings and leaves of maize, whereas the eggs, pupae, and cocoons were kept in plastic vials until the emergence of the parasitoids, the presence of mycosis and infection as sugested by Agboyi *et al.* (2020) and Idrees *et al*. (2023).

The parasitoids that emerged from larvae were recorded every 24 hours. Parasitoids were identified using a compound and stereoscope microscope and insect identification keys (Ordóñez-García *et al*., 2015).

The method described and employed by Jaber *et al*. (2016) and Zulfitri *et al*. (2018) was followed to record entomopathogens. Accordingly, larvae and pupae that died in the vials were examined for the presence of microbial pathogens using a stereomicroscope; signs of disease were recorded for all cadavers. Identification of entomopathogens was carried out at Ambo Agricultural Research Center Plant Protection Laboratory and Addis Ababa University.

After identification of all-natural enemies, voucher specimens of parasitoids and predators were kept in 70% ethanol, while nematodes and fungi were preserved on the dead cadaver of the insect, and were preserved in a refrigerator for further molecular identification.

3.2.2. Data Analysis

3.2.2.1. The Percentage of Infested Fields

The percentage of infested fields per district, zone, and region (FI) was determined by the formula:

3.2.2.2. Relative Infestation of Natural Enemies Species

Relative infestation of natural enemies species (Molina-Ochoa *et al*., 2004; Murúa *et al.*, 2009) was calculated using the following formula:

$$RA = \frac{N_i}{N_t} \times 100$$

Where the numerator (N_i) is the number of individuals of species, and the denominator (N_t) is the total number of individuals collected.

3.2.2.3. The Parasitism Rate of Natural Enemies

The parasitism rate (PA) (Zhou *et al*., 2003; Murúa *et al.*, 2009; Mailafiya *et al*., 2011) was estimated using the following formula:

$$PR = \frac{N_{pi}}{N_t} \times 100$$

Where the numerator is the number of parasitized FAW pi, and the denominator is the total number of individuals FAW collected

3.2.2.4. Data Transformation

Data on average infestation was transformed using arcsine and subjected to Proc GLM (SAS version 9.4) for analysis of variance and means separation using Tukey's Honestly Significant Difference (HSD) test. Graphs were plotted using Excel.

3.3. RESULTS

3.3.1. Distribution and Infestation Levels of FAW in Different Agro-ecological Zones of Ethiopia

The study was carried out in various agro-environmental Regions and Zones of Ethiopia (Gambela, Benishangul Gumuz, Amhara, Tigray, SNNPR, and Oromiya) during the 2018/19 and 2020/21 cropping season (Table 3). During 2018/19 cropping season, 434 farmers' fields, 97 districts from 31 zones, and 6 regional states were assessed for the possibility of FAW invasion (Table 2). In 2019/20 cropping season, 384 farmers' fields from 105 districts, 31 zones, and 8 administrative regions were also inspected. (Table 3).

The assessment results in 2018/19 cropping seasons showed that from a total of 434 farmers' fields 249 were infested and 185 farms fields were not infested. The overall mean average infestation of FAW for this year was estimated to be 16.38. In the 2019/20 cropping season, out of 384 farmers' fields observed, 310 farmers fields were found infested while 74 farmers fields were not infested and and the over all average infestation of FAW in this cropping season was scored for about 21.41.

As indicated by the study in the 2018 to 2019 crop season, FAW is broadly distributed across maize growing in 6 regions of Ethiopia (Table 2) and the event was noticed and recorded in many fields studied.

In the Gambela region, the assesment was carried out in the 3 zones, 4 districts, and 21 farmers fields and the highest FAW infestations (30 to 80) were recorded in Anywaa, Majang, and Nuer zones while the lowest invasion (0-10) was recorded in the Nuer zone (Table 2). From Benishangul-Gumuz, 3 zones, 5 districts, and 43 farmers' fields were assessed and the FAW invasion (30 to 90) were recorded in Kamashi zone, and the least attack (0-10) was recorded from Metekel zone. (Table 2).

From Amhara region, 4 zones, 13 districts, and 66 farmers' fields were also checked and in many farmers' fields the infestation of the FAW was recorded at the medium level (20-30) and while the most the areas showed reduced invasion level (0-10) in Gonder zones farmers' fields (Table 2).

In the Tigray region, 2 zones, 4 districts, 20 farmers' fields were assessedand for about 10-60 invasion of the FAW was recorded at the Northwest zone and the lowest infestation level was recorded 0-5% at South Tigray.

In Southern Nations, Nationalities, and Peoples' Region (SNNPR) 5 zones, 11 districts, 25 farmers fields were evaluated and the highest infestation of FAW (30-80) was recorded at Sidama, Hadiya, Wolayta, and Gamo Gofa, although the least (0-10) was recorded at Kembata Tembaro (Tables 2) In Oromiya, 14 zones, 60 districts, and 259 farmers' fields were evaluated. The highest infestation of FAW (20-80) was recorded in all districts except from Guji and North Showa (0-5). during 2018/2019 cropping season (Table 2).

The results showed that the FAW was widespread in all surveyed agroecologies during 2020/21 cropping seasons at varying levels (Table 2). From Afar region, 1 zone, 2 districts, and 9 farmers' fields were assessed and all farms were found infested by the FAW and the highest FAW infestation (30 to 100) was recorded from some farm areas, while the lowest (10-20) invasion was recorded in others (Tables 3). In Oromiya, 10 zones, 50 districts, and 192 farmers' fields were surveyed. Out of 192 farms, 163 were infested by FAW and 29 farmers' fields were not. The over all infestation situtions from surveyed areas showed about 30 to 90 in some different zones and the least attack (10-20%) were recorded in some surveyed areas.at the time of second cropping seasons. The mean average incidence in over all the surveyed areas was 22.03 (Table 3). Likewise, in Amhara, 6 zones, 10 districts, and a total of 26 farms were surveyed. From these , 20 of them were infested while 6 farms were not infested by FAW. At the time of assessment in this region, the highest infestation (30-80) was recorded in Mecha and Fogera districts, and the lowest infestation (10-20) was recorded in Jabi Tehnan and Bahir Dardistricts. The mean average incidence over all the surveyed areas was 18.8 (Table 3).

Table 2. Surveyed regions, zones, and number of farmers fields that were considered in cropping seasons of 2018/ 2019.

No	**Region**	**Zones**	**NfFS**	**Tot.**	**SpP**		**PI**	**IZ**	**IR**
					NfIF	NfNIF			
1	Gambella	Anywaa,	7	21	6	1	10-80	38.57	35.72
		Nuer,	8		5	3	0-60	18.75	
		Majang	6		6	0	30-70	55.0	
2	B/Gumuz	Asosa Zuria,	21	43	21	0	30-80	52.86	48.84
		Kamashi,	13		13	0	30-90	61.54	
		Metekel	9		8	1	0-70	21.11	
3	Amhara	West Gojam,	37	66	19	18	0-30	6.22	6.06
		Awi, South	13		9	4	10	7.69	
		Gojam, North	2		1	1	0-20	10	
		Gondar	14		3	11	0-20	3.57	
4	Tigray	Northwest,	17	20	5	12	0-60	9.41	8.5
		South Tigray	3		1	2	0-10	3.33	
5	SNNPR	Sidama,	11	25	8	3	0-70	25.45	34.16
		Hadiya,	2		2	0	30-60	45	
		Kenbata Tembaro,	1		1	0	10	10	
		Wolayta,	6		6	0	10-80	38.33	
		Gamo	5		4	1	0-80	42	
6	Oromia	Guji,	9	259	0	9	0	0.0	10.98
		Bedele,	4		1	3	0-40	10	
		Buno Bedele,	4		1	5	0-70	11.66	
		Jima,	9		0	7	0	0	
		East Showa,	15		10	3	0-20	11.54	
		East Arisi,	5		0	5	0-30	0	
		West Hararghe,	24		4	20	0-20	2.08	
		East Hararghe,	45		12	35	0-40	4.26	
		North Showa,	1		0	1	0	0.0	
		H/G/Wollega,	23		20	3	10-60	22.61	
		West Wollega,	21		20	1	10-40	17.14	
		East Wollega,	37		21	16	0-80	17.03	
		West Shoa,	52		32	20	0-70	12.88	
		Southwest Shoa	10		5	2	0-40	17.14	
	Sub Tot.		434	434					

NfFS= Number of farms surveyed; SpP= Status per Plot; NfIF= Number of infested fields; NfNIF= Number of not infested fields; PI= Plot incidence; IR= FAW average infestation on regions and IZ= FAW average infestation on Zones.

In Tigray region, 4 zones, 12 districts, and 24 farmers' fields were assessed where out of these, 13 fields were infested and the other 11 were not. At Tselemti and Laelay Adiabo, the highest infestation levels (30-40) were recorded and the lowest infestations (10-20) were recorded at Tahtay Koraro, Laelay Maichew, and Wukiro. The mean average incidence over all the surveyed areas was 11.25 (Table 3). In the SNNPR region, 6 zones, 15 districts, and 50 farmers' fields were noticed: out of these 35 were infested, and 15 were not infested by the pest. 30-60 of invasions of the FAW were recorded at Boloso sore, Arba Minch, and West Abaya district, whereas 10-20 infestation was recorded on other surveyed areas except Konso. The mean average infestation of the FAW over all surveyed areas this season was 13.6 (Table 2).

From Gambela region Zones such as Ayywaa, Nuer & Gambela were assessed. From these about 4 districts and 26 farmers' fields were evaluated and the highest infestation of FAW (20-40) was recorded, while the lowest infestation of FAW (0-10) was recorded during the survey period (Table 3).

In Benishangul-Gumuz a total of 1 zone, 4 districts, and 26 farmer's fields assessed and from Somali region 1 zone, 8 districts, and 34 farmers' fieldswere also assessed. Out of these 26 farmers fields, 24 farmers fields were found infested by the FAW and while 2 farmers fields were not infested.

A total of 60 farmers' fields were assessed in Benishangul-Gumuz and Somali. Out of 60 farmers' fields, 55 were infested by FAW, and the remaining 5 farmers' fields were not infested.. The over all infestation level of FAW in both regions showed that 10-60 in Benishangul while 20-90 from Somali. The mean average infestation over the surveyed areas 16.15 and 41.18 was recorded at Benishangul-Gumuz, Somali, respectively. The map of Ethiopia that shows the over all distribution of FAW in different regions and localities were illustrated in Fig 2. bellow

Table 3. Description of surveyed areas and number of farms surveyed in 2019 to 2020 cropping seasons in selected regions.

No.	Region	Zones	NfFS	Tot.	SpP		PI	IZ	IR
					NfIF	NfNIF			
1	Afar	Afar	9	9	9	0	10-100	46.67	46.67
2	Oromia	East Showa,	15	192	9	6	0-80	18.0	22.0
		East Arsi,	1		0	1	0	0.0	
		West Showa,	10		10	0	20-50	37.78	
		East Wollega,	31		26	5	0-50	15.81	
		West Wollega,	25		23	2	0-40	21.20	
		Buno Bedele,	16		10	6	0-60	15.0	
		Illubabore,	14		11	3	0-60	16.43	
		Jima,	40		35	5	0-80	19.0	
		Borena,	38		37	1	0-90	34.21	
		West Guji	2		2	0	10-30	20.0	
3	Amahara	East Gojam,	5	26	3	2	0-20	8.0	18.8
		West Gojam,	4		2	2	0-30	10.0	
		Awi Zone,	5		4	1	0-30	14.0	
		Bahidar, Zuriya,	8		8	0	10-70	27.5	
		South Gonder,	3		2	1	0-80	33.33	
		North Gonder	1		1	0	30	30.0	
4	Tigray	Nourth western,	13	24	9	4	0-40	14.62	11.3
		Southwest,	3		3	0	20-30	20.0	
		Centeral zone,	3		1	2	0-20	6.67	
		South zone	5		0	5	0	0	
5	SNNP	Gamo,	9	50	8	1	0-40	17.8	13.6
		Segen,	4		2	2	0-20	7.50	
		Konso,	6		0	6	0	30	
		Welayta Sodo,	16		16	0	10-60	6.9	
		Gofa,	13		8	5	0-20	5.0	
		Hadiya	2		1	1	0-10		
6	Gambella	Anywaa,	11	23	7	4	0-40	11.82	12.6
		Nuer,	9		8	1	10-40	17.78	
		Gambella	3		0	3	0	0.0	
7	B/Gumuz	Asosa	26	26	24	2	0-40	16.15	16.15
8	Somali	Fafen	34	34	31	3	0-85	41.18	41.2
	Total		384	384					

NfFS= Number of farms surveyed; SpP= Status per Plot; NfIF= Number of infested fields; NfNIF= Number of not infested fields; PI= Plot incidence; IR= FAW average infestation on regions and IZ= FAW average infestation on Zones.

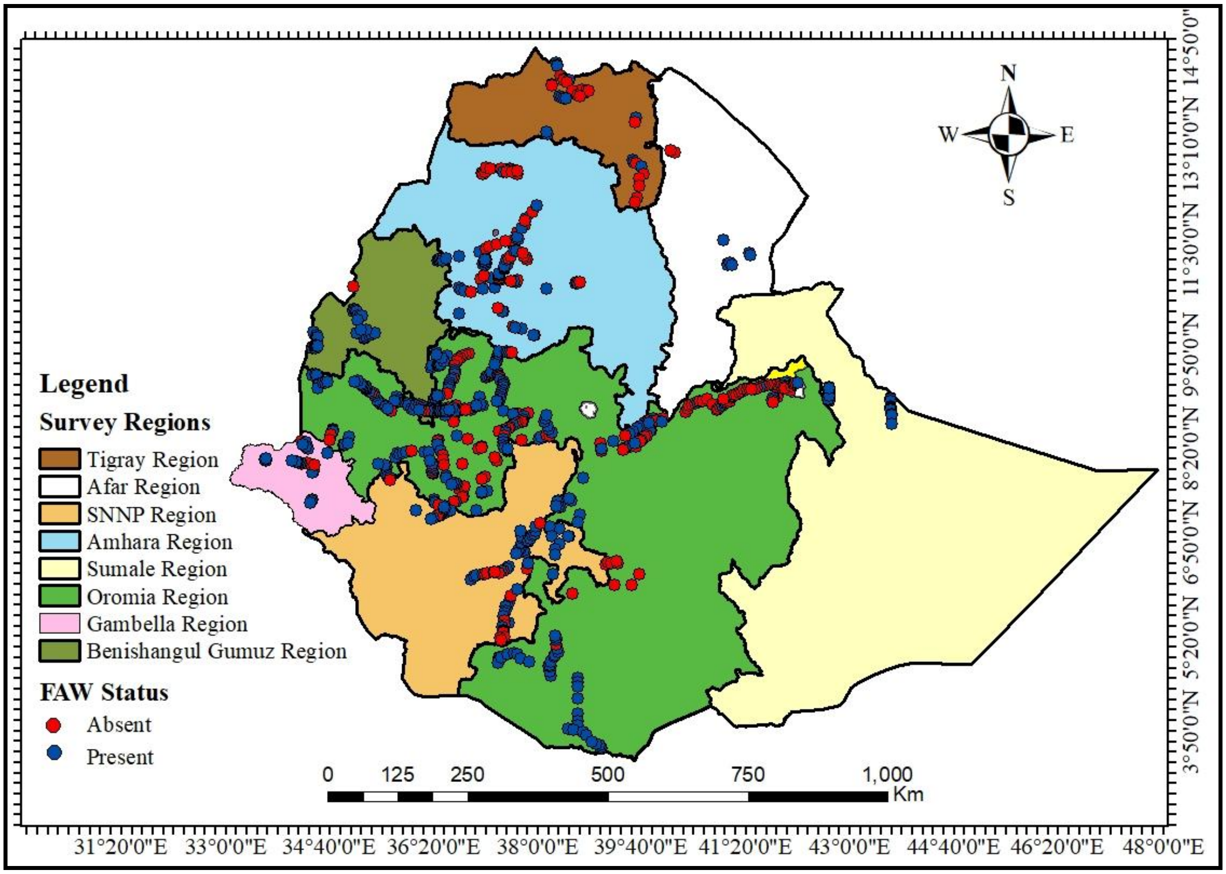

Figure 2. The map of Ethiopia showing FAW distribution for the 2018/19 to 2019/20 crop seasons in different regions**.**

3.3.2. Record of Natural Enemies Associated with FAW

During the assessments carried out for the determination of the FAW distribution in some regins, zones and localities of Ethiopia, attempts also made to collect natural enemies of FAW at its various growth stages. As a result, eight natural enemies were found and collected from different parts of surveyed areas in the country. These natural enemies were Ladybird beetle (Coccinelid) and Earwig (*Doru lineare*) as predators and feeding egg mass, first instars to third instar stages of the FAW were recorded (Figure 5 E&G) and their predation level was estimated for about 60 and 30%, respectively (Table 4). Trichogramma sp. was attacking the eggs up to 40%, and the larval parasitoid cotesia sp. were also parasitizing 36 to 38% of the larval population (Table 4). As indicated in Table 4, out of 30 dead cadaver insect samples, a total of 7 EPFs were isolated and identified. Out of seven different indigenous EPF only 4 *Metarhizium* species and 3 *Beauveria* species. However, Entomopathogenic Fungi and Entomopathogenic Nematodes, which attacked all stages of FAW larva and 80 to 100% parasitism level were observed.

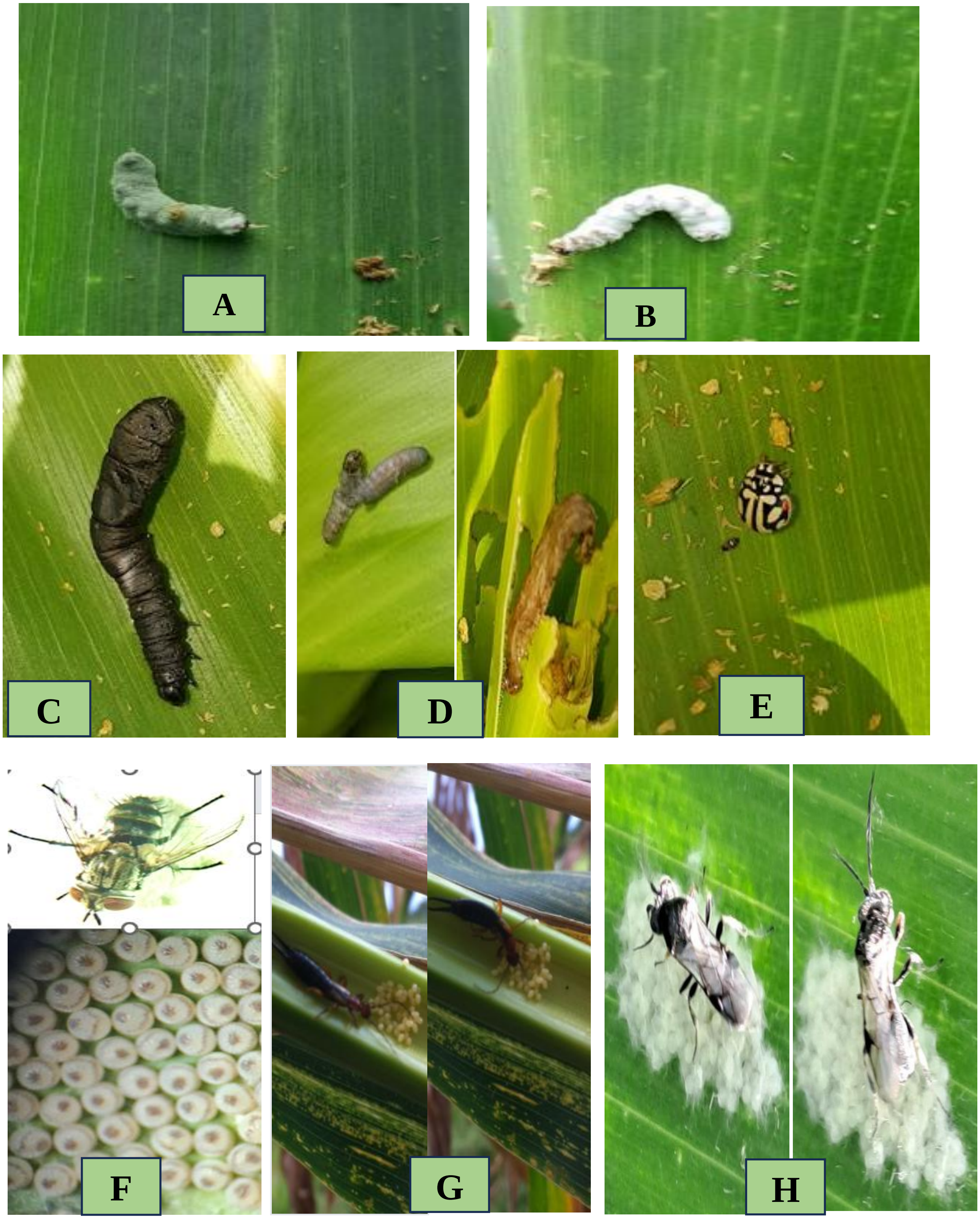

Figure 3. Natural enemies of FAW observed on FAW eggs and larvae: (A) Metarhizium species; (B) Beauveria species; (C) Heterorhabditis species; (D) Steinernema species; (E) Ladybird beetle; (F) Trichogramma (G) Earwig (H) Cotesia sp.

Table 4. Natural enemies associated with FAW (*Spodoptera fugiperda*) on maize collected from the survey areas in Ethiopia during 2018 to 2020.

No	Natural enemies	The more susceptible stage of FAW	Parasitism/ predaceous/ pathogenicity %age in 6 days on lab	Area of collection
1	Metarhizium species	3rd to 6th larval	80 to 100%	Borena, Guten, Jimma, Amhara,
2	Beauveria species			
3	Heterorhabditis species	3rd to 6th larval	80 to 100%	Borena, Guten, Bulehora, Amhara
4	Steinernema species			
5	Ladybird beetle (Coccinelids)	1st to 3rd	60%	Ambo, Bako, Melkasa
6	Earwig (*Doru lineare*)	1st to 3rd	30%	Ambo, Bako, Jima, Didesa
7	Trichogramma sp.	Egg	40%	Ambo, Bako, Jima, Goba, Assosa…
8	Cotesia sp.	2nd to 6th larval	36 to 38%	Jima, Melekasa, Borena, Andide (Amhara)

3.4. DISCUSSION

Currently, FAW has rapidly spread throughout most of the maize-producing agro-ecologies of Ethiopia. Following the first report of FAW in West Africa in January 2016, the insect pest was soon found in to East Africa in 2017 and the introduction into and infestation by FAW in some areas of Ethiopia, Kenya, and Tanzania moderate to high (33 to 100%) levels of infestation was recorded in the surveyed areas (Birhanu *et al*., 2019). A total of 434 farmers' fields were surveyed during the 2018/2019 crop season. Out of these fields, the number of infested farms was 249, and the number of uninfested farms was 185. The overall mean average infestation by FAW for that year was 16.38%. Furthermore, in the 20219/20 crop season, out of 384 farmers' fields assessed, the number of infested fields was 310, and the number of uninfested fields was 71. The mean average infestations by FAW in that period was 21.41%. Even so, larvae feed on both vegetative and reproductive maize (Capinera 2017; Day *et al*., 2017; Birhanu *et al*., 2019), attacking leaf tissue might not cause yield loss as the plant can tolerate such damage. Day *et al*.

(2017) reported that subsequent yield losses also depend on the growth stage of maize and the level of infestations.

Six natural enemies were found from different parts of Ethiopia in the current study. In the laboratory, Trichogramma sp. parasitized 40% FAW eggs within 6 days, the results agree with the previous findings (Pomari *et al*., 2012; Laminou *et al*., 2020). Ladybird beetle and earwig spp., within 6 days of laboratory evaluation they predated 60 and 30% of first to third-instar larvae of FAW. This is also in line with the study conducted by Birhanu *et al*. (2019) and Hoballah *et al*. (2004). In the case of entomopathogenic fungi and entomopathogenic nematodes, 80 to 100% pathogenicity was observed on the third to sixth-instar larvae of FAW (Birhanu *et al*., 2018).

Previously various efforts were made on natural enemies against FAW. Ten parasitoid species attacking the eggs and larvae of FAW were found in Ghana and Benin. In the same way, surveys carried out in Ethiopia, Kenya, and Tanzania, showed and collected seven parasitoid species were collected (Birhanu *et al*., 2018, 2019). Moreover, numerous previous records on parasitoids, predators, and pathogens are reported from Burkina Faso, Cameroon, Chad, DR Congo, Ethiopia, Madagascar, Nigeria the Sudan and Togo, and *Spodoptera* spp. recorded from Tanzania (Madl, 2014; Birhanu *et al*., 2018; Agboyi *et al.*, 2020).

3.5. CONCLUSIONS AND RECOMMENDATIONS

The assessment results revealed that FAW of maize was widely distributed in almost all surveyed areas of Ethiopia during 2018/19 and 2020/21 cropping seasons. Percentage of infested crop fields and plants by FAW vary from location to location within regional states (zones, districts, and kebeles) of Ethiopia. Variations observed may be due to differences in weather and maize variety planted by farmers during the survey year. The current study revealed that maize-producing farms in Ethiopia are under FAW pressure and consequently, management should be required to control the FAWt in an effective, affordable, and sustainable approach. From the asesment made sofar eight different types of indigenous natural enemies were recorded, and identified the potential for the management of FAW in an eco-friendly and sustainable manner.

CHAPTER IV

IV. SEASONAL DISTRIBUTION AND INFESTATION LEVEL OF FALL ARMYWORM, *S. frugiperda* IN ETHIOPIA

ABSTRACT

The alien invasive insect pest, Spodoptera frugiperda *Smith (Lepidoptera: Noctuidae), commonly referred to as fall armyworm (FAW), has been causing significant losses to maize production in Africa since its detection in 2016. Despite being the primary insect pest of the main food crop in the country (Ethiopia), researchers are more focused on management methods' development. There is no or little research done on the seasonality of the insect pest, which greatly helps in timing intervention time by farmers. The primary purpose of this research was to determine how FAW in maize fields changed with the seasons. Fall armyworm surveys were carried out in Ethiopia's major maize-growing regions. These included Afar, Amhara, Benishangul Gumuz, Oromia, Southern Nations, Nationalities and Peoples (SNNP, and Tigray Regions,in the dry and rainy seasons from 2018 to 2019 and 2019 to 2020, respectively. The survey sites were purposively selected based on production statistics of maize and FAW occurrence reports in Ethiopia. From different regions, a total of 480 maize-producing farmers' fields were surveyed, i.e. 240 fields in the dry season and 240 fields in the rainy season. Out of the 240 maize fields surveyed during the dry season, 218 fields were infested by FAW. Out of a total of 240 fields assessed, 146 fields were infested by FAW during the rainy season. The dry season had an average infestation of 42.5, while it was 25.8 for the rainy season. The average infestation percentage of plants per plot for the dry season was higher in Afar, Amhara, Oromia and Tigray, while the lowest was recorded at Benishangul Gumuz and SNNP. During the dry season, the average infestation by FAW in the regions ranged from 22.6 to 52.2, and during the rainy season, it ranged from 11.3 to 46.6. Afar (46.6), Oromia (22.0), Amhara (18.8), SNNP (13.6), Benishangul Gumuz (16.1), and Tigray had higher (11.3) average infestations during the wet season. In conclusion, the findings revealed that FAW is an economically important maize insect pest both in the dry season and in the rainy season, while the dry season infestation is slightly higher than in the rainy rainy season.*

Keywords: Cropping season, Dry season, Fall armyworm, Infestation, Invasive, Population density, Rainy season

4.1. INTRODUCTION

4.1.1. Background

Fall armyworm, *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae), is a polyphagous insect pest native to tropical and subtropical regions of the Americas (Cock *et al* 2017; FAO, 2017), which has recently invaded Africa (Goergen *et al.*, 2016; FAO, 2017). It is regularly intercepted in intercontinental trade (CABI, 2016), but has not previously become established outside of the Americas (Goergen *et al.*, 2016; Cock *et al.*, 2017). However, FAW was first reported in West and Central Africa in 2016 (Goergen *et al.*, 2016) and quickly spread to different parts of the African countries, causing significant damage to maize (Birhanu *et al.*, 2019; Feldmann *et al.*, 2019; Albasini *et al.*, 2020). The occurrence of FAW in Ethiopia was confirmed in February 2017 by the Ministry of Agriculture and Food Security (AKLDP, 2017).

In Ethiopia, maize is cultivated in both dry and rainy seasons. The rainy season starts from May 18 to the end of May to early June, and the planting of maize during dry or off-season starts in early November to late December. During the dry season, maize is cultivated mainly in areas with irrigation systems or in valleys and riverbanks. Approximately 88% of maize produced in Ethiopia is during the rainy season for food (Mandefro *et al.*, 2002). Several insect pests have limited the production and productivity of maize in Ethiopia from time immemorial since its cultivation started in Ethiopia in the 17th century. Most of the insect pests are indigenious in Ethiopia where maize is co-evolved. However, insect pests, like *Chilo partellus* Swinhoe and FAW are exotic and entered the country without any natural enemies.

Like other insect pests, FAW is known to be influenced by climate states of various seasons. The number of FAW in a particular area was influenced by various factors such as the time of year, weather condition and availability of the host plants. (Albasini *et al.*, 2020). In its native habitat, FAW occurs in temperate areas where only 2.3% of the area may allow the pest to survive year-round, although still subject to worrisome seasonal risks (Senay *et al.*, 2022). But in other places, such as the southeast region of the United States and African countries, FAW is considered a sporadic insect pest due to weather conditions of those regions, which are not suitable in some periods of the year (Nagoshi *et al.*, 2018; Albasini *et al.*, 2020). When weather conditions are not favorable for its development and reproduction, FAW is forced to migrate to more suitable locations for its survival (Westbrook *et al*., 2016; Albasini *et al*., 2020).

As indicated by Albasini *et al*. (2020) and Nboyine *et al*. (2020), there was a slight difference in temperatures between seasons. Unlike temperature, the difference in rainfall between seasons has significantly affected the development of the FAW (Albasini *et al.*, 2020). The articles suggest that rainfall was a key factor influencing the differences observed in the number of FAW egg masses and larvae per field between seasons in all surveyed areas. The survival of FAW was also not affected by the temperature. Despite the observed high infestation of FAW in the farmers' fields and commercial farms, little empirical information is available regarding the distribution, and importance of FAW in different maize-growing areas of Ethiopia.

4.1.2. Statement of the Problem

The previous studies on the seasonal distribution of FAW showed that it occurs throughout the year, both in dry and rainy seasons on maize (Albasini *et al*., 2020; Kumbhar *et al*., 2022). The newly occurred/emerged FAW in Ethiopia had challenged the production of maize and the crop was also produced in dry and rainy seasons. However, there is no available information regarding the distribution and damage level of FAW in the dry and rainy seasons. The purpose of this study was to assess the seasonal distribution of FAW in maize fields in the major maize-growing regions of Ethiopia. This will help maize growers develop an appropriate management strategy for sustainable maize production and productivity.

4.1.3. Scope of the Study

The scope of the study was to investigate the seasonal distribution of FAW in different maize-growing areas of Ethiopia. The field assessment was conducted during the 2018/19 dry and rainy seasons. Important data, like altitude, coordinates of the survey localities, and infestation levels for each farm were recorded.

4.1.4. Significance of the Study

The weather conditions in most of Ethiopia's areas are possibly favorable for maize production and maize is cultivated in dry and rainy seasons. However, one of the major constraints on maize production is insect pest problems, especially in the current days has been the impact of the newly occurring or emergent polyphagous insect pest, FAW causes significant yield losses. Studying the seasonal distribution of FAW is important in developing proper management of the FAW. Since the FAW is a recently occurring insect pest, there is lack of information on seasonal distribution in Ethiopia.

4.1.5. Objectives of the Study

To assess the seasonal distribution of FAW in maize fields across the maize-growing areas of Ethiopia.

4.2. MATERIALS AND METHODS

Survey of FAW were conducted in major maize-growing regions of Ethiopia. These include Afar, Benishangul Gumuz, Amhara, Tigray, South Nation, Nationality and Peoples (SNNP), and Oromia during dry and rainy cropping seasons of 2018/ 2019 dry season and 2019/ 2020. (Table 6). The study sites were purposefully selected based on production statistics of maize and FAW occurrence reports in the country (Birhanu *et al*., 2019).

From eight regions, a total of 26 zones; 76 districts) and 480 maize-producing farmers' fields were assessed in both cropping seasons (Table 6). Per season 240 farmers' fields were considered for the assessments at the interval distance of 5 to 10 km along the roadsides. At each selected sampling point, 20 plants were selected in a "W" pattern and checked for the presence of FAW eggs and larvae. Maize stalks and both the upper and lower surfaces of plant leaves were inspected for the presence of targeted insect. The number of larvae and egg masses present in each plant was recorded. The number of infested plants and plants damaged as a consequence of the FAW attack was also recorded. The plant damage was assessed using a visual scale ranging from 0 to 5 scores as described by Albasini *et al*., (2020): 0 = plant with no visual foliar damage; 1 = up to 10% of foliar damage; 2 = foliar damage between 10 to 25%; 3 = foliar damage between 25 to 50%; 4 = foliar damage between 50 to 75%; 5 = more than 75% of foliar damage or a dead plant due to FAW attack.

In addition, five to ten infested plants were randomly selected from each field and cut at ground level. Each plant was first externally checked for the presence of FAW. Leaves were then removed from each plant to check for the presence of FAW entry or exit holes. A plant with holes was dissected and the number of larvae and pupae was counted (Sokame *et al*., 2020). FAW numbers per plant (external and internal) were summed up and divided by the total number of dissected plants to calculate the mean density of FAW per infested plant (Sokame *et al*., 2020).

The average temperature, relative humidity, and precipitation of the study regions are presented in Table 5. Coordinates of the survey localities were recorded using GPS and collection localities were mapped using ArcGIS software to determine the distribution of FAW in Ethiopia.

The percentage of the infested fields (prevalence) per district, zone, and region (FI) was determined by the formula:

$$\mathbf{FI} = \frac{\mathbf{Fi}}{\mathbf{Ft}} * \mathbf{100}$$

Where FI is the percentage of the infested fields, Fi is the number of fields insect infestation reordered and Ft is the total number of fields surveyed (Albasini *et al*., 2020).

Table 5. Surveyed regions, zones, and districts with altitude for the dectation of FAW.

Regions	Rainy season			Dry season		
	Av. Temp (^{o}C)	Humidity (%)	Precipitation (mm)	Av. Temp (^{o}C)	Humidity (%)	Precipitation (mm)
Afar	40-41.0	31-23	0.8-0.1	36.8-34.4	37-43	0.5-0.20
Oromia	22.8-20.2	46-70	15.4-10.2	25.1-28.5	43-44	7.4-0.70
Amhara	23.3-24.1	58-78	4.1-114.0	26.3-23.4	71-62	2.4-0.70
Tigray	23.1-22.2	53-54	1.1-2.0	26.4-26.0	44-51	0.6-0.50
SNNP	25.7-26.3	70-81	17.6-8.0	27.7-28.3	71-63	15.3-11.7
B/Gumz	26.3-27.0	80-61	1.2-2.0	28.1-25.9	68-78	9.8-2.60

Table 6. Descriptions of survey regions, zones, and districts for FAW with altitudinal ranges in Ethiopia.

Region	**Zones**	**Districts**	**Altitude (m.a.s.l.)**
Afar	Afar	Asaita, Dubti	349-371
Oromia	East Showa	Fentale, Boset, Adama, Merti, Batu, Dugda Bora	959-1644
	East Arsi	Jeju	1238-1268
	West Showa	Gudeya Bila	1522-1643
	East Wollega	Gobu Seyo, Sibu Sire, Diga, Wayu Tuka, Nekemte, Leka Dulecha, Gutu Gida, Belo Gigafo	1187-2224
	West Wollega	Gimbi, Nejo, Lalo Asabi, Boji Dirmji, Leta Sibu, Kiltu kara, Mene Sibu	1552-1959
	Buno Bedele	Bedele, Chora, Gehi, Didesa	1519-2194
	Illubabore	Ale, Didu, Halu, Hurumu, Yayo	1557-1923
	Jima	Goma, Seka Chekorsa, Shebe Sombo Kersa	1366-2005
Amahara	East Gojam	Awobele, Andid, Gozamen	2170-2461
	West Gojam	Dembecha, Jabi Tehinan	1697-1832
	Awi Zone	Banja	1955-2502
	Bahidar Zuriya	Mecha	1222-1994
	South Gonder	Fogera, Libo Kemkem	1783-1876
	North Gonder	Adi Arkay	1216-1260
Tigray	Nourth western	Tselemti, Laelay Adiabo, Tahtay Adiabo, Wukiro, Hintale Wojerat, Adeigudem	1160-2482
	Southwest	Tahitay Koraro & Laelay Koraroa	1925-1958
	Central zone	Mayze Girmay & Laelay Maychew	2050-2136
	South zone	Wukiro	1978-2001
SNNP	Gamo	Arbaminch, West Abaya	1113-1237
	Segen	Derashe	1138-1269
	Konso	Konso, Abela Abeya	1190-1571
	Welayta Sodo	Sodo Zuriya, Humbo, Gesuba, Boloso Sore, Damot Gale	1270-2134
	Gofa	Kucha, Denba Gofa, & Zala	981-1234
	Hadiya	Bada Wacho	1779-2017
B/Gumuz	Asosa	Banbasi, Homosha, Asosa	1253-1720

4.3. RESULTS

4.1.6. Distribution and Infestation Level of FAW

A total of 240 maize fields were surveyed and out these 218 maize fields were found infested by FAW and 22 maize fields were not infested during the dry season (January to May) of 2018/2019 cropping season. During the rainy season(June to October) of 2019/2020, a total of 240 fields were surveyed in which 146 fields were found infested, and only 94 fields were not infested by the FAW (Table 7).

Table 7. Surveyed zones, number of farms, plot incidence, percent infestation at regional, zonal level during the dry season of 2018/2019 and 2019/2020 rainy seasons.

Zones	**NofD**	**Total**	**Dry Season**			**Rainy Season**		
			SpP		IZ	SpP		IZ
			NfIF	NfNIF		NfIF	NfNIF	
Afar	2	14	14	0	52.2	6	8	46.67
East Showa	6	100	15	0	37.3	9	6	18.0
East Arsi	1		2	0	25	0	2	0.0
West Showa	1		2	0	35	0	2	37.78
East Wollega	8		23	1	46.3	18	6	15.81
West Wollega	7		17	2	32.11	11	8	21.20
Buno Bedele	4		9	0	52.22	3	6	15.0
Illubabore	5		13	1	31.42	11	3	16.43
Jima	4		15	0	44.0	10	5	19.0
East Gojam	3	26	5	0	60	3	2	8.0
West Gojam	2		4	0	32.5	2	2	10.0
Awi Zone	1		5	0	48	4	1	14.0
Bahidar	1		8	0	60	8	0	27.5
Zuriya	2		3	0	75	2	1	33.33
South Gonder	1		1	0	0.25	1	0	30.0
North Gonder								
Northwestern	6	24	12	1	41.58	9	4	14.62
Southwest	2		3	0	37.5	3	0	20.0
Central zone	2		2	1	28.5	1	2	6.67
South zone	1		3	2	16.8	0	5	0
Gamo	2	50	8	1	23.33	4	5	17.8
Segen	1		2	2	10	1	3	7.5
Konso	2		0	6	0	2	4	5
Welayta Sodo	5		16	0	39.4	12	4	30
Gofa	3		10	3	17.69	8	5	6.9
Hadiya	1		1	1	10	1	1	5.0
Asosa	3	26	25	1	28.46	17	9	16.15
Total	76	240	218	22		146	94	

Notes: NofD= Number of districts per each zone, SpP= Status per Plot, NfIF= Number of infested fields, NfNIF= Number of not infested fields, IZ= FAW average infestation on Zones

The average plot infestation and percentage of infested fields for 6 regions and seasons of sampling are shown above (Table 7). In the dry season, the average percentage of infested plots ranged from 10 to 75, while in the rainy season, the values ranged from 5 to 46.6. The average percentage of infested plants per plot for the dry season was higher in the regions of Afar, Amhara, Oromina and Tigray although the lowest was recorded at Benishangul Gumuz and SNNPR. The average infestation due to FAW over the regions during the dry season ranged from 22.6 to 52.2 and for the rainy season, from 11.3 to 46.6. The average percentage infestation in the regions for the rainy season was higher in the regions of Afar (46.6), Oromia (22.0) Amhara (18.8), SNNP (13.6), Benishangul-Gumuz (16.1) and Tigray (11.3) (Figure 5). The results showed that FAW infestation was higher during the off-season than during the rainy season in all sampling plots and regions (Figure 4, 5).

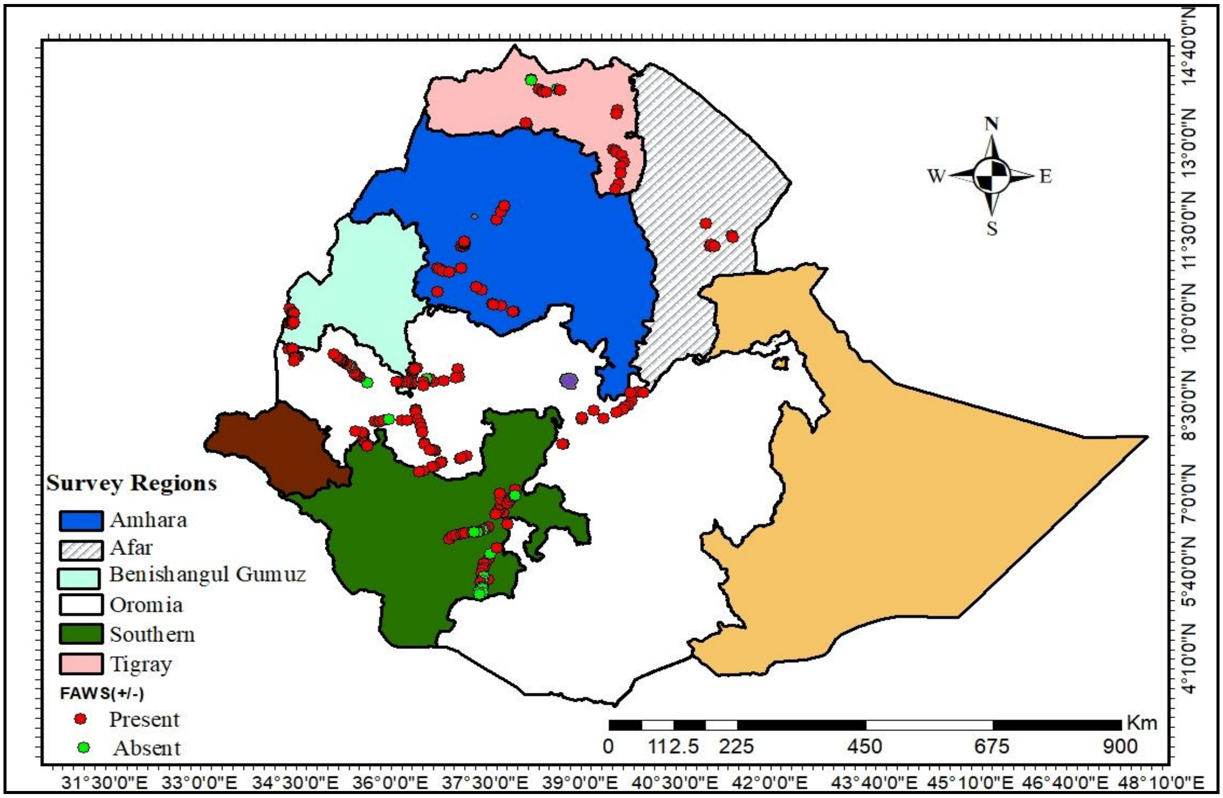

Figure 4. Distribution of FAW in Ethiopia during the dry crop season of 2018/2019.

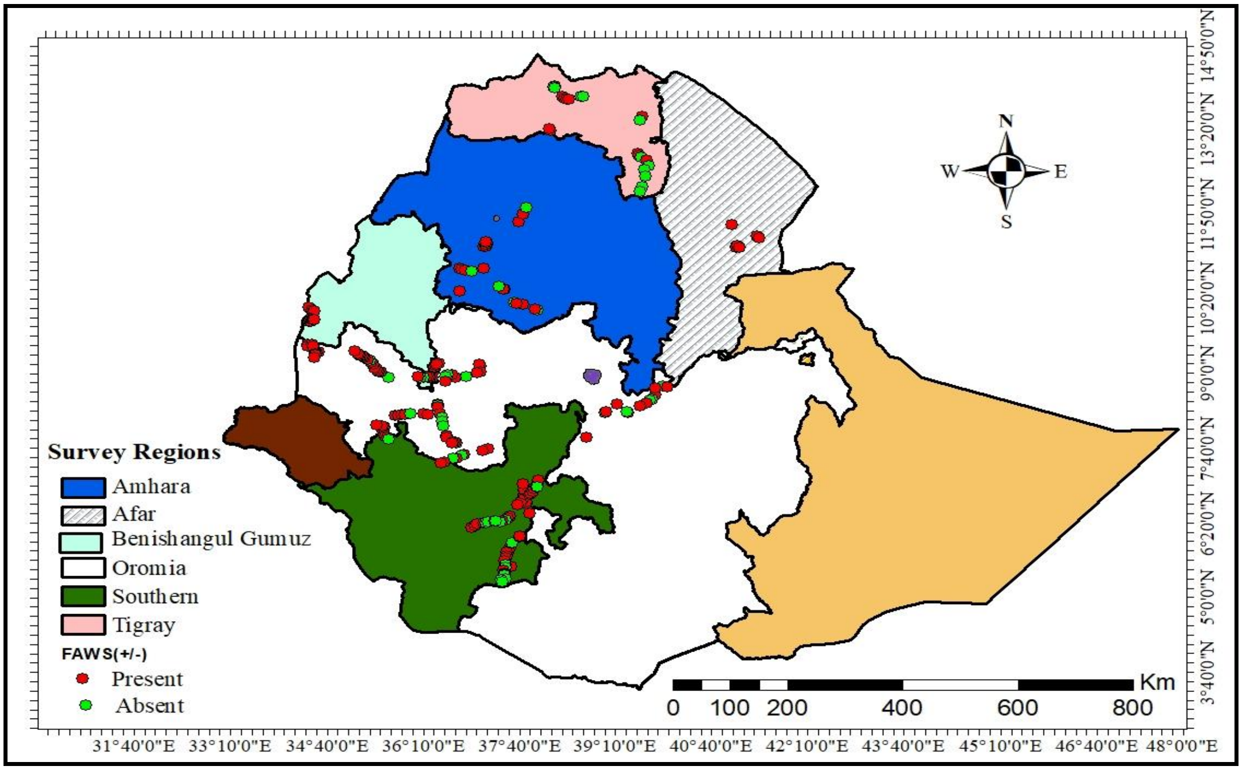

Figure 5. Distribution of FAW during the rainy crop season of 2019/2020 in Ethiopia.

4.3. DISCUSSION

The FAW was generally dispersed in all maize-producing localities of Ethiopia (Figures 4 & 5). Most of the farmers in these regions practice continuous maize production throughout the year due to the availability of residual moisture and irrigation water. From the cropping history, it was evident that rotating maize with other crop types decreased FAW infestation as compared to maize mono-cropping (Tanyi *et al*., 2020). The presence of maize crops in the field throughout the year provided a favorable environment for the preservation of this insect. Similarly, Albasini *et al*. (2020) reported that fields planted year-round with maize have mostly high FAW infestation.

According to Albasini *et al*. (2020), Nboyine *et al*. (2020), and Early *et al*. (2018), there was a slight distinction in temperatures between seasons in the regions (Table. 5). In contrast to temperature, the distinction in precipitation between seasons was recognizably huge. The current assessment results showed that precipitation was a key factor impacting the distinctions seen in the population of FAW larvae and occurrence per field between seasons in all regions and that temperature didn't influence the endurance of FAW. As indicated in the studies infestation level and high population of FAW were observed during dry season.

In Eastern Africa, especially in Kenya, Tanzania, Uganda, and Ethiopia, the occurrence of FAW was recorded in the same year (Birhanu *et al*., 2019), and the weather conditions in these areas were almost the same. The study conducted by Caniço *et al*. (2020) showed that the seasonal abundance of FAW in Florida was lower between December and April, and they suggest that the reduction of the amount of rain had a positive effect on the population of FAW. This is also with the agreement of our assessment results that indicated the high infestations level of FAW were recorded during the dry seasons when compared with the rainy season.

Concerning the rainy season, a few researchers (Early *et al*., 2018; García *et al*., 2018, and Albasini *et al*., 2020) recommended that the population of FAW is contrarily affected by rainy seasons. The findings of this assessment is align with the theory that the population of FAW decreases during the rainy season due to the adverse conditions it creates, which significantly impact the survival dynamics of FAW.

4.4. CONCLUSION AND RECOMMENDATIONS

The study results showed that the distribution and infestation level of FAW differs in the dry season and the rainy season. However, the infestation and damage level of the insect was higher in the dry season. Whereas the temperature may affect the performance of FAW, the slight variation of temperature between seasons did not have an impact on the changes in the population of larvae over time. Therefore, the results strengthen one of the current recommendations for the control of FAW that means early planting of maize is essential in the trimming season that may considerably decrease the population of FAW.

CHAPTER V

V. BIOLOGY AND HOST PREFERENCE OF FAW UNDER GREENHOUSE AND WIREHOUSE CONDITIONS

ABSTRACT

Although FAW is a polyphagous insect feeding on about 353 host plants in its native habitat, there is limited information regarding its alternative host plants in addition to maize, feeding preference and its biology on different hosts in Ethiopia. These studies were therefore carried out with the objectives to study the biology and host preferences of FAW in the greenhouse and wire-house of Ambo Agricultural Research Center from July 2021 to October 2021. The biology of FAW was studied on 5 commonlly cultivated host crops such as maize, sorghum, chickpeas, barley, and wheat. Data on duration of each developmental stag (egg incubation period, larval, pre-pupal, pupal, and egg to adult period), pupal weight, and survival rate percentage on different tested host crops were collected. The oviposition and larval feeding preference studies were carried out using choice and no-choice experiments on 23 major cultivated crops. The results of biological study revealed, the Egg incubation period was ranged between 5 to 8 days on tested crops with no significant variation among each other. The larval developmental period was completed comparatively within a short period on Chickpea (14.5 days) and longer on Sorghum (19.9 days). Similarly, the developmental period from egg to adult is short (31.5 days) and long (37.8 days) on the same crops, respectivelly. The highest and lowest eggs were laid on maize (125.5) and chickpeas (51), respectively. In terms of the cannibalism percentage, significant ($p<0.05$) variation was observed concerning larval cannibalism in different instar. The highest cannibalism percentage was on chickpeas (90), followed by barley (75), wheat (62), sorghum (36) and maize (25). The host preference study result discovered that maize, sorghum, Swiss chard, teff, elephant grass, and cabbage were the most preferred hosts for larval development and egg laying. The adult female laid eggs on faba bean, soya bean, haricot bean, garlic, and mustard plants, but the eggs did not hatch into larvae. In conclusion, these current findings indicated that FAW development and survival period varied in different host crops. This research results improve knowledge on FAW host preference and suggest that some of these host plants (faba bean, soya bean, haricot bean, garlic, and Ethiopian mustard) could offer potential for use as an intercropping in FAW management strategies.

Keywords: Eggs, Host crops, Larval cannibalism, Oviposition, Pupa, *Spodoptera frugiperda*

5.1. INTRODUCTION

5.1.1. Background

Fall armyworm (FAW) *Spodoptera frugiperda* (J.E. Smith) *(Lepidoptera: Noctuidae)* was first reported in America and recently spread throughout African countries, including Ethiopia, which is sometimes locally known as 'American temich in Amharic (Abrahams *et al*., 2017; Cock *et al*., 2017; Uzayisenga *et al*., 2018; Wu *et al*., 2019). According to Fall armyworm (FAW) is well distributed in 47 African countries, including Ethiopia, and can invade most of the Sub-Saharan Regions (Day *et al*., 2017; Birhanu, 2018; Prasanna *et al*., 2018; Birhanu *et al*., 2019).

More than 100 plant species, including cotton, maize, rice, sorghum, soybean, sugarcane and wheat were severely damaged by FAW (Kenis *et al*., 2022). However, recent studies confirmed that a total of 353 plant species are hosts of FAW (Montezano *et al*., 2018). Fall armyworm poses a serious challenge to the food and nutrition security and livelihoods of millions of farming households in Sub-Saharan Africa (SSA) due to its capacity to spread quickly and cause extensive damage across multiple crops (Baudron *et al*., 2019).

In maize, FAW attacks all the crop stages from seedling to ear development. It causes leaf defoliation, whorl, and ear damage; and reduces grain quality and overall yield (Anyanda *et al*., 2022). Recent estimates by CABI in 12 maize-producing countries showed that without control, FAW can cause maize yield losses ranging from 4.1 to 17.7 million tonnes per year, which is equivalent to an estimated loss ranging from 1088 million to US$4661 million annually (Rwomushana *et al*., 2018). Baudron *et al*. (2019) reported a yield loss of 11.57% due to FAW damage in smallholder maize fields in Zimbabwe, and which is relatively lower than the perceived losses reported by smallholder farmers in different countries, such as Ghana and Zambia where the losses were estimated at 26.6 and 35%, respectively (Rwomushana *et al*., 2018).

5.1.2. Statement of the Problem

The fact that FAW has a high reproductive rate, a very short generation time, and a high spread potential (Huang *et al*., 2021; Chen *et al*., 2022) has heightened public concern about this insect pest. The occurrence of FAW has significantly affected the economy, threatened food security, and is hazardous to the environment, humans and animals, and synthetic chemicals are used to manage the insect pest (Abro *et al*., 2021). To manage this huge infestation, basic knowledge of FAW biology and host preference studies are crucial and decisive. However, in Ethiopia findings that refer to the information on FAW biology and host rage study are still limited or scanty. Therefore, life cycle parameters and host rage studies were used to specify the survival of insect pest and to explain the variability in FAW damage symptoms on different crops. To determine the best application to manage FAW, it would be helpful to gather all the pieces of information on biology and host range general aspects.

5.1.3. Scope of the Study

The life cycle is important in managing insects because the habitats and appearance of an insect may change dramatically through the course of a life cycle, depending on the form of life cycle. Understanding the lifecycle of pests is important for effective pest control. It is important to target the pest during its most vulnerable stage, which is often the larva or pupa stage. During these stages, the pest is more susceptible to pest control measures, such as insecticides or biological controls. Host range tests aim to demonstrate if adult of FAW oviposit in a test host plant, or if the FAW s immature stages can feed and develop on a given host plants and complete their development.

5.1.4. Significance of the Study

The previous studies on FAW biology and host range have been deployed from various locations, including India (Praveen and Mallapur, 2019), Brazil (Silva *et al*., 2017), Indonesia (Nurkomar *et al*., 2023), and Africa (Kona *et al*., 2021). However, FAW is a new or emergent invasive insect pest in Ethiopia and no data or reports have been released in Ethiopia as of yet. Therefore, this study directly assessed the biology of FAW on selected major crops as well as host range study on 23 different crop plants. Studying the biology and host range of FAW is important for understanding the survival, population growth, and infestation by the insect throughout the year. Detecting FAW infestations before they cause economic damage is the key to develop its management strategies.

5.1.5. Objectives of the Study

Genera Objective:

- To provide a baseline information and standardize the FAW management strategies and ensures the end users know what has to be done in each growth phase and what is coming next.

Specific Objectives:

- To study the life cycle of FAW on five selected crops under greenhouse condition
- To study feeding suitability and cannibalism percentage of FAW within larvae on selected crops
- To study food attractiveness and oviposition preference of FAW on different crops in greenhouse and wirehouse condition

5.2. MATERIALS AND METHODS

5.2.1. Mass Rearing of FAW

FAW larvae were collected from infested maize fields arround Ambo areas. Mass rearing was done at the Ambo Agricultural Research Center (AmARC) under greenhouse conditions being fed on maize seedlings grown in pots according to Cruz *et al*. (2010), Birhanu (2018), and Tiwari (2022) procedures. The F2 generation obtained were used for research purposes. The third instar larvae were used for host range studies, while the eggs retrieved from the second generation were used for FAW biology study.

5.2.2. Biology of FAW

Five crops, viz. barley, chickpea, maize, sorghum and wheat were used for the biological study on FAW. Thus, FAW progenies were reared separately on each crop in a rearing cage with the dimension of 1*1*1.5 m. Each crop was grown in a pot with a diameter of 20 cm following the agronomic standard of the crops. Five to ten seeds of each crop were sown in each pot. The experiment was designed in a randomized complete block design with four replications. Similar size (For barley and wheat: more than 9 unfolded leaf stages; For maize and Sorghum weaning: 4-5 leaf stage; For chickpea: 9 multifoliate leaf stage) and cleaning of each crop were used for the experiment. Then, 10 pairs of pupae were introduced into each pot. A male pupa was distinguished from a female pupa by having a shorter abdomen and a larger head, while a female pupa had a longer abdomen with an ovipositor. Positions of genital and anal openings on the terminal segments were also the other morphological characters used for identification as described by Prasanna *et al*. (2018) and Babu *et al*. (2019).

The daily temperature and relative humidity values were recorded until the end of the experiment. To record the duration of oviposition and time of egg hatching, all pots were checked twice a day in early the morning and late afternoon. After egg hatching, the total period of the larval developmental stage, and pre-pupal stage were recorded. The pre-pupal collections were made through destructive sampling. The pre-pupa was separately kept in a small transparent plastic box (15 cm diameter) with moistened soil and covered with a white mosquito net. Pupal weight (g) was measured 24 hours after pupation; sex ratio, survival (%) (different stage of FAW) and days from pupa to adult emergence were also recorded. The number of newly-emerged adults was recorded and the adults were transferred into their separate crop plants in the pot and adult longevity was recorded too.

Total egg count data were log-transformed before subjecting to the analysis of variance (ANOVA) to ensure normality of the data. The number of days required for egg hatching, larval and pupal development, adult emergence, and adult longevity were analyzed using one-way ANOVA via a general linear model (PROC GLM, SAS Institute, 9.4) package. The least significant difference (LSD) at the 5% level was used to separate significant means.

5.2.3. Feeding Suitability Test

The FAW feeding suitability experiment was conducted using third instar starved larvae (but water-satiated) for 2 hrs under a growth chamber having 25±2 °C temperature, 60±10% relative humidity, and a photoperiod of 12:12 hours light-to-dark. The treatments were arranged in a completely randomized design (CRD) with 3 replications in the small buckets. The feed and larvae were weighed daily using a sensitive balance and the remaining feed and faeces were removed and stored separately. Then, up to sixth instar stage, the initial weight (mg), final weight (mg), amount of feed consumed (mg), amount of feces produced (mg), and feeding interval (days) were recorded. The amount of food consumed by the larvae was calculated using the following formula (Khan and Saxena, 1985). All data calculated for different hosts were analyzed using SAS software.

Digested feed = Feed consumed - Faeces weight:

$$\text{Food consumed} = \frac{\mathrm{W1}\,(C1 - C2)}{C1 + W2 - W1}$$

Where W1 is the initial weight of the starved larva, W2 is the final weight, C1 is the initial weight of the unstarred larva, and C2 is the final weight.

5.2.4. Cannibalism Rate Study on Different Host Plants

The study was conducted to compare the cannibalism percentage of FAW within larvae on different crops. Twenty 2nd to 3rd instar larvae were transferred to a plastic bucket (60 cm x 25 cm size) and 5 freshly-grown pot seedlings of different crops (barley, chickpea, maize, sorghum and wheat) and larvae were transferred to each plastic bucket (with the detached leafs) under room having 25±2 °C temperature, 60±10% relative humidity. Each treatment was replicated four times and arranged in a completely randomized design (CRD).

The cannibalism (C) was calculated as follows:

$$\mathrm{C} = \mathrm{N} - (\mathrm{Ns} + \mathrm{Nd})$$

Where N denoted the initial number of larvae in each container with different plant leaves, Ns = number of surviving larvae, and Nd = number of dead larvae (Sokame *et al*., 2023).

5.2.5. Host Preference Study of FAW

Twenty-three crop plants (Table 8) were used for choice and no-choice study under greenhouse and wirehouse conditions for oviposition and feeding preference studies of FAW. Five plants with 4 to 6 fully developed leaves were utilized in the trials.

The free-choice study was carried out in screened cages measuring 3 m long, 3 m wide, and 2.5 m high. The no-choice study was done inside individual cages having a size of 1 m × 1 m × 1.5 m, length, width, and height, respectively. The treatments were arranged in a randomized complete block design (RCBD) with four replications.

Twenty-five pairs of pupae with a 1:1 (male: female) ratio were released into each cage with test plants for the free-choice study and five pairs of pupae were placed/inserted/ into each cage with test plants for the no-choice study. On the ninth day, all pupae released for both experiments emerged as adults. The female adults started laying eggs after 3-4 days, and then the tested plant parts were examined by using a hand lens. The number of eggs, the egg's position on the plant (bottom, middle, and upper canopy), number of larvae, larval weight, feces weight, and percent plant damage were recorded.

The percentage (%) of leaves damaged by FAW larvae was calculated as the number of leaves with feeding damage divided by the total leaves, multiplied by 100 (Mersie *et al.*, 2018). SAS software was used for data analyses (SAS Institute, 2000). Before analysis, data were checked for normality. Treatment means were separated using LSD at $P \leq 0.05$.

Table 8. Plant species included for the host preference study of FAW under greenhouse and wirehouse conditions from July to October 2021.

No.	Plant species	Family	Common name	Variety	No.	Plant species	Family	Common name	Variety
1	*Zea mays*	Grasses	Maize	Jibat	13	*Daucus carota*	Umbellifers	Carrot	DARC/9
2	*Sorghum bicolor*	Grasses	Sorghum	Melkam	14	*Brassica oleracea*	Mustards	Cabbage	Tana
3	*Vicia faba*	Fabaceae	Faba bean	Walki	15	*Lactuca sativa*	Asteraceae	Lettuce	Paris Island
4	*Cicer arietinum*	Legumes	Chickpea	Worku	16	Swiss Chard	Amaranthaceae	Swiss Chard	
5	*Glycine max*	Legumes	Soybean	Pawe-1	17	*Capsicum*	Nightshade	Pepper	Vigro
6	*Hordeum vulgare*	Grasses	Barley	HB-1307	18	*Solanum lycopersicum*	Nightshade	Tomato	Venis
7	*Triticum*	Grasses	Wheat	Liben	19	*Solanum tuberosum*	Nightshade	Potato	Belete
8	*Phaseolus vulgaris*	Fabaceae	Haricot bean	Awash Melka	20	*Beta vulgaris*	Amaranthaceae	Beat root	Farida
9	*Eragrostis tef*	Poaceae	Teff	Dagim	21	*Allium cepa*	Amaranthaceae	Onion	Bombay Red
10	*Johnson grass*	Grasses	False sorghum		22	*Allium sativum*	Amaranthaceae	Garlic	HL
11	*Pennisetum purpureum*	Grasses	Elephant grass	Elephant grass	23	*Brassica carinata*	Brassicaceae	Ethiopian mustard	Abesha Gomen
12	*Chrysopogon zizanioides*	Grasses	Vetiver Grass						

5.3. RESULTS

5.3.1. Biology of FAW on Different Crops

The number of FAW egg mass laid varied significantly ($p \leq 0.05$) among the crop plants studied, but there was no significant variation ($p > 0.05$) in the time taken for the eggs to hatch. The lowest (32) number of eggs laid per FAW female was recorded on chickpeas, while the highest (122) number of eggs was laid on maize (Table 9). The incubation period of eggs ranged from 5 to 8 days (Table 11). The egg was pearly white when laid, but changed to black when aged. The larvae were green at hatching with black lines and stains. As larvae grew, they remained green but only had blacklines on the underside. The mean larval survival varied from 41.3 to 76.5% in the third instar and in the sixth-instar 13.8 to 52.2% were noted on reared host plants. The survival percentages from egg hatched to pupae were significantly different in the host crops, the highest (40.2) survival percentage was on maize, followed by wheat (30.1), sorghum (27.1) and barley (21.8), while the lowest survival was on chickpea (6.4) (Table 9). The larval period (first instar through sixth instar) was 17.6, 19.9, 14.5, 15.9, and 15.9 days on maize, sorghum, chickpea, barley, and wheat, respectively (Table 11). The pupal period ranged between 8.54 and 9.58 days (Table 11).

The time taken from larva to adult was significantly different ($p \leq 0.05$) among the tested crops. The longest (37.8 days) time from larva to adult was recorded on sorghum, followed by maize (35.89 days), while the lowest duration of 31.53 days, 33.12 days, and 33.27 days were recorded from chickpea, barley, and wheat, respectively (Table 11). The mean larval weights varied from 0.156 to 0.198 g for the third instar and from 0.212 to 0.269 g for the third instar when reared on different host plants. The heaviest (0.269 g) mean larval weights of sixth-instar larvae reared on maize. Similarly, the pupal weights ranged from 179 to 247 g. The maize plant supported the heaviest pupal weight, while the lightes pupal weight was for those larvae fed on chickpea and sorghum (Table 10). FAW adult counts varied from 2 to 36.8 on different host plants used for rearing and the highest were recorded on maize, followed by wheat, sorghum, and barley, whereas the smallest adult number was recorded on chickpea (Table 10).

Table 9. Oviposition and survival rate of FAW larvae on different host plants (Mean ± SE).

Crops	Number of egg masses	Neonate (number)	Egg Hatched (%)	3rd instar survival (%)	6th instar survival (%)	Survival %
Maize	122± 4.3a	92	73.4±3.5a	76.5±6.5a	52.2±7.2a	40.2±5.6a
Sorghum	84.0±2.8b	67.5	71.1±6.5a	73.1±9.6a	37.3±7.3b	27.1±7.9b
Chickpea	32.0±1.6e	28.5	55.9±8.2b	41.3±4.4b	13.8±5.6c	6.4±2.2c
Barley	42.5±2.5d	66.25	69.5±4.5a	62.9±9.1a	35.7±9.9b	21.8±5.6b
Wheat	51.0±5.5c	73.75	73.7±6.8a	63.8±5.1a	43.6±3.6ab	30.1±8.8b
CV (%)	11.7		9.1	14.9	13.8	9.7

Means in a column followed by the same letters are not statistically different, LSD, P≤ 0.05.

Table 10. Larval and pupal weight and emerged adult of FAW on the different crops.

Crops	Larval and pupal weight (g)[a]			Number of adults	Sex retio
	3rd instars	6th instars	Pupal		
Maize	0.198±0.001a	0.269±0.03a	0.247±0.0a	36.8±3.6a	0.52±0.10
Sorghum	0.156±0.028b	0.205±0.01c	0.193±0.01cd	17.8±3.8bc	0.44±0.28
Chickpea	0.190±0.004a	0.238±0.01b	0.179±0.01d	1.8±0.5d	0.48±0.26
Barley	0.186±0.001a	0.212±0.01c	0.205±0.03bc	14.5±4.1c	0.52±0.14
Wheat	0.192±0.00a	0.226±0.00bc	0.214±0.0b	21.5±3.1b	0.54±0.16
CV (%)	6.99	6.6	6.2	17.8	Ns

NB: To get the single larval weight, larval or pupal weight in the table was calculated from the weight of total larvae or pupae in each replication divided by the number of weighed larvae or pupae.

Table 11. Duration of the developmental stage of FAW on different host plants (Mean ± SE).

Treatment	**Duration (days)**				
	Egg to hatch	**1st to 6th instar**	**Pre-pupae**	**Pupae**	**Total development time from Egg to adult**
Maize	6–8	17.6±1.3ab	1.89±0.06a	9.58±0.16a	35.89±0.46b
Sorghum	5–8	19.9±2.2a	1.97±0.09a	9.44±0.19a	37. 8±0.50a
Chickpea	5–8	14.5±2.1c	1.89 ±0.08a	8.54±0.09c	31.53±0.15d
Barley	5–8	15.9±1.2bc	1.86±0.07a	8.86±0.24bc	33.12±0.27c
Wheat	5–8	15.9±2.2bc	1.69±0.07b	9.09±0.11abc	33.27±0.17c

Means in a column followed by the same letters are not statistically different, LSD, P≤ 0.05.

5.3.2. Feeding Preference of FAW

The research results of final larval weight, feed consumption, faeces weight, digested feed weight, and feeding time of FAW is tabulated below(Table 12). The final larval weight that was measured on various host crops varied significantly ($p \leq 0.05$) (Table 12). The larvae fed on maize had the heaviest ultimate weight (2.58 g), followed by wheat (2.39 g), and barley (2.33 g). The larvae fed on chickpeas had the lightest (2.17 g) recorded total weight (Table 12). The larvae fed to maize, wheat, barley, and chickpea showed the maximum feed consumption, whereas the larvae fed on sorghum had the lowest feed consumption. On the same crop plants used for testing, the largest faeces weight and digested feed were recorded on maize, sorghum, wheat, and barley and the lowest were noted on chickpeas (Table 12). The larvae that fed on wheat (6 days), barley (6.25 days), and chickpea (8 days) were comparatively took shorter time to finish the feed, while the larvae that were fed on maize, and sorghum took 10 days and 12.25 days to finish the feed (Table 12).

Crops	**Larval Initial weight (g) (2^{nd} instars) (n=20)**	**Larval Final weight (g) (6^{th} instars) (n=20)**	**Feed consumed (g)**	**Feces weight (g)**	**Digested feed**	**Feeding time (day)**
Maize	1.48	2.58±0.7a	298.5±1.2a	96.83±1.1a	201.67±1.6b	10.00±0.8b
Sorghum	1.49	2.25±1.9bc	262.3±4.6b	82.70±2.96c	179.4±7.0c	12.25±0.5a
Chickpea	1.49	2.17± 1.6d	298.6± 1.2a	67.41±2.3d	231.39±3.4a	8.00±0.4bc
Barley	1.47	2.33±1.5b	298.2±1.7ba	91.41±2.4a	206.79±2.8b	6.25±0.4d
Wheat	1.47	2.39±1.4b	298.06±0.8a	92.35±1.3a	205.9±1.1b	6.00±0.2
CV (%)		1.01	0.81	2.5	1.85	5.9

This means that columns separated by the same letters are not statistically different by LSD at $P \leq 0.05$. (NB: Initially, from each crop 300 g was used for feed suitability study).

5.3.3. Effects of Food Source on Larval Cannibalism

Effects of different host plants as a food source on larval cannibalism during rearing FAW is presented (Table 13). The cannibalism rate of FAW larvae was different for different crops (Table 13). Average cannibalism percentages of 8.8 to 86.3%, 2.8 to 25% and 0 to 25% were recorded on fourth, fifth, and sixth-instar, respectively. Cannibalism percentages were affected by the larval age and the host plant's feed for rearing. In the study, high cannibalism percentage was recorded on chickpeas, followed by barley and wheat in the third to sixth instars. The lowest cannibalism was recorded on maize and sorghum feed larvae (Table 13).

Table 12. Cannibalism percentage of FAW at each larval instar (3rd – 6th) reared on different crops.

Crops	Initial number of 3rd instar larvae	4th instar	5th instar	6th instar	3rd to 6th instars
Maize	20	8.8±4.9e	10.75±5.8bc	7.72±3.2cd	18.0±4.1e
Sorghum	20	17.5±6.5d	12.1±4.5abc	12.31±4.1bc	28.0±8.5d
Chickpea	20	86.3±2.5a	25.0±6.6a	0.0±0.0d	90.0±0.0a
Barley	20	58.8±4.8b	23.7±7.8ab	19.64±4.8ab	90.0±4.1b
Wheat	20	48.8±4.8c	2.8±5.6c	25.11±5.1a	75.0±6.5c
CV (%)		10.9	8.9	6.3	9.4

NB: Cannibalism % 3rd to 6th means cannibalism throughout the larval instars recorded which is calculated from the number of pupae/initial number of 3rd instars larvae*100.

5.3.4. Oviposition Preference of FAW Adults with Choice and Non-choice Test

To well understand the oviposition preference of FAW adults, various major crops grown in Ethiopia were tested under choice and non-choice test methods. There were significant differences among the tested crops in oviposition preference by FAW adult moth under both choice and non-choice tests. The number of eggs laid on different crops by FAW adult female moths on average ranged from 33.8 to 217.5 on the host range study in the no-choice test (Table 14). Among the crop plants tested with no-choice test, the highest (217.5) number of FAW eggs on maize, sorghum (210.5), Swiss chard (2010), and lettuce (2010) were recorded. Statistically, vetiver grass (202), cabbage (201.5), potato (198), elephant grass (193.5), and tef (191.5) showed equal attraction for FAW adult female oviposition. On the other hand, the lowest number of eggs was recorded on Ethiopian mustard (94), onion (49.5), garlic (40.5), and faba bean (33.75).

Similarly, FAW female adults significantly preferred among host crops for oviposition during the choice-test. The number of eggs laid on different crops during the choice test ranged from nil to 103 (Table 14). Among the crop plants tested with the choice-test, the highest number of FAW eggs were laid on maize, sorghum, and elephant grass, followed by lettuce, vetiver grass, wheat, and barley. Consistent with the non-choice test, the lowest number of adeult female eggs were laid on carrot, pepper, beetroot, onion, garlic, and Ethiopian mustard.

5.3.5. Hatchability, Larval Development, and Damage on Crops Under Non-choice Test

The hatchability percentage was highest on maize (81.5), sorghum (65.5), and wheat (61.25), followed by Swiss Chard (49), cabbage (49), tomato (48.5), tef (46), haricot bean (40.5), potato (40), lettuce (39.5), elephant grass (38.5) and lowest on faba bean (3.5), garlic (5), Ethiopian mustard (8.5), onion (15.5) and carrot (19). The percentage survivability recorded was found to be highest in the larvae hatched from the eggs laid by females fed on maize, sorghum, wheat, false sorghum, chickpea, and elephant grass, barley leaves, and lowest when females laid eggs on vetiver grass, pepper, faba bean, beetroot, carrot, onion, tomato, and Ethiopian mustard. Damage level was recorded at 25 days of eggs hatched and the highest damage was observed on wheat, chickpea, barley, tef, maize, and sorghum in that order. On the other hand, no damage was observed on vetiver grass and pepper, and comparatively, the lowest damage was recorded on carrot, Ethiopian mustard, faba bean, garlic and potato and tomato (Figure 6).

The mean six instars larval weights significantly varied when larvae reared on different host crops. The highest mean larval weights of sixth-instar larvae were recorded from the larvae reared on maize, followed by tef, sorghum, and wheat. In contrast, the lowest larval weight was recorded from vetiver grass, Ethiopian mustard, pepper, faba bean, and garlic. Similarly, based on the cumulative frass weight, the larvae reared on maize produced the highest frass weight, followed by chickpea, wheat, sorghum, tef, haricot bean, and barley. The lowest frass weight was recorded from the larvae reared on faba bean, pepper, and Ethiopian mustard (Table 14).

Table 13. Oviposition, number of larvae, and larval weight of FAW in choice and no-choice experiments in the greenhouse AmARC during 2021.

Treatment	Choice test		No-choice test			
	MNEA8Ds	MNEA8Ds	%MLH	LS%	MLW25Ds	FW25Ds
Maize	103.8±10.1a	217.6±25.9a	81.5±2.3a	72.3±2.1a	5.3±0.6a	17.66a
Sorghum	96.0±12a	210.5±43.9a	65.5±7.7b	70.5±1.3ab	3.7±0.2c	13.09cd
Faba bean	45.4±22.1fg	33.8±19.5i	3.5±0.1j	0.75±0.5m	0.9±0.2gh	0.03nm
Chick pea	54.8±6.1defg	114.8±13.3fg	30±2.2efg	62.0±1.4c	4.8±0.2b	15.11b
Soya bean	61±4.4cdef	140.3±17.3defg	21.5±4.9hfg	39.3±2.4f	2.0±0.2ef	8.22gh
Barley	74±10.5bcd	177.3±27.2cde	32.5±8.3ef	51.3±1.7d	2.1±0.3e	13.82bcd
Wheat	75±3.6bcd	136.5±61.7efg	61.25±5.4cb	69.5±1.3b	4.3±0.3bc	14.46bc
False sorghum	48.5±8.2efg	110.5±47.5fg	24±3.7hfg	68.8±2.1b	0.9±0.1h	9.79fg
Haricot bean	50.2±5.2efg	163.5±46.2cde	40.5±2.7ed	26.3±2.5i	1.2±0.4gh	8.62gh
Tef	54.4±3.6defg	191.5±55.3abc	46±2.3d	42.5±2.1e	4.3±0.4bc	13.96bcd
Elephant grass	99.4±6.8a	193.5±21.0abc	38.5±7.4ed	51.3±1.3d	1.3±0.2gh	7.11hi
Vetiver grass	78.8±4.6abc	202.0±12.1ab	30±5.2efg	0.3±0.5m	1.5±0.4fg	2.58lm
Carrot	0.03±0.0i	152±22.6cdef	19±5.0hig	4.5±1.3jkl	0.1±0.03i	5.99ij
Cabbage	68.4±8.0bcde	201.5±22.9ab	49±8.8cd	36.8±1.0g	2.9±0.3d	12.31de
Lettuce	73.8±18.2bcd	212.0±12.1a	39.5±5.5ed	35.5±0.6gh	3.0±0.1d	12.25de
Swiss chard	68.2±6.4bcde	210.0±23.4a	49±8.9cd	34.3±1.3h	3.0±0.7d	13.93bcd
Pepper	0.03±0.0i	108 ±10.6fg	25.5±1.1hfg	0.3±0.5m	0.1±0.03i	0.03nm
Tomato	39.8±11.2gh	179±36.9cde	48.5±2.5d	4.5±1.0jkl	0.2±0.1i	4.90jk
Potato	20.6±2.2hi	198±12.5abc	40±6.7ed	5.0±0.8jk	0.9±0.2h	5.34ijk
Beetroot	0.03±0.0i	187±18.9cd	29.5±4.2efg	2.8±1.0l	3.1±0.6d	10.80ef
Onion	0.03±0.0i	49.5±11.1hi	15.5±4.8hij	4.5±1.3jkl	0.8±0.3h	2.03lmn
Garlic	0.03±0.0i	40.5±26.6i	5±1.6j	3.5±0.6kl	0.1±0.0i	2.26lmn
Ethiopian mustard	0.03±0.0i	94.0±13.4gh	8.5±2.1ij	5.8±1.0j	0.1±0.6i	0.65n
CV (%)	31.77	22.0	25.7	4.66	36.69	13.08
LSD (0.05)	9.87	7.7	12.2	1.98	0.95	1.87

MNEA8Ds, mean number of eggs after 8 days; %MLH, percent mean larvae hatch; LS%, Larvae survival percent; MLW25d, Mean larval weight gram on 25 days (N=16), and FW25ds, Frass weight on 25th day.

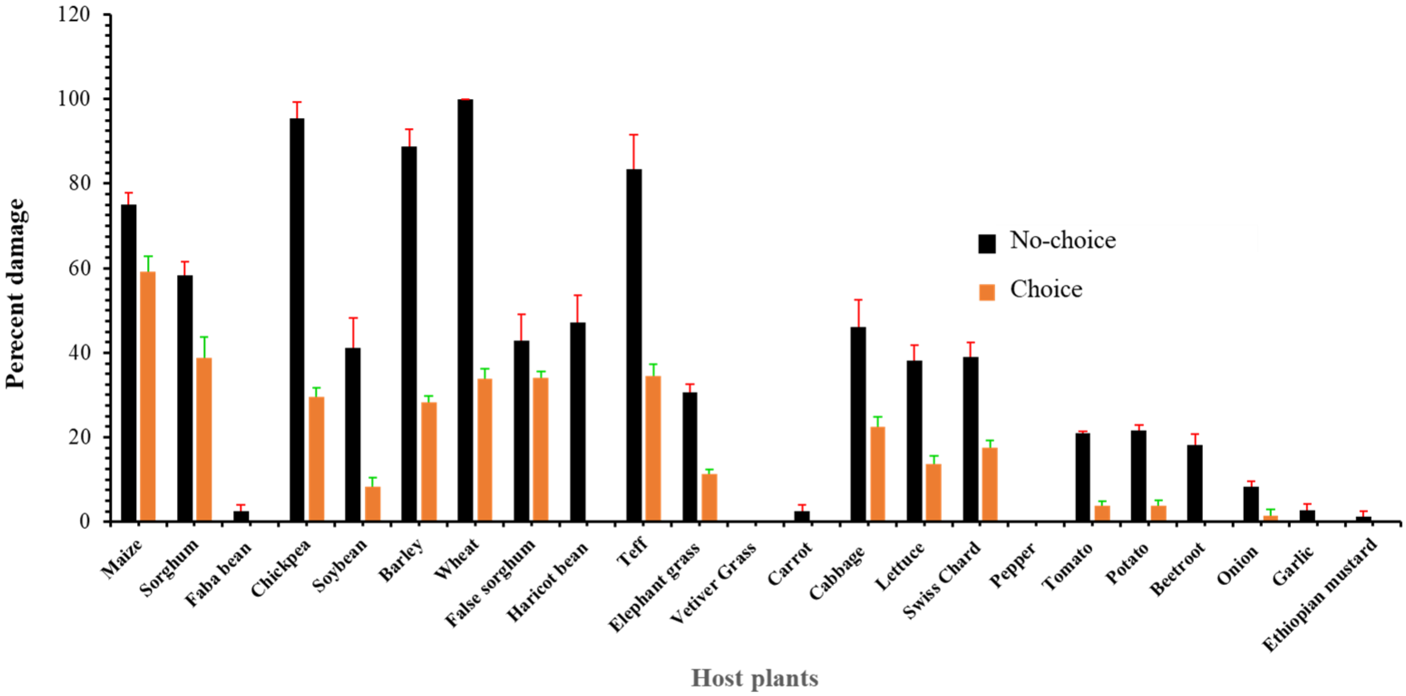

Figure 6. Percentage leaves damage of host plants caused by FAW under no-choice and choice tests.

5.4. DISCUSSION

The biology of FAW was investigated on barley, chickpea, maize, sorghum and wheat. The crop plants in the current study were selected based on their cultivation status in different parts of the country and the prevalence of FAW on the crops. The high number of eggs was laid on maize and sorghum, which were expected to be the main hosts of FAW. Similarly, FAW larvae highly survived on cereal crops (barley, maize, sorghum,and wheat), whereas a low number of survivals were observed on chickpeas. This phenomenon is directly related to the palatability and physiological or biochemically content of the crops (Hwang *et al*., 2008; Chen *et al.,* 2009; Wijerathna *et al*., 2021; Gopalakrishnan and Kalia, 2022). Therefore, the naturally occurring biochemicals, such as 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA) and 6-methoxy-benzoxazolin-2-one (MBOA) in maize, sorghum, and wheat than to the compounds produced by pulse and cash (cotton) crops (Raffa 1987; Hardke *et al*., 2015).

The percentage of cannibalism was very higher among FAW larvae confined on chickpea plants than it was among those fed on cereals (barley, maize, sorghum and wheat). These may be due to proliferation and vegetative biomass, on maize and sorghum the larvae tended to disperse throughout the plant and they get the chance to hide in or under leaf, leaf sheath, and shoot, these reduce cannibalism among larvae. Chickpeas have small biomass and larvae remain in very close proximity to each other, thus leads an increase in cannibalism percentage (Raffa 1987). The other reason may induce defenses in plants to reduce herbivory by increasing cannibalism (Orrock *et al.,* 2017).

The host preference study was also conducted using both choice and non-choice tests. Thus, major crops grown in Ethiopia were assessed for host preference of FAW under greenhouse and wire-house. Accordingly, Gramineae family, including maize, sorghum, Elephant grass, and Vetiver Grass, and vegetables, like cabbage, lettuce potato, and Swiss chard were highly preferred for oviposition both at choice and non-choice test. Oviposition preference of lepidopterans in general, FAW in particular, is influenced by leaves' surface and appearance, and chemical and tactile cues in host plants. With this regard, strong tactile stimuli for grooved and pitted surfaces for ovipositional behavior and preference were observed in FAW (Rojas *et al*., 2003), *Spodoptera exigua* (Greenberg *et al*., 2002), and *Epiphyas postvittana* ((Sotelo-Cardona *et al*., 2021). Moreover, the naturally occurring chemical compounds, terpenes, found in maize, sorghum, and wheat were reported as the most attractive odor for oviposition of FAW (Signoretti *et al*., 2012; Nandhini *et al*., 2022; Birhanu *et al*., 2023). Our current study justifies these findings as more eggs were oviposited on maize, sorghum, and wheat, which are characterized by grooved and pitted leaves surfaces. Similarly, the larval survival rate on these crops was high, which indicates the larval preference for the crops. This could be because of various reasons, such as the proliferation of biomass and nutritional content. The feeding and ovipositional responses of FAW on different host plants revealed that maize and sorghum are the most preferred crops (Wijerathna *et al*., 2021; Nandhini *et al*., 2022; Tiwari, 2022; Birhanu *et al*., 2023)

Oviposition preference does not necessitate the offspring's performance in the FAW (Sotelo-Cardona *et al*., 2021). In our current study, however, the oviposition preference towards vegetables was high during no-choice, it was comparatively low during choice. This is because, during the choice test, other more preferred hosts, like maize and sorghum, were available for preference. This indicates that the adult prefers host crops for oviposition. Consistent with the oviposition and feeding preference result, the grass family and vegetables (cabbage. lettuce, and Swiss chard) were highly damaged by the larvae.

In general, the choice and non-choice tests indicated that even though the ovipositional and feeding preference of FAW varied among the host plants, the insect can survive and complete its life cycle on both grass families and the same as the vegetables.

5.5. CONCLUSIONS AND RECOMMENDATIONS

The findings of the biological study of FAW showed that the insect pest can complete its life cycle on barley, chickpea, maize, sorghum, and wheat host crops. However, variations were observed in duration to larval developmental period, pupal durations, and survival percentage among the host plants. The shortest larval and pupal durations, high larval and pupal weight, and highest survival percentage were recorded from FAW fed on maize. In addition to the survival percentage, the cannibalism percentage of FAW was highest when rearing on chickpeas, and the lowest on maize and sorghum. The host preference study under no-choice and choice experiment on different crop plants showed variation in adult oviposition, larval feeding, larvae survival percentage, percent damage, and larval weight. As a result, some of the tested crop plants were not preferred by the FAW. Therefore, the utilization of those crop plants (faba bean, soya bean, haricot bean, garlic, and Ethiopian mustard) could be promising in cropping systems for crop rotation and inter-cropping to reduce the impact of FAW, and is important to develop integrated pest management.

CHAPTER VI

VI. OCCURRENCE AND PATHOGENIC POTENTIAL OF ENTOMOPATHOGENIC NEMATODE ISOLATES FROM MAIZE GROWING AREAS OF ETHIOPIA

ABSTRACT

*This study was conducted with the objective to asses and tet pathogenicity of native entomopathogenic nematodes (EPNs) from soils across maize-producing areas of Ethiopia for the control of fall armyworm (*Spodoptera frugiperda *J.E. Smith). Six hundred seventy-nine soil samples were collected from eight regional states of Ethiopia from August to October 2019/2020 and 28 different EPN isolates were isolated and identified. Out of these 28 indigenous EPNs isolated, 13 of them were found to be from Steinernema species and 15 were from Heterorhabditis species. Morphological characterizations of the isolates were performed for species identification All the 28 native EPN isolates identified from survey and 9 additional isolates from stock culture of Ambo Agricultural Research Center (i.e., HH, J-01, HI, APPRC-p20692, APPRC-p0508, AEH, HBWWM, Z9, and APPRC-PL 0697) were tested in a single dose (500 infective juveniles (IJ)/ml) for their efficacy under laboratory condition using 3rd instar larvae FAW* and the treatments were arranged in a completely randomized design with three replications. *Based on the laboratory results, most virulent isolates were further evaluated for their efficacy against FAW in a pot experiment under greenhouse condition using* Randomized Complete Block Design with four replications. *Among 28 Isolates tested Aso-Tes-287 from Steinernema sp. and Am-Ger-Tes-74, Am-Adm-Tes-369, and Z9 from Heterorhabditis sp. significantly caused larval mortality within a maximum of eight days. Moreover, the* LT_{50} *values i.e., 3.5 to 6.69 days showed that these isolates are more virulent. These five most virulent isolates were further tested for their potential in a pot experiment under wirehouse conditions at three different concentration levels (250, 400, and 600 IJ/ml). Based on results of the pot experiment, the highest larval mortality (78.3%, 74.7%) were achieved by Am-Ger-Tes-74 and Aso-Tes-287), respectivelly at the concentration of 600 IJ/ml. Therefore, the two isolates from Steinernema (Aso-Tes-287) and Heterorhabditis (Am-Ger-Tes-74), which caused higher mortalities within shorter periods, were promising bio-agents for the management of FAW.*

***Keywords**:* Bioassay, Entomopathogenic nematodes, Fall armyworm, *Heterorhabditis*, Mortality, *Steinernema*, Virulence.

6.1. INTRODUCTION

6.1.1. Background

Maize, (*Zea mays* L.) is an annual crop that is grown as a source of food primarily in the tropical and subtropical areas of the world, including several countries in Sub-Saharan Africa (SSA) and Ethiopia (Midega *et al*., 2015; Erenstein *et al*., 2022). Each year, maize is grown on over 33 million hectares of land in Sub-Saharan Africa (FAOSTAT, 2015). In SSA, maize is essential for economic and food security for over 208 million people (Abate *et al*., 2015). Similarly, maize is a major cereal crop in Ethiopia, ranking second both in yield and area coverage (CSA, 2020). In Ethiopia, maize occupies about 2 million hectares of land. Of these, smallholder farms accounted for more than 95% of the total area and production (CSA, 2020).

Despite its large production area and importance, maize has a very low average grain yield in SSA, with yields ranging from 2 to 3 t ha^{-1}, which is the lowest in the world (Cairns *et al*., 2013; Abate *et al*., 2015; Assefa *et al*., 2020;), which could be attributed to a variety of abiotic and biotic factors. The damage caused by insect pests is the most significant biotic component (Altaf *et al*., 2022). More than 40 insect species have been recorded on maize under field conditions in Ethiopia (Abraham *et al*., 1993; Tolera *et al*., 2018). The maize stem borer, *Busseola fusca* Fuller, the spotted stem borer, *Chilo partellus* Swinhoe, and several termite species (*Macrotermes* and *Microtermes* spp.) are known to be the most serious insect pests (Emana *et al*., 2008). More recently, however, the invasive and polyphagous insect pest called fall armyworm, *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae), has become a major insect pest causing substantial yield losses on maize (Birhanu *et al*., 2019; Kumela *et al*., 2019; De Groote *et al*., 2020). In Ethiopia, Uganda and Zimbabwe, the damage due to FAW ranged from 60–70%, 87.66% and 32%, respectively, on maize plants (Baudron *et al*., 2019; Sharon *et al*., 2020; Wondimu *et al*., 2021).

Chemical insecticides are the main means of management againstFAW worldwide. However, the use of synthetic insecticides to suppress lepidopterous pests has caused environmental contamination and the emergence of insecticide resistance in several insect species (Bloem and Carpenter, 2001). As a result, various microbial management methods are being investigated, including the use of transgenic cultivars harboring *Bacillus thuringiensis* toxins (Horikoshi *et al*., 2016), fungi (Birhanu *et al*., 2019; Kuzhuppillymyal-Prabhakarankutty *et al*., 2021) and viruses (Gómez-Valderrama *et al*., 2022). Entomopathogenic nematodes (EPNs) of the genus Heterorhabditidae and Steinernematidae are also used against insect pests (Gozel and Gozel, 2016). The infective juveniles (IJs) penetrate the insect hemocoel and release their symbiotic bacteria (*Xenorhabdus* spp. in Steinernematidae and *Photorhabdus* spp. in Heterorhabditidae), which multiply and generate metabolites that kill the insect pest and serve as a source of food for nematodes (Godjo *et al*., 2018; Danso *et al*., 2021; Wattanachaiyingcharoen *et al*., 2021). The susceptibility of FAW (Andaló *et al*., 2010; Acharya *et al*., 2020; Lalramnghaki *et al*., 2021) and *Mentaxya ignicollis* (Tesfaye *et al*., 2018) to EPNs has been reported.

6.1.2. Statement of the Problem

This study provides information regarding the occurrence and pathogenicity of EPNs in Ethiopia. Characterizing and evaluating EPN isolates in the laboratory and wire-house will help to select virulent strains among these species and use them for the most effective and environmentally friendly biological control program of different insect pests, including FAW, in the future. Furthermore, the best efficacy of having EPN isolates can also be mass-produced and commercialized for maize-producing farmers, who are suffering from different insect pests, mainly FAW. The distribution and pathogenic potential of EPNs against FAW have not been studied in Ethiopia so far. Isolating and evaluating the pathogenicity potential of these nematodes will help the efforts that are being made to reduce maize damage due to FAW.

6.1.3. Scope of the Study

Entomopathogenic nematodes (EPNs) of the families Heterorhabditidae and Steinernematidae are obligate parasites of insects and are used as biological control agents of economically important insect pests. Therefore, it is very important to collect, isolate, characterize, and evaluate the indigenous EPNs (*Heterorhabditidae* and *Steinernematidae*) isolates from different agro-ecologies of the country to look into effective strains against FAW.

6.1.4. Significance of the Study

The use of several chemical synthetic pesticides to manage the FAW is reduced when maize is produced using native and indigenous EPNs, i.e. an environmentally friendly and non-toxic alternative production method. These pesticidal chemicals are hazardous to the environment and human beings besides being expensive to the subsistence farmers. Although the research works on EPNs for biological control programs of insects are limited in our country, such important works that use environmentally friendly approaches and result in sustainable pest reduction are also supported by policymakers. Moreover, it can avoid or minimizes any pesticide resistance development and create a competitive market advantage by reducing the associated problems of agro-chemicals at large.

However, efforts to isolate, characterize, and evaluate EPNs (belonging to Heterorhabditidae and Steinernematidae) isolates from different agro-ecological zones will help to select the most virulent or effective strains among the isolates of EPNs and enable to use them for the biological control program of FAW and other insect pests. Moreover, the best-performing EPN isolates can also be working in techniques for mass production, appropriate formulation to keep the quality, and large-scale application for maize-producing farmers to reduce the insect pest problem, particularly FAW.

6.1.5. Objectives of the Study

General Objective:

- To reduce FAW infestation through the development of appropriate control strategies.

Specific Objectives:

1) To undertake a survey in order to collect and identify avialble native entomopathogenic nematode isolates from soils of maize-producing agroecologies of Ethiopia
2) To evaluate the efficacy of entomopathogenic nematode isolates against FAW larvae in *in-vitro* conditions
3) To evaluate the selected isolates against FAW on maize plants under pot culture

6.2. MATERIALS AND METHODS

6.2.1. Soil Sampling

Soil samples were randomly collected from Oromia, Afar, Amhara, Benishangul-Gumuz, Gambella, Somali, South Nation Nationality peoples (SNNP) and Tigiraymaize growing areas from August to Septemberb 2019. Accordingly, a total of 679 soil samples were collected from 10-15 cm depth using an auger at intervals of 5 to 10 kilometers based on the maize farm availability. From each farm, 5 soil samples were randomly collected and composited (approximately 1 kilogram). The soil samples were transported to the Ambo Agricultural Research Center (AmARC) Entomology Laboratory and kept at 12-15 °C for later use (Abdel-Razek *et al*., 2018; Ashenafi *et al*., 2019; Yuksel and Canhilal, 2019). Additionally, dead FAW cadavers and other insects were collected and transported to the same Laboratory for further isolation and identification of EPNs.

6.2.2. Isolation of Entomopathogenic Nematodes

The insect-bait method was used to isolate EPN from the soil samples (Orozco *et al*., 2014). Briefly, the larvae of the greater wax moth *Galleria mellonella* were used as bait to isolate entomopathogenic nematodes from the soil samples. Ten third instar larvae of *G. mellonella* were placed into small glass jars of 500 mL. The soil samples were moistened and placed on top of the larvae until approximately 2/3 of the glass jars were filled. The glass jars were maintained in the dark at 22 to 25 °C. Every day, the glass jars were inverted, so that the larvae continually had to move through the soil and were repeatedly exposed to infective juveniles in the soil. In the course of the experiment, data on FAW larval mortality was recorded daily for ten days. The dead larvae were collected and submerged in 70% ethanol for one minute and washed in sterile distilled water for three minutes to remove saprophytes, the non-infective stage, and the host tissues (Orozco *et al*., 2014; Abdel-Razek *et al*., 2018).

The disinfected cadavers were placed in a modified white trap for recovery of nematode progeny and when there was the emergence of infective juveniles (IJ’s), they were harvested and poured into the flask. The flask with nematode suspension was stored in an incubator between 10–20 °C for later use. The stored flasks were checked periodically for the availability of EPN. Usually, Steinernematids isolate was stored for 6-12 months without the need for sub-culturing (Orozco *et al*., 2014; Abdel-Razek *et al*., 2018).

6.2.3. Identification of EPN Isolates

The EPN IJs outgrown from the cadaver were recultured on *G. mellonella* and pure cultures were obtained through a successive transfer for identification. For newly-isolated EPNs, permanent slide was prepared using TAF (Triethanomine 2 mL, Formalin (40% formaldehyde) 7 mL, and 91 mL distilled water) (Ashenafi Kassaye, 2022; Gümüşsoy *et al*., 2022). Microscopic identification was made on live and slide-mounted specimens of IJs of EPNs using an Olympus 33 camera-mounted compound microscope and morphological identification was made based on growth stage and morphological characters of EPNs following the procedures employed by previous investigators (Orozco *et al*., 2014; Ashenafi *et al*., 2019; Yuksel and Canhilal, 2019) and the color of the dead cadavers that exhibited different colors due to the symbiotic bacteria associated with the EPN species, i.e. cadavers with a brown or ochre coloration, which is a sign of parasitization by Steinernematids, whereas brick red to dark purple cadavers parasitization by Heterorhabditids.

6.2.4. Preparation of EPN Suspension

The suspension of each EPN isolate was prepared for the experiments. The EPN isolates were screened for their efficacies against FAW larvae at 500 IJs and wirehouse experiment at three concentrations (250, 400, and 600 IJs/mL) were prepared using the dilution method, and the actual number of nematodes in the stock solution was calculated according to Abdel-Razek *et al*. (2018) as follow:

$$C = N \times (X + L) \times S$$

Where, C = Actual number of nematodes in the stock solution, N = Average number of nematodes per counted sample, S = Volume of original stock solution (mL), and (X + L) = Total volume (mL) in the diluted sample.

6.2.5. Mass Rearing of FAW Larvae

For mass rearing FAW larvae were collected from AmARC maize plantation fields and mass-reared at AmARC, Entomology Laboratory, on young seedlings of maize as a feed following the procedure of Mwamburi (2021). A total of 1140 3rd instar larvae of FAW of the second generation were used for the screening the efficacies of the EPN isolates, while a total of 780 seconds to third instar larvae of FAW were used for the pot experiment.

6.2.6. Laboratory Bioassay of EPNs

All EPN isolates were screened for their virulence against FAW 3rd instar larvae at the Entomology Laboratory of AmARC. The isolates included the 37 native nematode isolates (28 new and 9 existing at AmARC EPN) (Table 15, Appendix Table 2). Ten larvae were used in each Petri dish (150 mm * 15 mm size plastic Petri dish) with filter paper and young fresh chopped maize stems inside. For each isolate, an aqueous suspension containing 500 IJ/mL was prepared in distilled water and applied using a micro-pipette. Sterile distilled water (SDW) was used as a free control treatment. Treatments were incubated at 24 °C and 60% RH and maintained in a growth chamber for 10 days. The treatments were arranged in a completely randomized design (CRD) with three replications.

The mortality data were recorded daily starting from the first day for ten days. The dead larvae were collected and submerged into 70% ethanol for three seconds and 0.5% sodium hypochlorite (NAOCl) for two minutes (Orozco *et al*., 2014) and washed in sterile distilled water for three minutes to remove saprophytes and all conidia found on the outer surface of cadavers. The disinfected cadavers were allowed to dry for ten minutes on Watman No.1 filter paper. Cadavers were held under high humidity on Petri dishes containing damp filter paper to provide sufficient humid conditions to promote EPN outgrowth. A larva was considered dead by nematode when the cadavers burst and the nematodes were visible around it and those which showed pathogenic characteristics (i.e. the release of *Xenorhabdus* for *Steinernema* sp. and release of *Photorhabdus* for *Heterorhabditis* sp. of EPN isolates) of the entomopathogenic nematodes were recorded as infected.

Mortality data was corrected for the corresponding mortality (CM) by the Abbott’s formula:

$$CM\,(\%) = \frac{(T - C)}{(100 - C)} * 100$$

Where CM is corrected mortality, T is percent mortality in treated insects and C is percent mortality in untreated insects (Abbott, 1925).

Table 14. List of EPN isolates existed at Ambo Agricultural Research Center (AmARC) that tested against the FAW in single dose experiment.

No.	EPN isolate code	Habitat	Genus	Area of collection
1	HH	Soil	*Steinernematidae* sp.	Harge Hirna
2	J-01	Soil	*Steinernematidae* sp.	Jima
3	HI	Soil	*Steinernematidae* sp.	Bule Hora
4	APPRC-p20692	Soil	*Steinernematidae* sp.	Shambu
5	APPRC-p0508	Soil	*Heterorhabditis* sp.	Jima Sokoru
6	AEH	Soil	*Heterorhabditis* sp.	Ambo
7	HBWWM	Soil	*Hetrorabitidus bacteriophora*	South Africa
8	Z9	Soil	*Heterorhabditis s* sp.	Batu
9	APPRC PL 0697	Soil	*Heterorhabditis* sp.	Fincha

6.2.1. Efficacy of Selected *Steinernema* and *Heterorhabditis* Species against FAW Larvae under Wireheouse Conditions

The experiment was carried out at the AmARC wirehouse during 2019/2020. For this experiment, the popular maize variety Jibat was used and five maize seeds were planted in each pot (21 cm diameter and 19 cm height). The pots were initially filled with the composition of black soil, compost, and sand at a proportion of 2:1:1 and watered at three days intervals. Urea at the rate of 0.52 g per pot was applied one and a half months after planting. Treatments were applied after 50 days when the seedling reached a height of approximately 40 cm. Twenty-third instar larvae of FAW were transferred into each pot. Four hours after infestation with the larvae, four effective potential EPN isolates, namely Z9, Am-Aso-Tes-287, Am-Ger-Tes-74, and Am-Adm-Tes-369, were applied at a concentration of 250 IJs mL^{-1}, 400 IJs mL^{-1} and 600 IJs mL^{-1} using the handheld sprayers (Gonfa *et al*., 2016). Sterilized distilled water was used as a control.

Each treatment was placed in the wirehouse in separate cages. The treatments were applied randomly in a Randomized Complete Block Design (RCBD) with four replications.

Data on larval mortality and plant damage were recorded 12 days after artificial inoculation. Mortality was corrected using Abbot's formula given as:

$$\%CM = \frac{(\%T - \%C)}{(100 - \%C)} * 100$$

Where CM is corrected mortality, T is mortality in treated insects, and C is mortality in untreated insects (Abbott, 1925).

The percentage of the efficacies of the native isolates were determined using Abbott's formula (Abbott, 1925) given as Efficacy = (Cd – Td) X 10/ Cd).

Where: Cd: Number of live individuals in the control plots after the treatment. Td: Number of live individuals in the treated plots after the treatment.

Plant damage was scored on visual observation using 0-9 scale (0=no damage, 1= only pinhole lesions on whorl leaves, 2 = pinhole and shoot-hole lesions on whorl leaves, 3 = A few small (0.5-1 cm) elongated lesions on leaves, 4= several leaves with mid-sized (1-3 cm) lesions, 5= several leaves with large elongated lesions or small portions eaten away, 6= several leaves with large elongated lesions and large portions eaten away, 7= many elongated lesions and large portions eaten from leaves, 8= many elongated lesions and many portions eaten from leaves, 9= many leaves destroyed (Davis *et al*., 1992; Navik *et al*., 2021).

6.2.2. Statistical Analysis

The larval mortality and plant damage data were analyzed using a one-way analysis of variance (SAS Institute, 2012). Moreover, the LT_{50} value was also determined for all EPNs using daily records of percent mortality data. The data were arcsine transformed on % mortality and damage before analyses (Gomez and Gomez, 1984). Means were compared at the $P < 0.05$ level, and Duncan's test (for laboratory test) and LSD (wire-house pot experiment) was used to separate means.

6.3. RESULTS

6.3.1. Survey of Indigenous EPN in the Maize-Producing Regions of Ethiopia

A total of 679 soil samples were collected from different regions, including Afar, Amhara, Benishangul-Gumuz, Gambella, Somali, Southern Nations, Nationalities and People's Region (SNNPR) and Tigiray.Out of these samples, 28 new EPN isolates were found. However, no nematode was recovered in the soil samples collected from Gambella, Somali and SNNP regional states. The regions with the highest EPN positive soil samples were Oromia and Tigray, with proportions of 5.34 and 13.09%, respectively. In contrast, only 3.48% of soil samples collected from the Amhara region were positive for EPN. The soil samples collected from Afar and Benishangul-Gumuz had a 2.89% positivity rate for EPN (Table 16, Fig. 7).

Table 15. Summary of native entomopathogenic nematodes isolates obtained from the maize-producing regions of Ethiopia, August to October 2019/2020.

Site	No. of fields surveyed	Total No. of positive samples	EPN isolates obtained
Oromia	187	10	4 Steinernematidae & 6 Heterorhabditis
Amhara	86	3	2 Steinernematidae & 1 Heterorhabditis
SNNP	60	0	No
Tigray	84	11	4 Steinernematidae & 7 Heterorhabditis
B. Gumuz	86	2	2 Steinernematidae
Somali	54	0	No
Afar	69	2	1 Steinernematidae & 1 Heterorhabditis
Gambella	53	0	No

NB: B. Gumuz = Benishangul-Gumuz, SNNP = Southern Nation, Nationalities, and Peoples'

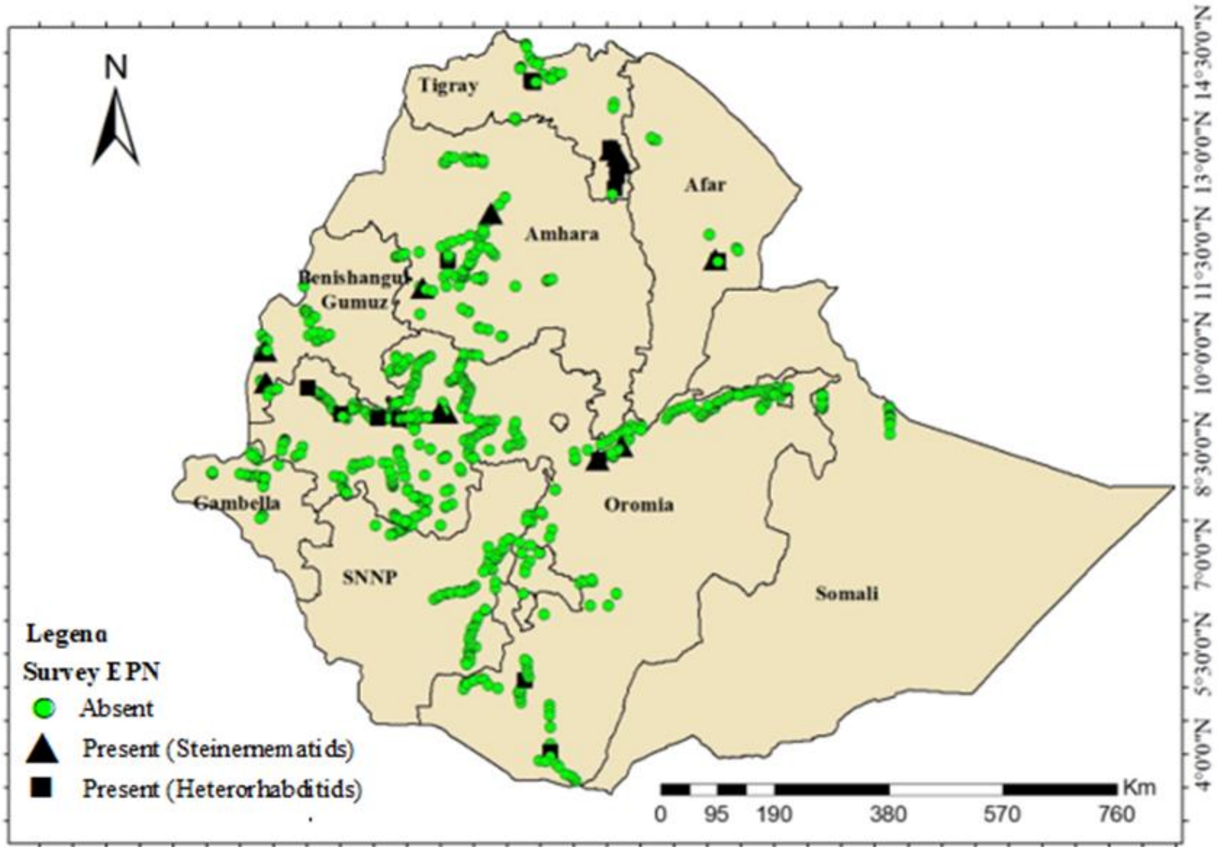

Figure 7. Soil collection sites for assessing indigenous EPN from eight maize-growing regions of Ethiopia during August to October 2019/20 main crop season.

6.3.2. Identification of EPN Isolates

For detailed identification, morphological characterization of the isolated EPN was done under microscopy. Primarily, the identification was made based on the cadaver's color. The cadavers parasitized by *Steinernematids* showed brown or ocher color, while cadavers with *Heterorhabditid* sp. were brick-red to dark purple (Figure 8). The morphometrics, such as observations on the reflex of the male spicule, total length, esophageal length, body width, distance from the anterior or posterior end of vulva, and vulva to tail, considered for the identification of the newly-isolated EPN and summarized and tabulated (Table 17).

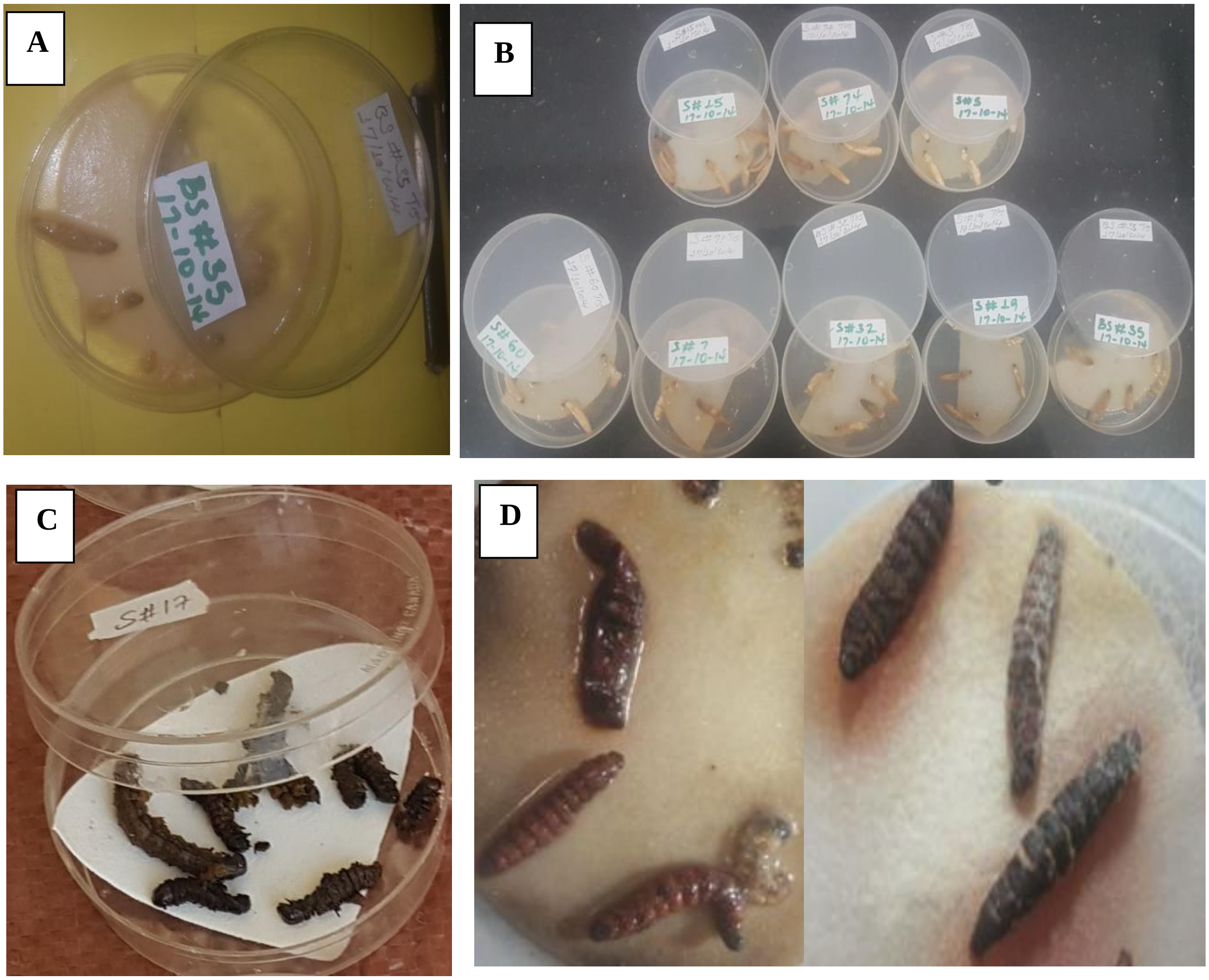

Figure 8. Mycosis on larvae of FAW due infection with EPN isolates of Steinernema species (A & B), Heterorhabditis species and, (C and D).

Am-Aso-Tes-287 isolate has a digitate, short, cone-shaped tail (Figure 8A), whereas Am-Adm-Tes-369 possessed a cone-shaped, short tail without digitation (Figure 8B). Furthermore, the microscopic morphological description of the spicules, vulva, tail, and oesophagus of the most effective isolates of *Heterorhabditis* spp. (Am-Aso-Tes-287 and Am-Adm-Tes-369) and Steinernematidae (Am-Ger-Tes-74) were investigated (Figure 8A-C and Figure 9A-C). Accordingly, spicules of Am-Aso-Tes-287 and Am-Adm-Tes-369 were formed as slender, erected paired emerged from opposite sides (Figure 9A and 9B), whereas spicules of Am-Ger-Tes-74 were attached from one point and they were flaccid (Figure 9C). Posterior part of the three male EPNs was cylindrical, narrow at the tip.

In Figure 10C, Steinernematidae (Am-Ger-Tes-74) was unique because it had a narrow and long tip tail. The vulva lips of Am-Aso-Tes-287 and Am-Adm-Tes-369 were swollen and protruded (Figures 10A and 10B). The female tail of Am-Aso-Tes-287 was conical and sharply-pointed with a thin width (Figure 10A). The female tail of Am-Adm-Tes-369 was also conical and sharply-elongated with a small width and a larger tail (Figure 10B). In contrast, the female tail of Am-Ger-Tes-74 concave and longer with a larger width that was hidden inside the vulva (Figure 10C).

The other structure used to characterize the isolated EPN was the oesophagus structure (Figure 11A-C). *Heterorhabditis* sp. (Am-Aso-Tes-287) isolates possessed pharyngeal region showing the procorpus, metacorpus, isthmus, and basal bulb (Figure 11A). Similarly, Am-Adm-Tes-369 possessed pharyngeal region, but the region formed a non-cylindrical shape, which extended to anterior concave part of the oesophagus (Figure 11B). Steinernematidae (Am-Ger-Tes-74) characterized by swollen procorpus, cylindrical metacorpus with dorsal gland orifice (Figure 11C).

Based on the morphological characterization, the newly-isolated EPN isolates belonged to the *Heterorhabditis* spp. and *Steinernematids* genus. Of the 28 positive soil samples, 15 (53.57%) were *Heterorhabditis* spp., while 13 (46.43%) were Steinernematids (Tables 16). The newly-isolated EPNs were morphologically similar to *Steinernema ethiopiense*, *Steinernema yirgalemense*, *Heterorhabditis indica*, and *Heterorhabditis bacteriophora*. Five isolates (Am-Waz-Tes-68, Am-Tse-Tes-70, Am-Haw-Tes-71, Am-DeD-Tes-76 and Am-AnD-Tes-80) were morphologically similar to *S. ethiopiense* and five isolates (Am-Kor-Tes-7, Am-Gum-Tes-15, Am-UlM-Tes-20, Am-AsA-Tes-37 and Am-SeG-Tes-50) were *S. yirgalemense*. In addition, seven samples (Am-AdG-Tes-59, Am-Bel-Tes-60, Am-Wez-Tes-67, Am-Adm-Tes-369, Am-Aad-Tes-72, Am-Huj-Tes-73 and Am-She-Tes-244) showed the same characters for *H. indica* and six isolates (Am-Kur-Tes-8, Am-KoD-Tes-19, Am-KuG-Tes-43, Am-Ben-Tes-292, Am-MeG-Tes-293 and Am-Tey-Tes-295) were identified as *H. bacteriophora*. The isolates Am-Amb-Tes-281 and Am-Aso-Tes-287 belonging to the *Heterorhabditis* genus and Am-Adm-Tes-69, Am-DiM-Tes-341 and Am-Ger-Tes-74 belonged to *Steinernematids* genus were found difficult to characterize them to the species level and thus require further study at the molecular level.

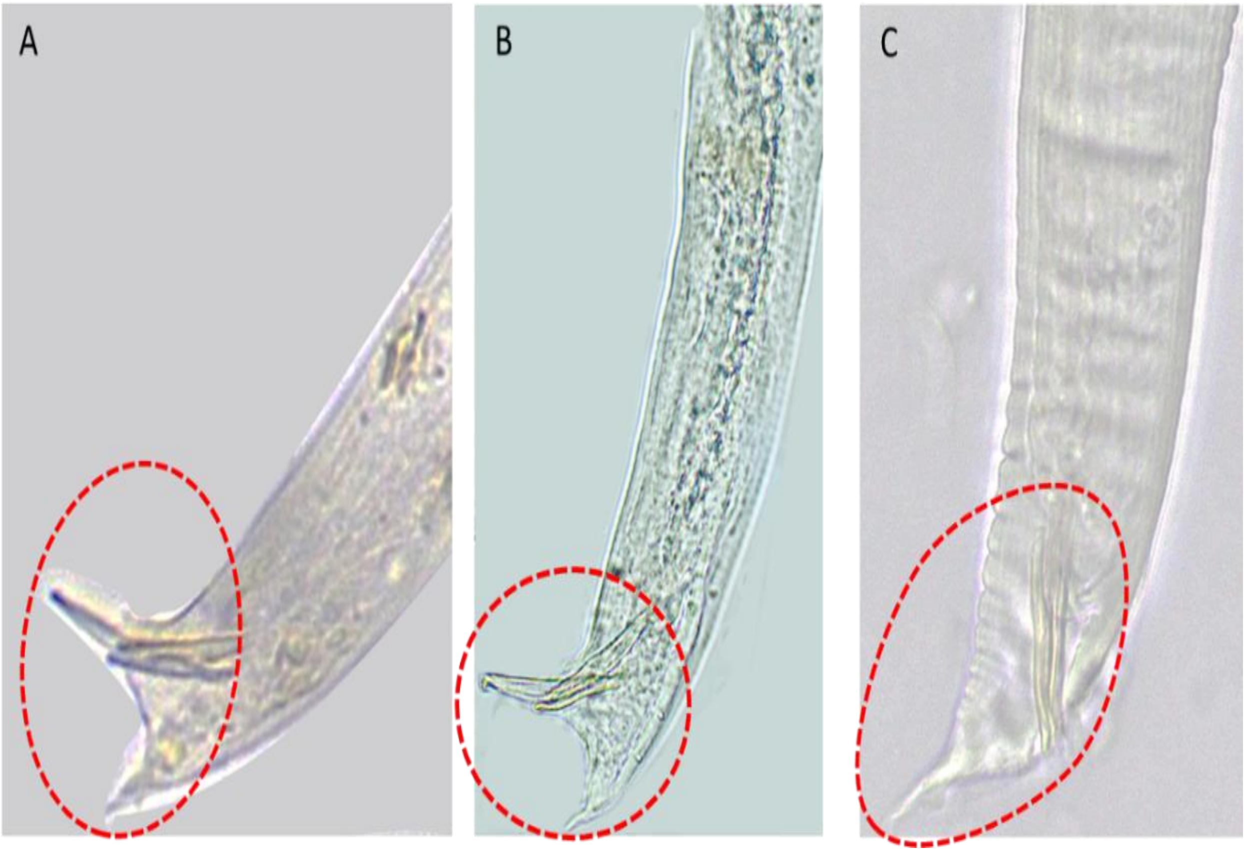

Figure 9. Morphological descriptions of isolated male EPN spicules and tail. Heterorhabditis: A) Am-Aso-Tes-287 and B) Am-Adm-Tes-369 Steinernematidae: C) Am-Ger-Tes-74. Red circles indicate male spicules and tail.

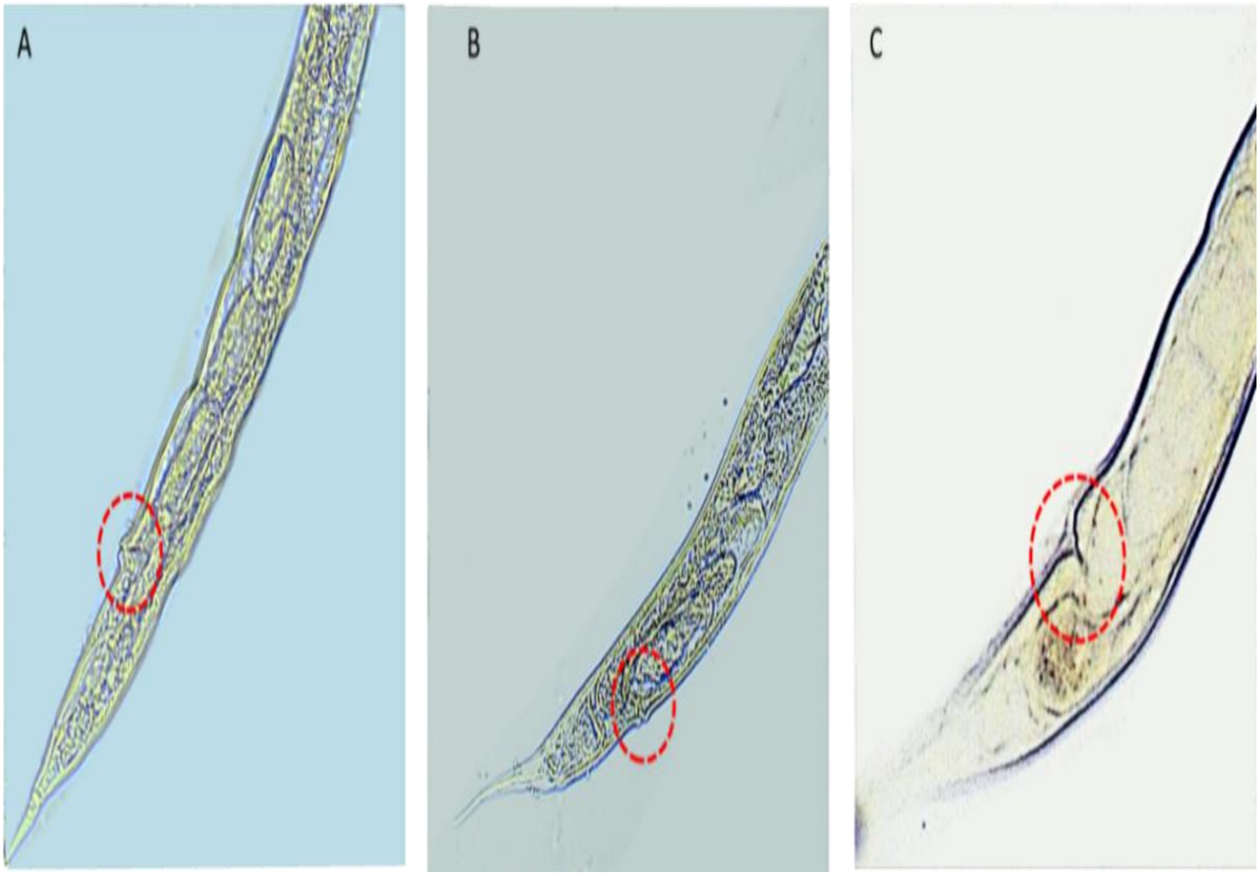

Figure 10. Morphological descriptions of isolated female EPN vulva and tail. Heterorhabditis: A) Am-Aso-Tes-287 and B) Am-Adm-Tes-369 Steinernematidae: C) Am-Ger-Tes-74. Red circles indicate female vulva.

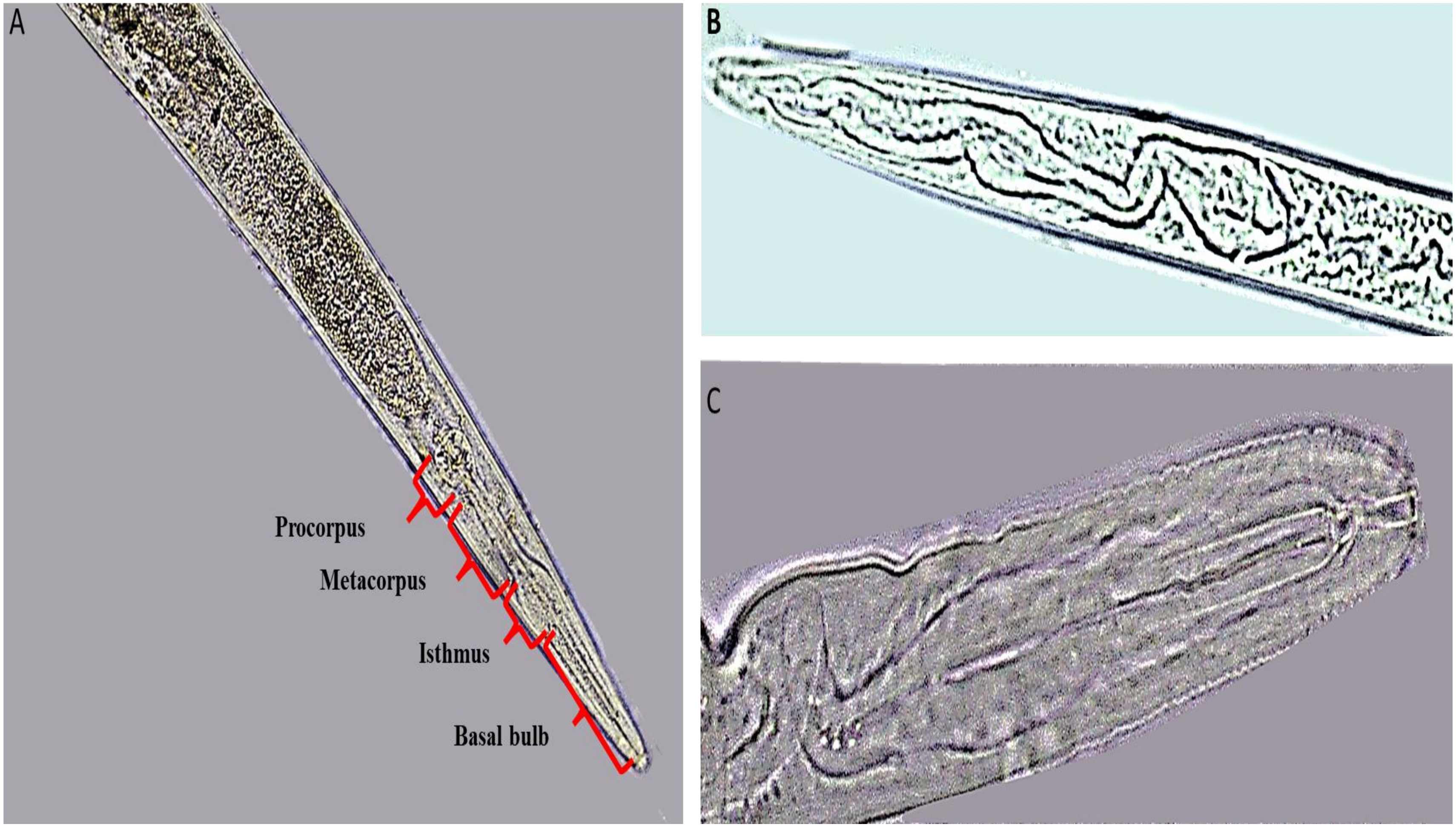

Figure 11. Male isolated EPN oesophagus, Heterorhabditis: A) Am-Aso-Tes-287 and B) Am-Adm-Tes-369 Steinernematidae: C) Am-Ger-Tes-74.

Table 16. Morphometric measurements of infective juveniles (IJs) of the newly-isolated entomopathogenic nematode (µm) (n = 5) from soil samples collected from areas growing maize in Ethiopia during August to October 2019.

Character	**Isolate Code**									
	Am-Waz-Tes-68	**Am-Tse-Tes-70**	**Am-Haw-Tes-71**	**Am-DeD-Tes-76**	**Am-AnD-Tes-80**	**Am-Kor-Tes-7**	**Am-Gum-Tes-15**	**Am-UlM-Tes-20**	**Am-AsA-Tes-37**	**Am-SeG-Tes-50**
Body length Mean ± SEM:										
Male	1222.2±0.6	1224.2±1.0	1225.9±0.4	1226.8±0.3	1223.1±1.2	1072.3±0.4	1072.2±0.2	1081.5±0.4	1098.1±0.5	1097.4±0.4
Female	1975.1±0.6	1970.8±0.2	1974.9±1.9	1975.3±0.1	1969.7±1.5	1374.9±1.0	1376.5±0.8	1388.5±0.4	1391.5±0.7	1390.4±0.1
Oesophagus Length (Mean ± std):										
Male	649.4±1.6	662.0±1.0	668.3±0.6	669.1±1.7	648.4±0.4	228.9±1.4	228.4±0.2	230.5±0.5	230.5±0.2	230.7±1.5
Female	205.3±0.4	201.9±0.5	206.2±0.7	206.7±1.3	202.0±0.9	412.6±0.3	413.6±0.5	422.5±0.4	422.7±0.1	422.4±0.2
Body width Mean ± std:										
Male	38.8±0.9	39.5±1.4	40.1±0.6	40.1±0.4	38.4±0.7	84.3±0.7	81.5±0.6	82.6±0.2	82.1±0.1	83.4±0.4
Female	72.1±0.8	73.0±0.1	74.5±1.0	75.4±0.7	74.2±0.4	119.2±1.0	120.0±0.9	124.4±0.3	124.4±0.4	124.4±0.4
DP	1524.0±0.1	1521.7±0.6	1517.7±0.9	1517.8±1.2	1520.6±0.8	796.7±0.5	796.3±0.7	798.3±0.3	800.5±0.5	799.5±0.5
VTL	246.0±0.6	247.2±0.9	250.6±1.6	251.5±1.2	247.4±1.1	165.6±0.6	166.7±0.3	167.7±0.2	168.3±0.4	168.5±0.4
SL	99.2±0.4	98.5±0.3	114.6±0.3	114.8±0.2	101.1±0.9	148.4±0.2	148.5±0.2	150.0±0.0	150.2±0.4	150.0±0.3
ST	405.5±0.7	406.1±1.0	408.4±0.4	408.5±0.5	408.3±0.4	498.3±0.7	495.2±1.0	500.0±0.7	598.5±0.2	500.5±0.5
Tail length Mean ± std:										
Male	148.3±1.1	148.1±0.4	151.4±0.6	152.7±0.4	148.2±0.2	55.7±0.1	56.0±0.0	62.4±0.2	62.8±0.1	62.4±0.2
Female	264.4±1.5	268.5±0.5	269.9±0.8	272.8±0.2	273.8±0.8	281.3±1.1	280.3±0.9	282.9±1.2	281.7±1.5	284.2±0.9

Table 17. cont'd

Character	**Isolate Code**								
	Am-AdG-Tes-59	**Am-Bel-Tes-60**	**Am-Wez-Tes-67**	**Am-Adm-Tes-369**	**Am-Aad-Tes-72**	**Am-Huj-Tes-73**	**Am-She-Tes-244**	**Am-Kur-Tes-8**	**Am-KoD-Tes-19**
Body length:									
Male	1101.9±0.9	1101.6±0.4	1115.5±0.5	1120.5±0.5	1115.7±0.7	1104.5±1.9	1135.6±0.6	1083.7±0.9	1082.9±1.2
Female	1498.6±0.6	1498.2±0.1	1502.8±0.8	1518.2±0.5	1510.9±0.8	1499.2±0.7	1515.8±0.8	1232.5±1.4	1232.5±0.5
Oesophagus length:									
Male	477.6±1.0	471.1±0.9	483.5±1.6	485.7±0.3	485.0±1.2	481.2±0.2	485.3±0.7	341.3±0.6	349.0±0.6
Female	307.9±0.2	308.0±0.1	310.5±0.4	311.4±0.5	310.3±0.6	308.4±0.4	311.4±0.4	204.3±0.6	204.3±0.6
Body width:									
Male	56.0±0.6	55.4±0.9	52.6±0.2	55.5±0.4	54.7±1.1	51.3±0.9	55.4±0.3	41.1±0.5	41.2±1.1
Female	176.0±1.2	179.3±0.5	175.2±0.5	179.4±1.0	175.6±1.5	163.1±1.4	125.1±1.1	87.5±1.1	91.3±1.1
DP	518.2±0.4	517.7±0.1	518.2±1.0	525.3±0.4	521.6±0.4	517.8±0.9	523.7±0.4	355.6±0.9	355.4±0.4
VTL	672.5±0.5	672.5±0.1	674.1±0.2	681.4±0.3	679.0±0.7	672.1±0.8	680.8±1.0	672.6±0.3	672.7±0.4
SL	93.8±1.7	91.3±0.6	94.0±0.9	96.3±1.4	95.3±0.3	91.4±1.0	96.6±0.3	40.4±0.4	40.7±2.0
ST	373.8±0.7	373.5±1.1	373.3±0.9	381.0±1.0	380.2±0.7	373.6±1.5	381.7±0.2	373.3±1.0	372.7±0.2
Tail length:									
Male	118.4±1.5	119.6±1.4	121.5±0.2	125.6±0.9	125.5±1.4	118.4±1.5	127.6±1.4	28.7±0.8	29.6±0.8
Female	369.7±0.8	370.8±0.7	371.6±1.2	384.8±0.7	389.4±0.7	369.4±0.7	395.9±0.8	148.5±1.0	149.1±1.2

Table 17. cont'd

Character	**Isolate Code**								
	Am-KuG-Tes-43	**Am-Ben-Tes-292**	**Am-MeG-Tes-293**	**Am-Tey-Tes-295**	**Am-Amb-Tes-281**	**Am-Aso-Tes-287**	**Am-Adm-Tes-69**	**Am-DiM-Tes-341**	**Am-Ger-Tes-74**
Body length:									
Male	1081.4±1.1	1084.7±1.8	1085.8±1.6	1071.2±2.0	1335.4±0.9	1363.0±1.2	1103.4±1.1	1121.4±1.2	1148.2±0.2
Female	1231.0±1.5	1248.7±1.3	1249.43±0.8	1245.4±1.2	2193.2±0.9	2262.0±0.2	1468.3±0.0	1506.8±0.2	1617.0±1.0
Osophagus length:									
Male	349.8±1.3	362.3±2.0	361.1±0.5	363.6±0.9	844.5±2.0	856.0±1.0	420.6±0.9	428.5±0.2	429.1±0.8
Female	204.6±0.7	212.6±0.3	212.2±0.2	212.5±0.4	728.5±0.5	740.7±0.3	289.6±0.4	291.0±0.9	392.3±0.5
Body width:									
Male	41.6±1.0	45.5±1.4	43.6±0.4	43.6±0.3	165.7±0.7	172.5±1.4	86.1±0.4	88.3±0.6	89.4±1.0
Female	94.6±1.2	97.4±0.9	92.3±1.0	91.7±1.3	197.8±1.2	198.6±0.6	149.3±0.9	154.5±0.6	126.8±1.5
DP	354.1±0.5	355.2±0.6	355.3±0.4	354.5±0.4	564.3±0.1	568.4±0.2	476.2±0.4	476.4±0.7	478.2±0.7
VTL	672.2±1.0	680.9±1.0	682.0±0.6	678.4±1.5	900.4±0.6	952.9±0.2	702.4±0.8	739.3±0.4	746.6±1.8
SL	40.7±0.3	42.9±1.2	42.4±0.5	42.8±0.5	376.7±0.7	381.3±0.3	410.6±1.7	114.2±0.8	416.4±1.8
ST	372.2±2.0	379.9±9.5	382.3±0.7	387.2±0.9	309.3±1.1	357.1±0.9	404.2±0.9	439.6±0.6	444.3±1.6
Tail length:									
Male	29.6±0.5	34.4±0.6	34.2±1.5	34.2±0.3	218.5±1.8	236.7±0.4	163.8±1.4	172.4±1.7	174.2±0.4
Female	138.7±1.0	154.9±1.7	152.2±1.0	152.8±1.3	397.1±1.6	416.6±1.6	324.7±1.7	371.0±1.5	378.4±0.2

DP= Distance from the anterior or posterior end of the vulva (μm), VTL= Vulva to tail length, SL= Spicule length, ST= Spicule to tail.

6.3.3. Virulence Screening of EPN Isolates Under Laboratory Conditions

All thirty-seven isolates of *Steinernematidae* sp. and *Heterorhabditis* sp. were pathogenic to FAW and caused varying levels of larval mortality within 4 to 10 days after treatment (DAT) (Table 18). The isolates had highly and significantly ($p \leq 0.01$) different virulence at 4, 6, 8, and 10 DAT (Table 18). At 4 DAT, Z9 (48.9%), Am-Ger-Tes-74 (46.9%), Am-Adm-Tes-369 (43.1%), Am-Aad-Tes-72 (43.0%), caused significantly higher cumulative mortality, followed by the isolates Am-Aso-Tes-287 (26.48%), Am-Kur-Tes-8 (22.26%), Am-SeG-Tes-50 (18.04%), APPRC-P20692 (18.04%), and Am-MeG-Tes-293 (13.7%). The rest 22 EPN isolates did not cause larval mortality similar to the control (Table 18). Besides, the percent mortalities due to isolates Am-Waz-Tes-68, Am-Tse-Tes-70, Am-Amb-Tes-281, Am-AsA-Tes-37, Am-Gum-Tes-15 and Am-Waz-Tes-68 were not significantly ($p>0.05$) different from each other (Table 18).

Out of thirty-seven isolates of EPNs, the highest cumulative mortality was observed due to Am-Aso-Tes-287 (89.0%) isolate, followed by Am-AdG-Tes-59 (68.9%), Am-Adm-Tes-369 (66.1%), Am-Ger-Tes-74 (66.1%), Am-SeG-Tes-50 (63.9%), Z9 (63.9%), Am-DiM-Tes-341 (61.2%), APPRC-p0508 (63.4%), Am-KoD-Tes-19 (59.0%), Am-Ben-Tes-292 (55.9%), Am-Huj-Tes-73 (55.4%), Am-Tey-Tes-295 (55.0%), Am-Tse-Tes-70, Am-Gum-Tes-15, and Am-Kur-Tes-8 (51.1%), HH and Am-Bel-Tes-60 (50.9%), respectively, at 6 DAT. The remaining EPN isolates of *Steinernematidae* and *Heterorhabditis sp*. caused the lowest mortality on FAW larvae (Table 18).

In most of the isolates, the mortality of FAW larvae was significantly higher in 6, 8 and 10 DAT when than the mortality in 4 DAT (Table 18). Most of the tested isolates (22 isolates) caused 50 to 75% FAW larval mortality within 8 DAT. Isolates Am-Aso-Tes-287, Am-Adm-Tes-369, Am-Ger-Tes-74, Z9, Am-SeG-Tes-50, Am-Gum-Tes-15, Am-AdG-Tes-59, APPRC PL 0697 and AEH caused significantly higher (71– 89%) larval mortality at 8 DAT, whereas isolates HBWWM, and APPRC-PL0697 caused significantly lower mortality than other treatments (Table 18).

Mortality levels of FAW larvae ranged between 26.1 and 89.0 at 10 DAT. The FAW larval mortality on this day was more or less similar to the 8 DAT. Am-Aso-Tes-287, Am-Adm-Tes-369, Am-Ger-Tes-74, and Z9 isolates resulted in higher mortality than the other isolates.

Differences between isolates for lethal time (LT$_{50}$) against FAW larvae at 500 IJs mL^{-1} were highly significant ($p \leq 0.01$) (Table 18). Isolate Am-Aso-Tes-287 resulted in significantly shorter LT$_{50}$ (3.5) than the rest of the treatments. Am-BuT- Tes-369 and Am-Ger-Tes-74 with LT$_{50}$ of 6.7, and the isolate Z9 with LT$_{50}$ of 6.6 resulted in significantly lower LT$_{50}$ than the rest of the treatments other than Am-Aso-Tes-287. On the other hand, the isolates HBWWM and APPRC PL 0697 with LT$_{50}$ of 16.7 gave significantly longer LT$_{50}$ values than the rest of the treatments (Table 18).

Table 17. Mortality percentage and LT$_{50}$ of FAW 10 days after treatment with isolates of Steinernematidae and Heterorhabditis sp. at the rate of 500 IJs mL^{-1}.

EPN isolates		Mortality ±S.E*				LT$_{50}$
		4DAT	6DAT	8DAT	10DAT	
Am-Aso-Tes-287*	Steinernematidae Sp	26.48±12.1cd	89.0±0.0a	89.0±0.0a	89.0±0.0a	3.5±0.3l
Am-Waz-Tes-68		9.56±4.8efg	26.6±0.0ghi	49.8±11.4defghi	49.8±11.4defgh	13.5±0.3c
Am-Tse-Tes-70		9.56±4.8efg	51.1±12.01cdef	62.4±0.9bcdef	62.4±0.9bcdef	11.75±0.3defg
Am-MeG-Tes-293		13.7±3.6cdef	53.1±9.42bcdef	53.5±10.4defgh	53.5±10.4cdefg	12.25±0.3de
Am-Kor-Tes-7		0.99±0.0g	38.9±12.1efgh	44.0±21.0fghij	53.1±4.8cdefg	12.5±0.3d
HH		9.52±4.8efg	50.8±0.0cdef	53.6±7.3defgh	53.6±7.1cdefg	12.5±0.3d
Am-SeG-Tes-50		18.04±7.8defg	63.9±24.9bc	71.4±15.2abc	71.4±15.2abc	11.25±0.3ghi
Am-Haw-Tes-71		0.99±0.0g	30.8±7.3fgh	31.3±10.5jk	31.3±10.5hi	15.32±0.2b
Am-AnD-Tes-80		0.99±0.0g	55.0±7.3bcde	62.4±0.9bcdef	71.4±15.2abc	11.08±0.4ghij
J-01		0.99±0.0g	39.0±12.1efgh	54.3±13.1cdefgh	54.3±13.1cdefg	12.25±0.4de
Am-DeD-Tes-76		0.99±0.0g	37.0±9.4fgh	55.5±7.6cdefgh	55.5±7.6cdefg	12.08±0.4def
Am-Gum-Tes-15		0.99±0.0g	51.1±12.1cdef	71.4±15.2abc	71.4±15.2abc	11.17±0.3ghij
HI		0.99±0.0g	9.5±4.7ij	45.5±17.8efghij	45.5±17.8efghi	13.73±0.8c
Am-UlM-Tes-20		0.99±0.0g	39.2±0.0efgh	43.1±4.6hijk	43.1±4.6fghi	14.07±0.2c
Am-Amb-Tes-281		9.56±4.8efg	9.5±4.7ij	45.2±5.2fghij	45.2±5.2efghi	13.64±0.4c
Am-AsA-Tes-37		9.56±4.9efg	55.4±13.9bcde	62.4±0.9bcdef	71.4±15.2abc	11.07±0.2ghij

Table 18. cont'd

EPN isolates		Mortality ±S. E*				LT_{50}
		4DAT	6DAT	8DAT	10DAT	
Am-Kur-Tes-8	Heterorhabditis Sp.	22.26±9.5cde	51.1±12.1cdef	61.1±9.9bcdefg	70.1±19.1abcd	11.4±0.2fgh
Am-Bel-Tes-60		0.99±0.0g	50.9±10.1cdef	63.1±6.9bcde	63.1±6.9bcdef	11.65±0.4efg
Am-AdG-Tes-59		0.99±0.0g	68.9±4.7b	74.3±13.2ab	74.3±13.2abc	10.82±0.1hij
Am-KoD-Tes-19		0.99±0.0g	59.0±3.8bcd	62.4±0.9bcdef	62.4±0.9bcdef	11.82±0.1defg
APPRC-p0508		0.99±0.0g	63.4±0.0bc	74.3±13.2ab	74.3±13.2abc	10.68±0.2ij
Am-KuG-Tes-43		0.99±0.0g	43.1±6.6defg	45.4±14.4efghij	45.5±14.4efghi	13.65±0.1c
AEH		0.99±0.0g	38.9±16.2efgh	74.3±13.2ab	74.3±13.2abc	10.7±0.2ij
Am-She-Tes-244		9.56±4.9efg	49.6±22.5cdef	57.2±16.2bcefgh	57.2±16.2cdef	12.14±0.3def
Am-BuT-Tes-369*		43.1±3.3ab	66.1±4.6bc	89.0±0.0a	89.0±0.0a	6.69±0.1k
Am-Huj-Tes-73		30.8±7.3bc	55.4±13.9bcde	61.1±9.9bcdefg	61.1±9.9bcdef	12.07±0.2def
Am-Wez-Tes-67		0.99±0.0g	36.1±16.3fgh	38.6±14.7ijk	35.6±14.7ghi	15±0.3b
HBWWM		0.99±0.0g	23.9±4.7hi	26.1±11.4k	26.1±11.4i	16.73±0.2a
Am-Ger-Tes-74*		46.9±3.3a	66.1±4.7bc	89.0±0.0a	89.0±0.0a	6.69±0.1k
Am-DiM-Tes-341		6.8±10.1gf	61.2±3.8bc	71.4±15.2abc	71.4±15.2abc	11.07±0.2ghij
Am-Adm-Tes-69		0.99±0.0g	32.3±12.0gh	31.3±10.5jk	31.3±10.5hi	15.55±0.6b
Z9*		48.9±6.8a	63.93±7.4bc	89.0±0.0a	89.0±0.0a	6.6±0.3k
APPRC PL 0697		0.99±0.0g	28.8±3.8gh	28.9±9.9jk	28.9±9.9hi	16.73±0.2a
Am-Tey-Tes-295		0.99±0.0g	55.0±7.3bcde	60.0±4.6bcdefg	60.0±4.6bcdef	11.75±0.2defg
Am-Aad-Tes-72		43.0±3.3ab	54.8±3.5bcde	58.2±6.4bcdefgh	67.2±19.7bcd	11.5±0.3efgh
Am-Ben-Tes-292		0.99±0.0f	55.91±7.3bcde	66.9±20.6bcd	80.5±14.8ab	10.5±0.3j
Control		0.99±0.0g	0.99±0.0j	0.99±0.0l	0.99±0.0j	
CV (%)		9.84	22.89	19.05	22.2	
F Value		7.19	8.52	9.26	6.8	

Means in the same column followed by similar letters are not significantly different according to the LSD test at 0.05. * Indicate isolates selected for further screening.

6.3.4. Effect of EPNs Isolates on Larval Mortality and Leaf Damage at Wirehouse

The percent FAW larval mortality increased with the increase in the concentration of the infective juveniles (IJs) of the EPNs. The lowest larval mortality of 36.5, 42.4, 46.7 and 48.9%, respectively, were recorded on the 12 DAT for Z9, Am-Adm-Tes-369, Am-Ger-Tes-74, and Am-Aso-Tes-287 at the lowest concentration of 250 IJs mL^{-1}, which increased on medium concentration (400 IJs mL^{-1}) to 51.2, 57.8, 65.3, and 68 %, for Z9, Am-Adm-Tes-369, Am-Ger-Tes-74, and Am-Aso-Tes-287, respectively (Table 19). The larval mortality increased to 53.2, 63.4, 74.3, and 74.6% at the concentration of 600 IJs mL^{-1} on the same isolates. On the other hand, the EPN concentration of 600 IJs mL^{-1} caused the highest (74.3 and 74.6%) larval mortality due to isolates Am-Ger-Tes-74 and Am-Aso-Tes-287.

Moreover, the percentage of damage decreased to isolate Z9 (50.8, 42.1 and 35.3%), Am-Aso-Tes-287 (46.0, 38.2 and 30.9%), Am-Ger-Tes-74 (45.0, 37.2 and 28.9%) and Am-Adm-Tes-369 (49.9, 41.2 and 40.0%) at the tested concentrations of 250, 400 and 600 IJs mL^{-1}, respectively. The percentage damage by FAW decreased with increase in the concentration of *Steinernema* sp. (Am-Aso-Tes-287) and *Heterorhabditis* sp. (Z9, Am-Adm-Tes-369 and Am-Ger-Tes-74) (Table 19). At the lowest concentration of the isolates (250 IJs mL^{-1}), the highest damage and the lowest mortality of FAW larvae were recorded on 4 isolates (Table 19).

Table 18. Percentage larval mortality and leaf damage due to FAW 12 days after inoculation with the entomopathogenic nematodes *Steinernematidae* sp. (Am-Aso-Tes-287) and *Heterorhabditis* sp. (Z9, Am-BuT-Tes-369 and Am-Ger-Tes-74), under wirehouse conditions.

Treatment	First Experiment		Second Experiment	
	Mean Mortality (%)	Mean Damage (%)	Mortality (%)	Damage (%)
Z9 250	36.5±15.8e	50.8±0.0b	28.9±1.8f	46.9±1.7bc
Z9 400	51.2±3.3cde	42.1±±2.9bcd	38.1±4.4def	39.2±2.9cd
Z9 600	53.2±4.5bcd	35.3±1.8def	50.2±4.9bc	35.2±3.5de
Am-Aso-Tes-287 250	48.9±11.9cde	46.0±4.4bc	44.3±10.7bcd	45.9±1.7bc
Am-Aso-Tes-287 400	68.0±5.3ab	38.2±1.7cdef	49.9±1.5bc	39.2±5.0cd
Am-Aso-Tes-287 600	74.6±6.2a	30.9±4.9ef	74.7±13.1a	28.9±1.9e
Am-Ger-Tes-74 250	46.7±10.8de	45.0±5.8bc	42.1±7.9cde	46.9±4.4bc
Am-Ger-Tes-74 400	65.3±4.6abc	37.2±4.5cdef	52.2±9.8bc	37.1±1.7cde
Am-Ger-Tes-74 600	74.3±13.2a	28.9±2.0f	78.3±18.6a	33.0±6.3de
Am-BuT-Tes-369 250	42.4±12.4de	49.9±6.1b	31.7±4.5ef	51.8±1.7b
Am-BuT-Tes-369 400	57.8±8.4bcd	41.2±3.3bcd	43.9±6.1bcd	41.1±1.7cd
Am-BuT-Tes-369 600	63.4±4.9abc	40.0±4.6cde	54.5±6.4b	40.9±7.1cd
Control	0.99±0.0f	66.1±4.7a	0.99±0.0g	65.2±7.6a
P Value	<.0001	<.0001	<.0001	<.0001
F Value	12.13	8.48	25.23	7
CV (%)	18.47	13.57	14.89	14.33

Means in the same column followed by similar letters are not significantly different according to the LSD test at 0.05.

6.4. DISCUSSION

Results of this study revealed the occurrence of EPNs with varying virulence against FAW in various maize-producing areas of Ethiopia, including Afar, Amhara, Benishangul-Gumuz, Oromia and Tigray regions. However, no EPNs were discovered in Somali and SNNP regions. The occurrence and distribution of EPNs are dependent on the soil's physico-chemical properties (Kandji *et al*. 2001). The absence of EPNs in Somali and SNNP could be related to the soil properties in those areas. As it was reported by Kour *et al*., (2020), EPNs said to be absent in clay, silty clay, silty loam, silty clay loam, and clay loam soil . Additionally, Gebremedhin *et al*. (2020) reported that the majority of the soils in the Somali region are clay loamy. Thus the current finding is also similar with those previous reports.

Based on morphological descriptions, 54% of the isolated EPNs belonged to *Heterorhabditis* species and the remaining 46% belonged to the family Steinernematidae. Moreover, cadavers parasitized by Steinernematidae showed brown or ochre coloration, whereas the cadavers parasitized by Heterorhabditids exhibited brick red to dark purple colors (Dolinski *et al*., 2012; Lalramnghaki, 2018).

The Steinernematids and Heterorhabditids species were the most commonly collected species in Ethiopia similar to reports from different countries, including Egypt, India, Mexico and Turkey (Mekete *et al*., 2005; Girón-Pablo *et al*., 2012; Tamiru *et al*., 2012; Gonfa *et al*., 2016; Devi *et al*., 2017; Abdel-Razek *et al*., 2018; Ashenafi *et al*., 2019; Yuksel and Canhilal, 2019). The isolates Am-Aso-Tes-287, Am-BuT-Tes-369, Am-Ger-Tes-74, and Z9 caused the highest mortality within 8 days. This indicates that these isolates have the potential to be used for the management of the insect pest FAW. Similarly, Steinernematids and Heterorhabditids species demonstrated the highest mortality against different insect pests, for instance, *Tuta absoluta*, *Phyllophaga vetula* (Batalla-Carrera *et al*. 2010; Girón-Pablo *et al*., 2012), *Spodoptera litura* (Adithya and Shivaprakash, 2021), and storage insect (Qader *et al*., 2021). In addition, Shahina *et al*. (2009) and Abbas (2010) reported that Steinernematids and Heterorhabditids species. caused 100% mortality in red palm weevil in the third and fifth instars at a concentration of 400 IJs mL^{-1}. Andaló *et al*. (2010) also reported 97.6 and 100% mortality of FAW larvae by *Steinernema* sp. and *Heterorhabditis* in the laboratory and greenhouse.

Isolates Am-Aso-Tes-287, Am-BuT-Tes-369 and Am-Ger-Tes-74, Z9 caused 50% mortality within 3.5, 6.7, 6.7 and 6.6 days, respectively, which is faster than the LT_{50} reported by Adithya and Shivaprakash (2021) on *Spodoptera litura* using the most active symbiotic bacterial of EPN and *Steinernematids.* Similarly, Bhairavi *et al*. (2021) reported significant variations in lethal time to 50% mortality using *Heterorhabditis bacteriophora* for the control of *Odontotermes obesus* and *Agrotis ipsilon*. In the current study, the intermediately virulent isolates Am-Aad-Tes-72, Am-AdG-Tes-59, Am-KuG-Tes-43, and AEH, had LT_{50} of 10.5, 10.82, 10.68, and 10.7 days, respectively, compared to those categorized as weakly virulent. Further evaluation of the four highly virulent EPN isolates on pot-planted maize showed that an increase in the concentration levels of the EPN increased mortality in FAW larvae. Similar results have been reported by Shahina *et al*. (2009) and Gonfa *et al*. (2016) using *Heterorhabditis* and *Steinernema* spp. isolates that caused the highest mortality against red palm weevil and diamondback moth under laboratory and greenhouse conditions. The current research data on plant damage revealed that there was less plant damage when the EPN isolate concentration was higher.

6.5. CONCLUSIONS AND RECOMMENDATIONS

Native strains of the entomopathogenic nematodes, Steinernematids, and Heterorhabditids species were isolated from soils of maize-producing areas of Ethiopia to identify efficient strains to parasitize and manage FAW. Among the newly-isolated EPN strains Am-Aso-Tes-287, Am-BuT-Tes-369, Am-Ger-Tes-74, and Z9 were found as the most pathogenic to FAW in both laboratory and wire-house pot experiments. The isolates Am-Aso-Tes-287 and Am-Ger-Tes-74 performed better than the other isolates at all concentrations, exposure times, and in cumulative mortality of the insect pest. Therefore, these isolates have the potential for the development of microbial insecticide (biopesticide) against FAW to be used as a component of an integrated management strategy against the pest. However, future studies are needed on a collection of EPNs from different agro-ecologies, molecular characterization of entomopathogenic nematode isolates, field evaluations using appropriate formulation under high insect population conditions, and more research on techniques of mass production, and shelf-life of the isolates.

CHAPTER VII

VII. EVALUATION OF ENTOMOPATHOGENIC FUNGI FOR THE BIOLOGICAL CONTROL OF FAW ON MAIZE UNDER LABORATORY AND WIREHOUSE CONDITIONS

ABSTRACT

Fall armyworm (FAW), Spodoptera frugiperda *J.E. Smith (Lepidoptera: Noctuidae), is one of the most serious pests of maize causing high economic losses in the maize-growing areas in Ethiopia. Management of FAW heavily relied on chemical pesticides, which posed serious safety concerns on the environment, and human and animal health. An integrated pest management (IPM) approach to the management of FAW should include biological control as a component of IPM. Hence, a survey for naturally occurring entomopathogenic fungi (EPF) was conducted in various maize-growing regions of Ethiopia, between August to October 2019/2020 cropping years. The EPFs were recovered from 69 (18.5%) of 679 soil samples. Thirty- six isolates were identified as* Beauveria *spp and the other thirty-three species were identified as* Metarthium *spp. However, for 47 (New 33+existing 14)* Beauveria *species and 41 (New 36+ existing 5)* Metarhizium *species, the efficacy of isolates was evaluated under laboratory against 3rd instars larvae of the FAW using $1*10^8$ spores' suspension per milliliter. Based on 10 days of cumulative larval mortality data, AMe26, ABe32, AMe34, SMe162, SMe173, OMe242, OMe291, OMe292, OMe296, ABe28, ABe38, ABe39, ABe42, OMe336, OBe245 and AMe38 were categorized as the most virulent isolates as compared to others. However, four (ABe28, ABe38, SMe162, and AMe32) out of the 16 isolates, and which grew quickly on artificial media, had good viability during subculturing, and their LT_{50} values only took 5.6 days to kill 50% of the overall FAW larval population. They were used for further testing of their potential on potted maize plants infested with second to third-instar larvae of FAW under lath-house conditions. The most virulent isolates, ABe28, ABe38, SMe162, and AMe32, were examined in a lath-house pot experiment at four different conidial concentration levels ($1x10^6$, $1x10^7$, $1x10^8$, and $1x10^9$ conidia per milliliter), as well as an untreated control. In comparison to the others, isolates ABe28 and SMe162 exhibited significantly increased larval mortality at the higher conidial concentration (i.e., $1x10^9$ conidia per milliliter) after two, four, six, and eight days after treatment application. Similarly, all fungal isolates, except for the untreated control, caused considerably lower larval mortality at their lowest conidial concentration ($1x10^6$ conidia per milliliter). In conclusion, when conidial concentration increased, all fungal isolates showed similar increases in control potential. Furthermore, it could prevent development of pesticide resistance and create a competitive market advantage by lowering agro-chemical-related difficulties in general. The findings suggested the prospects of FAW management through efficient utilization of biological control at large and EPF in particular, and farmers can effectively manage FAW actions while minimizing environmental impact. According to these study findings it is possible that EPF will play an important role as microbial agents against FAW. Future studies will focus on molecular characterization of entomopathogenic fungi isolates, technics of mass production procedures, optimal formulation to maintain purity, and large-scale application.*

Keywords: *Beauveria*, Entomopathogenic fungi, FAW, IPM, Maize, *Metarhizium,* Pathogenicity.

7.1. INTRODUCTION

7.1.1. Background

Maize, *Zea mays* L., is a major grain crop grown by smallholder farmers throughout the highlands, midlands, and lowlands of Sub-Saharan Africa (SSA) countries, including Ethiopia. Maize holds a crucial position in Ethiopia's agricultural sector, being one of the most important cereal crops in the country. It dominates in both yield per hectare and area coverage, taking the first and second positions, respectively (Midega *et al*., 2015). The crop is cultivated on over 2 million hectares of land in Ethiopia, and smallholder farms contribute to more than 95% to the total area and production as reported by the CSA (2017).

Despite its extensive production area and importance, maize has a very low average grain yield in SSA, with yields ranging from 1.0 t ha^{-1}, which are among the lowest in the world (Cairns *et al*., 2013). This could be attributed to a variety of abiotic and biotic factors. The damage caused by insect pests is the most significant biotic component (Emana *et al*., 2008; Sharon *et al*., 2020; Altaf *et al*., 2022). More than 40 bug species have been discovered in Ethiopian maize fields (Abraham *et al*., 1993). The maize stem borer *Busseola fusca* Fuller, the spotted stem borer *Chilo partellus* Swinhoe, and several termite species (*Macrotermes* and *Microtermes* spp.) are known to be the most serious insect pests (Emana *et al*., 2008). More recently, an invasive insect pest commonly known as fall armyworm (FAW), *Spodoptera frugiperda* J.E. Smith (Lepidoptera: Noctuidae), rapidly spread to different parts of Africa, including Ethiopia, starting in 2016 and became a major insect pest causing substantial yield losses on maize (Birhanu *et al*., 2019; Kumela *et al*., 2019). Birhanu *et al* (2019) reported that FAW is widely distributed in Ethiopia and Kenya on maize fields, and the percent infestation ranged from 80 to 100% and 82.2 to 100% in the two countries, respectively.

The FAW is a polyphagous insect pest that ravages and eats maize as well as 353 other crops, including cotton, millet, sorghum, sugarcane, wheat and vegetable crops. Fall armyworm is a global threat to food security, harming food production and the livelihoods of millions of rural residents (Montezano *et al*., 2018). Fall armyworm could result in the loss of up to 17.7 million tonnes of maize every year, costing between USD 2.5 and USD 6.2 billion and that is enough to feed tens of millions of people in the world.

Over the last 60 years, insect pests have been mostly subdued using synthetic insecticides that may result in pesticide resistance and detrimental consequences on non-target organisms, including humans, as well as the environment (Picimbon, 2019). As a result, other management choices, such as botanicals, natural enemies, and microbial insecticides are being tested and used by farmers all over the world.

The use of biological control agents, such as entomopathogenic fungi (EPF) to manage insect pests is a crucial component of integrated crop protection in modern agriculture (Meyling and Eilenberg, 2007; Solomon *et al*., 2019). The use of entomopathogenic fungi can help lessen the environmental impacts of chemical insecticides, while also increasing maize output. The application of EPF against maize stem borers and FAW has shown encouraging results in recent laboratory and greenhouse tests conducted in Ethiopia (Birhanu *et al*., 2018; Tesfaye *et al*., 2020; Dinberu *et al*., 2022).

7.1.2. Statement of the Problem

This study provides information regarding the occurrence and pathogenicity of EPF in Ethiopia. Characterizing and evaluating EPF isolates in the laboratory and wire-house will help to select virulent strains among these species and use them for the most effective and environmentally friendly biological control program of different insect pests. However, there is no scientific evidence that EPF is effective against FAW. Furthermore, the potential of bio-agents is not fully realized. As a result, the current investigation was carried out to: (1) Determine the *in vitro* efficacies of EPF isolates against FAW larvae; and (2) Evaluate the selected EPF isolates for the management of FAW in maize plants under pot culture.

7.1.3. Scope of the Study

Entomopathogenic fungi (EPFs) belongs to Beauveria and Metarhizium are parasitic specifically to insects and are used as biological control agents of economically important insect pests. Therefore, it is very important to collect, isolate, characterize, and evaluate the indigenous EPFs (Beauveria and Metarhizium) isolates from different agro-ecologies of the country to look into effective strains against FAW.

7.1.4. Significance of the Study

The recent occurrence of an invasive and polyphagous insect pest, FAW, has become a major threatening insect pest causing substantial yield losses on maize; and to manage the damage due to FAW maize producing farmers used different synthetic insecticides. However, the use of synthetic insecticides to manage these lepidopterous insect pests has caused environmental contamination and the development of insecticide resistance in several insect species. A recognition of the situation has diverted the attention of scientists to search alternatives to overcome this complex situation, in which biological control is the most desired approach of pest management that kept the damage below the economic injury level through least or no contamination of the environment. In different cropping areas of Ethiopia, the presence of naturally occurring biological control agents (bioagents) has been reported recently. The survey of Amha *et al.* (2021) and Gelana *et al*. (2022) revealed that EPF (especially *Beauveria bassiana* and *Metarhizium anisopliae*) pathogens were important mortality factors of larvae of spider mites and *Galleria mellonella*. However, efforts to isolate, characterize, and evaluate EPF isolates from different maize-growing agro-ecologies will help to select the virulent or effective strains among the isolates of EPF and use them for the biological control program of FAW and other insect pests. Moreover, the best-performing EPF isolates can also be working in techniques for mass production, appropriate formulation to keep the quality, and large-scale application on crop farms of maize-producing farmers to reduce the insect pest problem, particularly FAW.

7.1.5. Objectives of the Study

General Objective:

- To increase production and productivity of maize in different agro-ecologies through the use of effective bio-control agnets against FAW.

Specific Objectives:

1) To undertake a survey, collect and identify native entomopathogenic fungi isolates from soils of maize-producing agroecologies of Ethiopia
2) To evaluate the efficacy of entomopathogenic fungi isolates against FAW larvae *in in-vitro*r
3) To evaluate the selected isolates of EPF against FAW on maize plants under pot culture

7.2. MATERIALS AND METHODS

7.2.1. Survey and Sample Collection

To detect and isolate local EPF species, representative samples were taken from maize fields, roadsides, across forests, and virgin land. A total of 679 soil samples were collected at 5 to 10 km intervals from various locations. About 1 kg of soil samples were taken from each site and carried in plastic bags to the Ambo Agricultural Research Center Plant Protection Laboratory, where they were stored at 12-15 °C for later use (Tesfaye *et al*., 2012; Dhanapal *et al*., 2020). Other dead insects and dead FAW cadavers identified in the survey area were also collected.

7.2.1.1. Isolation of Entomopathogenic Fungi

The larvae of the greater wax moth *Galleria mellonela* were used as bait to isolate entomopathogenic fungi from the soil samples. Ten third instars larvae of *G. mellonela* were placed into small glass jars of about 500 mL capacity. Soil samples collected from different locations and farming practices were placed on top of the larvae until approximately 2/3 of the glass jars were filled. The glass jars were incubated at 22 °C. Every two days, the glass jars were inverted, so that the larva continually had to move through the soil and was thus repeatedly exposed to infective conidia. In the course of the experiment, data on mortality was recorded daily for ten days. The dead larvae were collected and submerged into 70% ethanol for one minute and washed in sterile distilled water for three minutes to remove saprophytes and all conidia found on the outer surface of the larval body (Zimmermann, 1986; Tesfaye *et al.*, 2012; Kim *et al*., 2018; Sularno and Fefiani 2019; Dhanapal *et al*., 2020; Vivekanandhan *et al*., 2020).

The disinfected cadavers (dead larvae) were allowed to dry on filter paper for three minutes. This step was added to ensure that mycosis observed on the surface of the cadavers would not be attributed to spores used during the treatment but rather to growth from the interior to the exterior of the insect after colonization of internal organs (Meyling, 2007). Cadavers were held under high humidity on Petri dishes containing damp filter paper to provide sufficient humid conditions to promote fungal outgrowth. Petri dishes were sealed with parafilm to maintain greater than 95% RH and incubated in the dark at 27 °C (Tesfaye *et al.*, 2012; Dhanapal *et al*., 2020; Vivekanandhan *et al*., 2020). It was considered larval mycosis when the growth of the fungus is visible on the external surface and those that show hyphal growth characteristic of the entomopathogenic fungi were recorded (Zimmermann, 1986; Meyling, 2007).

Fungi samples that were outgrowing and sporulating on the cadaver were cultured on artificial media (SDYA) and pure cultures were acquired for identification through a subsequent transfer. The identification was done based on macro and micro growth and morphological features of fungi and using an identification guideline for soil fungi (Zimmermann, 1986).

7.2.1.2. Preparation of Fungal Isolates

Slant cultures of different isolates from the collections were sub-cultured onto Sabouraud dextrose agar with yeast extract to provide inocula for experiments (SDAY). Then cultures were incubated at 27 °C and 75% RH for 10 days. A sterile scalpel was used to scrape the surface of ten-day-old cultures, which were then suspended in an aqueous solution of 0.01% Tween 80. To remove mycelia, the fungal suspension was vortexed for one minute to break up conidial chains or clumps, and then filtered through multiple layers of sterile cheesecloth. A hemocytometer was used to quantify the concentration of conidia in the filtrate under a light microscope (40x magnification). For single-dose concentration assay or screening of EPF experiment, $1*10^8$ conidia per milliliter from stock suspension was used and for dose response lathhouse experiments $1*10^6$, $1*10^7$, $1*10^8$ and $1*10^9$ conidia per milliliter were prepared from each isolate.

7.2.2. Bioassay

7.2.2.1. *In vitro* Eefficacy of EPF Isolates Against FAW Larvae

In this bioassay, all the different native isolates (new 70 and 19 existing EPF supplied by the Ethiopian Institute of Agricultural Research (EIAR), Ambo Agricultural Research Center (Table 20 and 21) were assayed for pathogenicity against FAW at Ambo Agricultural Research Center in Plant Protection Laboratory. The 3rd instar FAW larvae were used for this experiment and treated on a Petri dish. Ten larvae were introduced in each Petri dish with filter paper and young fresh chopped maize stems inside. For each isolate an aqueous suspension containing $1.0*10^8$ conidia per milliliter was prepared in 0.01% Tween 80. Inoculation was made by directly spraying one milliliter of $1.0*10^8$ conidia per milliliter of each isolate using micropipette

(Tesfaye *et al.,* 2012; Belay *et al*., 2016; Dhanapal *et al*., 2020; Vivekanandhan *et al*., 2020). For the control group, the same number of FAW larvae were treated with sterile distilled water containing 0.01% Tween 80. The treatments were incubated at 27 °C and 70 ± 5% RH and maintained for 10 days. The treatments were randomly arranged using a completely randomized design (CRD) with three replications. Mortality was observed every 24 hours after treatment application, for ten consecutive days.

The dead larvae were collected and submerged into 70% ethanol for one minute and washed in sterile distilled water for three minutes to remove saprophytes and all conidia found on the outer surface of the larval body. The disinfected cadavers of FAW were allowed to dry for ten minutes. This step was added to ensure that mycosis observed on the surface of the cadavers would not be attributed to spores used during the treatment but rather to growth from the interior to the exterior of the insect after colonization of internal organs. Cadavers were held under high humidity on Petri dishes containing damp filter paper to provide sufficient humid conditions to promote fungal outgrowth. The Petri dish was sealed with Parafilm to maintain greater than 95% RH and was incubated in the dark at 27 °C. It was considered larval mycosis when the growth of the fungus was visible on the external surface and those which show hyphal growth characteristic of the entomopathogenic fungi were recorded as infected.

Mortality resulting due to the fungal isolates was confirmed based on the visual observation of fungal outgrowth (mycosis) on the surface of larval cadavers as indicated by Aysheshim (2002) and Chang *et al*., (2021). All larvae killed by the fungal isolates became tough and rigid upon death. The *Beauveria* isolates commenced white sporulation gradually covering the insect body when the cadavers were placed in Petri dishes and a comparison was made to the respective shape and color of the isolates already preserved on SDAY medium (Figure 19). Similarly, those larval cadavers killed by *Metarhizium* isolates produced green sporulation while placed on Petri dishes, and a comparison was made with the respective isolates cultured on SDAY medium (Figure 20).

Mortality data was corrected for the corresponding control mortality by the formula:

$$\%CM = \frac{(\%T - \%C)}{(100 - \%C)} * 100$$

Where CM is corrected mortality, T is mortality in treated insects, and C is mortality in untreated insects or control (Abbott, 1925).

Table 19. List of EPF isolates existed at AARC were tested against the FAW in single dose experiment; 2019/2020.

No.	Species	Isolate code	No.	Species	Isolate code
1	*Metarhizium anisopliae*	PPRC 2	11	*Metarhizium anisopliae*	PPRC 60
2	*Metarhizium anisopliae*	PPRC 61	12	*Metarhizium anisopliae*	PPRC 61
3	*Metarhizium anisopliae*	PPRC 19	13	*Metarhizium anisopliae*	PPRC 67
4	*Metarhizium anisopliae*	PPRC 4	14	*Metarhizium anisopliae*	ICIPE-30
5	*Metarhizium anisopliae*	DLCO-76	15	*Beauveria bassiana*	9614
6	*Metarhizium anisopliae*	DLCO-23A	16	*Beauveria bassiana*	9604
7	*Metarhizium anisopliae*	DLCO-90	17	*Beauveria bassiana*	9609
8	*Metarhizium anisopliae*	DLCO-131	18	*Beauveria bassiana*	9601
9	*Metarhizium anisopliae*	PPRC- 51	19	*Beauveria bassiana*	PPRC-56
10	*Metarhizium anisopliae*	PPRC-4	----	----	----

7.2.2.2. Evaluation of the EPF Against Larvae of FAW in Wirehouse

The experiment was conducted at the Ambo Agricultural Research Center inside the Plant Protection Wirehouse during 2019/2020 main cropping seasons. For this experiment, one known cultivar of maize (Jibat) was used and five maize seeds were planted in each pot (21 cm diameter and 19 cm height). The pots were initially filled with the composition of black soil, compost, and sand at a proportion of 2:1:1 and watered at three-day intervals. Urea at a rate of 0.52 g per pot was applied at one and a half months of age. A dose mortality wirehouse experiment at 4 concentration ($1*10^6$, $1*10^7$, $1*10^8$, and $1*10^9$ spore ^{per} milliliter) was conducted for those effective isolates (AMe32, SMe162, ABe28, and ABe38), selected dased on laboratory screening experiment. Controls were treated with sterilized distilled water (SDW) containing 0.01% Tween 80. Second to third-instar larvae of FAW of the second generation were used for the wirehouse efficacy experiment. Third instars larvae of FAW were inoculated into the maize seedlings. After four hours of larval inoculation, treatments were applied using the handheld sprayers. Each treated pot plants were placed in the wirehouse in separate cages. The treatments were arranged in a randomized complete block design (RCBD) with four replications.

For wirehouse experiments, data on larval mortality was taken 15 days after artificial inoculation. Larval mortality was corrected for control mortality using Abbot's formula given by:

$$\%CM = \frac{(\%T - \%C)}{(100 - \%C)} * 100$$

Where CM is corrected mortality, T is mortality in treated insects, and C is mortality in untreated insects (Abbott, 1925).

The percentage of the efficacy of the native isolate was determined using Abbott (1925) formula:

$$\%\ \text{Efficacy} = \frac{(\text{Cd} - \text{Td})}{\text{Cd}} \text{ X } 100$$

Where: Cd: Number of live individuals in the control plots after the treatment period. Td: Number of live individuals in the treated plots after the treatment period.

7.2.3. Statistical Analysis

Tge data were subjected to one-way analysis of variance using the SAS software package (SAS Institute, 2004). Before undertaking data analysis arcsine transformation was made on percentage mortality and damage to stabilize variance (Gomez and Gomez, 1984). The means were separated using a DMR test (laboratory bioassay experiment) and LSD (wirehouse pot experiment).

7.3. RESULTS

7.3.1. Collection and Assessment of Indigenous EPF from Maize-Producing Areas of Ethiopia

A total of eight regions of maize-producing areas were surveyed during 2019/2020 to get native entomopathogenic fungi, and a total of 679 soil samples were collected for the identification of the native fungal isolates through examination of the growth, morphology, and microscopic features of conidiogenous cells using cover slip and scotch tape techniques. Out of 679 soil samples, a total of 69 EPF isolates were purified and identified (Table 21). Out of seventy different indigenous EPF obtained, only 33 Metarhizium species and 36 Beauveria species were identified and confirmed using a Manual of Soil Fungi (Amha *et al*., 2021). Isolates that produced a white powdery mass of spores on the external surface of *Galleria* larval cadavers were identified as Beauveria species (Figure 12 and 14). whereas those isolates that infected the *Galleria* larvae and produced green crust-like velvet on the walls of the cuticle were identified as Metarhizium species (Figure 13 and 15).

Among the newly isolated and identified entomopathogenic fungi; twenty-one Metarhizium spp. and twenty-one Beauveria spp. were found from Oromia regional state, six Metarhizium spp. and seven Beauveria spps were from Amhara and two Metarhizium spp. and two Beauveria spp. were from SNNPR and six Beauveria spp. were from Tigray and four Metarhizium spp. were from Benishangul Gumuz Regional States of maize-growing areas (Table 21 and 22). However, from Afar, Gambella and Somali Regional States 9, 23 and 34, respectively, soil samples were collected and no EPF isolates were found. Beauveria species were the dominant (26.24%) EPF in the maize-growing areas collection as compared to Metarhizium species (24.14%). Higher proportions of entomopathogenic fungi were obtained from the Oromia regional state than the other maize-growing regional states. This showed the importance of exploring the unique characteristics of entomopathogenic fungi in different geographic areas.

Figure 12. A) Beauveria. species of fungal outgrowth on Galleria cadaver and B) different isolates of Beauveria cultured on SDYA medium.

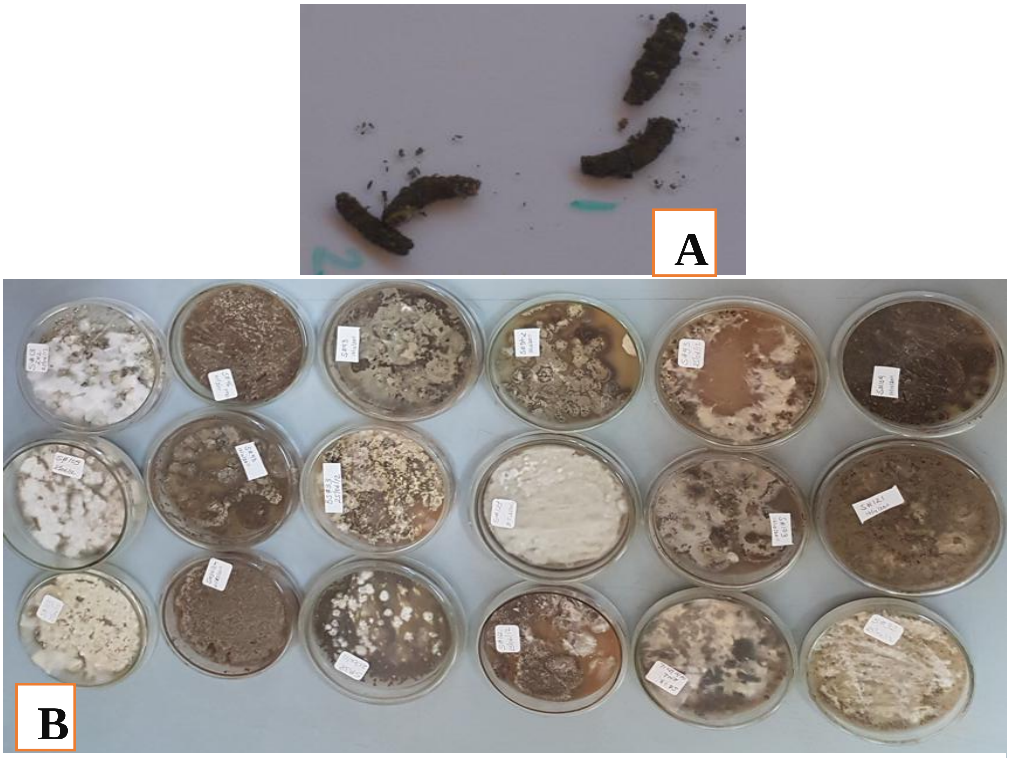

Figure 13. A) Metarhizium species outgrowth on Galleria cadaver and B) different isolates of Metarhizium cultured on SDYA medium.

Table 20. Description of the survey of indigenous EPF in five maize-growing regions of Ethiopia during 2019 to 2021.

S. No	Sample Code	Regions	Zones	Districts	Locations	Altitude	Latitude	Longitude	Remarks
1	OMe13	Oromia	East Showa	Boset	Huluko Horota	1033	08.844746	039.796383	*Metarhizium* spp.
2	OBe19	Oromia	East Showa	Boset	Kona Degaga	1526	08.405732	039.369478	*Beauveria* spp.
3	OBe25	Oromia	East Showa	Dugda Bora	Beri Malima	1602	08.420385	039.024900	*Beauveria* spp.
4	AMe26	Amhara	East Gojam	Awobele	Yekeyit	2461	10 15.432	037 55.829	*Metarhizium* spp.
5	ABe27	Amhara	East Gojam	Andid	Gud Alema	2358	10 15.595	037 54.397	*Beauveria* spp.
6	ABe28	Amhara	East Gojam	Gozamen	Yebeka	2377	10 21.149	037 42.803	*Beauveria* spp.
7	AMe32	Amhara	West Gojam	Dembecha	Wengi	1773	10 37.703	037 24.181	*Metarhizium* spp.
8	AMe34	Amhara	West Gojam	Jabi Tehinan	Bir Sheleko	1697	10 35.305	037 10. 307	*Metarhizium* spp.
9	ABe38	Amhara	Awi Zone	Banja	Zigo Marta	2186	10 57.589	036 46.867	*Beauveria* spp,
9	AMe38	Amhara	Awi Zone	Banja	Zigo Marta	2186	10 57.589	036 46.867	*Metarhizium* spp.
10	ABe39	Amhara	Awi Zone	Banja	Bida Jegola	2502	10 56.422	036 53.010	*Beauveria* spp.
10	AMe39	Amhara	Awi Zone	Banja	Bida Jegola	2502	10 56.422	036 53.010	*Metarhizium* spp.
11	ABe42	Amhara	Bahidar Zuriya	Mecha	Kudme	1984	11 23.845	037 06.809	*Beauveria* spp.
12	ABe44	Amhara	Bahidar Zuriya	Mecha	Enguti	1991	11 25.296	037 07.466	*Beauveria* spp.
13	AMe46	Amhara	Bahidar Zuriya	Mecha	Kolela	1931	11 27.588	037 07.286	*Metarhizium* spp.
14	ABe51	Amhara	Nourth Gonder	Adi Arkay	Aba Mar	1216	13 29.950	038 07.611	*Beauveria* spp.
15	TBe52	Tigray	Tigray	Tselemti	Medihaniyalem	1171	13 31.468	038 06.879	*Beauveria* spp.
16	TBe54	Tigray	Tigray	Laelay Adiabo	Deba	1769	14 17.527	038 12.055	*Beauveria* spp.
17	TBe63	Tigray	Tigray	Laelay Maichew	Dura	2050	14 06.602	038 39.238	*Beauveria* spp.
18	TBe64	Tigray	Tigray	Laelay Maichew	Dura	2040	14 06.444	038 39.894	*Beauveria* spp.
19	TBe65	Tigray	Tigray	Wukiro	Korir	1978	13 45.570	039 35.838	*Beauveria* spp.
20	TBe75	Tigray	Tigray	Alamata	Ayer Marfiya	1511	12 23.316	039 34.106	*Beauveria* spp.
21	OBe85	Oromia	East Wollega	Diga	Jirata	2194	09 02.169	036 29.201	*Beauveria* spp.
22	OMe88	Oromia	Buno Bedele	Bedele	Bashure	1855	08 31.809	036 21.989	*Metarhizium* spp.
23	OMe93	Oromia	Illubabora	Didu	Gordomo	1791	07 59.103	035 32.199	*Metarhizium* spp.
24	OBe96	Oromia	Illubabora	Didu	Lallo Megalla	1724	07 54.661	035 36.099	*Beauveria* spp.
24	OMe96	Oromia	Illubabora	Didu	Lallo Megalla	1724	07 54.661	035 36.099	*Metarhizium* spp.
25	OBe97	Oromia	Illubabora	Ale	Gumero	1684	08 09.030	035 30.124	*Beauveria* spp.
25	OMe97	Oromia	Illubabora	Ale	Gumero	1684	08 09.030	035 30.124	*Metarhizium* spp.
26	OBe99	Oromia	Illubabora	Hurumu	Hurumu	1767	08 20.311	035 42.246	*Beauveria* spp.
27	OMe105	Oromia	Buno Bedele	Chora	Abdela	1870	08 21.462	036 13.384	*Metarhizium* spp.
28	OMe109	Oromia	Buno Bedele	Didessa	Yembro Haro	2177	08 09.191	036 28.052	*Metarhizium* spp.
29	OMe121	Oromia	Jima	Shebe Sombo	Alo Sebaka	1366	07 28.462	036 25.899	*Metarhizium* spp.
30	OMe124	Oromia	Jima	Kerssa	Etimbile	1694	07 42.854	037 05.128	*Metarhizium* spp.

Table 21 con't

S.No	Sample Code	Regions	Zones	Districts	Locations	Altitude	Latitude	Longitude	Remark
30	OBe124	Oromia	Jima	Kerssa	Etimbile	1694	07 42.854	037 05.128	*Beauveria* spp.
31	SBe134	SNNP	Segen	Derashe	Gato	1269	05 32.828	037 25.022	*Beauveria* spp.
32	SBe137	SNNP	Konso	Konso	Teshemale Ateya	1190	05 27.195	037 26.294	*Beauveria* spp.
33	SMe162	SNNP	Welayta Sodo	Gesuba	Wachiga Esho	1880	06 43.984	037 38.415	*Metarhizium* spp.
34	SMe173	SNNP	Hadiya	Bada Wacho	Wera Lolo	2017	07 10.483	037 57.456	*Metarhizium* spp.
35	OBe242	Oromia	East Wollega	Diga	Oda	1330	09. 02 047	036. 13 607	*Beauveria* spp.
35	OMe242	Oromia	East Wollega	Diga	Oda	1330	09. 02 047	036. 13 607	*Metarhizium* spp.
36	OBe244	Oromia	West Wollega	Gimbi	Shene	1252	09. 03 677	036. 05 921	*Beauveria* spp.
37	OMe245	Oromia	West Wollega	Gimbi	Abasena	1647	09. 01 448	035. 59 339	*Metarhizium* spp.
38	OMe248	Oromia	West Wollega	Lalo Asabi	Aroji Harowa	1886	09. 13 991	035. 41 971	*Metarhizium* spp.
38	OBe248	Oromia	West Wollega	Lalo Asabi	Aroji Harowa	1886	09. 13 991	035. 41 971	*Beauveria* spp.
39	BGMe279	Benishangul	Asosa	Asosa	Amba 14	1509	10. 00 783	034. 36 289	*Metarhizium* spp.
40	BGMe281	Benishangul	Asosa	Asosa	Amba 16	1386	09. 57 100	034. 39 446	*Metarhizium* spp.
41	BGMe285	Benishangul	Asosa	Asosa	Amba 5	1611	10. 06 249	034. 35 739	*Metarhizium* spp.
42	BGMe287	Benishangul	Asosa	Asosa	Amba 23	1562	10. 04 683	034. 38 339	*Metarhizium* spp.
43	OMe291	Oromia	West Wollega	Mene Sibu	Bengua	1418	09. 47 113	034. 55 897	*Metarhizium* spp.
44	OMe292	Oromia	West Wollega	Mene Sibu	Teyibaba	1608	09. 48 166	035. 00 998	*Metarhizium* spp.
45	OMe296	Oromia	East Wollega	Wayu Tuka	Worebabo Migna	1884	09. 02 061	036. 41 034	Metarhizium spp.
45	OBe296	Oromia	East Wollega	Wayu Tuka	Worebabo Migna	1884	09. 02 061	036. 41 034	*Beauveria* spp.
46	OMe336	Oromia	Borena	Yabelo	Darito	1539	04^{0} 44.237	038^{0} 11.559'	*Metarhizium* spp.
47	OBe337	Oromia	Borena	Dubuluk	Kersa	1553	04^{0} 39.781	038^{0} 14.169'	*Beauveria* spp.
48	OMe341	Oromia	Borena	Dire	Dida Mega	1549	04^{0} 01.932	038^{0} 20.707'	*Metarhizium* spp.
49	OMe344	Oromia	Borena	Mio	Melbana	1307	03 53.554	038 33.991	Metarhizium spp.
50	OBe345	Oromia	Borena	Mio	Boku	1285	03 51.187	038 43.806	*Beauveria* spp.
51	OBe348	Oromia	Borena	Moyale	Digalu	1166	03 37.273	039 00.334	*Beauveria* spp.
52	OBe354	Oromia	Borena	Yabelo	Dida Yabelo	1564	04 55.285	038 10.026	*Beauveria* spp.
53	OBe356	Oromia	Borena	Teltele	01 Kebele	1462	05 03.319	037 22.233	*Beauveria* spp.
54	OBe365	Oromia	Borena	Elwaye	Elwaye Golbe	1220	04 58.969	037 51.427	*Beauveria* spp.
55	OMe366	Oromia	Borena	Yabelo	Dida Yabelo	1531	04 56.065	038 10.698	*Metarhizium* spp.
56	OMe367	Oromia	Borena	Yabelo	Beke Haro	1534	04 59.304	038 12.524	*Metarhizium* spp.
57	OMe369	Oromia	Borena	Gomole	Buya Tika	1602	05 06.068	038 16.039	*Metarhizium* spp.
58	OBe376	Oromia	West Showa	Melkasa	MARC cage-1	1550	08 419675	039 32 761	*Beauveria* spp.
59	OBe377	Oromia	West Showa	Melkasa	MARC cage-2	1550	08 419675	039 32 761	*Beauveria* spp.
60	OBe377	Oromia	West Showa	Melkasa	MARC cage-2	1550	08 419675	039 32 761	*Metarhizium* spp.
61	OBe378	Oromia	West Showa	Melkasa	MARC cage-3	1550	08 419675	039 32 761	*Beauveria* spp.

Table 21. Native entomopathogenic fungi obtained from Eight maize producing regions of Ethiopia, 2019/20.

Site	No. of fields surveyed	Total No. of EPF	Fungal isolates obtained	Common fungi spp.
Oromia	187	42	21M.spp., 21B.spp.	Both species
Amhara	86	13	6 M.spp., 7 B.spp.	Both species
SNNP	60	4	2 M.Spp., 2 B.spp.	Both species
Tigray	84	6	6 B.spp.	*Beauveria* species
B. Gumuz	86	4	4 M.Spp.,	*Metarhizium* species
Somali	54	0	0	0
Afar	69	0	0	0
Gambella	53	0	0	0

NB: *B*. spp. and *M*. spp. implies *Beauveria* and *Metarhizium* species, respectively.

7.3.2. Screening of Indigenous Fungal Isolates of *Beauveria* and *Metarhizium* Species Against Larvae of FAW Under Laboratory

All of the 69 indigenous fungal isolates collected from survey areas and the additional 19 entomopathogenic fungal agents obtained from AARC, were capable of infecting and killing the larval stages of FAW under laboratory conditions (Tables 23, 24).

All the native entomopathogenic fungal isolates showed a reduction in the overall larval population of FAW as compared to the untreated check (Table 23, 24). Four days of cumulative larval mortality results indicated that AMe32, SMe162, ABe28, and ABe38 produced above 40% larval population reduction as compared to the other isolates (Table 23, 24). Only two fungal isolates of *Metarhizium* species (SMe162 and AMe32) showed the highest (100%) virulence potential within the first six-day period (Tables 22, 23) as compared to the others. Similarly, two fungal isolates from *Beauveria* species, (ABe32 and ABe28) caused the highest (100%) mortality over six days period. Based on the cumulative mortality, over the first ten days, it can be seen that 12 of the isolates of *Metarhizium* and *Beauveria* species (AMe26, AMe32, AMe34, SMe162 SMe173, OMe242, OMe291, OMe292, OMe296, ABe28, AMe38, and ABe39,) killed 100% of the larval population of FAW and only four isolates (ABe42, OMe336, OBe245, and AMe38) caused 70% larval population. Most of the selected isolates killed more than 50% of the larval population of FAW (Tables 23, 24).

The highly virulent isolates ABe28, ABe38, SMe162, and AMe32 had the lowest LT_{50} value (5.6 days), followed by AMe34, OMe242, OMe292 (8.1 days), AMe26, SMe173, OMe291, OMe296, and ABe39 (9.21 days) (Table 25), and the other group of highly virulent isolates AMe38, OBe245, OMe336 and ABe42 showed a relatively short LT_{50} ranging from 11.29 to 12.29 days (Table 23). The most intermediate and weakly virulent isolates had LT_{50} values ranging from 13 to 22.4 days. This clearly shows that, in addition to mortality, LT_{50} values can effectively measure the virulence of the isolates.

However, four of the 16 isolates (ABe28, ABe38, SMe162, and AMe32) grew quickly on artificial media, had good viability during sub-culturing, and their LT_{50} values only took 5.6 days (Table 25) and they were used for further testing on potted-maize plants infested with second to third instar larvae under lath-house conditions.

Depending on the bioassay efficacy result, the isolates were categorized as highly (70-100%), intermediately (30-69%), and weakly virulent (10-29%) (Figure 16). Isolate of *Metarhizium* spp. AMe26, AMe32, AMe34, AMe38, SMe162, SMe173, AMe242, OMe245, OMe291, OMe292, OMe296 and SOMe336, and *Beauveria* spp. ABe28, ABe38, ABe39 and ABe42 caused the highest mortality with corrected mortalities of 70 to 100%, in 10 days after treatment (Figure 16).

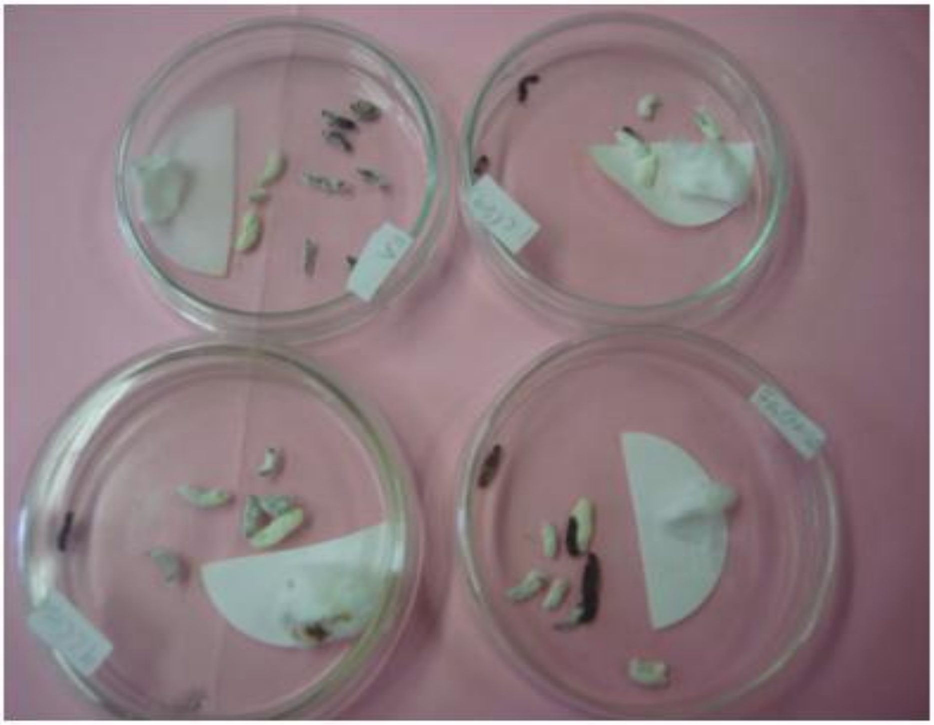

Figure 14. a & b. FAW larvae killed by native Beauveria isolates (a) AMe28 and (b) ABe38

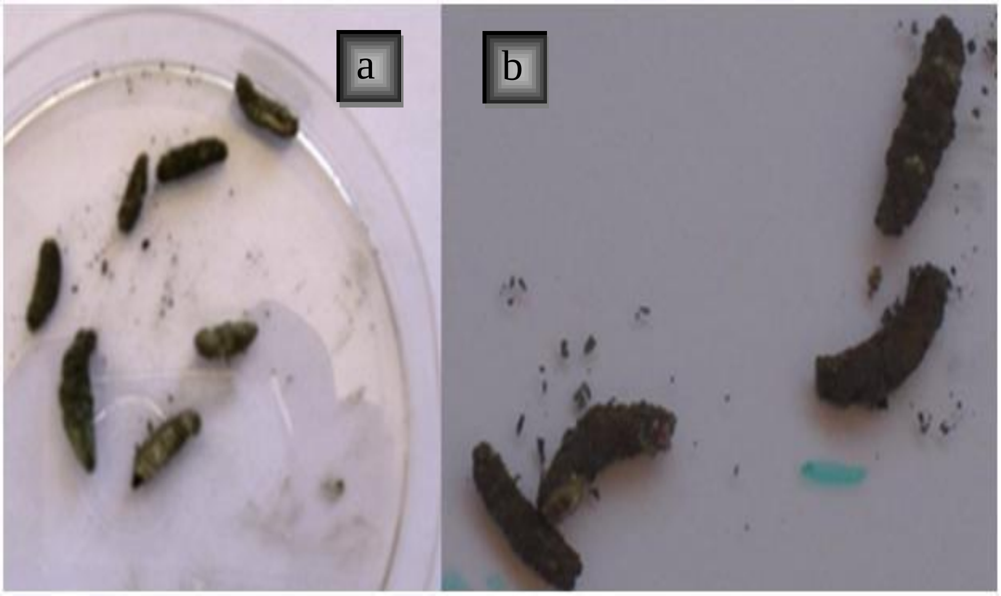

Figure 15. a & b FAW larvae killed by native Metarhizium isolates (a) SMe162 and (b) AMe32

Table 22. Mortality percentage of FAW larvae that were treated with different fungal isolates of Beauveria and Metarhizium species under lab conditions.

No	Isolates	%Mortality10 DAT ±SE		No	Isolates	%Mortality 10 DAT ±SE		No	Isolates	%Mortality 10 DAT ±SE	
1	AMe26	86.64 ±1.28a	Metarhizium spp	31	OMe367	36.93±1.02bc	Metarhizium spp	61	OBe96	27.71±0.47c	Beauveria spp
2	AMe32	86.64 ±1.28a		32	OMe369	36.15 ±0.77bc		62	OBe97	27.71±1.66c	
3	AMe34	86.64 ±1.28a		33	OMe377	36.15 ±0.77bc		63	OBe99	22.48±1.46c	
4	AMe38	70±1.10ab		34	OMe13	32.71 ±1.10bc		64	OBe124	22.48±1.46c	
5	AMe39	36.93±1.02bc		35	PPRC 2	36.93±1.02bc		65	SBe134	19.41±1.31e	
6	AMe46	36.93 ±1.02bc		36	PPRC 61	36.93 ±1.02bc		66	SBe137	18.26± 1.26e	
7	OMe88	36.93±1.02bc		37	PPRC 19	19.41±1.31e		67	OBe242	18.02±1.78e	
8	OMe93	36.93±1.02bc		38	PPRC 4	31.93±0.85bc		68	OBe244	14.18±1.55f	
9	OMe96	36.15 ±0.77bc		39	DLCO-76	22.48±1.46c		69	OBe248	9.96±1.26f	
10	OMe97	36.15 ±0.77bc		40	DLCO-23	27.71±0.47c		70	OBe296	31.93±0.85bc	
11	OMe105	32.71 ±1.10bc		41	DLCO-90	27.71±0.47c		71	OBe337	31.93±0.85bc	
12	OMe109	32.47±1.93bc		42	DLCO-131	27.71±0.47c		72	OBe345	30.55±1.69bc	
13	OMe121	31.93±0.85bc		43	PPRC- 51	31.93±0.85bc		73	OBe348	27.71±0.47c	
14	OMe124	31.93±0.85bc		44	PPRC-4	31.93±0.85bc		74	OBe354	27.71±0.47c	
15	SMe162	86.64 ±1.28a		45	OBe19	32.47±1.93bc	Beauveria spp	75	OBe356	27.71±1.66c	
16	SMe173	86.64 ±1.28a		46	OBe25	31.93±0.85bc		76	OBe365	22.48±1.46c	
17	OMe242	86.64 ±1.28a		47	ABe27	31.93±0.85bc		77	OBe376	22.48±1.46c	
18	OMe245	70±1.10ab		48	ABe28	86.64 ±1.28a		78	OBe377	19.41±1.31e	
19	OMe248	36.93±1.02bc		49	ABe38	86.64 ±1.28a		79	OBe378	18.26± 1.26e	
20	BGMe279	36.93 ±1.02bc		50	ABe39	86.64 ±1.28a		80	PPRC 60	22.48±1.46c	
21	BGMe281	36.93±1.02bc		51	ABe42	70±1.10ab		81	PPRC 61	31.93±0.85bc	
22	BGMe285	36.93±1.02bc		52	ABe44	36.93±1.02bc		82	PPRC 67	30.55±1.69bc	
23	BGMe287	36.15 ±0.77bc		53	ABe51	36.93 ±1.02bc		83	ICIPE-30	31.93±0.85bc	
24	OMe291	86.64 ±1.28a		54	TBe52	36.93±1.02bc		84	9614	19.41±1.31e	
25	OMe292	86.64 ±1.28a		55	TBe54	36.93±1.02bc		85	9604	19.41±1.31e	
26	OMe296	86.64 ±1.28a		56	TBe63	36.15 ±0.77bc		86	9609	18.26± 1.26e	
27	OMe336	70±1.10ab		57	TBe64	31.93±0.85bc		87	9601	22.48±1.46c	
28	OMe341	36.93±1.02bc		58	TBe65	31.93±0.85bc		88	PPRC-56	31.93±0.85bc	
29	OMe344	36.93 ±1.02bc		59	TBe75	30.55±1.69bc		89	Control	6.42±1.22f	
30	OMe366	36.93±1.02bc		60	OBe85	27.71±0.47c					

Table 23. Summary of percentage mortality of the FAW larvae treated with different fungal isolates of *Beauveria* and *Metarhizium* species in the laboratory screening experiment.

No.	Fungal Isolates	Mortality* ±SE 4DATAppl.	Mortality *±SE 6DATAppl.	Mortality* ±SE 8DATAppl.	Mortality* ±SE 10DATAppl.
1	AMe26	0±0.00e	30±0.00d	40±2.62ab	100%
2	AMe32	40±0.00a	60±0.00b	0±0.00f	100%
3	AMe34	20±2.71c	70±2.21a	10±3.66e	100%
4	AMe38	0±0.30e	30±4.22d	30±3.22c	70%
5	SMe162	40±0.00a	60±0.00b	0±0.00f	100%
6	SMe173	0±0.00e	30±4.22d	50±2.71a	100%
7	OMe242	20±2.71c	60±0.00b	20±3.66b	100%
8	OMe245	0±0.00e	20±2.21e	50±2.71a	70%
9	OMe291	30±2.21b	10±0.00f	50±2.71a	100%
10	OMe292	30±2.21b	50±1.92c	20±3.66d	100%
11	OMe296	10±0.00d	20±0.00e	50±2.71a	100%
12	OMe336	10±0.00d	10±0.00f	40±2.62ab	70%
13	ABe28	40±0.00a	60±0.00b	0±0.00f	100%
14	ABe38	40±0.00a	60±0.00b	0±0.00f	100%
15	ABe39	30±2.21b	20±2.21e	40±2.62ab	100%
16	ABe42	10±0.00d	30±4.22d	20±3.66d	70%
17	Un treated	0±0.00e	0±0.00f	0±0.00f	10%

* Values followed by the same letter in the same column do not differ significantly (P>0.05) according to DMRT
Note: 4 DATAppl = four days after treatment application.

Table 24. Percentage mortality and LT_{50} of FAW larvae at ten days after treatment with isolates of indigenous *Beauveria* and *Metarhizium* species at the rate of 1×10^8 conidia per milliliter.

Fungal isolates	**CM10 days**	**LT_{50} (days)**	**95% Fiducial limits**	**Intercept ± S. E**	**X2**	**P-value**
AMe26	100	9.21±0.44	9.62-10.30	-0.22±0.37	2.35	0.0001
AMe32	100	5.60±1.57	4.74-6.96	3.48±1.10	15.41	0.0001
AMe34	100	8.10±0.54	7.82-8.24	-0.11±0.43	1.16	0.0022
AMe38	70	12.29±0.37	11.89-12.03	-.54±11.23	1.34	0.0001
SMe162	100	5.60±1.57	4.74-6.96	3.48±1.10	15.41	0.0001
SMe173	100	9.21±0.44	9.62-10.30	-0.22±0.37	2.35	0.0001
OMe242	100	8.12±1.11	7.8-8.32	-2.59±0.62	9.08	0.0001
OMe245	70	12.29±0.37	11.89-12.03	-.54±11.23	1.34	0.0001
OMe291	100	9.12±0.59	9.04-10.62	2.11±0.44	18.01	0.0001
OMe292	100	8.10±0.45	7.82-8.42	-0.11±0.43	1.16	0.0022
OMe296	100	9.21±0.44	9.62-10.30	-0.22±0.37	2.35	0.0001
OMe336	70	12.29±0.37	11.89-12.03	-.54±11.23	1.34	0.0001
ABe28	100	5.60±1.57	4.74-6.69	3.48±1.10	15.41	0.0001
ABe38	100	5.60±1.57	4.74-6.96	3.48±1.10	15.41	0.0001
ABe39	100	9.12±0.59	10.22-9.87	2.11±0.44	18.01	0.0001
ABe42	70	11.29±0.37	11.89-12.03	-.54±11.23	1.34	0.0001
Control	10	22.4±4.32	16.42-28.1	-0.10±0.94	1.001	0.0242

Note: CM10 days = Cumulative Mortality (±SE) (10 days).

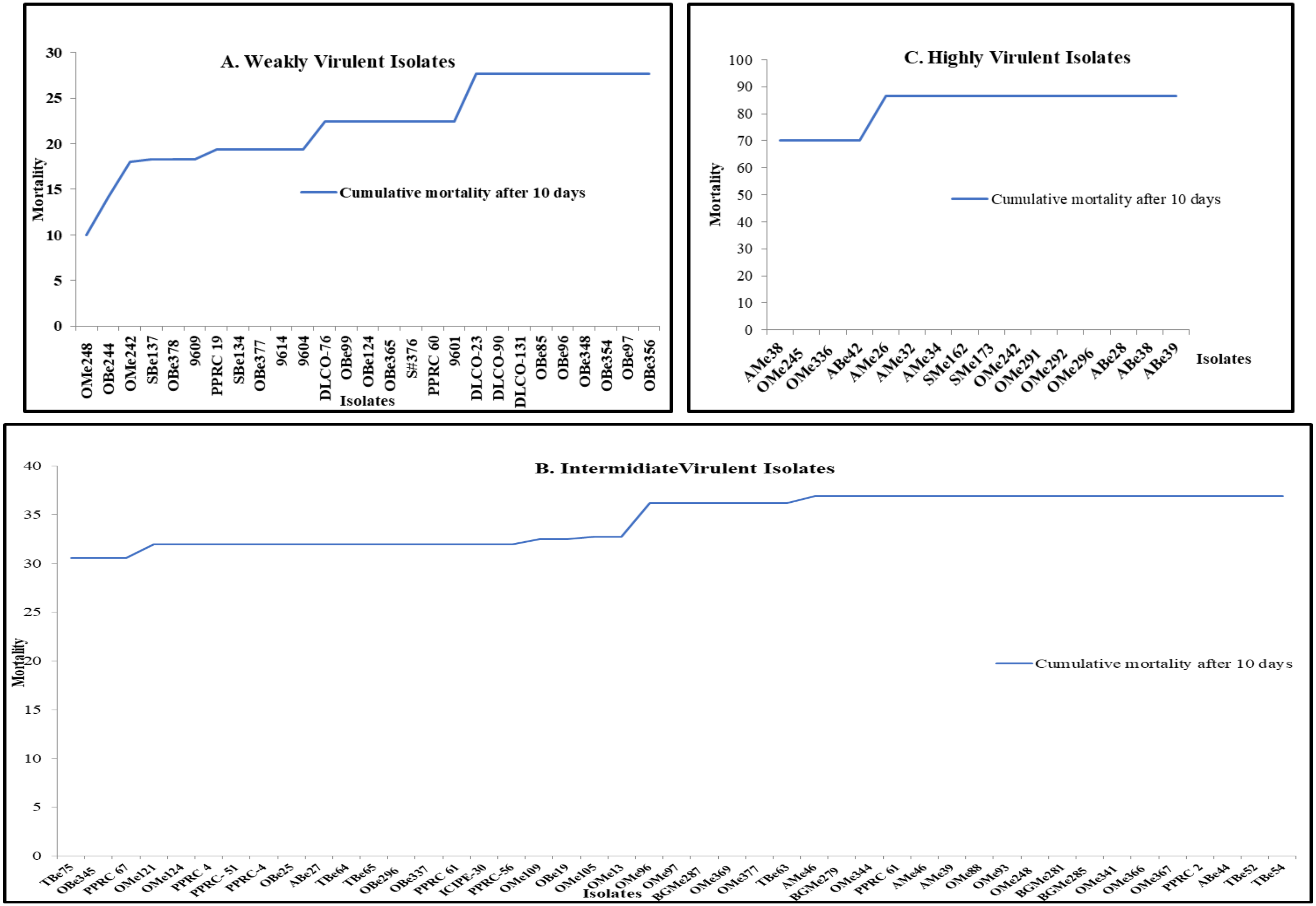

Figure 16. Percentage cumulative mortality of FAW larvae following treatment with different native Beauveria and Metarhizium isolates, (a) weakly, (b) intermediate and (c) highly virulent isolates

7.3.3. Pot Experiments Using Different Concentation Levels of Virlent Isolates

The study was conducted using the four most virulent native EPF isolates, namely AMe32, SMe162, ABe28, and ABe38 at four different levels of concentrations: 1 x 10 ^{6}, 1 x 10 ^{7}, 1x10^{8,} and 1x 10^{9} conidia per milliliter including untreated check indicated that there was a significant difference in mortality due to isolates inoculated at different concentrations (F=15.3804, DF=16, $p \leq 0.0001$) two days after treatment (F=7.0423, DF=16 $p \leq 0.0001$), four days after treatment (F= 6.025, DF=4, $p \leq 0.0001$), six days after treatment and (F=3.075, DF=16, $p \leq 0.0001$) and eight days after treatment application (F=2.023, DF=16, $p \leq 0.0001$) (Table 26). The lowest (10%) mortality was recorded in the untreated control, even eight days, after treatment application. This low record of mortality in the control was a natural death as it was observed that there was no growth of any one of the above four fungi when kept in moist chambers at 25±3 ^{o}C for about 3-6 days.

All the tested native EPF isolates caused significantly higher FAW larval mortality as their concentration and duration increased (Table 26). The native entomopathogenic fungal isolates ABe28 (i.e. 70.26, 82.42, 93, and 100%) and SMe162 (i.e., 70.26, 84.24, 96.24, and 100%) produced significantly superior mortality at the highest concentration (1 x 10^{9} conidia per milliliter) in two, four, six and eight days after treatment applications, respectively, as compared to the other isolates. Similarly, the fungal isolates ABe38, and AMe32 produced significant mortality at their higher concentration levels two, four, six, eight, and ten days after treatment applications (Table 26). Almost all of the tested native fungal isolates produced the lowest larval mortality in their respective lower concentrations. On average, the indigenous four fungal isolates AMe32, SMe162, ABe28, and ABe38 produced more than 84-100% larval population reduction over the first ten-day period.

The plant height and the percentage damage to the crop plant on the experimental plots were negatively correlated. The plants that had the least percentage damage values showed the tallest plant height and *vice versa*. Regarding the percentage damage of maize plants, eight days after treatment application, there were significant (P = 0.024, DF = 16, F = 1.43) differences among different concentrations and isolates. The least leaf damages 20.47 and 14.29% were exhibited due to isolate ABe28 and SMe162, respectively, at a concentration of 1*10^9 conidia per milliliter and for ABe38 and AMe32 26.19 and 24.16%, respectively, at 1*10^9 conidia milliliter, while the highest damage ranged from 74.14 to 78.75%, recorded on all control treatments (Table 26). However, there were highly significant ($P = 0.0001$, DF = 16, $F = 0.240$) differences in plant height eight days after treatment application. The tallest plant heights were 51.37 and 51.98 cm due to isolates ABe28 and SMe162, respectively, at 1*10^9 concentration and, due to the rest treatments, it ranged from 40.4 to 49.6 cm (Table 26).

Table 25. Percentage cumulative mortality of larval stage and percent damage of FAW treated with different conidial concentrations of indigenous *Beauveria* and *Metaharizium* species.

Isolates	Conc.(mL^{-1})	% Mortality (days)				IPH	FPH	%DA10D
		A4Ds	A6Ds	A8Ds	A10Ds			
AMe28	1×10^6	52.28c	56.61de	56.64de	58.31ef	40	49.1ab	37.44bcd
	1×10^7	60.22b	68.40b	75.83bc	86.67b	44	49.32ab	33.39cdef
	1×10^8	68.42a	80.20a	88.00a	98.00a	40	46.7cde	31.06bcde
	1×10^9	70.26a	82.42a	93.00a	100.00a	40	51.37a	20.47fg
ABe38	1×10^6	38.68efg	48.00e	58.42de	64.50de	42	46.7cde	40.6bc
	1×10^7	48.42cd	58.50cd	64.17c	76.62cd	42	43.6ef	34.56bcde
	1×10^8	62.00b	66.40b	78.00b	80.00bc	40	47.9abcd	34.56bcde
	1×10^9	64.00b	68.80b	81.00b	84.00bc	44	49.6ab	26.19def
SMe162	1×10^6	38.34fg	38.86f	44.2ef	56.38ef	40	49.1ab	39.11bc
	1×10^7	44.28cde	61.11cd	64.22c	88.42b	44	48.6b	37.73bcde
	1×10^8	62.88b	78.52ab	90.20a	94.00a	40	49.62ab	32.84bc
	1×10^9	70.26a	84.24a	96.24a	100.00a	40	51.98a	14.29g
AMe32	1×10^6	30.68g	34.35g	40.29g	48.33f	42	43.9def	39.17bc
	1×10^7	38.42def	46.42e	46.75ef	70.42d	42	47.0cde	34.28bcde
	1×10^8	60.62b	72.26b	78.80b	82.26bc	40	47.0cde	43.56b
	1×10^9	64.00b	76.42ab	82.20b	88.42bc	42	47.3bcde	24.16efg
Control		0.00h	10.00h	10.00h	20.00g	40	40.4f	78.75a
		0.00h	10.00h	10.00h	10.00g	42	41.8f	72.1a
		0.00h	10.00h	10.00h	10.00g	40	41.3f	74.14a
		0.00h	10.00h	10.00h	20.00g	40	40.4f	74.14a
LSD (0.05)							4.12	11.45
CV (%)							6.25	19.66
P-value							0.0001	0.024

Notes: A4Ds = %mortality after four days, IPH = Initial Plant Height, FPH = Final Plant Height, %DA10D = Percent Damage after 10 Days of Treatment application.

7.4. DISCUSSION

In the present study, the natural occurrence and/or distribution of EPFs in several maize-producing areas of Ethiopia were assessed. Accordingly, EPFs recovered from Amhara, Benishangul Gumuz. Oromia, SNNP and Tigray regions, were evaluated for their biocontrol potential or efficacies against the larvae of FAW. There was no EPF isolated from the soil samples from the Afar, Gambella and Somali areas. The occurrence and distribution of EPF are highly dependent on the soil's physico-chemical properties (Sharma *et al*., 2021). In this regard, the absence of EPF in Afar, Gambella and Somali might be related to the soil properties of the specific survey areas. Soil texture, pH or soil reaction, soil electrical conductivity, and temperature generally play a key role in the occurrence and distribution of EPF (Qayyum *et al*., 2021). The probability of occurrence of EPF is increased in sandy soils and clay soil (Tkaczuk *et al*., 2014). Entomopathogenic fungi were absent in silty clay, silty loam, silty clay loam, and clay loam soil types. In supporting this finding, several investigators have similarly reported that most of the Afar, Gambella and Somali,region soil property is clay loamy (Yitbarek *et al*., 2016; Gebremedhin *et al*., 2020; Yared *et al*., 2023).

Based on morphological descriptions, the isolated EPFs belonged to Beauveria species (52.17%) and Metarhizium species (47.83%) (Aysheshim *et al*., 2003; Tesfaye *et al*., 2012; Amha *et al*., 2021). Moreover, cadavers parasitized by *Beauveria* spp. showed white to cream colonies with irregular edges and powdery appearance, typical macroscopic traits, such as septate mycelium, conidiogenous cells size, conidia emerge and hyaline structure, whereas the cadavers parasitized by *Metarhizium* species were green and cylindrical in shape of conidia (Tesfaye *et al*., 2012; Bich *et al*., 2021). The *Beauveria* and *Metarhizium* species were the most commonly collected EPF species in Ethiopia and in agreement with studies conducted in Egypt, India, Indonesia, and Mexico (Abdullah *et al*., 2020; Amha *et al*., 2021; Gandarilla-Pacheco *et al*., 2021; Ahmed *et al*., 2022; Beemrote *et al*., 2023).

The EPF isolates AMe28, ABe38, SMe162, and AMe32 caused the highest mortality within 10 days. This indicates that these isolates have the potential to be used for the management of FAW. Similarly, *Beauveria* and *Metarhizium* species *exihibited or* demonstrated the highest mortality against different insect pests; for instance, Asian citrus psyllid, khapra beetle, stem borer, and

Pachnoda marginata (Belay *et al*., 2016; Tesfaye *et al*., 2020), *Spodoptera exigua* (Gandarilla-Pacheco *et al*., 2021), and storage insect pests (Mantzoukas *et al*., 2019). In addition, Gürlek *et al*. (2018) reported that *Beauveria* and*Metarhizium* spp. caused 83% mortality in codling moth larvae at a concentration of 1×10^8 conidia per milliliter. Ramanujam *et al*. (2020) also reported 88.30% mortality of FAW larvae due to treatment with *Beauveria bassiana* and *Metarhizium anisopliae* in both laboratory and field conditions.

The highly virulent isolates AMe28, ABe38, SMe162 and AMe32 caused 50% mortality of FAW larvae within 5.6 days, which was faster than the LT_{50} reported by Akutse *et al*. (2020) on *Spodoptera frugiperda* using the most effective *Metarhizium anisopliae* and *Beauveria bassiana*. Similarly, Ullah *et al*. (2022) reported significant variations in lethal time to 50% mortality using *B. bassiana* and *M. anisopliae* for control of peach-potato aphid, green peach aphid, and FAW. In the current study, the other group of highly virulent isolates AMe34, OMe242, OMe292, AMe26, SMe173, OMe291, OMe296, ABe39, AMe38, OBe245, OMe336, and AMe42, had LT_{50} of 8.1-12.29 days (Usman *et al*., 2021). Further evaluation of the four highly virulent EPF isolates on pot-planted maize showed that an increase in the concentration levels of the EPN increased mortality in FAW larvae. In line with this study, Idrees *et al*. (2023) studied different EPF isolates using different concentrations ($1*10^6$, $1*10^7$and $1*10^8$) against six instar FAW and they confirmed that EPF isolates caused the highest egg and larval mortality of 40, 70, and 85.6% at $1*10^6$, $1*10^7$, and $1*10^8$ conidia per milliliter, respectively. In similar other studies, several investigators reported that all fungal strains tested at different concentrations on dose/response experiment caused significant mortality on thrips, two-spotted spider mites and *Spodoptera litura* (Asi *et al*., 2013; Roza *et al*., 2017; Gelana *et al*., 2022). Similar results have also been reported by Ramanujam *et al*. (2020) and Montecalvo and Navasero (2021) using *Metarhizium* sp. isolates that caused the highest mortality against FAW under laboratory conditions.

7.5. CONCLUSIONS AND RECOMMENDATIONS

Entomopathogenic fungi have the potential as successful biological control, which is a cost-effective, environmentally safe alternative to chemicals for FAW management. In the current study, an attempt was undertaken to: (1) isolate native strains of the Entomopathogenic fungi *Metarhizium* and *Beauveria* spp. from soils of maize-producing areas of Ethiopia; and (2) find out the efficient strain(s) that could suppress FAW insect pest. Among 70 soil strains, 16 (AMe26, AMe32, AMe34, SMe162, SMe173, OMe242, OMe291, OMe292, OMe296, ABe28, AMe38, ABe39, ABe42, OMe336, OBe245 and AMe38) were found to be the most pathogenic to FAW. However, out of 16 isolates, four (ABe28, ABe38, SMe162, and AMe32) performed better than the other isolates in artificial medium growth character and their conidial production, exposure times, and 10 days cumulative larval mortality data on laboratory screening experiment.

In conclusion, based on the pot experiment, two of the isolates (SMe162 and ABe28) performed better than the other isolates at all concentrations, exposure times, and in cumulative mortality of the insects. Therefore, isolates SMe162 and ABe28 were found to be potential candidates for the development of microbial insecticide against FAW as a component of an integrated pest management (IPM) strategy. However, future studies need to focus on the molecular characterization of entomopathogenic fungi isolates, and field evaluation using appropriate formulation under high insect population conditions. Further researches are also needed on mass production characteristics and shelf-life studies of the identified isolates.

CHAPTER VIII

VIII. GENERAL SUMMARY, CONCLUSIONS AND FUTURE RESEARCH OUTLOOK

8.1. GENERAL SUMMARY

The findings of this current study showed that FAW occurred in different maize-producing areas in both on off-season and rainy seasons and caused significant losses of maize, but infestation and damage levels were higher in the off-season than in the rainy season. Fall armyworm was an economically important maize pest both in the off-season and in the rainy season, while the off-season infestation was slightly higher than in the rainy season.

The natural enemies of FAW were assessed from maize-growing areas of Ethiopia and documented the complex of native natural enemies of FAW in Ethiopia. Among the identified natural enemies of FAW, larval parasitoids (*Cotesia* sp.), egg parasitoids (*Trichogramma* sp.), and predators (ladybird beetle and earwig). The entomopathogenic nematodes (*Steinernema* species (13 in number) and *Heterorhabditis* species (15 in number) and Entomopathogenic fung (*Beauveria* species (33 in number) and *Metarhizium* species (36 in number)) were the most common bioagents. Moreover, the isolated EPF and EPN were evaluated against the FAW both at the laboratory and wirehouse.

Among the tested, four fungal *Beauveria* (i.e., ABe32 and ABe28) and *Metarhizium* (SMe162, AMe32) and nematode isolates [one isolate of *Steinernema* species (Aso-Tes-287) and three isolates of *Heterorhabditis* species (Am-Ger-Tes-74, Am-Adm-Tes-369, and Z9)] produced significantly superior or high larval mortality and were categorized as the most virulent isolates as compared to the other isolates. These most virulent isolates were further tested for their potential on FAW larvae on pot-planted maize plants that were already infested with second to third instars larvae under wirehouse conditions at AmARC.

The biology of the FAW insect pest was studied on different crops and the insect completed its life cycle on barley, chickpea, maize, sorghum and wheat host crops. However, variations were observed in duration to larval developmental period, pupal durations, and survival percentage of the pest larvae among the host plants. The host preference study using 23 different crop plants under no-choice and choice experiments showed variation in adult oviposition, larval feeding, larvae survival percentage, percent damage, and larval weight. As a result, some of the tested crop plants were not preferred by the FAW.

8.2. CONCLUSIONS

Our research results revealed that FAW was severely and widely distributed in almost all surveyed maize-producing areas in both off-season and rainy seasons. FAW Percentage of infested fields and percentage of infested plants varied from location to location within the agro-ecologies of Ethiopia, which indicated that weather conditions affected the distribution and maize damage levels by FAW. The biology and host preference study indicated that the FAW development, survival, and maize damage level varied in different host crops, whereas some of the tested crop plants were not preferred by the FAW. Even though the insect was believed to be a polyphagous pest, the current study indicated that host preference and damage level by FAW varied among the test crops.

In the present study, the indigenous EPF and EPN isolates identified from the maize-growing fields showed the potential for the management of FAW insect pest in an eco-friendly and sustainable manner and it could also be used as an alternative for the synthetic pesticides verifying their efficacies in the fields. The results indicated extremely fruitful prospects for insect pest management through efficient utilization of biological control at large and EPF and EPN in particular.

8.3. FUTURE RESEARCH OUTLOOK

- Variation in virulence among the identified few entomopathogenic fungal and nematode isolates indicated the requirement for larger collection and screening in future research activity
- Further studies are also needed to insight the key toxins which are responsible for affecting the physiological function of FAW and slowing down the feeding performance

- The infestation and damage level within the agro-ecologies may be correlated with the weather conditions in the study areas. To investigate the real reason, the generation number of FAW within different agroecology is crucial
- It is desirable to conduct pertinent molecular characterization studies on entomopathogenic nematode and fungal isolates and field evaluations using appropriate formulation
- Research works are needed on the development of techniques for mass production, shelf-life, appropriate formulation to keep or maintain the quality, and means of large-scale application and
- Faba bean, soya bean, haricot bean, garlic, and Ethiopian mustard were less affected by FAW in the current study and the mechanism responsible for slowing down the feeding performance of these crops /resisted needs to be further studied
- Modeling work is vital to predict FAW occurrence and to characterize the habitat suitability.

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APPENDICES

Appendix 1: Table 1. Details of FAW assessment point, natural enemies' collection areas, and soil samples collected for EPN and EPF isolation

SC	Regions	Zones	Districts	Locations	Altitude	Latitude	Longitude	Previous Crop	PI (%)
S#1	Afar	Afar	Asayta	Kere Guda	355	011.784951N	041.024159E	Maize	70
S#2	Afar	Afar	Asayta	Kere Guda	358	011.576139N	041.433147E	Tomato + Onion	30
S#3	Afar	Afar	Asayta	Berga	349	011.54167N	041.456469E	Maize	10
S#4	Afar	Afar	Dufti	Gurumudale korle	371	011.39544N	041.09089E	Fallow	80
S#5	Afar	Afar	Dufti	Kulimudali	367	011.39502N	041.09246E	Maize	60
S#6	Afar	Afar	Dufti	Gurumudale korle	365	011.38338N	041.09208E	Maize	30
S#7	Afar	Afar	Dufti	Korile	365	011.41122N	041.11304E	Fallow	20
S#8	Afar	Afar	Dufti	Kurule	361	011.38189N	041.15543E	Maize	100
S#9	Afar	Afar	Dufti	Kurule	360	011.38109N	041.15040E	Fallow	20
S#10	Oromia	East Showa	Fentale	Godofa Fate	959	08.871121N	039.920062E	Onion	0
S#11	Oromia	East Showa	Fentale	Dire Seden	992	08.857918N	039.995502E	Maize	40
S#12	Oromia	East Showa	Fentale	Dire Seden	1112	08.714276N	039.808277E	Maize + Onion	80
S#13	Oromia	East Showa	Boset	Huluko Horota	1033	08.844746N	039.796383E	Onion	10
S#14	Oromia	East Showa	Merti	Gologota	1175	08.633357N	039.754656E	Maize	0
S#15	Oromia	East Showa	Merti	Gologota, MAI	1204	08.613125N	039.712297E	Soyabean	0
S#16	Oromia	East Showa	Merti	Wersha kona	1213	08.563448N	039.668345E	Maize	10
S#17	Oromia	East Arsi	Jeju	Dore Huruta	1238	08.538750N	039.642873E	Maize	0
S#18	Oromia	East Showa	Boset	Doni 01	1259	08.512162N	039.564080E	Maize	20
S#19	Oromia	East Showa	Boset	Kona Degaga	1526	08.405732N	039.369478E	Paper+Tomato	10
S#20	Oromia	East Showa	Adama	Ulaga Melkaoba	1395	08.407085N	039.361730E	Tomato	0
S#21	Oromia	East Showa	Batu	Abine Germama	1637	07.956680N	038.718676E	Maize	0
S#22	Oromia	East Showa	Batu	Abune Germama	1604	07.955440N	038.714409E	Onion	30
S#23	Oromia	East Showa	Dugda Bora	Beroda	1644	8.549258N	039.204537E	Wheat	30
S#24	Oromia	East Showa	Dugda Bora	Ashewa	1602	08.395840N	039.012952E	Maize+Onion	0
S#25	Oromia	East Showa	Dugda Bora	Beri Malima	1602	08.420385N	039.024900E	Onion+Tomato	40
S#26	Amara	East Gojam	Awobele	Yekeyit CT	2461	10 15.432	037 55.829	Teff	0
S#27	Amara	East Gojam	Andid	Gud Alema	2358	10 15.595	037 54.397	Maize	10
S#28	Amara	East Gojam	Gozamen	Yebeka	2377	10 21.149	037 42.803	Cabbage	10

SC	Regions	Zones	Districts	Locations	Altitude	Latitude	Longitude	Previous Crop	PI (%)
S#29	Amara	East Gojam	Gozamen	Yewela	2170	10 23.591	037 34.617	Teff	0
S#30	Amara	East Gojam	Gozamen	Lay Addis	2213	10 22.283	037 36.301	Teff	20
S#31	Amara	West Gojam	Dembecha	Angot Yedegra	1783	10 37.701	037 24.775	Maize	0
S#32	Amara	West Gojam	Dembecha	Wengi	1773	10 37.703	037 24.181	Maize	30
S#33	Amara	West Gojam	Jabi Tehinan	Hod Anshi	1832	10 40.702	037 19.535	Maize	0
S#34	Amara	West Gojam	Jabi Tehinan	B.Sh.Ag.I	1697	10 35.305	037 10. 307	Maize	10
S#35	Amara	Awi Zone	Banja	Askuna Abo	1955	11 00.250	036 41.751	Potato	20
S#36	Amara	Awi Zone	Banja	Askuna Abo	1976	11 00.117	036 64.831	Onion	10
S#37	Amara	Awi Zone	Banja	Askuna Abo	2012	10 59.127	036 44.070	Onion	30
S#38	Amara	Awi Zone	Banja	Zigo Marta	2186	10 57.589	036 46.867	Potato	10
S#39	Amara	Awi Zone	Banja	Bida Jegola	2502	10 56.422	036 53.010	Potato	0
S#40	Amara	Bahidar Zuriya	Mecha	Abo meski	1944	11 23.604	037 04.500	Maize	70
S#41	Amara	Bahidar Zuriya	Mecha	Shona block	1994	11 24.144	037 07.248	Maize	10
S#42	Amara	Bahidar Zuriya	Mecha	Kudme	1984	11 23.845	037 06.809	Maize	60
S#43	Amara	Bahidar Zuriya	Mecha	Kudm Georgise	1974	11 23.287	037 06.584	Maize	20
S#44	Amara	Bahidar Zuriya	Mecha	Enguti	1991	11 25.296	037 07.466	Maize	20
S#45	Amara	Bahidar Zuriya	Mecha	Enguti	1962	11 26.216	037 07.195	Maize	10
S#46	Amara	Bahidar Zuriya	Mecha	Kolela	1931	11 27.588	037 07.286	Finger millet	10
S#47	Amara	Bahidar Zuriya	Mecha	Keldebi	1222	11 28.437	037 06.875	Maize	20
S#48	Amara	South Gonder	Fogera	Shena	1783	11 50.578	037 38.271	Fallow	80
S#49	Amara	South Gonder	Fogera	Awa Kokit	1790	11 59.042	037 42.591	Maize	20
S#50	Amara	South Gonder	Libo Kemkem	Selkisana Ginaza	1876	12 05.937	037 45.667	Maize	0
S#51	Amara	North Gonder	Adi Arkay	Aba Mar	1216	13 29.950	038 07.611	Fallow	30
S#52	Tigray	Tigray	Tselemti	Medihaniyalem	1171	13 31.468	038 06.879	Finger millet	30
S#53	Tigray	Tigray	Tselemti	Boreke	1160	13 31.889	038 06.465	Maize	40
S#54	Tigray	Tigray	Laelay Adiabo	Deba	1769	14 17.527	038 12.055	Fallow	30
S#55	Tigray	Tigray	Laelay Adiabo	Deba	1763	14 17.731	038 11.846	Maize	30
S#56	Tigray	Tigray	Laelay Adiabo	Adey Abagi	1796	14 16.647	038 11.423	Maize	0
S#57	Tigray	Tigray	Laelay Adiabo	Adey Abagi	1799	14 16.258	038 11.688	Teff	0
S#58	Tigray	Tigray	Tahtay Adiabo	Mey Adrasha	1914	14 06.618	038 19.400	Onion + maize	10
S#59	Tigray	Tigray	Tahtay Koraro	Adi Gidad	1972	14 05.127	038 21.776	Teff	20
S#60	Tigray	Tigray	Tahtay Koraro	Beles	1937	14 03.977	038 23.508	Teff	20
S#61	Tigray	Tigray	Laelay Koraro	Ademenabir	1959	14 03.709	038 25.857	Teff	20
S#62	Tigray	Tigray	May Zegiray	Mahasiye	2136	14 07.214	038 37.065	Onion	0
S#63	Tigray	Tigray	Laelay Maichew	Dura	2050	14 06.602	038 39.238	Teff	20
S#64	Tigray	Tigray	Laelay Maichew	Dura	2040	14 06.444	038 39.894	Teff	0

SC	Regions	Zones	Districts	Locations	Altitude	Latitude	Longitude	Previous Crop	PI (%)
S#65	Tigray	Tigray	Wukiro	Korir	1978	13 45.570	039 35.838	Teff	20
S#66	Tigray	Tigray	Wukiro	Agulay	2001	13 41.238	039 34.733	Teff	0
S#67	Tigray	Tigray	Hintale Wojerat	Wezada	2132	13 04.599	039 32.000	Onion	10
S#68	Tigray	Tigray	Hintale Wojerat	Wezada	2264	13 02.935	039 33.335	Teff	10
S#69	Tigray	Tigray	Adeigudem	Admesino	2482	13 00.950	039 35.059	Teff	0
S#70	Tigray	Tigray	Adeigudem	Tsehafti	2092	12 58.073	039 40.147	Teff	5
S#71	Tigray	Tigray	Raya Azebo	Hawleti	1742	12 51.158	039 42.195	Teff	0
S#72	Tigray	Tigray	Raya Azebo	Abadu	1758	12 47.195	039 38.482	Teff	0
S#73	Tigray	Tigray	Raya Azebo	Hujira	1614	12 39.933	039 38.838	Teff	0
S#74	Tigray	Tigray	Alamata	Gerjeli	1460	12 28.499	039 36.289	Sorghum	0
S#75	Tigray	Tigray	Alamata	Ayer Marfiya	1511	12 23.316	039 34.106	Teff	0
S#76	Oromia	West Showa	Bako	Denbi Dima	1643	09 06.559	037 05.834	Maize	40
S#77	Oromia	East Wollega	Gudeya Billa	Hagllo Gidami	1873	09 14.324	037 02.156	Teff	30
S#78	Oromia	East Wollega	Gudeya Billa	Darbes	1896	09 15.834	037 01.716	Maize	20
S#79	Oromia	East Wollega	Gobu Seyo	Sombo Kejo	1613	09 07.697	037 02.858	Tomato + cabbage	10
S#80	Oromia	East Wollega	Gobu Seyo	Angobo Dembi	1757	09 06.508	036 59.459	Teff	30
S#81	Oromia	East Wollega	Sibu Sire	Cheri Jarsso	1750	09 02.864	036 48.509	Maize	0
S#82	Oromia	East Wollega	Wayu Tuka	Gute Badiya	1862	09 01.763	036 38.324	Potato	30
S#83	Oromia	East Wollega	Nekemte	Gari	2089	09 04.820	036 35.621	Potato	5
S#84	Oromia	East Wollega	Nekemte	09 Kebele	2054	09 04.982	036 31.731	Maize	0
S#85	Oromia	East Wollega	Diga	Jirata	2194	09 02.169	036 29.201	Maize	0
S#86	Oromia	East Wollega	Leka Dulecha	Ale Kewissa	2208	08 57.841	036 29.110	Maize	10
S#87	Oromia	Buno Bedele	Bedele	Kere Lillo	1856	08 32.939	036 22.304	Tomato	20
S#88	Oromia	Buno Bedele	Bedele	Bashure	1855	08 31.809	036 21.989	Tomato+paper	0
S#89	Oromia	Buno Bedele	Bedele	Digaja	1882	08 29.506	036 21.876	Maize	5
S#90	Oromia	Illubabora	Ale	Segi	1808	08 07.023	035 32.116	Maize	0
S#91	Oromia	Illubabora	Ale	Segi Baki	1908	08 06.083	035 31.530	Maize	20
S#92	Oromia	Illubabora	Ale	Segita Geta	1923	08 02.086	035 31.353	Maize	10
S#93	Oromia	Illubabora	Didu	Gordomo	1791	07 59.103	035 32.199	Maize	10
S#94	Oromia	Illubabora	Didu	Gordomo Badiya	1725	07 57.268	035 33.197	Maize	10
S#95	Oromia	Illubabora	Didu	Dabeno Gute	1724	07 56.069	035 35.269	Maize	10
S#96	Oromia	Illubabora	Didu	Lallo Megalla	1724	07 54.661	035 36.099	Maize	0
S#97	Oromia	Illubabora	Ale	Gumero	1684	08 09.030	035 30.124	Maize	60
S#98	Oromia	Illubabora	Hallu	Amuma	1652	08 09.564	035 24.175	Maize	20
S#99	Oromia	Illubabora	Hurumu	Hurumu	1767	08 20.311	035 42.246	Fallow	5
S#100	Oromia	Illubabora	Hurumu	Gaba	1557	08 19.792	035 45.352	Onion + tomato	30

SC	Regions	Zones	Districts	Locations	Altitude	Latitude	Longitude	Previous Crop	PI (%)
S#101	Oromia	Illubabora	Yayu	Mesengo	1558	08 20.563	035 48.181	Maize	5
S#102	Oromia	Illubabora	Yayu	Bondo Megala	1633	08 21.143	035 50.981	Maize	40
S#103	Oromia	Illubabora	Yayu	Wetete	1606	08 22.446	035 56.136	Maize	0
S#104	Oromia	Buno Bedele	Chora	Gule	1873	08 21.854	036 08.719	Maize	60
S#105	Oromia	Buno Bedele	Chora	Abdela Badiya	1870	08 21.462	036 13.384	Maize	60
S#106	Oromia	Buno Bedele	Gechi	Gissa	1932	08 22.751	036 24.345	Maize	20
S#107	Oromia	Buno Bedele	Gechi	Gito	2133	08 18.688	036 26.408	Maize	0
S#108	Oromia	Buno Bedele	Gechi	Bido Jire	2161	08 14.100	036 26.852	Cabbage	0
S#109	Oromia	Buno Bedele	Didessa	Yembro Haro	2177	08 09.191	036 28.052	Potato	0
S#110	Oromia	Jima	Guma	Afo Yachi	1711	07 58.368	036 30.468	Maize	65
S#111	Oromia	Jima	Goma	Gechi Hureche	1721	07 57.051	036 30.388	potato + paper	20
S#112	Oromia	Jima	Goma	Gembe	1570	07 50.200	036 40.514	Maize	0
S#113	Oromia	Jima	Goma	Kesso Hiti	1542	07 51.420	036 39.200	Maize	20
S#114	Oromia	Jima	Goma	Bulbulo	1606	07 51.206	036 37.138	Maize	20
S#115	Oromia	Jima	Goma	01 kebele	1647	07 50.970	036 35.655	Tomato + onion	20
S#116	Oromia	Jima	Seka Chekorsa	Biyo Kechema	1802	07 38.618	036 46.889	Maize	20
S#117	Oromia	Jima	Seka Chekorsa	Biyo Kechema	1767	07 37.531	036 45.598	Teff	0
S#118	Oromia	Jima	Seka Chekorsa	Shashemne	1904	07 34.012	036 39.792	Maize	30
S#119	Oromia	Jima	Shebe Sombo	Sombo	1924	07 33.565	036 37.549	Maize	0
S#120	Oromia	Jima	Shebe Sombo	Soba Wala	1718	07 29.478	036 29.050	Teff	20
S#121	Oromia	Jima	Shebe Sombo	Alo Sebaka	1366	07 28.462	036 25.899	Maize	10
S#122	Oromia	Jima	Kerssa	Kitibile	1685	07 42.010	037 04.422	Maize	10
S#123	Oromia	Jima	Kerssa	Bela Wajo	1689	07 44.692	037 10.312	Maize	60
S#124	Oromia	Jima	Kerssa	Etimbile	1694	07 42.854	037 05.128	Potato	20
S#125	SNNP	Gamo	Arbaminch	Ganta Kanchama	1237	05 59.159	037 32.416	Cotton	30
S#126	SNNP	Gamo	Arbaminch	Ganta Kanchama	1132	05 36.528	037 31.691	Maize	10
S#127	SNNP	Gamo	Arbaminch	Ganta Kanchama	1113	05 54.005	037 30.120	Maize	20
S#128	SNNP	Gamo	Arbaminch	Shele M.L. farm	1134	05 52.630	037 27.818	Maize	10
S#129	SNNP	Gamo	Arbaminch	Zeyise Elgo	1114	05 48.571	037 27.860	Maize	10
S#130	SNNP	Gamo	Arbaminch	Wezeka Zeeyise	1131	05 46.494	037 26.653	Fallow	10
S#131	SNNP	Segen	Derashe	Holte	1188	05 42.953	037 26.018	Sorghum	20
S#132	SNNP	Segen	Derashe	Holte	1138	05 39.318	037 27.232	Maize	0
S#133	SNNP	Segen	Derashe	Shelele	1237	05 35.350	037 26.655	Teff	0
S#134	SNNP	Segen	Derashe	Gato	1269	05 32.828	037 25.022	Maize	5
S#135	SNNP	Konso	Konso	Choro	1227	05 29.541	037 26.076	Maize	0
S#136	SNNP	Konso	Konso	Baede	1204	05 28.251	037 25.985	Maize	0

SC	Regions	Zones	Districts	Locations	Altitude	Latitude	Longitude	Previous Crop	PI (%)
S#137	SNNP	Konso	Konso	Teshemale Ateya	1190	05 27.195	037 26.294	Sorghum	0
S#138	SNNP	Konso	Konso	Dokotu	1300	05 23.235	037 26.708	Maize	0
S#139	SNNP	Konso	Konso	Sorobo	1462	05 22.625	037 22.611	Maize	0
S#140	SNNP	Konso	Konso	Gocha	1571	05 20.474	037 23.654	Maize	0
S#141	SNNP	Gamo	Arbaminch	Lemat school	1228	06 03.132	037 33.422	Maize	0
S#142	SNNP	Gamo	West Abaya	Omo Lante	1184	06 09.033	037 39.924	Banana	30
S#143	SNNP	Gamo	West Abaya	Omo Lante	1177	06 09.652	037 40.035	Maize	40
S#144	SNNP	Welayta Sodo	Abela Abeya	Abala Mareka	1270	06 34.593	037 49.661	Onion	60
S#145	SNNP	Welayta Sodo	Humbo	Gututu Larena	1805	06 45.171	037 46.328	Maize	40
S#146	SNNP	Welayta Sodo	Sodo Zuriya	Humbo Larena	1800	06 46.934	037 45.902	Maize	30
S#147	SNNP	Gofa	Kucha	Kucha Gale	1279	06 31.275	037 31.055	Maize	5
S#148	SNNP	Gofa	Kucha	Wezete	1339	06 29.241	037 28.742	Haricot bean	10
S#149	SNNP	Gofa	Kucha	Baso	1470	06 27.360	037 26.459	Maize	0
S#150	SNNP	Gofa	Kucha	Baso	1616	06 26.758	037 23.722	Maize	10
S#151	SNNP	Gofa	Kucha	Morka	1458	06 25.323	037 21.784	Maize	0
S#152	SNNP	Gofa	Denba Gofa	Suka	1299	06 18.936	036 54.650	Maize	10
S#153	SNNP	Gofa	Denba Gofa	Awande	1234	06 21.507	036 57.797	Maize	10
S#154	SNNP	Gofa	Denba Gofa	Zala Selo	1134	06 22.816	037 00.219	Maize	20
S#155	SNNP	Gofa	Zala	Kawisa	1083	06 23.785	037 05.871	Maize	10
S#156	SNNP	Gofa	Zala	Kawisa	1002	06 24.438	037 08.223	Maize	0
S#157	SNNP	Gofa	Zala	Wageshu	980.2	06 25.093	037 10.357	Maize	0
S#158	SNNP	Gofa	Kucha	Giya Kor	1015	06 26.028	037 16.089	Teff	5
S#159	SNNP	Gofa	Kucha	Morka	1202	06 25.811	037 17.906	Maize	0
S#160	SNNP	Welayta Sodo	Sodo Zuriya	Wareza Girira	1838	06 49.633	037 43.036	Maize	20
S#161	SNNP	Welayta Sodo	Sodo Zuriya	Haba	1823	06 47.170	037 40.884	Maize	20
S#162	SNNP	Welayta Sodo	Gesuba	Wachiga Esho	1880	06 43.984	037 38.415	Maize	30
S#163	SNNP	Welayta Sodo	Sodo Zuriya	Warza Lasho	1965	06 54.697	037 43.832	Maize	20
S#164	SNNP	Welayta Sodo	Boloso Sore	Gurumu Koyisha	1898	06 58.800	037 44.719	Maize	30
S#165	SNNP	Welayta Sodo	Boloso Sore	Dola	1844	07 00.162	037 44.033	Haricot bean	20
S#166	SNNP	Welayta Sodo	Boloso Sore	Areka 02	1690	07 06.193	037 42.774	Cabbage	30
S#167	SNNP	Welayta Sodo	Damot Gale	Fate	2134	06 55.505	037 49.894	Maize	20
S#168	SNNP	Welayta Sodo	Damot Gale	Fate	2106	06 56.027	037 50.271	Potato	5
S#169	SNNP	Welayta Sodo	Damot Gale	Agaze Doge	1974	06 58.381	037 52.360	Maize	20
S#170	SNNP	Welayta Sodo	Damot Gale	Abota Ulto	1906	07 01.802	037 53.906	Teff	20
S#171	SNNP	Welayta Sodo	Damot Gale	Buge	1864	07 03.096	037 55.833	Haricot bean	5
S#172	SNNP	Welayta Sodo	Damot Gale	Buge	1911	07 05.761	037 56.829	Maize	5
S#173	SNNP	Hadiya	Bada Wacho	Wera Lolo	2017	07 10.483	037 57.456	Teff	5

SC	Regions	Zones	Districts	Locations	Altitude	Latitude	Longitude	Previous Crop	PI (%)
S#174	SNNP	Hadiya	Bada Wacho	Hultegna Chafa	1779	07 13.218	038 01.528	Haricot bean	0
S#175	Gambella	Agnuwak	Abobo	Mender-7	540	08. 07 324	034. 34 859	Maize	10
S#176	Gambella	Agnuwak	Abobo	Mender-7	539	08. 03 667	034. 35 098	Maize	0
S#177	Gambella	Agnuwak	Abobo	Mender-7	502	08. 00 989	034. 34 324	Maize	40
S#178	Gambella	Agnuwak	Abobo	Mender-13	475	07. 58 178	034. 33 474	Maize	20
S#179	Gambella	Agnuwak	Abobo	Mender-13	478	07. 57 560	034. 33 750	Maize	20
S#180	Gambella	Agnuwak	Abobo	Mender-13	472	07. 55 973	034. 33 298	Sorghum	20
S#181	Gambella	Agnuwak	Abobo	Mender-17	460	07. 53 215	034. 30 492	Maize	10
S#182	Gambella	Agnuwak	Abol	Pinykew	430	08. 14 514	034. 29 466	Maize	10
S#183	Gambella	Agnuwak	Abol	Kebele-02	472	08. 15 990	034. 27 728	Maize	0
S#184	Gambella	Agnuwak	Abol	Kebele-01	452	08. 15 949	034. 27 106	Maize	0
S#185	Gambella	Agnuwak	Abol	Pinykew	456	08. 17 021	034. 24 008	Maize	0
S#186	Gambella	Nuer	Itang	Pulkot	437	08. 15 960	034. 15 673	Maize	0
S#187	Gambella	Nuer	Itang	Pulkot	435	08. 16 565	034. 14 016	Maize	40
S#188	Gambella	Nuer	Itang	Wathgach	434	08. 17 589	034. 10 583	Maize	10
S#189	Gambella	Nuer	Itang	Wathgach	427	08. 18 039	034. 08 798	Maize	10
S#190	Gambella	Nuer	Itang	Ware	421	08. 18 400	034. 07 256	Maize	40
S#191	Gambella	Nuer	Lare	Kuthunys	417	08. 19 319	034. 00 129	Maize	10
S#192	Gambella	Nuer	Lare	Koatmanchung	415	08. 19 737	033. 58 536	Maize	20
S#193	Gambella	Nuer	Lare	Teluth	414	08. 20 581	033. 57 367	Maize	20
S#194	Gambella	Nuer	Lare	Bilinkun	420	08. 22 685	033. 58 571	Maize	10
S#195	Gambella	Gambella	Gambella	New land	447	08. 14 472	034. 36 207	Maize	0
S#196	Gambella	Gambella	Gambella	New university	457	08. 14 452	034. 36 877	Maize	0
S#197	Gambella	Gambella	Gambella	Kebele-01	455	08. 14 317	034.37 232	Maize	0
S#198	Oromia	Jimma	Gumay	Lima Tawo	1686	08. 00 884	036. 28 318	Maize	30
S#199	Oromia	Jimma	Gumay	Yefo Yachi	1660	07. 59 453	036. 30 641	Teff	30
S#200	Oromia	Jimma	Gumay	Dabu	1767	07. 55 841	036. 30 790	Maize	10
S#201	Oromia	Jimma	Gomma	Kilole	1664	07. 54 529	036. 33 253	Maize	10
S#202	Oromia	Jimma	Gomma	Bubulo	1583	07. 51 580	036. 38 625	Maize	0
S#203	Oromia	Jimma	Seka Chekorsa	Kofe	1790	07. 38 948	036. 47 517	Maize	80
S#204	Oromia	Jimma	Seka Chekorsa	Buyo Kechema	1782	07. 37 139	036. 45 664	Maize	10
S#205	Oromia	Jimma	Seka Chekorsa	Gibe Boso	1880	07. 34 980	036. 42 178	Maize	30
S#206	Oromia	Jimma	Seka Chekorsa	Shashemne	1903	07. 33 975	036. 39 654	Fallow	30
S#207	Oromia	Jimma	Seka Chekorsa	Shashemne	1917	07. 33 901	036. 38 501	Maize	30
S#208	Oromia	Jimma	Seka Chekorsa	Atiro Gefere	2001	07. 33 511	036. 36 332	Sorghum	0
S#209	Oromia	Jimma	Shebe Sombo	Halo Sebaka	1787	07. 29 812	036. 29 578	Teff	5
S#210	Oromia	Jimma	Shebe Sombo	Sebak Wala	1680	07. 29 200	036. 28 799	Haricot bean	20

SC	Regions	Zones	Districts	Locations	Altitude	Latitude	Longitude	Previous Crop	PI (%)
S#211	Oromia	Jimma	Shebe Sombo	Halo sebaka	1445	07. 28 807	036. 26 889	Sorghum	5
S#212	Oromia	Jimma	Shebe Sombo	Kishe	1388	07. 28 104	036. 25 455	Sorghum	20
S#213	Oromia	Jimma	Kersa	Merewa	1807	07. 40 754	036. 54 104	Maize	20
S#214	Oromia	Jimma	Kersa	Merewa	1810	07. 41 703	036. 54 955	Maize	10
S#215	Oromia	Jimma	Kersa	Babo Sete	1812	07. 42 299	036. 57 454	Maize	20
S#216	Oromia	Jimma	Kersa	Tikur Balto	1778	07. 42 749	037. 00 182	Maize	5
S#217	Oromia	Jimma	Kersa	Kitimbile	1734	07. 42 621	036. 02 420	Sorghum	5
S#218	Oromia	Jimma	Mana	Gudeta Bula	1992	07. 43 750	036. 46 795	Maize	5
S#219	Oromia	Jimma	Mana	Gube Muleta	2005	07. 44 299	036. 46 377	Maize	5
S#220	Oromia	Jimma	Mana	Somodo	1989	07. 44 668	036. 47 532	Maize	10
S#221	Oromia	Jimma	Mana	Somodo	1988	07. 46 321	036. 47 999	Maize	10
S#222	Oromia	Buno Bedele	Mana	Belida	1863	07. 48 457	036. 47 784	Maize	5
S#223	Oromia	Buno Bedele	Didesa	Sobo	1519	08. 02 859	036. 28 629	Teff	5
S#224	Oromia	Buno Bedele	Didesa	Sobo	1825	08. 03 928	036. 27 421	Teff	0
S#225	Oromia	Buno Bedele	Didesa	Saso	2067	08. 06 290	036. 27 529	Teff	5
S#226	Oromia	Buno Bedele	Didesa	Yembero	2194	08. 09 357	036. 28 367	Teff	20
S#227	Oromia	Buno Bedele	Gechi	Bido	2127	08. 12 776	036. 26 736	Teff	0
S#228	Oromia	Buno Bedele	Gechi	Keko	2170	08. 17 815	036. 26 941	Teff	5
S#229	Oromia	Buno Bedele	Gechi	Jisa	1925	08. 22 818	036. 24 318	Maize	20
S#230	Oromiya	East Wollega	Guto Gida	Ambeleta	1560	09. 16 289	036. 31 089	Groundnut	10
S#231	Oromiya	East Wollega	Guto Gida	Mede Jalela	1380	09. 18 901	036. 30 665	Groundnut	10
S#232	Oromiya	East Wollega	Guto Gida	Uke	1368	09. 22 078	036. 31 024	Maize	5
S#233	Oromiya	East Wollega	Guto Gida	Uke Badiya	1364	09. 21 947	036. 31 074	Maize	5
S#234	Oromiya	East Wollega	Guto Gida	Uke Badiya	1342	09. 24 164	036. 32 317	Soyabean	45
S#235	Oromiya	East Wollega	Guto Gida	Meti	1325	09. 26 163	036. 34 354	Groundnut	20
S#236	Oromiya	East Wollega	Guto Gida	Horo	1366	09. 27 430	036. 38 426	Maize	40
S#237	Oromiya	East Wollega	Guto Gida	Meti	1320	09. 26 143	036. 34 587	Sesame	5
S#238	Oromiya	East Wollega	Guto Gida	Jirata	2224	09. 01 563	036. 28 897	Maize	10
S#239	Oromiya	East Wollega	Diga	Gudisa	2057	09. 02 121	036. 24.495	Maize	40
S#240	Oromiya	East Wollega	Diga	Bikila	1697	09. 04 291	036. 22 200	Maize	20
S#241	Oromiya	East Wollega	Diga	Bikila	1436	09. 03 077	036. 19 296	Maize	20
S#242	Oromiya	East Wollega	Diga	Oda	1330	09. 02 047	036. 13 607	Groundnut	0
S#243	Benishangul	East Wollega	Belo Gigafo	Shene	1187	09. 02 196	036. 08 741	Maize	0
S#244	Oromiya	West Wollega	Gimbi	Shene	1252	09. 03 677	036. 05 921	Groundnut	5
S#245	Oromiya	West Wollega	Gimbi	Abasena	1647	09. 01 448	035. 59 339	Maize	0
S#246	Oromiya	West Wollega	Gimbi	Lelisa Yesus	1877	09. 11 532	035. 46 766	Maize	10

SC	Regions	Zones	Districts	Locations	Altitude	Latitude	Longitude	Previous Crop	PI (%)
S#247	Oromiya	West Wollega	Lalo Asabi	Gerjo Siben	1839	09. 12 308	035. 43 572	Maize	5
S#248	Oromiya	West Wollega	Lalo Asabi	Aroji Harowa	1886	09. 13 991	035. 41 971	Maize	40
S#249	Oromiya	West Wollega	Boji Dirmeji	Bobine	1743	09. 16 845	035. 39 287	Maize	20
S#250	Oromiya	West Wollega	Boji Dirmeji	Leta Bobine	1907	09. 18 390	035. 37 299	Maize	30
S#251	Oromiya	West Wollega	Boji Dirmeji	Bikiltu Dila	1903	09. 26 158	035. 34 394	Maize	20
S#252	Oromiya	West Wollega	Nejo	Kumbi	1879	09. 28 944	035.31 663	Maize	30
S#253	Oromiya	West Wollega	Nejo	Woligelte	1896	09. 31 643	035. 29 220	Maize	0
S#254	Oromiya	West Wollega	Nejo	Kote Genasi	1908	09. 33 647	035. 25 623	Maize	5
S#255	Oromiya	West Wollega	Leta Sibu	Gori keble 01	1778	09. 36 990	035. 20 265	Maize	20
S#256	Oromiya	West Wollega	Leta Sibu	Gori keble 02	1647	09. 38 196	035. 19 146	Maize	20
S#257	Oromiya	West Wollega	kiltu Kara	Dandi Gudi	1637	09. 39 655	035. 18 754	Maize	20
S#258	Oromiya	West Wollega	kiltu Kara	Kiltu kara Badiya	1579	09. 40 943	035. 16 912	Maize	30
S#259	Oromiya	West Wollega	kiltu Kara	Bato Dale	1611	09. 40 647	035. 14 697	Maize	20
S#260	Oromiya	West Wollega	Mene Sibu	Biyo Sechi	1577	09. 42 458	035. 12 234	Maize	5
S#261	Oromiya	West Wollega	Mene Sibu	Rega Sechi	1623	09. 44 174	035. 10 009	Fallow	10
S#262	Oromiya	West Wollega	Mene Sibu	Kersa	1750	09. 49 655	035. 04 662	Maize	20
S#263	Benishangul	Asosa	Banbasi	Dabus	1396	09. 45 952	034. 48 002	Maize	20
S#264	Benishangul	Asosa	Banbasi	Mutsa	1440	09. 44 716	034. 45 393	Maize	20
S#265	Benishangul	Asosa	Banbasi	Mender-44	1424	09. 47 737	034. 42 857	Groundnut	20
S#266	Benishangul	Asosa	Banbasi	Mender-46	1444	09. 50 859	034. 41 354	Maize	0
S#267	Benishangul	Asosa	Banbasi	Mender-16	1426	09.56 752	034. 39 751	Soya bean	0
S#268	Benishangul	Asosa	Banbasi	Shekole	1253	10. 22. 909	034. 35 822	Maize	20
S#269	Benishangul	Asosa	Homosha	Shula	1328	10. 24 636	034. 34 073	Maize	5
S#270	Benishangul	Asosa	Homosha	Bemdon	1448	10. 27 574	034. 32 368	Maize	20
S#271	Benishangul	Asosa	Homosha	Gima	1720	10. 21 347	034. 37 156	Maize	30
S#272	Benishangul	Asosa	Homosha	Alegala	1387	10. 19 014	034. 38 479	Maize	30
S#273	Benishangul	Asosa	Homosha	Dare Selam	1382	10. 19 537	034. 40. 275	Maize	5
S#274	Benishangul	Asosa	Asosa	Asosa 01	1567	10. 03 278	034. 31 629	Maize	5
S#275	Benishangul	Asosa	Asosa	Mengele 32	1513	10. 01 870	034. 31 343	Teff + Haricot bean	20
S#276	Benishangul	Asosa	Asosa	Mengele 33	1504	09. 59 532	034. 31 546	Maize	20
S#277	Benishangul	Asosa	Asosa	Mengele 37	1515	09. 57 272	034. 31 649	Haricot bean + Maize	10
S#278	Benishangul	Asosa	Asosa	Shedriya	1548	10. 02 339	034. 33 816	Maize	20
S#279	Benishangul	Asosa	Asosa	Amba 14	1509	10. 00 783	034. 36 289	Maize	10
S#280	Benishangul	Asosa	Asosa	Gambella	1484	09. 59 836	034. 37 368	Maize	5
S#281	Benishangul	Asosa	Asosa	Amba 16	1386	09. 57 100	034. 39 446	Maize	10

SC	Regions	Zones	Districts	Locations	Altitude	Latitude	Longitude	Previous Crop	PI (%)
S#282	Benishangul	Asosa	Banbasi	Bambasi Badiya	1482	09. 44 004	034. 43 268	Maize	40
S#283	Benishangul	Asosa	Banbasi	Keshimando	1455	09. 41 635	034. 42 628	Maize	10
S#284	Benishangul	Asosa	Banbasi	Keshmando 2	1388	09. 36 652	034. 41 507	Maize	10
S#285	Benishangul	Asosa	Asosa	Amba 5	1611	10. 06 249	034. 35 739	Maize	10
S#286	Benishangul	Asosa	Asosa	Baro	1569	10. 05 343	034. 36.652	Teff	30
S#288	Benishangul	Asosa	Asosa	Selega 22	1540	10. 04 192	034. 40 450	Maize	10
S#289	Oromia	West Wollega	Mene Sibu	Kela Dabus	1371	09. 45 410	034. 49 235	Maize	30
S#290	Oromia	West Wollega	Mene Sibu	Kela Dabus	1959	09. 46 497	034. 52 658	Maize	40
S#291	Oromia	West Wollega	Mene Sibu	Bengua	1418	09. 47 113	034. 55 897	Maize	5
S#292	Oromia	West Wollega	Mene Sibu	Teyibaba	1608	09. 48 166	035. 00 998	Maize	20
S#293	Oromia	West Wollega	Gimbi	Melka Gasi	1911	09. 08 841	035. 51 451	Maize	20
S#294	Oromia	West Wollega	Gimbi	Shone	1804	09. 05 070	035. 54 278	Maize	80
S#295	Oromia	East Wollega	Wayu Tuka	Gute Badiya	1896	09.01 838	036. 38 795	Maize	5
S#296	Oromia	East Wollega	Wayu Tuka	Worebabo Migna	1884	09. 02 061	036. 41 034	Maize	30
S#297	Oromia	East Wollega	Sibu Sire	Chingi	1796	09. 02 868	036. 43 665	Maize	10
S#298	Oromia	East Wollega	Sibu Sire	Jalele	1828	09. 03 234	036. 47 170	Maize	5
S#299	Oromia	East Wollega	Sibu Sire	Lelisa	1845	09. 02 705	036. 53 464	Maize	10
S#300	Oromia	East Wollega	Sibu Sire	Chiri Jarso	1760	09. 02 647	036. 50 309	Maize	20
S#301	Oromia	East Wollega	Sibu Sire	Lelisa	1730	09. 04 397	036. 55 081	Faba Bean	10
S#302	Somali	Fafen	Gursum	Alahago	1478	09^0 16.857'	042^0 34.685'	Maize	0
S#303	Somali	Fafen	Gursum	Halago	1476	09^0 11.014'	042^0 34.646'	Maize	30
S#304	Somali	Fafen	Gursum	Gulmurud	1419	09^0 13.720'	042^0 36.515'	Maize	40
S#305	Somali	Fafen	Gursum	Ufeyesa	1411	09^0 12.950'	042^0 37.033'	Maize	0
S#306	Somali	Fafen	Gursum	Ufeyesa	1407	09^0 12.098'	042^0 37.292'	Maize	20
S#307	Somali	Fafen	Gursum	Tikidem	1412	09^0 10.580'	042^0 37.770'	Maize	60
S#308	Somali	Fafen	Dendema	Elbahay	1334	09^0 11.548'	042^0 24.814'	Maize	60
S#309	Somali	Fafen	Dendema	Anodi	1331	09^0 11.405'	042^0 24.234'	Maize	30
S#310	Somali	Fafen	Dendema	Anodi	1418	09^0 11.318'	042^0 23.235'	Maize	60
S#311	Somali	Fafen	Babile	Doyo	1634	09^0 12.570'	042^0 21.304'	Maize	70
S#312	Somali	Fafen	Jigjiga	Gerbase	1749	09^0 20.597'	042^0 50.872'	Maize	0
S#313	Somali	Fafen	Jigjiga	Degahiyahad	1791	09^0 19.711'	042^0 53.471'	Maize	20
S#314	Somali	Fafen	Jigjiga	Holey	1788	09^0 18.224'	042^0 55.030'	Onion	80
S#315	Somali	Fafen	Jigjiga	Ahimedle	1737	09^0 16.781'	042^0 57.599'	Sorghum	20
S#316	Somali	Fafen	Jigjiga	Ahimedle	1696	09^0 15.738'	042^0 59.459'	Maize	60
S#317	Somali	Fafen	Jigjiga	Hare	1631	09^0 12.540'	043^0 04.065'	Maize	40
S#318	Somali	Fafen	Jigjiga	Hare	1657	09^0 11.866'	043^0 05.089'	Maize	20

SC	Regions	Zones	Districts	Locations	Altitude	Latitude	Longitude	Previous Crop	PI (%)
S#319	Somali	Fafen	Kebiribeya	Gerbe	1701	09^0 09.857'	043^0 07.561'	Maize	80
S#320	Somali	Fafen	Kebiribeya	Kebribeya	1701	09^0 04.704'	043^0 11.971'	Maize	20
S#321	Somali	Fafen	Kebiribeya	Kebribeya	1675	09^0 03.833'	043^0 12.266'	Maize	60
S#322	Somali	Fafen	Kebiribeya	Estabud	1641	09^0 02.393'	043^0 12.979'	Maize	60
S#323	Somali	Fafen	Kebiribeya	Estabud	1643	09^0 01.662'	043^0 13.297'	Maize	60
S#324	Somali	Fafen	Ararso	Guyo	1580	08^0 57.527'	043^0 15.843'	Maize	40
S#325	Somali	Fafen	Ararso	Gilo	1563	08^0 ^{5}6.227'	043^0 16.484'	Maize	70
S#326	Somali	Fafen	Ararso	Darselam	1532	08^0 47.317'	043^0 19.838'	Maize	20
S#327	Somali	Fafen	Haruris	Gilegelbi	1727	09^0 22.694'	042^0 53.868'	Sorghum	70
S#328	Somali	Fafen	Haruris	Harta Alibele	1764	09^0 ^{23}.518'	042^0 57.358'	Sorghum	10
S#329	Somali	Fafen	Haruris	Harta Alibele	1775	09^0 22.803'	042^0 58.234'	Maize	70
S#330	Somali	Fafen	Haruris	Harta Alibele	1793	09^0 24.531'	043^0 00.229'	Sorghum	10
S#331	Somali	Fafen	Haruris	Harta Alibele	1813	09^0 25.238'	043^0 02.480'	Sorghum	60
S#332	Somali	Fafen	Haruris	Gobi Yerfa	1808	09^0 25.811'	043^0 04.141'	Maize	40
S#333	Somali	Fafen	Wuchale	Gobi Yerfa	1761	09^0 28.112'	043^0 08.639'	Maize	20
S#334	Somali	Fafen	Wuchale	Gobi Yerfa	1668	09^0 30.719'	043^0 11.595'	Maize	40
S#335	Somali	Fafen	Wuchale	Gobi Yerfa	1647	09^0 31.335'	043^0 12.286'	Maize	60
S#336	Oromia	Borena	Yabelo	Darito	1539	04^0 44.237'	038^0 11.559'	Maize	30
S#337	Oromia	Borena	Dubuluk	Kersa	1553	04^0 39.781'	038^0 14.169'	Maize	75
S#338	Oromia	Borena	Dubuluk	Kersa Denbi	1561	04^0 35.070'	038^0 14.628'	Maize	50
S#339	Oromia	Borena	Dubuluk	Goro Dada	1528	04^0 24.361'	038^0 16.394'	Fallow	40
S#340	Oromia	Borena	Dire	Medecho	1595	04^0 08.909'	038^0 16.416'	Maize	40
S#341	Oromia	Borena	Dire	Dida Mega	1549	04^0 01.932'	038^0 20.707'	Fallow	60
S#342	Oromia	Borena	Dire	Haralo	1400	03^0 57.655'	038^0 24.847'	Maize	10
S#343	Oromia	Borena	Mio	Melbana	1347	03 54.072	038 28.689	Maize	10
S#344	Oromia	Borena	Mio	Melbana	1307	03 53.554	038 33.991	Fallow	10
S#345	Oromia	Borena	Mio	Boku	1285	03 51.187	038 43.806	Haricot bean	35
S#346	Oromia	Borena	Mio	Hidbabo	1271	03 48.098	038 46.438	Maize	30
S#347	Oromia	Borena	Moyale	Til Mado	1132	03 35.706	039 01.607	Fallow	85
S#348	Oromia	Borena	Moyale	Digalu	1166	03 37.273	039 00.334	Fallow	80
S#349	Oromia	Borena	Moyale	Sebante	1223	03 38.793	038 58.593	Maize	20
S#350	Oromia	Borena	Moyale	Bokara	1160	03 40.280	038 55.480	Maize	30
S#351	Oromia	Borena	Moyale	Bokala	1226	03 41.747	038 52.212	Fallow	20
S#352	Oromia	Borena	Yabelo	Deritu	1573	04 46.196	038 11.928	Tef	10
S#353	Oromia	Borena	Yabelo	Deritu	1599	04 50.140	038 11.372	Maize	10
S#354	Oromia	Borena	Yabelo	Dida Yabelo	1564	04 55.285	038 10.026	Fallow	20

SC	Regions	Zones	Districts	Locations	Altitude	Latitude	Longitude	Previous Crop	PI (%)
S#355	Oromia	Borena	Yabelo	Dida Yabelo	1531	04 55.074	038 11.929	Maize	85
S#356	Oromia	Borena	Teltele	01 Kebele	1462	05 03.319	037 22.233	Maize	10
S#357	Oromia	Borena	Teltele	Bila	1455	05 02.017	037 22.522	Haricot bean	20
S#358	Oromia	Borena	Teltele	Bila	1409	05 00.761	037 21.691	Tef	30
S#359	Oromia	Borena	Teltele	Bule Korma	1410	04 58.257	037 21.010	Maize	10
S#360	Oromia	Borena	Teltele	Dignehan	1406	05 03.571	037 24.562	Maize	50
S#361	Oromia	Borena	Teltele	Gololi	1060	05 06.132'	037 30.280	Fallow	10
S#362	Oromia	Borena	Elwaye	Brindad	927	05 07.790	037 36.487	Fallow	10
S#363	Oromia	Borena	Elwaye	Brindad	880	05 07.133	037 39.397	Maize	80
S#364	Oromia	Borena	Elwaye	Samba	981	05 02.137	037 43.177	Maize	70
S#365	Oromia	Borena	Elwaye	Elwaye Golbe	1220	04 58.969	037 51.427	Tef	55
S#366	Oromia	Borena	Yabelo	Dida Yabelo	1531	04 56.065	038 10.698	Tef	85
S#367	Oromia	Borena	Yabelo	Beke Haro	1534	04 59.304	038 12.524	Maize	30
S#368	Oromia	Borena	Gomole	Buya Gudo	1590	05 04.484	038 15.153	Maize	10
S#369	Oromia	Borena	Gomole	Buya Tika	1602	05 06.068	038 16.039	Maize	10
S#370	Oromia	Borena	Gomole	Surupa badiya	1619	05 09.309	038 19.093	Maize	10
S#546	Oromia	Guji	Bore	kola barana	2765	06.35683N	038.67104E	Maize	0
S#547	Oromia	Guji	Bore	Baro gosa	2625	06.34586N	038.70967E	Maize	0
S#548	Oromia	Guji	Bore	Shelemele	2078	06.35014N	038.76656E	Maize	0
S#549	Oromia	Guji	Bore	Eshro Alayo	2066	06.37183N	038.76003E	Maize	0
S#550	Oromia	Guji	Adola	Litu	1686	05.84384N	038.97967E	Maize	0
S#551	Oromia	Guji	Shakiso	Litu	1795	05.73583N	038.90469E	Potato	0
S#552	Oromia	Guji	Sora	Murin Burato	2355	06.13603N	038.73991E	Field pea	0
S#553	Oromia	Guji	Sora	Muri	2199	06.05267N	038.32623E	Faba bean	0
S#554	Oromia	Bedele	Kerka	Bilu	1419	08.66938N	036.39113E	Maize	0
S#555	Oromia	Bedele	Bedele	Diba Bate	1901	08.55622N	036.36613E	Potato	0
S#556	Oromia	Bedele	Buno Bedele	Bube Korsa	1878	08.52515N	036.36876E	Maize	30
S#557	Oromia	Bedele	Buno Bedele	kilenso Babicho	1909	08.50978N	036.36585E	Maize	0
S#558	Oromia	Buno Bedele	Gachi	Dabo Hana	1921	08.38040N	036.40493E	Maize	80
S#559	Oromia	Buno Bedele	Didesa	Kare Lilo	2064	08.10526N	036.45942E	Potato	0
S#560	Oromia	Buno Bedele	Didesa	Banchure	1717	07.94157N	036.50939E	Maize	0
S#561	Oromia	Buno Bedele	Didesa	Urgesa	1610	07.85327N	036.61787E	Teff	0
S#562	Oromia	Buno Bedele	Goma	Dangu Waji	1592	07.87685N	036.64751E	Teff	0
S#563	Oromia	Buno Bedele	Goma	Saso	1590	07.85935N	036.64387E	Maize	0
S#564	Oromia	Jima	Mana	Yachi Hurache	1991	07.74138N	036.78901E	Maize	0

SC	Regions	Zones	Districts	Locations	Altitude	Latitude	Longitude	Previous Crop	PI (%)
S#565	Oromia	Jima	Mana	Suse	2010	07.76773N	036.77171E	Teff	0
S#566	Oromia	Jima	Seka Chekorsa	Bulbulo	1870	07.57502N	036.68243E	Maize	0
S#567	Oromia	Jima	Seka Chekorsa	Bulbulo	1917	07.56411N	036.64579E	Maize	0
S#568	Oromia	Jima	Shebe Sombo	Somodo Abujadi	1383	07.46845N	036.42388E	Maize	0
S#569	Oromia	Jima	Shebe Sombo	Garuke	1669	07.48529N	036.47581E	Maize	0
S#570	Oromia	Jima	Shebe Sombo	Gibe boso	1688	07.48609N	036.48151N	Maize	0
S#571	Oromia	East Showa	Adama	Shashemene	1443	08.24339N	039.22022E	Maize	20
S#572	Oromia	East Showa	Adama	Kishe	1340	08.26092N	039.24022E	Maize	20
S#573	Oromia	East Showa	Boset	Sebaka Wala	1365	08.26889N	039.26246E	Teff	10
S#574	Oromia	East Showa	Boset	Sebaka Wala	1444	08.28269N	039.28487E	Maize	20
S#575	Oromia	East Showa	Boset	Ulaga Melekauwa	1772	08.29054N	039.31181E	Maize	20
S#576	Oromia	East Arisi	Jeju	Batu Degaga	1269	08.29392N	039.34688E	Maize	0
S#577	Oromia	East Arisi	Jeju	Kechachile Guji	1228	08.27185N	039.34868E	Teff	0
S#578	Oromia	East Arisi	Jeju	Sara Areda	1313	08.27710N	039.35047E	Teff	0
S#579	Oromia	East Arisi	Jeju	Sara Areda	1243	08.31079N	039.35.056E	Maize	0
S#580	Oromia	East Arisi	Jeju	Uruta Dore	1194	08.34752N	039.41536E	Maize	0
S#581	Oromia	East Showa	Fentale	Tibila GB-A4	1100	08.43498N	039.49355E	Teff	0
S#582	Oromia	East Showa	Fentale	Tibila GB-A91	978	08.54641N	040.00838E	Maize	0
S#583	Oromia	West Hararghe	Gumbi Bordede	Huruta Dore	1060	09.01340N	040.22857E	Maize	0
S#584	Oromia	West Hararghe	Gumbi Bordede	Achamo Geluwenze	1123	09.03986N	040.25178E	Maize	0
S#585	Oromia	West Hararghe	Gumbi Bordede	Dire Seden	1165	09.06626N	040.26221E	Maize	0
S#586	Oromia	West Hararghe	Gumbi Bordede	Benti	1336	09.07964N	040.29779E	Maize	0
S#587	Oromia	West Hararghe	Gumbi Bordede	Kela Bordede	1354	09.09184N	040.32201E	Maize	0
S#588	Oromia	West Hararghe	Miheso	Buri Arba	1384	09.10322N	040.34616E	Maize	10
S#589	Oromia	West Hararghe	Miheso	Dire Kalu	1418	09.11145N	040.37020E	Teff	0
S#590	Oromia	West Hararghe	Miheso	Aneno	1476	09.11621N	040.39847E	Maize	0
S#591	Oromia	West Hararghe	Miheso	Chitu Kora	1339	09.13726N	040.44436E	Maize	0
S#592	Oromia	West Hararghe	Chiro	Harmero Dima	1632	09.08075N	040.49496E	Maize	0
S#593	Oromia	West Hararghe	Chiro	Dega Daku	2235	09.03347N	040.54615E	Teff	20
S#594	Oromia	West Hararghe	Chiro	Tokuma	2004	09.03240N	040.53460E	Teff	0
S#595	Oromia	West Hararghe	Chiro	Areda Fayo	2278	09.03907N	040.54984E	Maize	0
S#596	Oromia	West Hararghe	Chiro	Kololo	2199	09.05997N	040.56830E	Maize	0
S#597	Oromia	West Hararghe	Chiro	Arebrekete	2260	09.07384N	040.58718E	Teff	10
S#598	Oromia	West Hararghe	Tulo	Kuliso	2179	09.08858N	041.01190E	Maize	0
S#599	Oromia	West Hararghe	Tulo	Alberekete	2233	09.10189N	041.02541E	Maize	20
S#600	Oromia	West Hararghe	Tulo	Shola	2008	09.10137N	041.04100E	Maize	0
S#601	Oromia	West Hararghe	Tulo	Shola	1730	09.10797N	041.06342E	Maize	0

SC	Regions	Zones	Districts	Locations	Altitude	Latitude	Longitude	Previous Crop	PI (%)
S#602	Oromia	West Hararghe	Tulo	Odakebena	1873	09.13818N	041.06901E	Maize	0
S#603	Oromia	West Hararghe	Tulo	Chefe	2148	09.15129N	041.08095E	Maize	10
S#604	Oromia	West Hararghe	Tulo	Terekanfeta	2368	09.16016N	041.08305E	Maize	0
S#605	Oromia	West Hararghe	Doba	Medegidu Basha	2313	09.16925N	041.10223E	Teff	0
S#606	Oromia	West Hararghe	Doba	Dadi	2400	09.17404N	041.13171E	Maize	0
S#607	Oromia	East Hararghe	Goro Gutu	Geda	2508	09.19859N	041.15524E	Maize	0
S#608	Oromia	East Hararghe	Goro Gutu	Koreke	2276	09.20594N	041.17701E	Maize	0
S#609	Oromia	East Hararghe	Goro Gutu	Efabelem	2211	09.22150N	041.19888E	Maize	0
S#610	Oromia	East Hararghe	Goro Gutu	Chefebante	2225	09.22927N	041.23195E	Maize	0
S#611	Oromia	East Hararghe	Goro Gutu	Boru Abdi	2115	09.23025N	041.25646E	Maize	0
S#612	Oromia	East Hararghe	Goro Gutu	Gursum	2046	09.23310N	041.27313E	Maize	10
S#613	Oromia	East Hararghe	Kersa	Umir Ali	2129	09.27653N	041.54045E	Maize	10
S#614	Oromia	East Hararghe	Kersa	Midisa Jalela	2027	09.26902N	041.51567E	Maize	0
S#615	Oromia	East Hararghe	Kersa	Umer	2068	09.26902N	041.49034E	Maize	0
S#616	Oromia	East Hararghe	Kersa	Usuman	2021	09.26443N	041.46373E	Maize	0
S#617	Oromia	East Hararghe	Meta	Dengago	2245	09.26604N	041.43293E	Maize	0
S#618	Oromia	East Hararghe	Meta	Metekoma	2419	09.25515N	041.40572E	Maize	0
S#619	Oromia	East Hararghe	Meta	Dalemirga	2263	09.25723N	041.38166E	Maize	0
S#620	Oromia	East Hararghe	Meta	Mechayesa	2240	09.24619N	041.35872E	Maize	10
S#621	Oromia	East Hararghe	Meta	Hawibilusuma	2260	09.23840N	041.32831E	Maize	0
S#622	Oromia	East Hararghe	Goro Gutu	Kulubi	2084	09.22793N	041.30240E	Maize	0
S#623	Oromia	East Hararghe	Goro Gutu	Dusegemechu	2083	09.22843N	041.27788E	Maize	0
S#624	Oromia	East Hararghe	Kersa	Kelata	2082	09.27234N	041.55419E	Maize	0
S#625	Oromia	East Hararghe	Kersa	Chelenko	2108	09.23064N	041.56023E	Maize	40
S#626	Oromia	East Hararghe	Haromaya	Ademyerer	2009	09.21194N	041.54734E	Maize	0
S#627	Oromia	East Hararghe	Haromaya	Kelada	1980	09.20205N	041.53283E	Maize	0
S#628	Oromia	East Hararghe	Kurfa Chele	Abidibiftu Geda	1755	09.17208N	041.52494E	Maize	0
S#629	Oromia	East Hararghe	Kurfa Chele	Gola Muda	1978	09.14889N	041.52435E	Maize	0
S#630	Oromia	East Hararghe	Kurfa Chele	Awmer	2265	09.14309N	041.49834E	Maize	0
S#631	Oromia	East Hararghe	Kurfa Chele	Kersa Biftu	2413	09.13856N	041.48764E	Maize	20
S#632	Oromia	East Hararghe	Gurawa	Ula Jalela	2490	09.12034N	041.47311E	Maize	20
S#633	Oromia	East Hararghe	Gurawa	Jiri Gemechu	2451	09.09497N	041.47967E	Maize	0
S#634	Oromia	East Hararghe	Gurawa	Orde Kure	2460	09.09011N	014.48690E	Maize	0
S#635	Oromia	East Hararghe	Gurawa	Jiru Balina	2546	09.13279N	041.46.628E	Maize	0
S#636	Oromia	East Hararghe	Haromaya	Ula Jalela	2045	09.23623N	041.57453E	Maize	0

SC	Regions	Zones	Districts	Locations	Altitude	Latitude	Longitude	Previous Crop	PI (%)
S#637	Oromia	East Hararghe	Haromaya	Ula Jalela	2024	09.24897N	042.01813E	Maize	0
S#638	Oromia	East Hararghe	Haromaya	Ula Jalela	2071	09.25674N	042.02462E	Maize	0
S#639	Oromia	East Hararghe	Haromaya	Lencha	2033	09.27439N	042.02228E	Maize	0
S#640	Oromia	East Hararghe	Haromaya	Haramaya University	2089	09.29847N	042.02119E	Maize	20
S#641	Oromia	East Hararghe	Haromaya	Bate	2073	09.28669N	042.00593E	Maize	20
S#642	Oromia	East Hararghe	Haromaya	Finkile	2056	09.25326N	041.59848E	Maize	10
S#643	Oromia	East Hararghe	Haromaya	Kerensa	2028	09.25285N	041.56860E	Maize	0
S#644	Oromia	East Hararghe	Haromaya	Amuma	2026	09.24174N	041.59283E	Maize	10
S#645	Oromia	East Hararghe	Haromaya	Tuji Gebisa	1957	09.20603N	042.05043E	Maize	0
S#646	Oromia	East Hararghe	Dire Tiyara	Adele	2106	09.23083N	042.06.367E	Maize	0
S#647	Oromia	East Hararghe	Kombolcha	Adele	2170	09.24851N	042.06653E	Maize	0
S#648	Oromia	East Hararghe	Kombolcha	Sukulu	2071	09.26933N	042.08296E	Maize	0
S#649	Oromia	East Hararghe	Kombolcha	Berkete	2119	09.27274N	042.09089E	Maize	0
S#650	Oromia	East Hararghe	Kombolcha	Kombolcha	2097	09.27861N	042.10817E	Maize	0
S#651	Oromia	East Hararghe	Kombolcha	Kalu Tulu	2260	09.28776N	042.12189E	Maize	10
S#652	Oromia	East Showa	Fentale	Burka Dini	967	08.55129N	039.51563E	Maize	30
S#653	Oromia	East Showa	Fentale	Wore Mohamed	1112	08.52341N	039.46010E	Maize	0
S#654	Oromia	North Showa	Minjar Shenkora	Wore Mohamed	1316	08.50721N	039.43530E	Maize	0
S#655	Oromia	East Showa	Boset	Elala	1353	08.48801N	039.42125E	Maize	20
S#656	Oromia	East Showa	Boset	Tututi	1256	08.47112N	039.39249E	Maize	20
S#657	Oromia	East Showa	Boset	Amora Bet	1211	08.45163N	039.35630E	Maize	30
S#658	Oromia	East Showa	Boset	Kawa Hara	1251	08.41924N	039.31566E	Maize	20
S#659	Oromia	East Showa	Boset	Kawara Mergesa	1406	08.40497N	039.28207E	Maize	10
S#660	Oromia	East Showa	Boset	Borchota	1453	08.38513N	039.24582E	Maize	0
S#661	Oromia	H/G/Wollega	Horro	Kere Nuraera	2398	9.57786	37.14013	Maize	60
S#662	Oromia	H/G/Wollega	Horro	Tedecha	2400	9.58725	37.1661	Maize	70
S#663	Oromia	H/G/Wollega	Horro	Walenchiti	2390	9.60247	37.19227	Maize	70
S#664	Oromia	H/G/Wollega	Abaycoman	Doyo	2326	9.61246	37.22589	Maize	0
S#665	Oromia	H/G/Wollega	Abaychoman	Doyo	2326	9.62812	37.24831	Maize	0
S#666	Oromia	H/G/Wollega	Abaychoman	Didibe Kistane	2358	9.64622	37.26672	Maize	60
S#667	Oromia	H/G/Wollega	Abaychoman	Diga Arba	2405	9.65295	37.229168	Maize	70
S#668	Oromia	H/G/Wollega	Abaychoman	Homi	2418	9.64829	37.32645	Maize	40
S#669	Oromia	H/G/Wollega	Abaychoman	Dambal	2368	9.60895	37.3396	Maize	50
S#670	Oromia	H/G/Wollega	Abaychoman	Dambal	2319	9.59086	37.35186	Maize	80
S#671	Oromia	H/G/Wollega	Abaychoman	Hacane Migiru	2308	9.56938	37.36232	Maize	0
S#672	Oromia	H/G/Wollega	Guduru	Jare Irgi	2257	9.53104	37.37236	Maize	50

SC	**Regions**	**Zones**	**Districts**	**Locations**	**Altitude**	**Latitude**	**Longitude**	**Previous Crop**	**PI (%)**
S#673	Oromia	H/G/Wollega	Guduru	Jare	2262	9.50963	37.42002	Maize	40
S#674	Oromia	H/G/Wollega	Guduru	Jare	2285	9.47394	37.43919	Maize	60
S#675	Oromia	H/G/Wollega	Guduru	Gobu	2251	9.44722	37.4285	Maize	80
S#676	Oromia	H/G/Wollega	Choman Guduru	Gudane Siban	2302	9.40871	37.42448	Maize	10
S#677	Oromia	H/G/Wollega	Choman Guduru	Gudane kobo	2229	9.36073	37.40461	Maize	20
S#678	Oromia	H/G/Wollega	Jima rare	Gudane Kobo	2275	9.32268	37.3875	Maize	60
S#679	Oromia	H/G/Wollega	Jima rare	Welkituma	2272	9.28619	37.38308	Maize	40
S#680	Oromia	H/G/Wollega	Jima rare	Dale	2267	9.25803	37.3742	Maize	80
S#681	Oromia	H/G/Wollega	Jima rare	Jaraf Shumba	2439	9.21601	37.36135	Maize	70
S#682	Oromia	H/G/Wollega	Jima rare	Gemeda	2559	9.18951	37.34066	Maize	60
S#683	Oromia	H/G/Wollega	Jima rare	Gemachisa	2622	9.18333	37.31998	Maize	10
S#684	Oromia	West Wollega	Gimbi	Keku kelo	1862	9.11319	35.46458	Maize	0
S#685	Oromia	West Wollega	Gimbi	Haro Guta	1815	9.1997	35.76419	Maize	20
S#686	Oromia	West Wollega	Gimbi	Haro Guta	1815	9.20358	35.7378	Maize	30
S#687	Oromia	West Wollega	Gimbi	Lalisa	1852	9.20449	35.72655	Maize	40
S#688	Oromia	West Wollega	Gimbi	Lalisa	1861	9.19986	35.73079	Maize	50
S#689	Oromia	West Wollega	Gimbi	Lalisa	1911	9.1399	35.86654	Maize	80
S#690	Oromia	West Wollega	Gimbi	Garjo Siban	1764	9.06744	35.53 001	Maize	70
S#691	Oromia	West Wollega	Gimbi	Lelisa Yesus	1758	9.11247	35.88358	Maize	60
S#692	Oromia	West Wollega	Gimbi	Melka Gose	1695	9.04878	35.94124	Maize	40
S#693	Oromia	West Wollega	Gimbi	Cuta Goci	1644	9.03153	35.96976	Maize	50
S#694	Oromia	West Wollega	Gimbi	Bikiltu Tokuma	1267	9.05181	36.08792	Maize	60
S#695	Oromia	West Wollega	Laloasabi	Nango Dambal	1896	9.23239	35.7098	Maize	20
S#696	Oromia	West Wollega	Laloasabi	Aba sena	1882	9.13938	35.42 089	Maize	50
S#697	Oromia	West Wollega	Laloasabi	Tole	1892	9.224098	35.69504	Maize	40
S#698	Oromia	West Wollega	Laloasabi	Haroji Serdo	1876	9.24986	35.69594	Maize	80
S#699	Oromia	West Wollega	Laloasabi	Haroji Serdo	1773	8.27083	35.68146	Maize	80
S#700	Oromia	West Wollega	Laloasabi	Warajiru	1875	9.18629	35.713	Maize	70
S#701	Oromia	West Wollega	Laloasabi	Harojiharawa	1900	9.16714	35.708	Maize	80
S#702	Oromia	West Wollega	Laloasabi		1834	9.16534	35.67682	Maize	50
S#703	Oromia	West Wollega	Laloasabi	Dongoro	1816	9.1732	35.65866	Maize	80
S#704	Oromia	East wollega	Diga	Gurji siban	1993	09^{0}.01'.158"	036^{0}.09'.874"	Maize	60
S#705	Oromia	East wollega	Diga	Waababosiban	1274	09^{0}.02'.228"	036^{0}.14'.179"	Maize	70
S#706	Oromia	East wollega	Diga	Barko	1247	09^{0}.03'.621"	036^{0}.24'.691"	Maize	0
S#707	Oromia	East wollega	Diga	Tasiomole	1376	09^{0}.03'.005"	036^{0}.16'.526"	Maize	0

SC	Regions	Zones	Districts	Locations	Altitude	Latitude	Longitude	Previous Crop	PI (%)
S#708	Oromia	East wollega	Diga	Degaga Didesa	1493	09^{0}.04'.139"	036^{0}.20'.101"	Maize	20
S#709	Oromia	East wollega	Diga	Arjo Gudetu	1497	09^{0}.04'.139"	036^{0}.20'.166"	Maize	10
S#710	Oromia	East wollega	Diga	Arjo Gudetu	1701	09^{0}.04'.371"	036^{0}.22'.103"	Maize	80
S#711	Oromia	East wollega	Diga	Arjo Gudetu	2018	09^{0}.02'.858"	036^{0}.23'.992"	Maize	60
S#712	Oromia	East wollega	Kiremu	Bikila	2084	09^{0}.57'.324"	036^{0}.50'.927"	Maize	0
S#713	Oromia	East wollega	Kiremu	Bikila	2007	09^{0}.56'.096"	036^{0}.48'.760"	Maize	0
S#714	Oromia	East wollega	Kiremu	Bikila	1994	09^{0}.54'.965"	036^{0}.45'.021"	Maize	0
S#715	Oromia	East wollega	Gida Ayana	Gudisa	1973	09^{0}.53'.314"	036^{0}.43'.082"	Maize	0
S#716	Oromia	East wollega	Gida Ayana	Kiremu	2060	09^{0}.51'.623"	036^{0}.40'.543"	Maize	0
S#717	Oromia	East wollega	Gida Ayana	Burka Soruma	2216	09^{0}.49'.426"	036^{0}.39'.804"	Maize	0
S#718	Oromia	East wollega	Gida Ayana	Kofekofe	2139	09^{0}.45'.967"	036^{0}.38'.297"	Maize	0
S#719	Oromia	East wollega	Gida Ayana	Dima	1535	09^{0}.41'.294"	036^{0}.37'.928"	Maize	20
S#720	Oromia	East wollega	Gida Ayana	Ejere	1436	09^{0}.39'.021"	036^{0}.37'.925"	Maize	20
S#721	Oromia	East wollega	Gida Ayana	Gatira	1375	09^{0}.36'.738"	036^{0}.37'.915"	Maize	30
S#722	Oromia	East wollega	Gida Ayana	Doro Hobora	1322	09^{0}.32'.623"	036^{0}.36'.914"	Maize	0
S#723	Oromia	East wollega	Gida Ayana	Handode Dicho	1366	09^{0}.31'.659"	036^{0}.35'.115"	Maize	10
S#724	Oromia	East wollega	Gida Ayana	Meke Lemesa	1391	09^{0}.29'.175"	036^{0}.32'.972"	Maize	60
S#725	Oromia	East wollega	Gida Ayana	Tulu Lencha	1317	09^{0}.26'.474"	036^{0}.32'.439"	Maize	40
S#726	Oromia	East wollega	Guto Gida	Mender-1	1328	09^{0}.24'.247"	036^{0}.32'.340"	Maize	50
S#727	Oromia	East wollega	Guto Gida	Mender-11	1385	09^{0}.22'.230"	036^{0}.31'.306"	Maize	60
S#728	Oromia	East wollega	Guto Gida	Mender-10	1394	09^{0}.18'.608"	036^{0}.30'.716"	Maize	0
S#729	Oromia	East wollega	Diga	Lelistu Hanger	2094	09^{0}.01'.887"	036^{0}.25'.088"	Maize	40
S#730	Oromia	East wollega	Diga	Uke	2237	09^{0}.01'.509"	036^{0}.28'.763"	Maize	0
S#731	Oromia	East wollega	Wayu tuka	Uke Badiya	2067	09^{0}.03'.224"	036^{0}.36'.199"	Maize	40
S#732	Oromia	East wollega	Wayu tuka	Mede Jalela	1914	09^{0}.01'.869"	036^{0}.39'.671"	Maize	30
S#733	Oromia	East wollega	Sibu Sire	Gudisa	1888	09^{0}.02'.170"	036^{0}.41'.362"	Maize	10
S#734	Oromia	East wollega	Sibu Sire	Jirata	1722	09^{0}.02'.689"	036^{0}.45'.388"	Maize	20
S#735	Oromia	East wollega	Sibu Sire	Ariya Jawi	1811	09^{0}.03'.307"	036^{0}.47'.607"	Maize	0
S#736	Oromia	East Wollega	Sibu Sire	Were Babo	1784	09^{0}.02'.499"	036^{0}.49'.570"	Maize	30
S#737	Oromia	East Wollega	Sibu Sire	Were Babo Begna	1834	09^{0}.02'.617"	036^{0}.53'.397"	Maize	40
S#738	Oromia	East Wollega	Sibu Sire	Adamu	1741	09^{0}.04'.345"	036^{0}.55'.011"	Maize	0
S#739	Oromia	East Wollega	Gobu Seyo	Chefe Jalela	1857	09^{0}.05'.650"	036^{0}.58'.711"	Maize	20
S#740	Oromia	East Wollega	Gobu Seyo	Cheri Jarso	1764	09^{0}.06'.529"	036^{0}.00'.089"	Maize	30
S#741	Oromia	West shoa	Bako Tibe	Lelisa	1623	09^{0}.07'.541"	036^{0}.02'.955"	Maize	60
S#742	Oromia	West shoa	Bako Tibe	Moto Chekorsa	1659	09^{0}.06'.540"	036^{0}.05'.252"	Maize	70
S#743	Oromia	West shoa	Bako Tibe	Hango Bekenisa	1670	09^{0}.05'.306"	036^{0}.08'.101"	Maize	0
S#744	Oromia	West shoa	Bako Tibe	Meki	1708	09^{0}.04'.554"	036^{0}.10'.131"	Maize	30

SC	Regions	Zones	Districts	Locations	Altitude	Latitude	Longitude	Previous Crop	PI (%)
S#745	Oromia	West shoa	Bako Tibe	Denbi Gobu	1691	09^0.02'.480"	036^0.11'.781"	Maize	20
S#746	Oromia	West shoa	Bako Tibe	Denbi Dima	1708	09^0.01'.386"	036^0.13'.925"	Maize	50
S#747	Oromia	West shoa	Ilu Gelan	Seden Kite	1742	09^0.01'.056"	036^0.16'.103"	Maize	40
S#748	Oromia	West shoa	Ilu Gelan	Tulu Sengota	1769	09^0.00'.615"	036^0.18'.076"	Maize	0
S#749	Oromia	West shoa	Ilu Gelan	Oda Haro	1740	09^0.57'.873"	036^0.19'.762"	Maize	60
S#750	Oromia	West shoa	Ilu Gelan	Gudina Wolkite	1707	09^0.55'.711"	036^0.19'.937"	Maize	60
S#751	Oromia	West shoa	Dano	Siba Biche	1630	09^0.53'.397"	037^0.19'.014"	Maize	0
S#752	Oromia	West shoa	Dano	Goba Washemo	1639	09^0.52'.052"	037^0.18'.432"	Maize	40
S#753	Oromia	West shoa	Dano	Seden Ilu	1739	09^0.50'.426"	037^0.18'.505"	Maize	30
S#754	Oromia	West shoa	Dano	Abeko Ano	1600	09^0.48'.734"	037^0.17'.991"	Maize	50
S#755	Oromia	West shoa	Dano	Dano Shenen	1668	09^0.47'.258"	037^0.16'.792"	Maize	60
S#756	Oromia	West shoa	Ilu Gelan	Dano Shenen	1769	09^0.59'.628"	037^0.21'.404"	Maize	70
S#757	Oromia	West shoa	Ilu Gelan	Dano Shenen	1859	09^0.00.052"	037^0.23'.462"	Maize	50
S#758	Oromia	West shoa	Cheliya	Gida Abu	2140	09^0.01'.574"	037^0.25'.085"	Maize	60
S#759	Oromia	West shoa	Cheliya	Dire Harayu	2561	09^0.58'.924"	037^0.27'.970"	Maize	40
S#760	Oromia	West shoa	Liben Jawi	Jato Dirki	2455	09^0.58'.223"	037^0.29'.530"	Maize	40
S#761	Oromia	West shoa	Liben Jawi	Hobora Beneso	2269	09^0.58'.065"	037^0.31'.202"	Maize	50
S#762	Oromia	West shoa	Liben Jawi	Sokondo	2295	09^0.58'.543"	037^0.33'.745"	Maize	0
S#763	Oromia	West shoa	Toke Kutaye	Wegidi Kortu	2369	08^0.58'.685"	037^0.36'.097"	Maize	0
S#764	Oromia	West shoa	Toke Kutaye	Liben Gamo	2347	08^0.59'.062"	037^0.37'.670"	Maize	30
S#765	Oromia	West shoa	Toke Kutaye	Liben Gamo	2370	08^0.58'.523"	037^0.39'.550"	Maize	20
S#766	Oromia	West shoa	Toke Kutaye	Wabo	2410	08^0.58'.885"	037^0.41'.603"	Maize	0
S#767	Oromia	West shoa	Toke Kutaye	Meti	2273	08^0.59'.096"	037^0.34'.493"	Maize	40
S#768	Oromia	West shoa	Toke Kutaye	Chancho Obi	1947	08^0.59'.322"	037^0.47'.043"	Maize	0
S#769	Oromia	West shoa	Ambo	Kele Berodo	2053	08^0.59'.580"	037^0.49'.321"	Maize	0
S#770	Oromia	West shoa	Toke Kutaye	Lencha	2056	08^0.56'.922"	037^0.44'.988"	Maize	20
S#771	Oromia	West shoa	Toke Kutaye	Kolba	2178	08^0.53'.844"	037^0.44'.858"	Maize	30
S#773	Oromia	West shoa	Dire Inchini	Senkele	2413	08^0.51'.218"	037^0.43'.125"	Maize	0
S#774	Oromia	West shoa	Dire Inchini	Birbirsafi Dogoma	2465	08^0.50'.452"	037^0.39'.569"	Maize	0
S#775	Oromia	West shoa	Dire Inchini	Birbirsafi Dogoma	2451	08^0.48'.841"	037^0.37'.449"	Maize	0
S#776	Oromia	West shoa	Dire Inchini	Melke Dera	2470	08^0.47'.955"	037^0.35'.174"	Maize	40
S#777	Oromia	West shoa	Dire Inchini	Bole Germama	2491	08^0.45'.858"	037^0.32'.235"	Maize	0
S#778	Oromia	West shoa	Shenen Jibat	Nano Jidu	2495	08^0.44'.668"	037^0.30'.526"	Maize	0
S#779	Oromia	West shoa	Shenen Jibat	Weldo Hine	2565	08^0.44'.152"	037^0.27'.158"	Maize	0

SC	Regions	Zones	Districts	Locations	Altitude	Latitude	Longitude	Previous Crop	PI (%)
S#780	Oromia	West shoa	Shenen Jibat	Buyema Dalfo	2378	08^{0}.43'.085"	037^{0}.25'.151"	Maize	20
S#781	Oromia	West shoa	Shenen Jibat	Bilo Abeyi	2171	08^{0}.41'.988"	037^{0}.21'.515"	Maize	0
S#782	Oromia	West shoa	Shenen Jibat	Mogno Hitete	2251	08^{0}.41'.003"	037^{0}.27'.295"	Maize	40
S#783	Oromia	West shoa	Shenen Jibat	Maru Jibat	2000	08^{0}.33'.197"	037^{0}.43'.637"	Maize	0
S#784	Oromia	West shoa	Nono	Maru Beha	2062	08^{0}.38'.046"	037^{0}.26'.168"	Maize	0
S#785	Oromia	West shoa	Nono	Abeyi Raji	1886	08^{0}.34'.198"	037^{0}.25'.925"	Maize	20
S#786	Oromia	West shoa	Nono	Maru Kombolcha	1818	08^{0}.31'.257"	037^{0}.25'.171"	Maize	30
S#787	Oromia	West shoa	Nono	Maru Kombolcha	1679	08^{0}.28'.698"	037^{0}.25'.199"	Maize	80
S#788	Oromia	West shoa	Nono	Hurumu Jibat	1525	08^{0}.26'.379"	037^{0}.27'.015"	Maize	70
S#789	Oromia	West shoa	Nono	Hurumu Gebireal	1563	08^{0}.25'.799"	037^{0}.30'.149"	Maize	60
S#790	Oromia	Southwest shoa	Welliso	Chefe Oda	2174	08^{0}.35'.186"	038^{0}.00'.536"	Maize	40
S#791	Oromia	Southwest shoa	Welliso	Omboro	2337	08^{0}.37'.379"	038^{0}.02'.175"	Maize	80
S#792	Oromia	Southwest shoa	Welliso	Alo Dinki	2342	08^{0}.38'.628"	038^{0}.07'.614"	Maize	0
S#793	Oromia	Southwest shoa	Becho	Kondala	2268	08^{0}.38'.145"	038^{0}.10'.455"	Maize	0
S#794	Oromia	Southwest shoa	Becho	Obikoji	2159	08^{0}.41'.286"	038^{0}.12'.443"	Maize	40
S#795	Oromia	Southwest shoa	Dawo	Adami Gotu	2175	08^{0}.44'.263"	038^{0}.09'.499"	Maize	50
S#796	Oromia	Southwest shoa	Dawo	Doyo Kora	2186	08^{0}.47'.191"	038^{0}.08'.336"	Maize	60
S#797	Oromia	West shoa	Dendi	Soyoma Genji	2592	08^{0}.51'.391"	038^{0}.05'.544"	Maize	20
S#798	Oromia	West shoa	Dendi	Sedekina Dega Guda	2370	08^{0}.55'.166"	038^{0}.06'.498"	Maize	10
S#799	Oromia	West shoa	Dendi	Nano Gebreal	2257	08^{0}.58'.277"	038^{0}.07'.235"	Maize	20
S#780									
S#781									
S#782									
S#783									
S#784									
S#785									
S#786									
S#787									
S#788									
S#789									
S#790									
S#791									
S#792									

Note: SC=Sample code, Gologota MAI= Gologota, Merti Agricultural industry, PI= **Plot infestation,** Yekeyit CT= Tabiya Yekeyit chigigne Tabiya, B.Sh.Ag. I= Bir Sheleko Agro industry, Tibila GB-A4=Tibila Genet Block-A4, Tibila GB-A91=Tibila Genet Block-A91

Appendix 2: Table 2. Description of indigenous EPN from eight maize-growing regions of Ethiopia collected soils.

New code	**Regions**	**Zones**	**Districts**	**Locations**	**Altitude**	**Latitude**	**Longitude**	**EPN isolate**
Am-Kor-Tes-7	Afar	Afar	Dufti	Korile	365	011.41122	041.11304	*Strenematodes* Sp
Am-Kur-Tes-8	Afar	Afar	Dufti	Kurule	361	011.38189	041.15543	*Hetrorabitidus Sp.*
Am-Gum-Tes-15	Oromiya	East Showa	Merti	Gumai	1204	08.613125	039.712297	*Strenematodes Sp*
Am-KoD-Tes-19	Oromiya	East Showa	Boset	Kona Degaga	1526	08.405732	039.369478	*Hetrorabitidus Sp.*
Am-UlM-Tes-20	Oromiya	East Showa	Adama	Ulaga Melkaoba	1395	08.407085	039.361730	*Strenematodes Sp*
Am-AsA-Tes-37	Amhara	Awi Zone	Banja	Askuna Abo	1994	11 24.144	037 07.248	*Strenematodes Sp*
Am-KuG-Tes-43	Amhara	Bahidar Zuriya	Mecha	Kudm Georgise	1974	11 23.287	037 06.584	*Hetrorabitidus Sp.*
Am-SeG-Tes-50	Amhara	South Gonder	Libo Kemkem	Selkisana Ginaza	1876	12 05.937	037 45.667	*Strenematodes Sp*
Am-AdG-Tes-59	Tigiray	Tigiray	Tahtay Koraro	Adi Gidad	1972	14 05.127	038 21.776	*Hetrorabitidus Sp.*
Am-Bel-Tes-60	Tigiray	Tigiray	Tahtay Koraro	Beles	1937	14 03.977	038 23.508	*Hetrorabitidus Sp.*
Am-Wez-Tes-67	Tigiray	Tigiray	Hintale Wojerat	Wezada	2132	13 04.599	039 32.000	*Hetrorabitidus Sp.*
Am-Waz-Tes-68	Tigiray	Tigiray	Hintale Wojerat	Wezada	2264	13 02.935	039 33.335	*Strenematodes Sp*
Am-Adm-Tes-69	Tigiray	Tigiray	Adeigudem	Admesino	2482	13 00.950	039 35.059	*Hetrorabitidus Sp.*
Am-Tse-Tes-70	Tigiray	Tigiray	Adeigudem	Tsehafti	2092	12 58.073	039 40.147	*Strenematodes Sp*
Am-Haw-Tes-71	Tigiray	Tigiray	Raya Azebo	Hawleti	1742	12 51.158	039 42.195	*Strenematodes Sp*
Am-Aad-Tes-72	Tigiray	Tigiray	Raya Azebo	Abadu	1758	12 47.195	039 38.482	*Hetrorabitidus Sp.*
Am-Huj-Tes-73	Tigiray	Tigiray	Raya Azebo	Hujira	1614	12 39.933	039 38.838	*Hetrorabitidus Sp.*
Am-Ger-Tes-74	Tigiray	Tigiray	Alamata	Gerjeli	1460	12 28.499	039 36.289	*Hetrorabitidus Sp.*
Am-DeD-Tes-76	Oromiya	West Showa	Bako	Denbi Dima	1643	09 06.559	037 05.834	*Strenematodes Sp*
Am-AnD-Tes-80	Oromiya	East Wollega	Gobu Seyo	Angobo Dembi	1757	09 06.508	036 59.459	*Strenematodes Sp*
Am-She-Tes-244	Oromiya	West Wollega	Gimbi	Shene	1252	09. 03 677	036. 05 921	*Hetrorabitidus Sp.*
Am-Amb-Tes-281	Benishangul Gumuz	Asosa	Asosa	Amba 16	1386	09. 57 100	034. 39 446	*Strenematodes Sp*
Am-Aso-Tes-287	Benishangul Gumuz	Asosa	Asosa	Amba 23	1562	10. 04 683	034. 38 339	*Strenematodes Sp*
Am-Ben-Tes-292	Oromiya	West Wollega	Mene Sibu	Bengua	1419	09. 47 114	034. 55 898	*Hetrorabitidus Sp.*
Am-MeG-Tes-293	Oromiya	West Wollega	Gimbi	Melka Gasi	1911	09. 08 841	035. 51 451	*Hetrorabitidus Sp*
Am-Tey-Tes-295	Oromiya	West Wollega	Mene Sibu	Teyibaba	1609	09. 48 167	035. 00 999	*Hetrorabitidus Sp.*
Am-DiM-Tes-341	Oromiya	Borena	Dire	Dida Mega	1549	04 01.932	038 20.707	*Hetrorabitidus Sp.*
Am-BuT-Tes-369	Oromiya	Borena	Gomole	Buya Tika	1602	05 06.068	038 16.039	*Hetrorabitidus Sp.*

Appendix 3: Figure 1. FAW larvae damage in the different crop plants tested in the no-choice experiment. A: Ethiopian mustard, B: Onion, C: Swiss chard, D: Cabbage, E: Soyabean, F: Haricot bean, G: Chickpea, H: Faba bean, I: False sorghum, J: lettuce, K: Garlic, L: Tomato, M: Potato, N: Pepper, O: Vetiver Grass, P: Wheat, Q: Barley, R: Sorghum, S: maize, T: Elephant grass

Appendix 4: Figure 2. FAW larvae damage in the different crop plants tested in the choice experiment.