

**EVALUATION OF ISOLATED FUNGAL ENTOMOPATHOGENS
AND SEED EXTRACTS OF *Piper guineense* AGAINST LARVAE OF
Helicoverpa armigera (Lepidoptera: Noctuidae) IN THE LABORATORY**

BY

NIMLIN DICKSON GABUIN

(10435554)

**BSc. (HONS) ZOOLOGY AND MEDICAL LABORATORY TECHNOLOGY
(UNIVERSITY OF BUEA, CAMEROON)**

**A THESIS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN
PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF
MASTER OF PHILOSOPHY (MPHIL) DEGREE IN ENTOMOLOGY**

**AFRICAN REGIONAL POSTGRADUATE PROGRAMME IN INSECT SCIENCE
(ARPPIS) UNIVERSITY OF GHANA, LEGON GHANA**

**JOINT INTER-FACULTY INTERNATIONAL PROGRAMME FOR THE
TRAINING OF ENTOMOLOGISTS IN WEST AFRICA**

**COLLABORATING DEPARTMENTS: ANIMAL BIOLOGY AND
CONSERVATION SCIENCE AND CROP SCIENCE (COLLEGE OF BASIC AND
APPLIED SCIENCES)**

JULY 2015

DECLARATION

I hereby declare that this thesis is the result of the original work personally done by me for the award of a Master of Philosophy Degree in Entomology at the African Regional Postgraduate Programme in Insect Science (ARPPIS) in the University of Ghana. All the references to other people's work have been duly acknowledged and this thesis has not been submitted in part or whole for the award of a degree elsewhere.

Signature..... Date.....

NIMLIN DICKSON GABUIN (Student)

SUPERVISORS:

Signature..... Date.....

REV. DR. WINFRED SETH KOFI GBEWONYO

Signature..... Date.....

DR. VINCENT YAO EZIAH

Signature..... Date.....

DR. WINFRED-PECK DORLEKU

ARPPIS COORDINATOR:

Signature..... Date.....

DR. ROSINA KYEREMATEN

ABSTRACT

A major constraint to increased and sustainable production of tomatoes is its infestation by insect pests particularly the tomato fruit worm *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae). The control of this insect relies heavily on the use of synthetic insecticides which have negative environmental effects. Some insects have developed resistance to certain insecticides. Efforts are therefore being made to look for alternative control strategies that are environmentally-safe and sustainable. This study determined the insecticidal effects of West African black pepper, *Piper guineense* (Schum and Thonn) seed extracts in combination with some indigenously isolated fungal entomopathogens on the larvae of tomato fruit worm, *H. armigera* and monitored the population trend of the pest on tomato fields during the major and minor tomato growing seasons. The entomopathogenic fungi were isolated from soil samples collected from around the University of Ghana, Legon, Accra and its neighbouring areas, using larvae of *Tribolium* spp. as baits. The results obtained in this study showed that there were more tomato fruit worms on tomato fields in the first cropping season than the second season. Only one of the entomopathogenic fungi was re-isolated from dead *H. armigera* and this was identified as *Aspergillus* sp. The pure isolate of fungus (*Aspergillus* sp.) and methanolic seed extract of *P. guineense* were bioassayed on *H. armigera* using topical application. *Aspergillus* sp. and the seed extract induced significant levels of mortality to the *H. armigera* larvae. *Aspergillus* sp. and seed extract induced 20% and 50% mortality, respectively. After six days of treatment but the seed extract inhibited the growth of *Aspergillus* sp. The study showed that fungi isolates and *P. guineense* seed extract could be potential control agents in the management of the tomato fruit worm.

DEDICATION

This work is dedicated to my caring grandmother Mami Helena Njarhang and to the Agricultural, Research and Scientific community.



ACKNOWLEDGEMENTS

My sincere gratitude goes to my supervisors Rev. Dr. Winfred Seth Kofi Gbewonyo, Dr. Winfred-Peck Dorleku and Dr. Vincent Yao Eziah for their suggestions, directions, guidance, development and active supervision of this work. Without which I would not have gone through.

I thank Mr. Henry Davis (at ARPPIS), Mrs. Esther Odoi-Barko, Mr. Godwin Behcha, Mr. Jacob Kwaku Donkor, Mr. Daniel Oduro Mensah and Mrs. Doris Amanor (at the Department of Biochemistry, Cell and Molecular Biology), Mr. Appiah (at Crop Science, Pathology Division), and Mr. Kojo Sakyi Yerinkyi and Dr. Michael (at Noguchi Memorial Institute) for their technical and professional assistance in the course of this project. I also thank the ARPPIS Teaching Assistants, Ms. Hettie Boafo and Mr. Thomas Buxton and the receptionist, Mr. Kodjo Benjamin Atta-Mensah for their encouragement and help.

My immense gratitude goes to the ARPPIS advisors for the theoretical and practical courses taught in the first year that prepared me for this thesis writing.

I thank Dr. Fokam Eric for giving me information on this study opportunity in Ghana and Associate Professor Kimbi Helen-Mbufong, Dr. Konje Christina, Dr. Sumbele Irene and Mr. Tange Denis Achiri for their assistance and cooperation in my being admitted into this University.

I am very grateful to my parents and relatives for their moral, financial and social support in bringing me up to this stage in life.

I appreciate all my mates and friends within and without ARPPIS for their assistance, words of encouragement, suggestions and positive criticisms towards this work. I am so grateful for having you.

My sincere appreciation also goes to the German Academic Exchange Service (DAAD) for the sponsorship of this M.Phil. Programme in the University of Ghana.

Finally and above all, I give much thanks and praises to the Almighty Creator for life and the abilities, providing all the requirements to go through all the stages I have been through.

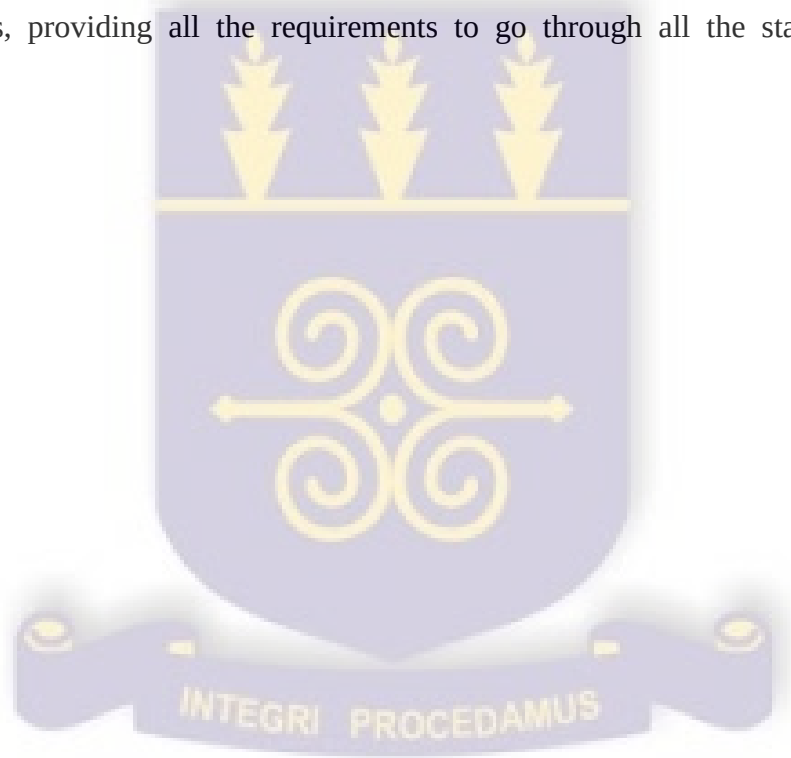


TABLE OF CONTENTS

DECLARATION	i
ABSTRACT.....	ii
DEDICATION	iii
ACKNOWLEDGEMENTS.....	iv
LIST OF FIGURES	ix
LIST OF PLATES	x
LIST OF TABLES.....	xi
LIST OF APPENDICES.....	xii
CHAPTER ONE	1
1.0 INTRODUCTION	1
1.1 JUSTIFICATION	5
1.2 OBJECTIVES.....	7
1.2.1 General objective	7
1.2.2 Specific objectives	8
CHAPTER TWO	9
2.0 LITERATURE REVIEW	9
2.1 The tomato plant	9
2.1.1 Agronomic practices	10
2.1.2 Economic and nutritional Importance of tomato	11
2.2 Pests and Diseases of tomato	12
2.2.1 Diseases of tomato	13
2.2.2 Pests of tomato.....	15
2.3 The tomato fruit worm (<i>Helicoverpa armigera</i>) (Hubner)	19
2.3.1 Biology and description of the tomato fruit worm.....	19
2.3.2 Tomato fruit worm generation	23
2.3.3 Origin and distribution of tomato fruit worm	23
2.3.4 Host range of tomato fruit worm	25
2.3.5 Pest status of the tomato fruit worm and how it causes damage to crops	26

2.3.6 Economic importance of <i>Helicoverpa armigera</i>	28
2.4 Management of the tomato fruit worm (<i>Helicoverpa armigera</i>)	29
2.4.1 Chemical control.....	30
2.4.2 Host plant resistance	30
2.4.3 Cultural control.....	31
2.4.4 Biological Control.....	31
2.5 Phyto-insecticides or Botanical insecticides used for insect pest control	34
2.5.1 West African Black pepper, <i>Piper guineense</i> (Schum and Thonn)	36
2.6 Compatibility of insecticidal plant extracts with entomopathogenic fungi.....	39
CHAPTER THREE	41
3.0 MATERIALS AND METHODS.....	41
3.1 Study Area	41
3.2 Establishment of tomato field	41
3.3 Population trend of tomato fruit worm	42
3.4 Insect collection and rearing	43
3.4.1 Collection of tomato fruit worm from the field	43
3.4.2 Laboratory culture of <i>H. armigera</i>	43
3.4.3 Laboratory culture of <i>Tribolium</i> spp.....	45
3.5 Isolation of entomopathogenic fungi	46
3.5.1 Soil sampling and the baiting of entomopathogenic fungi.....	46
3.5.2 Isolation of fungi from insect cadavers.....	49
3.5.3 Selection of entomopathogenic fungi	50
3.5.4 Myco-insecticidal effect of entomopathogenic fungi on <i>H. armigera</i> larvae.....	51
3.6 Morphological identification of entomopathogenic fungus.....	52
3.7 <i>Piper guineense</i> seed methanol extract preparation and testing	53
3.7.1 Collection and preparation.....	53
3.7.2 Methanol Extraction of <i>P. guineense</i> seeds	54
3.7.3 Insecticidal effect of plant extract.....	55

3.7.4 Compatibility of <i>P. guineense</i> seed extract with entomopathogenic fungus	55
3.9 Data Analyses	56
CHAPTER FOUR.....	57
4.0 RESULTS	57
4.1 Tomato fruit worm population dynamics.....	57
4.1.1 Population of larvae on tomato field in two planting seasons in 2014/2015	57
4.1.2 Relationship between tomato fruit worm population and some environmental factors.....	58
4.2 Isolation and confirmation of entomopathogenic fungi	60
4.2.1 Fungal isolation.....	60
4.2.2 Confirmation of entomopathogenicity of isolated fungi on larvae of <i>Tribolium</i> spp.	61
4.2.3 Myco-insecticidal effect of isolated fungi on <i>H. armigera</i> larvae	62
4.3 Insecticidal Effect of seed extract of <i>Piper guineense</i> seed on <i>H. armigera</i>	64
4.4 Insecticidal effect of seed extract and entomopathogenic fungus.....	65
4.5 LT ₅₀ of seed extract and SV3 fungus	66
4.6 Compatibility of entomopathogenic fungus with seed extracts	66
4.7 Morphological identification of the SV3 fungus	68
CHAPTER FIVE	69
DISCUSSION	69
CHAPTER SIX.....	74
CONCLUSIONS AND RECOMMENDATIONS	74
REFERENCES	76
APPENDICES	93

LIST OF FIGURES

Figure 1: Global distribution of <i>Helicoverpa armigera</i>	25
Figure 2: <i>Piper guineense</i> , a climber, and growing wild in the forests of Amedzofe, Ghana. The plant is shown clasping unto the trunk of a big tree.....	37
Figure 3: Population of tomato fruit worm per tomato plant during major and minor cropping seasons.....	57
Figure 4: Relationship between larval population and environmental temperature in the major cropping season.....	59
Figure 5: Relationship between larval population and rainfall in the major cropping season	59
Figure 6: Relationship between larval population and environmental temperature in the minor cropping season.....	59
Figure 7: Relationship between larval population and rainfall in the minor cropping season	60
Figure 8: Survival of larvae of <i>Tribolium</i> spp. inoculated with entomopathogenic fungi.	62
Figure 9: Percent survival of <i>H. armigera</i> after exposure to fungi for 11 days	63
Figure 10: Percent survival of <i>H. armigera</i> larvae treated with seed extract over 8 days period.....	65
Figure 11: Percent survival of tomato fruit worm after exposure fungus and extract for 9 days.....	66

LIST OF PLATES

Plate 1: Eggs of <i>H. armigera</i>	20
Plate 2: Larvae of <i>H. armigera</i>	21
Plate 3: Pupae of <i>H. armigera</i>	22
Plate 4: Adults of <i>H. armigera</i>	23
Plate 5: Tomato fruit worm feeding on leaves, flower and fruits.....	28
Plate 6: Three week old tomato field for population dynamic study	42
Plate 7: <i>H. armigera</i> rearing set up. at ARPPIS laboratory.....	44
Plate 8: Set up for rearing <i>Tribolium</i> spp. at ARPPIS laboratory	45
Plate 9: Soil samples for fungi isolation at Medicinal Plant Laboratory.....	48
Plate 10: A: Saprophytic fungal culture and B: Fungal culture of interest	50
Plate 11: Fungal treatment of tomato fruit worm	52
Plate 12: A. Fresh <i>P. guineense</i> seeds and B. Powdered <i>P. guineense</i> seed	53
Plate 13: A. Extraction on a stirrer plate B. Concentration of extract using a rotavapor and C. Drying of samples by vacuum.	54
Plate 14: Microapplicator used for topical application.....	55
Plate 15: Fungal growth on dead tomato fruit worm larvae.....	64
Plate 16: Morphological structure of <i>Aspergillus</i> sp. under compound microscope.....	68

LIST OF TABLES

Table 1: Locations for soil samples collection	47
Table 2: Geographic location of sample collection sites in isolated putative entomopathogenic fungi	61
Table 3: Effect of seed extract on <i>H. armigera</i> larvae after 6 days of treatment	64
Table 4: Lethal time (days) for cypermethrin, seed extract and SV3 fungus on <i>H. armigera</i>	66
Table 5: Compatibility of entomopathogenic fungus with acetone and aqueous solutions of seed extract	67

LIST OF APPENDICES

Appendix 1. T-test for larval population during the major and minor tomato cropping season	93
Appendix 2. Comparison of Survival Curves for larvae of <i>Tribolium</i> spp. when treated with fungi.....	93
Appendix 3. Comparison of Survival Curves of <i>H. armigera</i> when treated with fungi	93
Appendix 4 ANOVA for insecticidal effect of <i>Piper guineense</i> seed extract on <i>H. armigera</i>	94
Appendix 5. ANOVA for comparing effects of fungus and plant extract on <i>H. armigera</i> .	94
Appendix 6. ANOVA for compatibility of acetone seed extract and fungus.....	94
Appendix 7. ANOVA for compatibility of aqueous seed extract and fungus.....	95

CHAPTER ONE

1.0 INTRODUCTION

Vegetables are very important sources of nutrients including minerals and vitamins which are components of a balanced diet (Sajjan, 2006). The production of these vegetables, especially tomatoes (*Solanum lycopersicum*), is faced with the challenge of insect pests damage. In Ghana, tomato is cultivated all-year round under irrigation in the dry season and under rain-fed conditions in the rainy season. Yield is however low, with an average of about 10 tons per hectare. This is due to diseases, attack by pests and environmental factors (Kollie *et al.*, 2014). Most farmers practice seasonal tomato production with some farmers getting high yields during some periods. Tomato farming/production is therefore a source of nutrients, employment and finance and enhances standards of living for most families (Elizabeth and Shashi, 2010). It is cultivated in all the ten regions of Ghana but more extensively in the Ashanti and Brong Ahafo regions which contribute about 43% of total tomato produced in the country. Tomato provides nutrients like vitamins A and C, minerals (potassium, phosphorus, magnesium, calcium) and antioxidants such as lycopene which reduces risks of cancers and neurodegenerative diseases.

Pests and diseases of Tomato:

Among other reasons like low capital and soil fertility, diseases and arthropod pests' attack are the major constraints to increased productivity of tomato in the growing regions. Viral diseases, fungal infections and insect pest attacks are the most important setbacks in tomato cultivation. Insect pests of importance usually associated with tomato include whiteflies (*Bemisia tabaci*), aphids (*Aphis gossypii*), thrips (*Thrips tabaci*), leaf miners

(*Lyriomyza* spp.), and tomato fruit worms (*Helicoverpa armigera*) (Kolle *et al.*, 2014). Other pests of tomato include root-knot nematode, rust mite, red spider mite, plusia looper, cutworm, erinose mite, blight and wilt causing bacteria and fungi.

The whiteflies and the tomato fruit worms are major insect pests of tomato in the tropical tomato-producing regions probably because whiteflies transmit viral diseases on tomatoes and fruit worms cause serious direct damage to the tomato fruits, reducing the marketable tomato yield. The tomato fruit worm is also commonly called tomato fruit borer, African cotton worm, corn ear worm, cotton bollworm or old world bollworm or American bollworm. The tomato fruit worm, *H. armigera* is a noctuid moth with four main life stages, egg, larva, pupa and adult. The moths lay eggs singly or in groups on a variety of host plants. The eggs hatch into larvae in 3 to 6 days which have 5 to 7 instars but usually go through six instars. They feed on young twigs, leaves, flowers, buds and mostly on young fruits. The last larval instars drop off the host plant to pupate in soil. Adults emerge from the pupae to start a new generation.

The larval stage is the damaging stage of this insect pest, making it a very serious and important crop pest, particularly to tomato, corn, and cotton. They prefer feeding on their hosts' reproductive parts (flowers and fruits) though they may also feed on foliage. They feed on a variety of crops including chickpea, tomato, cotton, maize, and peanut. The feeding activity of this pest results in holes bored into reproductive structures and within leaves and twigs. On tomatoes, they invade young fruits, making holes in them as they feed. Sometimes larger larvae bore into older fruits. Secondary infection by other organisms on the infested fruits cause rotting and falling off of the fruits. This damage

results in yield loss, loss of product quality and hence low market value which leads to financial loss (Sullivan and Molet, 2007).

The pest is distributed through Asia, Europe, Oceania, and Africa. In Africa, affected countries include Nigeria, Ghana, Tunisia, Cameroon, Algeria, Benin and Ethiopia.

Control of the pest:

There are efforts made to control *H. armigera*. Both the use of bio-pesticides and chemical insecticides are among methods put in place to manage the pest. Synthetic insecticides have been widely and frequently used and are heavily relied on to control pests of tomato. These include Karate[®] (lambda cyhalothrin), Carbaryl, Combat[®] (hydamethylnon), Malathion, Attack[®] (emamectin benzoate) and Sunpyrifos (chlorpyrifos-ethyl). Over half of the insecticide application on cotton in India until the advent of Bt cotton was directed towards the cotton bollworm (Armes *et al.*, 1996).

The tomato fruit worms have been found to develop resistance to some chemical insecticides especially in areas where excessive application of these insecticides is common. For example, they have been found to be pyrethroid-resistant in the cotton and pulse growing areas of the Indian sub-continent. They are also developing resistance to organophosphates and other groups of insecticides (Armes *et al.*, 1996 and Borchert *et al.*, 2003).

Some Ghanaian tomato farmers have stopped the use of Combat[®] and Attack[®] and have moved to using Karate[®] and Sunpyrifos[®] due to ineffectiveness of the insecticides. Their ineffectiveness could be due to resistance by (Adu-Dapaah and Oppong-Konadu, 2002).

The adverse effects of these insecticides (environmental contamination, health problems etc.) have led to the focus of research on safer methods of pest control. These methods include use of botanicals and biological control agents such as parasitoids, predators and entomopathogens.

Botanicals are extracts of whole plants or plant parts which exhibit insecticidal activity. Neem and West African black pepper (*Piper* spp.) are examples of botanical insecticides used in insect pest control. Other commercially available formulations of botanicals include Biospark[®], Bipel[®], Biodos[®], Neemgold[®] and Exodos[®] (Sahayaraj *et al.*, 2011).

Entomopathogens are naturally-occurring microbes that are detrimental to insect pests. These include some bacteria, viruses and fungi. Most are highly specific with high virulence. *Beauveria bassiana* for instance occurs worldwide, with over 200 insect species mainly in the orders Lepidoptera and Coleoptera, being recorded as its hosts (Malarvannan *et al.*, 2010). Others include *Metarhizium anisopliae* and *Bacillus thuringiensis* (Bt), which are commercially available.

Some bio-control organisms and insecticidal plant products have therefore been used to control insect pests. These have led to some good results but this sector has not been deeply exploited, especially on the tomato fruit worms. Some of these products in use to control pests include the fungus *B. bassiana*, *B. thuringiensis* (Bt), Neem extracts (Azadirachtin) and *Piper* spp. extracts and powder. These have been tested on a number of insect pests including weevils, diamondback moth, and variegated grasshopper (Enkerli *et al.*, 2008 and Reddy and Mueller, 2015). Limited study has been done on the insecticidal

effects of entomopathogens on the tomato fruit worm and there have not been any study on the insecticidal effect of *P. guineense* seed extracts on *H. armigera*.

Organic farming and sustainable agriculture have therefore been found to be important in reducing the use of synthetic pesticides on produce like vegetables which are directly consumed by humans (Sajjan, 2006). Botanicals and entomopathogens are natural bio-pesticides which are ecologically-friendly, safe and cost effective.

1.1 JUSTIFICATION

The tomato fruit worm, *H. armigera* is a major pest of many crops and vegetables. Young larvae (2nd and 3rd) of *H. armigera* which can cause about 65% and 100% yield losses in cotton and pigeon pea, respectively at some locations in East Africa, Latin America and South Asia (Ting, 1986 and Thomas *et al.*, 1997), is also a major pest of tomato (Obeng-Ofori *et al.*, 2007). Globally, tomato fruit worm causes loss of more than \$300 million in pigeon pea per year (Thomas *et al.*, 1997).

H. armigera attacks the ripe and pre-ripe fruits of tomatoe, contaminating them with frass and exposing them to fungi and bacteria infection (Srinivasan, 2010). As a consequence, many farmers rely heavily on synthetic chemical insecticides like Combat®, Attack®, and Karate® to manage *H. armigera* and other insect pests. Much reliance on broad spectrum pesticides has been condemned in many parts of the world due to their adverse impacts on the environment. Pest resistance to synthetic pesticides, pest resurgence due to indiscriminate and untimely chemical pesticide use and their impacts on ecological imbalance make the need for sustainable alternative control methods necessary

(Malarvannan *et al.*, 2010). *H. armigera* has been developing resistance to most synthetic insecticides used against it, including pyrethroids (Sullivan and Molet, 2007).

High cost of synthetic insecticides, their persistence in the environment, etc. limit or prevent many farmers from purchasing them to protect their crops, though sometimes they are effective. The processes of finding effective and safer pest control methods as alternative to conventional pesticides are therefore ongoing. Fungal entomopathogens (e.g. *B. bassiana*) have been reported to be effective in reducing the weight and length of insects' larvae (*Spodoptera litura* larvae) hence effective in larval mortality (Malarvannan *et al.*, 2010). Fungal entomopathogens are commercially available and affordable. They can even be locally isolated since they are naturally-occurring.

Botanicals and entomopathogens are among the effective and sustainable agents of pest control. It is therefore important to study the insecticidal effects of botanicals with entomopathogens to maximize their efficacy against insect pests. Some studies have been undertaken on combining entomopathogens with botanicals and promising results have been achieved. For example, Sajjan (2006) obtained more mortality of *Plutella xylostella* Linnaeus (Lepidoptera: Plutellidae) when they were exposed to a mixture of *B. bassiana* and neem seed extract than with individual treatment. Therefore the combined use of pathogens and botanicals, if compatible may give better results.

The West African black pepper has been found to be a good alternative source of insecticide. It is native to Ghana, used as a spice and has medicinal properties, hence safe. Many species of *Piper* such as *P. guineense* and *P. nigrum* have been found to have insecticidal properties (Miyakado *et al.*, 1980). Piperine and guineensine have been found

as the main amides active in *P. guineense*, with the pipericide and guineensine reported to have more lethal effect on insects than pellitorine and kalecide (Miyakado *et al.*, 1980, Gbewonyo *et al.*, 1993 and Adosraku *et al.*, 2013).

Single applications of entomopathogens sometimes do not show any improvement in pest control. Single treatment of chickpea field with *M. anisopliae* and *B. bassiana* at a dose of 1×10^7 conidia/ml for example did not record any significant difference with the control treatment in reducing the number of *H. armigera* larvae (Rijal *et al.*, 2008). There is therefore the requirement for better formulation and application methods for fungal entomopathogens in which combination with botanicals could help in improving the effectiveness of either of them for pest control. Sajjan (2006) reported increase in cumulative mortality of diamondback moth treated with a combination of *M. anisopliae*, *B. bassiana* with neem seed kernel extract and cow urine. Sahayaraj (2011) found that *B. bassiana* tolerates (is compatible with) most commercial plant based pesticides and extracts. This study also found that aqueous extracts of most plants were more compatible with fungal entomopathogens compared to ethanol extracts.

Though some work has been done on the effect of seed extracts of *P. guineense* and entomopathogens on some insect pests, no study has been done on their effect on *H. armigera*.

1.2 OBJECTIVES

1.2.1 General objective

The goal of this research is to measure the population structure of tomato fruit worm, *Helicoverpa armigera* (Hubner) on tomato fields during the major and minor tomato

cultivation seasons and test the insecticidal effects of West African black pepper, *Piper guineense* (Schum and Thonn) seed extracts in combination with some isolated indigenous fungal entomopathogens on its larvae.

1.2.2 Specific objectives

The specific objectives of this study are:

1. To monitor the population of *H. armigera* larvae on tomato crops during the major and minor seasons of tomato cultivation.
2. To isolate entomopathogenic fungi from soils and determine their myco-insecticidal effect on *H. armigera* larvae.
3. To determine the insecticidal effect of methanolic extracts of *P. guineense* seed extracts on the larvae of *H. armigera*.
4. To test the compatibility of the isolated fungal entomopathogen with *P. guineense* seed extract for any synergistic effect.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The tomato plant

Tomato is said to have originated from the native peoples of South and Central America (today's Peru). European explorers brought the tomato from the Americas and disseminated it throughout Europe, Asia, Africa and other parts of the world sometime during the 16th century (Naika *et al.*, 2005). The tomato plant is now cultivated world-wide in a variety of environments. There are possibly more than 10,000 varieties in existence today and it is said to be the most widely eaten vegetable in the world.

In 1753 Linnaeus placed tomatoes in the genus *Solanum*, calling the cultivated tomato *Solanum lycopersicon* under a large and diverse Solanaceae family (Planet Natural, 1991 and Mueller, 2014).

Tomato is grown on more than five million hectares globally with nearly 129 million tons produced. China, the world's top grower of tomato accounts for more than one-quarter of the world's tomato acreage. Africa and Asia account for about 79% of world's tomato area with a global output of about 65% (Srinivasan, 2010). Tomato production in Africa is on the rise, with Egypt being the first tomato producing country in 2008 with an annual production of 9,204,097 tons and Morocco being among the top ten exporters of tomatoes in 2010 (FAOSTAT, 2010). There is a growing cross-border tomato trade arrangement between Burkina Faso – Ghana and Tanzania – Kenya. Tomato is grown in all African countries as its domestic consumption and demand is increasing with high yields recorded

in Nigeria, Ghana, Cameroon, Sudan and Benin. Tomato production in Ghana is a flourishing farming activity in the savanna and forest-savanna transitional belt. It is the most important crop in most established dry season gardens in most places. About 16,000 ha of Ghana's arable land are under tomato farming and mainly in the Ashanti (mostly in the Akumadan district) and Brong Ahafo (mostly in the Wenchi district) regions. Some levels of production also occur in the Greater Accra area, where co-operative tomato growing is concentrated around Amasaman, Dodowa, the Central region, around Mankesim and Swedru, the Eastern region, around Nsawam and Mangoase and the Volta region, around Sege and Ayikai-Dablo.

Overall annual production of tomato in Ghana is generally low and therefore inadequate for the nation. Average yield ranges from 7.5-15.0 tons per hectare (Adu-Dapaah and Oppong-Konadu, 2002 and Obeng-Ofori *et al.*, 2007).

2.1.1 Agronomic practices

Tomato is mainly cultivated in a few months of the year: major season during the rainy season i.e. June to October and minor season through irrigation during the dry season i.e. December to May with little rain. Within the calendar year in Ghana, different regions of the country produce tomato at different times of the year. Farming practices vary regionally, depending on farmers' choice, agro climatic conditions, opportunities and culture, and are acquired by farmers through colleagues, experience on the field and/or from agricultural extension officers in their localities. The ability to adhere to good farm management practices by farmers is very much influenced by a variety of factors, the most prominent of them being financial constraints (Adu-Dapaah and Oppong-Konadu, 2002).

Tomato is adapted to a wide range of climatic conditions, requiring fairly high day time temperatures (about 21 - 28°C) and low night temperatures of 15 - 20°C with high relative humidity to grow well. It does well on deep friable soils, rich in organic matter, well drained and with a high water-retaining capacity. Most growers raise tomatoes on beds based on the field's drainage. The field is tilled to form beds 0.15 – 0.18 m high and 1.68 m wide, especially in the rainy season or they are planted on flat grounds or furrows in the dry season. Tomato seeds are sown in nurseries and after 25 to 45 days, at five to seven-leaf stages, seedlings are transplanted into the fields mostly in rows. Fertilizer applied after about two weeks of transplant (Talekar *et al.*, 2003; Srinivasan, 2010).

Staking is done mainly for tomato cultivars with an indeterminate growth habit. Staking is done to prevent direct contact between fruits and the soil. Mulching of crop is done mostly in drier areas. This is to reduce evaporation, weed growth and to minimize possible erosion during the wet season. Irrigation is very necessary in the dry season most importantly when the crop is blooming and during early fruit development. Weeding is carried out when weeds are growing on the field.

The maturity stage at which the fruits are picked depends on the purpose for growing the crop and the farm to market distance. The first harvest takes about 45 to 56 days after transplanting and fruits may remain in crop for 4 to 5 weeks. Harvesting is done in the morning and/or in the evening (Obeng-ofori *et al.*, 2007).

2.1.2 Economic and nutritional Importance of tomato

Tomato is an important source of vitamins and an important cash crop for small-holders and medium-scale commercial farmers. As it is a relatively short duration crop, it fits into

different cropping systems and gives a high yield, it is economically attractive and the area under cultivation is increasing (Del Valle *et al.*, 2007)

Tomato fruits are consumed fresh in salads or cooked in sauces, soup and meat or fish dishes. They can be processed into purées, juices and ketchup. Canned and dried tomatoes are economically important processed products. Tomatoes contribute to a healthy, well-balanced diet. They are rich in micronutrients content which includes minerals, vitamins, essential amino acids, sugars, some antioxidants and dietary fibers. Tomato contains much vitamin B and C, iron, potassium, calcium, magnesium and phosphorus (Naika *et al.*, 2005). It contains high levels of lycopene that reduces the risks associated with several cancers and neurodegenerative diseases (Miller *et al.*, 2002).

Tomato also boosts income and hence standard of living of producers. Farming tomato has been a major source of employment and income generation for farmers and all other agents involved in its production and marketing. In Ghana, the high levels of tomato consumption coupled with the high production levels in the Upper East region for example, make the sector appear economically viable, supplying the country's tomatoes in the dry season (Adimabuno, 2010). Some farmers in Ghana achieve higher tomato yields, profitable production, and continue to choose to grow tomatoes as compared to other crops (Elizabeth and Shashi, 2010).

2.2 Pests and Diseases of tomato

Tomato production is negatively affected by a large number of diseases and pests that lead to poor yield of the crop hence causing food loss and financial loss due to fall in yield and the cost of managing the pests and diseases. Disease and pest attacks are some of the major

constraints to increased productivity in all tomato growing locations. Infection and infestation of tomato are caused by microorganisms like nematodes and macro organisms like insect pests (Akemn *et al.*, 2000; Jones *et al.*, 2004; Srinivasan *et al.*, 2010)

2.2.1 Diseases of tomato

Tomato plants are susceptible to several fungi, bacteria and viruses. Fungi and bacteria cause foliar (leaf), fruit, stem or root diseases (Anastacia *et al.*, 2011). A virus infection often leads to dwarfed growth and decreased production (Nouhoheflin *et al.*, 2009). Damage caused by diseases can result in considerable yield losses to farmers.

Bacterial diseases

Bacteria almost always infect the plant through weak spots, such as scars, stomata and lenticels (small openings on the surface of stems and roots) and wounds (e.g. from pruning) or other mechanical injuries. Once they have penetrated the plant, bacteria usually end up in the vascular system of stems, roots and leaves, often causing the latter to wilt (Akemn *et al.*, 2000; Tumwine *et al.*, 2002). Some bacterial diseases commonly found in tomatoes are as follows:

Bacterial wilt caused by *Ralstonia solanacearum*, which causes plant parts and subsequently the whole plant to wilt.

Bacterial spot caused by *Xanthomonas axonopodis* pv *vesicatoria*. The pathogen affects leaves, fruits and stems. Small spots appear on the leaves and on the fruit of infected plants. These spots are generally brown and circular. Leaves turn yellow and drop off. Elliptical lesions are found on stems and petioles.

Bacterial canker caused by *Clavibacter michiganensis*. The leaves of infected plants become yellow, wilt and dry up. Long, brown stripes, which can split open, appear on the stem. Adventitious roots may develop on the stems. Stems may also display cankers under some conditions. Internally, the vascular tissues of the stems display light yellow to brown streaks (Akemn *et al.*, 2000; Tumwine *et al.*, 2002; Naika *et al.*, 2005).

Viral Diseases:

Tomato is very sensitive to viral diseases. Viruses are often spread in tomato farms by insect vectors such as whiteflies, thrips and aphids. The damage caused by the viruses is usually much greater than the mechanical injury caused by the insect vector. Viruses affecting tomato include:

Tobacco mosaic virus (TMV). Machinery or workers transmit the virus mechanically to healthy plants. Symptoms include yellow-green spotted leaves, rolled-up leaves, stunted growth and discolouration of fruits (Kamba, 1986; Abel *et al.*, 1986).

Cucumber mosaic virus (CMV). It is transmitted by different aphid species to tomato plants from weeds or neighbouring crops. Infected tomato leaves may show a mild green mottling or more shoestring symptoms in which the leaf blades are greatly reduced. Infected fruits are small in size and often misshapen (Chabbouh and Cherif, 1990).

Tomato spotted wilt virus (TSWV). TSWV is transmitted by several thrips species. Infected plants are stunted and display yellow leaves. Fruits show characteristic green, yellow and red, slightly raised bulls-eye rings (Sherwood *et al.*, 2003).

Tomato yellow leaf curl virus (TYLCV). This virus is transmitted by whiteflies. Infected plants are erect and stunted. Leaves are yellow and curl upward or downward. An entire

field can be destroyed if plants are infected in the nursery (Naika *et al.*, 2005; Matete and Ndung'u, 2011).

Fungal diseases

A fungal infection is often caused by fungal spores that land on any surface of a plant part, germinate there and penetrate the plant tissue through its stomata, wounds, or sometimes even directly through the plant's surface. The harmful effects of the fungus are usually limited to the affected area, but there are some types of fungi that invade the plant's vascular tissues (xylem) and thus spread throughout the plant (Srinivasan *et al.*, 2010). The most important fungal infections in tomato include:

Early blight, caused by *Alternaria solani*. It is spread via seeds, wind, rain and infected plant remains. Plants that have been damaged are more susceptible to this fungus. Round, brown spots (with concentric rings) appear on the infected leaves. Sometimes small lumps can be found on the stem or on leaves, causing leaves to turn yellow and wilt. Infected flowers and small fruits fall off (Pscheidt and Stevenson, 1986; 1987).

Fusarium wilt, caused by *Fusarium oxysporum*. From the bottom up, leaves wilt, turn yellow and curl at the edges. Pink fungus fluff is found on dead plant parts (Mohamed *et al.*, 1994; Ploetz, and Pegg, 1997.).

Anthrachnose, caused by *Colletotrichum coccodes*. Transmission is most common via infected plant material (especially the fruit). Signs of infection by this disease are grey-brown spots (dents) on the fruit and, in humid weather, salmon-pink spores (Naika *et al.*, 2005, Srinivasan *et al.*, 2010).

2.2.2 Pests of tomato

Nematode Pests

Nematode infestation and transmission can occur in many ways: via infected plant material, farming tools, rainwater and irrigation water, strong winds (which carry infested soil particles), and contaminated soil carried on shoes, or animal feet (Corbett *et al.*, 2011). Some nematodes feed from the outside of plants, others enter the plant. All nematodes feed on the plant's sap, which can reduce the plant's productive capacity. Greater damage can occur if viruses or fungi enter the plant as a result of the injuries caused by the nematodes, and then proceed to hamper the plant's health, and the plant eventually dies. Nematode infestation usually begins in a small, limited part of the cultivated area, and spreads slowly throughout the farm.

Root-knot nematodes are of major importance in tomato cultivation. They cause galls (infected swellings) on plant roots. Three common types of root-knot nematodes are: *Meloidogyne incognita*, *M. javanica* and *M. arenaria*. Affected plants remain small, and are liable to soil-borne fungal and bacterial diseases. Nematodes cause yield losses of about 30% in tomato in the tropics (Naika *et al.*, 2005).

Major insect pests

The tomato plant is associated with a lot of insects most of which are serious damaging pests, a few are biological control/pollinating agents and others are neither pest nor of any benefit to the plant. Several kinds of insect pests attack tomato leaves, flower buds and fruits during tomato cultivation. These include:

Aphids: Winged aphids migrate into tomato fields from wild hosts and other crop fields and begin to establish colonies on the plants. Two species of aphids are common on

tomatoes, the potato aphids (*Macrosiphum euphorbiae*) and green peach aphids (*Myzus persicae*) (Hebert *et al.*, 2007; Kaushik, 2011; Mohamed *et al.*, 2014). The Aphids suck up sap from the plant with their piercing-sucking mouthparts. Tomato plants can tolerate large numbers of aphids without suffering yield loss but however, severe infestations can cause leaves to curl and may stunt plants. Decreased leaf area can increase sun scald to the fruit. Aphids are also vectors of certain plant viruses mentioned above.

Colorado potato beetle (*Leptinotarsa decemlineata*): It is an infrequent pest of newly set tomato plants. The adult and larva feed on the leaves and terminal growth of tomato plants, but typically only cause serious damage to young plants (Hunt and Whitefield, 1996). Once plants reach eight inches, adult or larval feeding, regardless of the apparent severity of damage, does not reduce fruit yield.

Tomato Fruit worm (*Helicoverpa armigera*): This is a serious and an important insect pest of the tomato plant (Firempong and Zalucki, 1990; Sharma, 2001; Talekar *et al.*, 2006). The adult moths lay eggs at night on flowers and/or on leaves near green fruit at the outer edges of the plant. After the eggs hatch, the larvae feed short periods of time on the foliage before attacking the fruit. They prefer to feed on green fruit and usually do not enter ripe fruit (Sharma, 2001; Talekar *et al.*, 2006). Damage consists of deep watery cavities frequently in the stem end of the fruit. During its development, one larva may injure several fruits. Their feeding results in a messy, watery, internal cavity filled with cast skins and feces. Damaged fruits will ripen prematurely. Late in the season, small larvae will also enter ripe fruit. Tomato fruit worm has a wide host range but tomatoes are preferred for egg-laying especially over corn. Other moths infest tomato in a similar manner.

Cabbage looper: Cabbage loopers (*Trichoplusia ni*) are common in tomato fields but they rarely cause serious damage. They are foliage feeders, and very rarely directly attack the fruit. When large populations are present they can lower yields by reducing plant vigor and increasing sun scald of fruit through foliage loss (Bessin, 2003).

Armyworms: Beet armyworms (*Spodoptera exigua*) and common armyworms (*Spodoptera litura*) are a widespread pest found in tomato fields every year. In some areas they may be the most important caterpillars attacking tomato (Kennedy, 2002; Heuvelink, 2005). The armyworm attacks both foliage and fruit, creating single or closely grouped circular or irregular holes. The common armyworms are nocturnal and actively feed during night hours. In fresh market tomatoes, the presence of such holes results in unmarketable fruits. The beet armyworms occasionally develop inside the fruit, causing damage similar to that of the tomato fruit worms whereas the common armyworms do not bore holes into tomato fruits.

Whitefly (*Bemisia tabaci*, *B. argentifolii*, *Trialeurodes abutilonia*, etc.): Whiteflies are found mostly on the undersides of leaves. Some species of whitefly (both adults and nymphs) cause damage to leaves by feeding (suck plant sap), which causes reduced plant vigour, curling and yellowing of leaves, and the insects produce honeydew, which causes leaves to appear blackened (from sooty mould growing on the honeydew), hence reducing photosynthetic efficiency of plants. Feeding by silverleaf whitefly is particularly damaging because it also causes fruit to ripen unevenly. Some whiteflies have been found to be the vectors of tomato infectious chlorosis virus, a virus capable of causing heavy losses in the production of fresh-market and greenhouse tomatoes. *Bemisia* species of whiteflies transmit Gemini viruses such as Tomato Yellow Leaf Curl (Zalom *et al.*, 2009). In 2003,

yellow leaf curl devastated the crop resulting in big losses for farmers in Ghana which made farmers reluctant to grow tomatoes, causing buyers and consumers to travel to Burkina-Faso to source tomatoes as they could not get sufficient quantity from the Upper East region (Elizabeth and Shashi, 2010).

Thrips: Several thrips also attack tomato but *Thrips tabaci* is the most important one. Thrips chafe the surfaces and suck the sap from broken cells on flowers and young fruit. Additional damages include blossom drop, scarring of fruit and malformation of the leaves. Thrips also act as vectors of kromnek (spotted wilt virus).

2.3 The tomato fruit worm (*Helicoverpa armigera*) (Hubner)

2.3.1 Biology and description of the tomato fruit worm

The tomato fruit worm occurs worldwide. It under goes complete life cycle, i.e. egg, larva, pupa and adult, with many generations annually. The adults are nocturnal, feeding on nectar. Eggs are laid on flowers or leaves which hatch to larvae. The larvae have five to six instar stages and feed on young leaves, stems, floral parts and fruits. The last instar drops into the soil to pupate, from which the adults emerge. The total life cycle takes between 44 and 65 days but the complete life cycle can be completed in just over a month if conditions are favourable (Evenson and Basinski, 1973; Fitt, 1989).

Eggs: Females of this pest lay hundreds of eggs on their host leaves and flowers/young fruits singly, at night. The freshly laid eggs of *H. armigera* are spherical in shape with a flattened base, yellowish-white and glistening at first but changes to dark brown shortly before hatching. The incubation period of eggs is reported to averagely be 4.02 ± 0.64 days with a range of 3 to 5 days, but takes longer in colder weather (Kumar *et al.*, 2009).

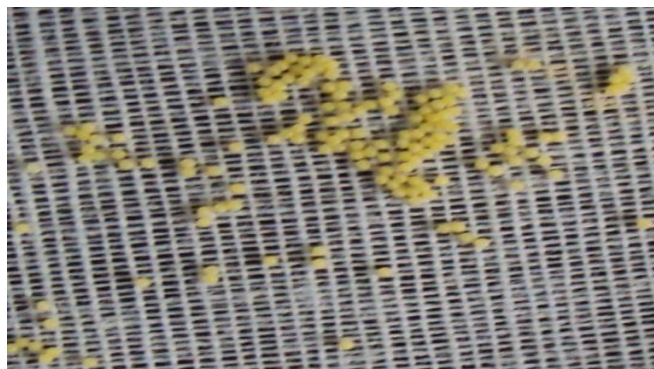


Plate 1: Eggs of *H. armigera* (photo credit: Nimlin Dickson)

Larva: The larval period of *H. armigera* is completed through five to six distinct instars. The colours of the larvae vary very much due to diet content, ranging from greenish brown, yellowish-brown, light black brown to brown with longitudinal stripes. The newly hatched larvae are tiny, active having light brown body with dark brown head. The first and second larval instars are yellowish-white to reddish-brown with dark-brown to black head capsule. The full grown larva is straw-yellow to green with lateral brown strips and the head as well as prothoracic legs are dark brown to black in color. The full grown larvae (pre-pupal stage) become sluggish, wrinkled with suspended feeding and movement. The pre-pupa is light green yellowish in color but later on turns to dark-brown. This stage lasts for 1-3 days with an average of 2.15 ± 0.16 days. When fully fed, the larvae descend to the soil and, after 1-7 days, pupate in an earthen cell, 2-8 cm below the surface. The total larval duration is reported to be between 22 to 40 days on various diets.



Plate 2: Larvae of *H. armigera* (photo credit: Nimlin Dickson)

Pupa: The freshly formed pupa is light green yellowish in colour, obtect type and becomes mahogany-brown colour. It gets further darkened prior to the emergence of moth. The surface is smooth and rounded, broad anteriorly and tapering posteriorly, with two tapering parallel spines at the posterior tip. The abdomen is distinctly marked into ten segments with spiracles located on fourth and ninth segments. The pupae normally develop inside a silken cocoon in soil. This stage takes minimum and maximum period of 9 and 24 days, respectively.



Plate 3: Pupae of *H. armigera* (photo credit: Nimlin Dickson)

Adult: The adult moth is stout bodied with broad thorax. The forewings have a series of dots on the margins and a black comma-shaped marking in the middle underside of each forewing. However, the hind wings are lighter in colour with a broad dark-brown border at the apical end; they have yellowish margins and strongly marked veins. The tip of abdomen of females is marked by a tuft of hair. They mate during early hours of the night. The average longevity of the adults ranges between two to twelve days, with females living longer than males (Ali *et al.*, 2009, Sharma *et al.*, 2011 and Shivanna *et al.*, 2012). Females of the tomato fruit worm start laying eggs 2-6 days after emergence.



Plate 4: Adults of *H. armigera* (photo credit: Nimlin Dickson)

2.3.2 Tomato fruit worm generation

The tomato fruit worm goes through three to twelve generations annually. The number of generations varies and depends on the place, the host plants of the insect and environmental and physical conditions. Sharma *et al.*, (2011) reported three generations from October to May on tomato. According to Borchert *et al.*, (2003), the tomato fruit worm is extremely well adapted to agro-ecosystems and can exhibit up to 11 generations a year under good conditions. The pest exhibits overlapping generations in the field.

2.3.3 Origin and distribution of tomato fruit worm

The tomato fruit worm, *H. armigera* (Hübner) (Lepidoptera: Noctuidae) is geographically widespread, present in Europe, Asia, Africa, Oceania and Brazil. Historically, it is thought to have colonized the American continents around 1.5 to 2 million years ago, leading to the current *Helicoverpa zea* populations in the continents. This species divergence history is evident in the mating compatibility between *H. zea* and *H. armigera* under laboratory conditions (Tay *et al.*, 2013). The wide distribution of the pest is as follows:

In Africa, it is found in Algeria, Angola, Benin, Botswana, Burkina Faso, Burundi, Cameroon, Cape Verde, Central African Republic, Chad, Congo, Côte d'Ivoire, Egypt, Ethiopia, Gabon, Gambia, Ghana, Guinea, Kenya, Lesotho, Libya, Madagascar, Malawi, Mali, Mauritania, Mauritius, Morocco, Mozambique, Namibia, Niger, Nigeria, Rwanda, Senegal, Seychelles, Sierra Leone, Somalia, South Africa, Sudan, Tanzania, Togo, Tunisia, Uganda, Zaire, Zambia, Zimbabwe etc. (Zalucki et al 1986, Guo 1997; EPPO, 2006).

In Asia, it is found in Afghanistan, Armenia, Azerbaijan, Bangladesh, Bhutan, Cambodia, China (widespread), Cyprus (locally established), Georgia, Hong Kong, India (widespread), Indonesia (widespread), Iran, Iraq, Israel, Japan, Jordan, Kazakhstan, Korea Democratic People's Republic, Korea Republic, Kuwait, Kyrgyzstan, Lao, Lebanon, Malaysia, Myanmar, Nepal, Pakistan, Philippines, Saudi Arabia, Singapore, Sri Lanka, Syria, Taiwan, Tajikistan, Thailand, Turkey, United Arab Emirates, Uzbekistan, Viet Nam, Yemen (Zalucki et al 1986, Guo 1997; EPPO, 2006).

In Oceania, it is found in American Samoa, Australia (north of 17°S and along the east coast), Cocos Islands, Fiji, Guam, Kiribati, Marshall Islands, Micronesia, New Caledonia, New Zealand, Norfolk Island, Northern Mariana Islands, Palau, Papua New Guinea, Samoa, Solomon Islands, Tonga, Tuvalu, Vanuatu (Zalucki *et al.*, 1986, Guo 1997; EPPO, 2006).

H. armigera is established in the following European states: Bulgaria, Greece, Portugal, Romania, Spain (widespread) and Cyprus, France, Hungary and Italy (restricted distribution).

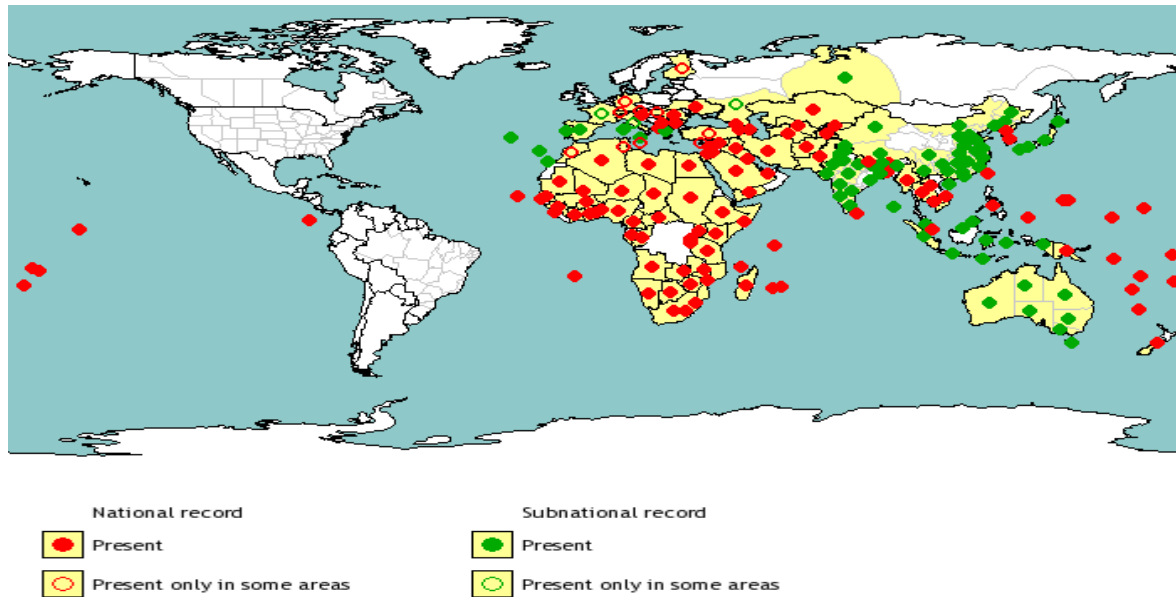


Figure 1: Global distribution of *Helicoverpa armigera*

Source: EPPO, 2006. Distribution maps of Quarantine Pests. *Helicoverpa armigera*.

2.3.4 Host range of tomato fruit worm

The tomato fruit worm is polyphagous and omnivorous, with the larvae attacking at least 60 cultivated and 67 wild host plants from numerous families including Asteraceae, Fabaceae, Malvaceae, Poaceae and Solanaceae. It primarily feeds on ornamental plants and flowers. Among the numerous hosts, the economically important and major hosts of the insect are: cotton, tobacco, tomatoes, potatoes, maize, soya bean, sorghum, lucerne, Phaseolus, chickpeas, and other Leguminosae. Not all host plants are equally preferred for oviposition but can be utilised in the absence of a preferred host. Female moths tend to choose pubescent (hairy) surfaces for oviposition rather than smooth leaf surfaces (King, 1994). Tall plants also tend to attract heavier oviposition than shorter plants. Pigeon pea and corn are considered to be the most suitable hosts for this insect, when compared to sorghum, red ambadi, marigold, and artificial diet (Bantewad and Sarode, 2000).

The larvae prefer to feed on reproductive parts of hosts (flowers and fruits) but may also feed on foliage. Although feeding larvae can sometimes be seen on the surfaces of plants, they are often hidden within plant organs (i.e. flowers or fruits), in which case bore holes may be visible (Smith-Pardo, 2014).

2.3.5 Pest status of the tomato fruit worm and how it causes damage to crops

This moth is a major pest threat because the larva can feed on a wide range of economically important crops as mentioned above. In addition to feeding on high value crops, the tomato fruit worm is an extremely serious and dangerous pest because its high fecundity, it has a short generation time, it can sustain itself on so many different plant species, it can undergo diapause during adverse conditions and it can migrate over long distances (Shanower and Romeis, 1999). To make matters worse, the pest is reported to have evolved a high degree of resistance to organophosphate and pyrethroid insecticides (Armes *et al.*, 1996). The tomato fruit worm has evolved two major strategies for adapting to adverse conditions. First, it has excellent migratory abilities and can fly up to 155 miles (250 km) in search of a viable food source. Secondly, it has the ability to enter into a facultative diapause when conditions become too hot or cold. These allow the tomato fruit worm to survive adverse conditions until environmental conditions improve (Borchert *et al.*, 2003). *H. amigera* larvae are extremely damaging because they prefer to feed and develop on the reproductive structures of crops (flowers, buds, immature fruits) which are rich in nitrogen. These structures are often the part of the crop that is harvested. Depending on the crop, tomato fruit worm induced damage can range from 50 to 90 percent of yield loss (Wang and Li 1984; Arnó *et al.* 1999; Lammers and MacLeod, 2007).

Feeding damage results in holes bored into reproductive structures and feeding within the plant. Secondary pathogens (fungi, bacteria) usually develop due to the wounding of the plant. The insect frass also occur alongside the feeding hole from larval feeding within.

The greatest effect on exotic conifers is in nurseries and newly planted areas, where seedlings may be extensively defoliated. This attack on seedlings usually occurs after populations have built up on a preferred alternative host, eaten out this food supply, and then transferred to the pines to complete their development. On pigeon pea, flower buds and flowers bored by small larvae may drop; larger larvae bore into locules of pods and consume developing seed. On cotton, bore holes are visible at the base of flower buds, and the buds are hollowed out. Bracteoles are spread out and curled downwards. Leaves and shoots may also be consumed by larvae. Larger larvae bore into maturing green bolls; young bolls fall after larval damage. On corn, eggs are laid on the silks, larvae invade the ears and developing grain is consumed. Secondary bacterial and fungal infections are common. On chickpea, larvae feed on foliage; sometimes the entire small plants are consumed. Larger larvae bore into pods and consume developing seed. On tomatoes, foliage, flowers, buds and young fruits are invaded and the fruits fall; larger larvae sometimes bore into older fruits (Zalucki *et al.*, 1986, Guo 1997. The pest therefore damages the tomato fruits directly, leading to huge loss. Secondary infections by other microorganisms lead to rotting. In areas where extensive defoliation occurs, terminal buds may be eaten out and, rarely, bark is also chewed.



Plate 5: Tomato fruit worm feeding on leaves, flower and fruits.

2.3.6 Economic importance of *Helicoverpa armigera*.

The severity of *H. armigera* attack varies not only among crops and regions, but also on temporal scale. This pest is of major economic importance in Africa and other affected regions, highly destructive and causing serious damage. Regional and local differences in hosts' preference of the pest can give rise to differences in pest status on particular crops. *H. armigera* has been reported to cause serious losses throughout its range, in particular to tomatoes, corn, and cotton. Because the larvae feed mainly on the flowers and fruit of high value crops, high economic damage can be caused at low population densities. On cotton, two to three larvae on a plant can destroy all the bolls within 15 days (CABI, 2007). Young larvae (second and third instar) can cause up to 65% loss to cotton yields (Ting, 1986). The *H. armigera* causes significant losses to tobacco during growth and reproductive stage of the crop, by feeding on growing buds in early stage and developing capsules at later stage (Shivanna *et al.*, 2012). Losses of over 900 million U.S. dollars on chickpea and pigeon pea and an estimate of 5 billion U.S. dollars on total crop losses due to *H. armigera* infestation have been recorded globally (Sharma, 2001) and an addition of about 1 billion U.S dollars spent on insecticides for control. In pigeon pea, an important grain legume in

South Asia, East Africa, and Latin America, this single pest causes yield losses of up to 100% in some years and locations (Thomas *et al.*, 1997). In Spain, *H. armigera* is one of the most harmful pests in tomato crops destined for industry (Arnó *et al.*, 1999).

Tomato yield losses due to the pest infestation in Ghana can be as high as 64 % without the use of insecticides (Gianessi, 2009). In the Old World, the annual cost of the damages caused by *H. armigera* has been estimated to be more than two billion U.S dollars. In Brazil, the economic loss caused by infestation of *H. armigera* in two crop growing seasons is estimated to be up to ten billion U.S dollars (Warren, 2013).

The activity of the tomato fruit worms vary throughout the year, peaking and declining during certain periods. The varied moth activity might be due to the weather factors, availability of other hosts and the preferred crop's availability/stage on the field (Shivanna *et al.*, 2012).

2.4 Management of the tomato fruit worm (*Helicoverpa armigera*)

Due to the damage caused by this pest on economically important crops, its control is therefore very important to curb the losses it causes. Management of tomato fruit worm requires careful monitoring for eggs and small larvae. When control is needed, it is essential to treat before large numbers of larvae enter fruit, where they are protected from sprays.

Management strategies have been proposed for *H. armigera*, such as use of biological control; pest monitoring; reduction of the seeding window for some crops; and adoption of refuge areas of conventional plants near transgenic cultivars. The pest can also be managed using integrated pest management techniques where all the control measures are integrated

in managing the pest like insecticide use, use of biological control measures, cultural practices and sanitary measures. However, chemical control is still the form of control heavily relied on by farmers (Sharma *et al.*, 2006). Some of the ways of managing the tomato fruit worm are as follows:

2.4.1 Chemical control: *H. armigera* is mainly controlled by synthetic insecticides in most areas and it has differing susceptibilities to many insecticides. Information obtained from tomato farmers in Sege, Akumadan, and Ada in Ghana, the commonly used synthetic insecticides for controlling the tomato fruit worm in these areas are Karate[®] (lambda-Cyhalothrin), Combat[®] (fipronil), Attack[®] (emamectin benzoate), Carbaryl and Chlorpyrifos.

These insecticides are applied on crops using knapsack sprayers and mist blowers. Authorities and production companies use social electronic media and extension officers to inform and educate farmers who probably do not have adequate training and knowledge on accurate use of these insecticides (Karim, 2000).

2.4.2 Host plant resistance: Plant cultivars which are resistant and tolerant to the feeding damage of *H. armigera* are very important in the management of the pest. Some crops display characters that reduce attractiveness to ovipositing adults and suitability for larvae to feed. This plant-based defense mechanism depends on factors like temporal avoidance, physical and chemical defense. Recently, genetic engineering of crops is able to make a major contribution in the production of inherently resistant/tolerant varieties. Tomato inhibitor 11 gene for instance, when expressed in tobacco has been shown to confer insect

resistance (Johnson *et al.*, 1989). These transgenic plants provide good control for pests difficult to reach with sprays.

2.4.3 Cultural control: Many agronomic practices have been suggested in the management of tomato fruit worm in many cropping systems. These practices include manipulation of planting dates, destruction of crop residues, destruction or manipulation of alternate hosts, trap cropping, and monitoring pest population by physical observation of plants or by use of light, sticky traps, pheromone traps, for accurate and timely control, weeding of crop fields, and intercropping. All these have provided some degree of control (Karim, 2000).

2.4.4 Biological Control: This occurs when a pest population is limited by another organism using various means like feeding, competition, substrate for oviposition, etc. These biocontrol agents could be predators, parasites or pathogens like fungi, viruses, bacteria and nematodes. This method stands today as a cornerstone of integrated pest management and the foremost alternative to the use of synthetic insecticides. Natural enemies have the potential of maintaining the population of *H. armigera* to sub-economic levels in the absence of insecticides.

Most of them are safe to humans and animals and have no adverse effects on non-targets and the environment, meaning that they are highly specific (Cherry *et al.*, 2003). Microbial agents that infect and kill insects are termed entomopathogens.

Bacterial entomopathogens: Bacterial biopesticide preparations are key products for crop protection without the use of chemicals. The active ingredient in such formulations is based on living organisms. *Bacillus thuringiensis* (Bt), a gram positive and spore forming

bacterium is the most widely used microbial agent in the control of insect pests of agriculture (Roh *et al.*, 2007). It is known for its pesticidal proteins (delta endotoxin) that kill insects. Bt products have been successfully used in China to control food and crop pests including *H. armigera* with an estimated 8 million hectares of farmland being protected with Bt (Xie *et al.*, 1990). In most developing countries, the use of Bt formulations is gradually rising with the products mostly imported.

Fungal entomopathogens: The role of fungal entomopathogens in pest control is already known and is in practice. They are widely distributed and naturally occurring in various fields, in the soil and on some organisms. Most fungal entomopathogens including *B. bassiana* and *M. anisopliae* are isolated from soil samples collected from crop, orchard and forested fields, sometimes using insects like the larvae of *Musca domestica*, *Boophilus microplus* ticks as baits (Mythili *et al.*, 2010). Investigating the occurrence and distribution of native entomopathogenic fungi is critical for their use as pest control agents in a given location. There is resurgence in the exploration of their potentials in pest control especially due to the awareness that chemical pesticides pose serious problems to the environment and are expensive to use. They are mostly used in integrated approach to pest control.

The effectiveness of fungal entomopathogens is determined by environmental factors like temperature, relative humidity and U.V. light. Therefore, exotic strains of entomopathogenic fungi that have been developed for use as pest control agents in a different country could be ineffective due to strain and environmental differences. They are slow in action and therefore act slowly in killing their targets. *B. bassiana* have been reported to cause mortality in *H. armigera* larvae at fourth instar stage when doses are topically sprayed on them. The treated larvae die mainly due to spread of fungal infections

into different body parts. In infested larvae, usually, the entire body is covered by fungal mycelia. The insect chitin is the main target of fungal attack (Prasad *et al.*, 2010). The cyclodepsipeptidic mycotoxin, beauvericin, produced by *B. bassiana* has been documented to be most effective for its larvicidal properties. Formulations of *B. bassiana* can thus serve as an effective broad spectrum biocontrol agent for soya bean and various other cash crops (Ritu *et al.*, 2012). *Metarhizium anisopliae* isolated from soybean field have also been reported to be viable and effective in controlling *H. armigera* larvae. Following the superior performance by *Nomuraea rileyi* compared to other fungi, Hatting (2012) suggested that this species had a high potential for development as a myco-insecticide against *H. armigera*. Some of these entomopathogenic fungi (*B. bassiana*, *M. anisopliae*, etc.) have been reported to be potential biocontrol agents against *H. armigera* and other insect pests on various crop fields like chickpea (Rijal *et al.*, 2008). *Aspergillus flavus* and Ghanaian indigenous *Aspergillus* sp. are also reported to be entomopathogenic to mosquito and diamondback moth respectively and have proven effective when used even in combination with chemical insecticide against some mosquito species (Anaisie *et al.*, 2011; Bhan *et al.*, 2013). *A. flavus* was recorded for the first time as a pathogen of the predator *Rhynocoris marginatus* (Fab.) (Hemiptera: Reduviidae) in natural agro-ecosystems of Tirunelveli District, Tamil Nadu, India (Sahayaraj *et al.*, 2011). Therefore, it may be pathogenic to other insect pests.

Viral entomopathogens: Many viruses have been identified in insect hosts and found to be pathogenic to these insect hosts. These viruses include baculoviruses, proxiviruses, cytoplasmic polyhedrosis viruses, and Nuclear Polyhedrosis Virus (NPV) (Ibarra and Del Rincon-Castru, 2008). These are being used and tried experimentally to control insect pests

including *Helicoverpa* species mostly in the developed countries like the United States. NPV is being sprayed in large cotton fields in China (Guangyu, 1989). Most of the viruses take about five days or more to kill their insect hosts. In Pakistan, baculovirus has a great potential to be used to control *H. armigera* in integrated pest management.

Entomopathogenic nematodes: Some freely existing nematodes (Steinernematidae and Heterorhabditidae) are so virulent and can easily kill their insect hosts. They have a broad host range and are safe to vertebrates, plants and other non-targets (Akhurst, 1990). Nematodes tend to be quickly inactivated by adverse environmental condition. This makes them good entomopathogens under laboratory conditions but they seldom impact insects on the fields. In the case of *H. armigera*, nematodes tend to be opportunists rather than biocides (Glazer and Navon, 1990). They are therefore not widely used as insect pest control agents as they do not meet their fundamental mission to any significant degree.

2.5 Phyto-insecticides or Botanical insecticides used for insect pest control

The use of chemical insecticides in the control of insect pests on crops has increased problems of pollution, contamination, development of insecticide resistance by pests, emergence of secondary pests, and has necessitated the search for alternative means of insect pest control such as the use of botanicals. Botanicals are safer than synthetic insecticides, easily biodegradable, environmentally safe, non-persistent and easily processed. Biopesticides extracted from plant materials, some of which are now commercially available can be used against insect pests including the tomato fruit worm. A number of plants possess pesticidal activity and investigations by various research groups in different parts of the world have confirmed this (Al-shannaf *et al.*, 2012). From various investigations, it has been established that pesticidal activity is usually distributed in most

cases among the various parts of the same plant though the lethality and quantities of the active components may vary (Rajapake and Ratnaseka, 2008). Of particular economic significance among the plants in common use today is the tropical plant *Azadirachta indica*, popularly known as the neem tree. Its insecticidal activity has been associated with the presence of azadirachtin, which is said to be highest in the kernel than in the leaves and other tissues of the plant. Other plants which have insecticidal properties include *Piper guineense* (Schum & Thonn), *Eucalyptus globules* (Labill), *Chrysanthemum coccineum*, *Cannabis sativa*, *Allium sativum*, *Aframomum melegueta*, *Zingiber officinale* and *Carica papaya* with the leaves, seeds, barks, roots, or fruits being insecticidal (Okwute, 2012).

Aqueous extract of individual and mixed form of *Azadirachta indica* A. Juss seeds kernel and leaves of *Milletia ferruginea*, Hochst and *Croton macrostachyus* Hochst have been tested against, *H. armigera* and shown to be lethal, hence effective in insect pest control (Lulie and Raja, 2012).

Powders of four plant materials, namely: *Dennettia tripetala* (Baker), *Eugenia aromatica* (Hook), *Monodora myristica* (Dunal) and *Piper guineense* (Schum and Thonn) were evaluated for the control of dermestid beetle, *Dermestes maculatus* (Coleoptera: Dermestidae) and each of the four plant powders were reported to cause significantly high mortality in both the adults and larvae of the fish beetle at all concentrations (Akinwumi, 2011). Many other plant extracts have been shown to be insecticidal to most insect pests though a few have given negative results.

Some commercially available products of insecticidal plant extracts include NeemAzal[®], XenTari[®], Spinosad[®], Biospark[®], Phytophrate[®], Exodos[®], Bidos[®] and Sumicidin[®]. These

products have been reported to significantly reduce the populations of insect pests like white flies, aphids and the tomato fruit worm on tomato fields (El Shafie and Abdelraheem, 2012).

2.5.1 West African Black pepper, *Piper guineense* (Schum and Thonn)

Piper guineense, the West African black pepper is an economically and ecologically important genus in the family Piperaceae. This tropical plant family has provided many past and present civilizations with a source of diverse medicines and food grade spice. It is considered to be among the most archaic of pan-tropical flowering plants. The genus *Piper* contains approximately 1,000 species of herbs, shrubs, small trees and hanging vines. Several *Piper* spp. from India, South-East Asia and Africa are of economic importance since they are used as spices and traditional medicines (Simpson and Ogorzaly, 1995). Hence the associated health risk to humans is generally regarded as low. *P. guineense* is the most familiar medicinal Piperaceae in Africa (Iwu, 1993). The unripe fruit is the source of black pepper, while the ripened fruit is the source of white pepper.

The West African black pepper (*Piper guineense*) is classified under the Kingdom Plantae, Division Magnoliophyta, Class Magnoliopsida and Order Piperales.

Piper species have a pan tropical distribution, and are most commonly found in the understory of lowland tropical rainforests, but can also occur in clearings and in higher elevation life zones such as cloud forests (Chahal *et al.*, 2011). Various classifications of use include abortifacients, antibiotic, arrow or fish poisons, diuretic, toothache remedy, tobacco snuff substitute and insect repellent (Scott *et al.*, 2008). Important species commonly used for the purpose of pest control include *Piper guineense*, *P. longum* and

Piper nigrum. *Piper guineense*, the West African black pepper, also called Ashanti pepper is an evergreen climbing shrub producing woody stems from 4-20 meters tall. It supports itself on other plants by means of adventitious roots produced along the stem. The plant is popular in Africa, especially in the West African region where it is often harvested from the wild, semi-cultivated and sometimes cultivated for its use. It is sold in local markets, used as spice in most dishes. Its distribution in Africa ranges from Senegal to Sudan, South Africa to DR Congo, Zambia, Tanzania, Cameroon and Ghana (Fern, 2014).

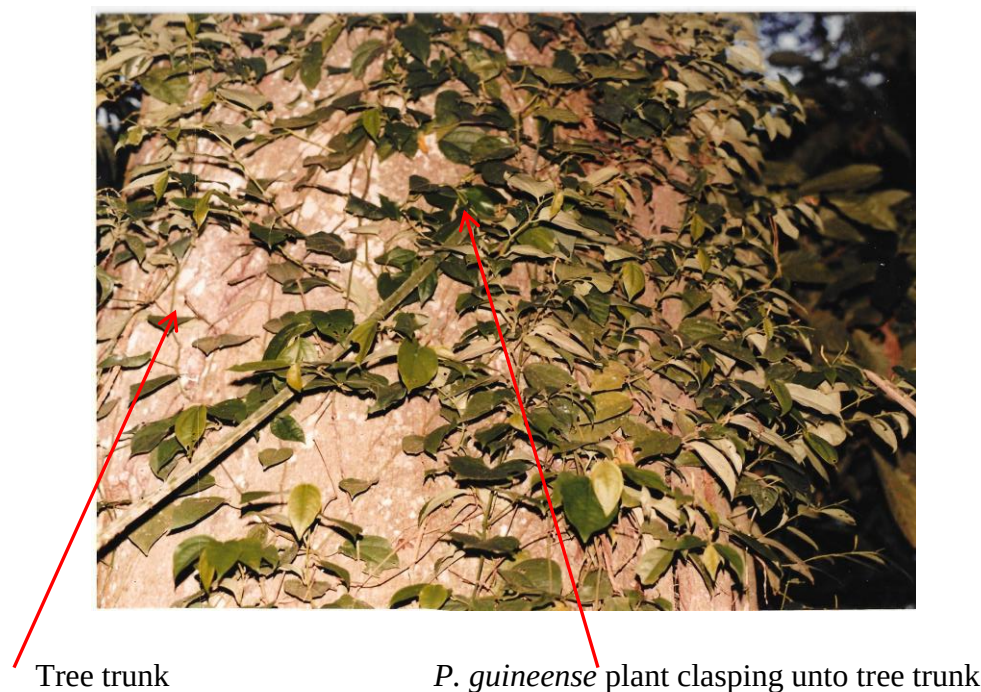


Figure 2: *Piper guineense*, a climber, and growing wild in the forests of Amedzofe, Ghana. The plant is shown clasping unto the trunk of a big tree.

Source: Gbewonyo (1991).

The efficacy of *Piper* extracts as botanical insecticides has been correlated with the concentration of piperamides in the extract. Piperamides are known to act as neurotoxins in insects (Gbewonyo *et al.*, 1993). Piperamides singly, or more importantly in combination,

may replace contact insecticides, specifically neurotoxic compounds such as carbamates, organophosphates and pyrethroids, for which resistance has developed (Scott *et al.*, 2008).

Pellitorine among other amides was isolated and identified as an insecticidal amide/component after petroleum ether extract of *Piper guineense* male roots showed insecticidal activity when tested against *Musca domestica* (Gbewonyo and Candy, 1992). The insecticidal amides in *P. guineense* include guineensinamide, kalecide, guineensine, pipericide and pellitorine of which pipericide and guineensine are reported to be more lethal than their counterparts (Gbewonyo *et al.*, 1993). *Piper guineense* has been in use as insect control agent for decades. Its seed oil extract impregnated in millet have been reported to help in controlling rust-red flour beetles under prevailing tropical storage conditions (Lale and Alaga, 2001). Ethanol extracts of *P. guineense* seeds and leaves have also proven lethal to mosquito larvae with the seed extract reported to be more active as a larvicide (Ihemanma *et al.*, 2014). A lot of tests have been carried out using this botanical especially on stored product insects. In post-harvest protection, *Piper guineense* seed powder has been reported to be effective in the control of *Sitophilus zeamais* in corn grains and *Callosobruchus subinnotatus* on bambara groundnut due to its lethal effect on the insects and reduction in their progeny. Finer particles are said to be more effective with results almost equivalent to those obtained using chemical insecticides (Asawalam and Emosairue, 2006; Oparaeke and Bunmi, 2006).

From the works that have been done so far, *Piper guineense* appears therefore to be a potential source of botanical insecticides that can be used to control many insect pests both in stored products and on field crops.

2.6 Compatibility of insecticidal plant extracts with entomopathogenic fungi

The need to preserve entomopathogens that occur naturally, or are introduced for insect control necessitates a proper understanding of the compatibility of entomopathogenic fungi with other crop protection techniques such as the use of insecticides, which may inhibit to a smaller or larger extent the development and reproduction of pathogen. A combination of entomopathogenic fungi with plant-based insecticides may provide a more sustainable pest management strategy at reduced cost. It is therefore, necessary to determine the compatibility of botanicals with entomopathogenic fungi of present and future importance to maximize their combined efficacy.

Some combination tests have been carried out between *B. bassiana* and some commercial botanicals and plant extracts and have proven to be compatible (Sahayaraj *et al.*, 2011). These tests showed that phytophorate and biodos were compatible with *B. bassiana*. Some plant extracts were also reported to be compatible with *B. bassiana* but *Annona squamosa* extract was reported not to be compatible. Sajjan (2006) reported an increase in efficacy of mycopathogens (*M. anisopliae* and *B. bassiana*) in the mortality of third instar larvae of *Plutella xylostella* when combined with cow urine and neem seed kernel extract in laboratory and field conditions.

Not all extracts from *Piper* spp. might be compatible with fungi cultures. Some *Piper* spp. are reported to contain some insecticidal amides which are also antifungal in nature. *Piper hispidum* and *Piper tuberculatum* for example have been shown to accumulate amides bearing isobutyl, pyrrolidine, dihydropyridone and piperidine moieties that have antifungal activities (Navickiene *et al.*, 2000).

Thus *P. guineense* plant extracts and fungal entomopathogens appear to be safe and sustainable insecticides in the control of insect pests. But the insecticidal effects of *P. guineense* seed extracts with fungal entomopathogens have not been tested on the larvae of *H. armigera* which is a very important insect pest of tomatoes and other important crops. This therefore has led to the importance of this study which evaluates their insecticidal effects on the larvae of *H. armigera*.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Area

This study was carried out at the Department of Biochemistry, Cell and Molecular Biology (Medicinal Plants Laboratory), African Regional Postgraduate Programme in Insect Science (ARPPIS) laboratory and at the School farm of the University of Ghana-Legon, Ghana.

3.2 Establishment of tomato field

Tomato was cultivated on the University of Ghana School Farm during the major and minor cultivation seasons on three plots, constituting three replicates with an area of 24 m² each. The plots were ploughed to a fine tilth and seedlings from Peto mech seeds were transplanted on the level surface in three lines. Each line contained eleven tomato plants, 45 cm apart and the three lines were 60 cm apart. NPK (200-150-225) fertilizer was applied to the soil two weeks after transplanting and during flowering of the tomato plants. The plots were watered when needed until the plants fruited and weeding done when necessary by hoe. The plots were bordered by grass and other weeds, garden eggs, okra and cabbage plants in the major season and weeds, garden egg plants and okra plants in the minor season. The field was established for monitoring of tomato fruit worm population and to have feeding materials for laboratory cultured *H. armigera*.



Plate 6: Three week old tomato field for *H. armigera* population trend study.

3.3 Population trend of tomato fruit worm

The population of the larvae was monitored on tomato plants cultivated during August to October 2014 and February to April 2015 cropping seasons with no insecticides applied. The tomato was planted to coincide the main and lean planting season of tomato. This is important as it determined the trend and peak period of the pest. This information helps to determine when it is best to apply the products being assayed on tomato field should they be found effective against the tomato fruit worms in the laboratory.

Monitoring started two weeks after transplanting. There were three plots constituting three replicates. On each plot, leaves, branches, stems, flowers and fruits were physically examined for the presence of the fruit worm. Five plants were examined on each line in a plot and the number of larvae on each plant counted. Monitoring was done twice a week for eight weeks.

3.4 Insect collection and rearing

3.4.1 Collection of tomato fruit worm from the field

Insect larvae (third to fifth instars) together with infested tomato fruits were collected in August and September from tomato farms in Akumadan, Sege, Ada and the University of Ghana school farm into plastic bowls lined with tissue papers and covered with white muslin cloth. The insects were taken to ARPPIS laboratory and reared at $26 \pm 1^\circ\text{C}$ and $85 \pm 5\%$ RH.

3.4.2 Laboratory culture of *H. armigera*

The tomato fruit worm larvae brought from the field were placed inside 2500 ml Crystal Clear Square[®] (225 cm²) plastic bowls, 9 cm in height, filled with soil sterilized in Heratherm OMS100 oven at 50°C to the height of 4cm for pupation. The larvae were fed with insecticide-free immature tomato fruits, young tomato leaves and twigs. The plastic bowls were covered with white muslin cloth. These food materials were changed every day and replaced with fresh materials collected from tomato fields as the larvae fed.

Pupae were collected into Maxi Box[®] plastic buckets (25×18 cm), lined with cardboard paper. The plastic buckets were covered with white muslin cloths and were used in rearing the adults.

Adults that emerged were fed on 10% sugar solution soaked in cotton wool inside plastic cups placed inside the plastic buckets. The females laid most of their eggs on the muslin cloth.

The muslin cloths containing eggs were removed and replaced by new ones. The eggs on the muslin cloths were placed inside 1L transparent plastic containers covered with muslin

cloth and perforated lid till they hatched. Hatched neonates (1st instar larvae) were picked using soft brush and introduced on to young tomato fruits, leaves and twigs inside the plastic bowls lined with soil. The neonates fed on the food materials, some crawled up to the muslin cloth to moult as they developed and the last larval instars pupated inside the soil.

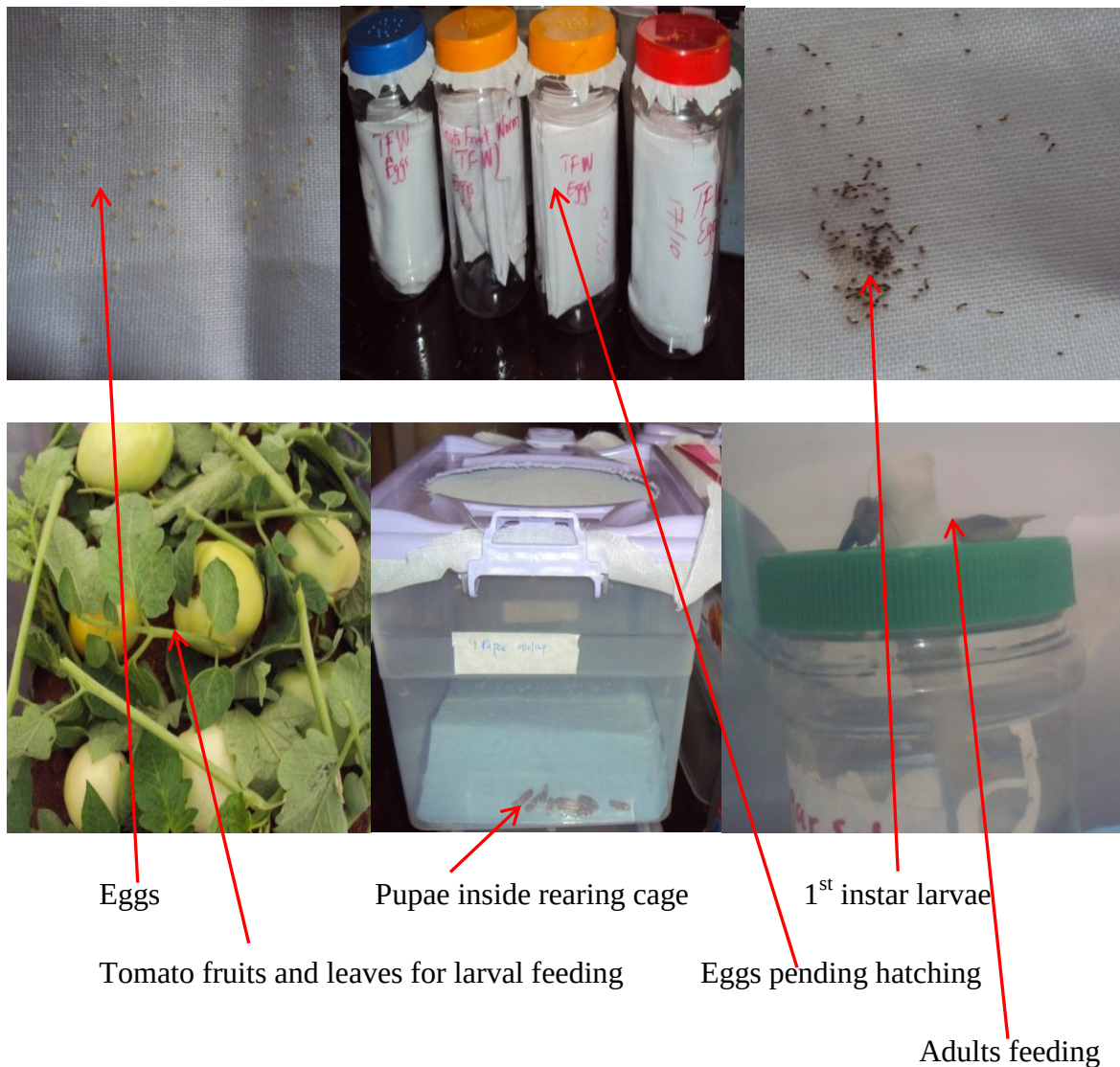


Plate 7: *H. armigera* rearing set up. at ARPPIS laboratory.

3.4.3 Laboratory culture of *Tribolium* spp.

Adults of *Tribolium* spp. were collected from infested maize bought from the Madina market in Accra, Ghana. These adults were placed in plastic bottles half-filled with ground maize and maintained at the insectary for two weeks to oviposit (Plate 8) and then placed in other plastic bottles containing ground maize. Larvae that hatched were removed using soft brush and used as bait for the isolation of entomopathogenic fungi because they last long and are very susceptible to entomopathogenic fungal infection.



A

B

C

Plate 8: Set up for rearing *Tribolium* spp. at ARPPIS laboratory

A: Plastic bottles containing ovipositing adults,

B: Removing larvae from ground

maize and C: Selected larvae to be used as baits.

3.5 Isolation of entomopathogenic fungi

The fungi used in this study were isolated from cadavers of tomato fruit worm, (TW), the common meadow katydid, *Orchelimum vulgare* (a green grasshopper) (GG) and from soil samples collected from around the University Ghana, Legon and neighbouring communities.

3.5.1 Soil sampling and the baiting of entomopathogenic fungi

The soil samples used for the study were collected from ten locations within the University of Ghana, Legon and neighbouring communities (Table 1). For each location, the android mobile phone App, SnooCode, was used to generate a unique code for the GPS location for the exact position of samples collection. These locations are the University School Farm (SF) 5°39'38.6"N 0°11'24.8"W, a tomato and maize cultivation area, Botanical Garden (BG) 5°39'30.1"N 0°11'14.0"W, a grass covered area, Assow Garden (AG) 5°39'17.8"N 0°11'16.4"W, an area covered with shrubs and grass, School of Engineering Science (SE) 5°39'24.8"N 0°11'01.6"W, a maize cultivation area, Sinna Garden (SG) (Crop Science) 5°38'58.6"N 0°11'04.8"W, a research field where a variety of crops are cultivated, Botany Department area (BD) 5°39'15.5"N 0°11'07.8"W, a potato cultivation area, Bush Canteen area (BC) 5°38'57.8"N 0°11'04.8"W, a maize and okra cultivation area, Staff Village (SV) 5°39'00.1"N 0°10'39.2"W, besides a football court, Near East Legon (EL) 5°38'48.5"N 0°10'10.6"W, a grass covered area, and Okponglo Area (OA) 5°38'23.1"N 0°10'40.1"W, a refuse dumping site. In each location samples were collected from five different places, about five meters apart. During the collection process, the soil was dug 4 cm deep and then soil particles beyond this point, with some level of moisture content was collected into 250 ml disposal plastic cups (about two-third filled) with perforated lids created by a heated 1

inch nail. The samples were taken to the Medicinal Plant Laboratory at the Department of Biochemistry, Cell and Molecular biology for fungi isolation.

Table 1: Locations for soil samples collection.

Location	GPS Coordinates	Site code
School Farm (SF)	5°39'38.6"N 0°11'24.8"W	SF1
		SF2
		SF3
		SF4
		SF5
Botanical Garden (BG)	5°39'30.1"N 0°11'14.0"W	BG1
		BG2
		BG3
		BG4
		BG5
Assow Garden (AG)	5°39'17.8"N 0°11'16.4"W	AG1
		AG2
		AG3
		AG4
		AG5
School of Engineering Science (SE)	5°39'24.8"N 0°11'01.6"W	SE1
		SE2
		SE3
		SE4
		SE5
Sinna Garden (SG)	5°38'58.6"N 0°11'04.8"W	SG1
		SG2
		SG3
		SG4
		SG5
Botany Department (BD)	5°39'15.5"N 0°11'07.8"W	BD1
		BD2
		BD3
		BD4
		BD5

Table 1 continued: Locations for soil samples collection

Bush Canteen (BC)	5°38'57.8"N 0°11'04.8"W	BC1
		BC2
		BC3
		BC4
		BC5
Staff Village (SV)	5°39'00.1"N 0°10'39.2"W	SV1
		SV2
		SV3
		SV4
		SV5
Near East Legon (EL)	5°38'48.5"N 0°10'10.6"W	EL1
		EL2
		EL3
		EL4
		EL5
Okponglo Area (OA)	5°38'23.1"N 0°10'40.1"W	OA1
		OA2
		OA3
		OA4
		OA5


Plate 9: Soil samples for fungi isolation at Medicinal Plant Laboratory.

A total of seven larvae of *Tribolium* spp. were placed in each soil sample and monitored for fungal infection and subsequent growth. Larvae of *Tribolium* spp. are generally very susceptible to entomopathogenic fungal infection and have a long larval stage (approximately 16 days) making them ideal candidates for use as baits for isolation of entomopathogens. When the soil sample got dry, tap water was used to moisten the soil samples ensuring the soil is not water-logged.

Monitoring for fungal growth on the *Tribolium* spp. larvae was done after three days of larval inoculation on the soil samples. Larval cadavers were removed from the soil and surface sterilized in 1% Sodium Hypochlorite solution prepared using household bleach (Power Zone®) for about 10 seconds and rinsed in tap water for two minutes in a petri dish. The sterilized cadavers were placed on moist tissue papers in petri dishes to observe fungal growth, if there was internal infection. Fungal growths on sterilized cadavers were cultured on Sabouraud Dextrose Agar (SDA) media amended with 500 µg/ml tetracycline to avoid bacterial growth.

3.5.2 Isolation of fungi from insect cadavers

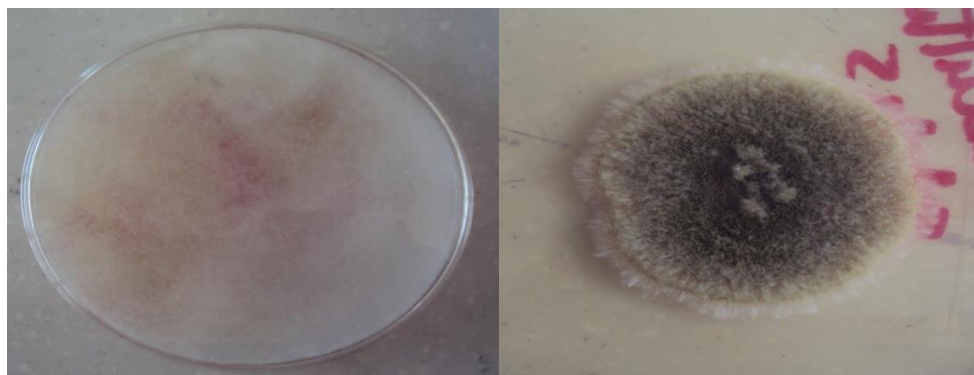
A cadaver of common meadow katydid (a green grasshopper) (GG) and a tomato fruit worm (TW) collected from the botanical garden and the school farm, respectively were surface sterilized in 1% sodium hypochlorite prepared from the household bleach (Power Zone®) solution as previously described. The surface sterilized cadavers were placed in petri dishes lined on the inside with moist tissue papers and incubated in a dark cupboard. Fungal growth on the cadavers were cultured on SDA media plates by picking up a few spores and introducing them into the media using sterilized wooden probes.

Cultured fungi were purified by sub-culturing the fungal colonies into new media plates. The fungal cultures were sub-cultured four times during the purification process.

3.5.3 Selection of entomopathogenic fungi

Entomopathogenic fungi can also grow on artificial media in the laboratory and need to come in contact with susceptible host to successfully grow and proliferate. They will therefore grow on media slowly and in a spreading manner. Opportunistic fungi trying to grow on dead insect host will therefore quickly grow in a fluffy manner on media since there is much food source on the media (Hasan *et al.*, 2012).

This was used as a basis for selection of fungi of interest during the isolation process. Fungi growing gradually in a spreading mode were selected as those of interest while those growing faster in a fluffy manner were discarded as opportunistic fungi.



A

B

Plate 10: A: Saprophytic fungal culture and B: Fungal culture of interest.

To confirm their entomopathogenicity and that they were actually isolated from the larvae of *Tribolium* spp., all the fungal cultures of interest isolated were tested against the larvae of *Tribolium* spp. The larvae were placed directly on all the fungal cultures on the media plates and left partially opened for 12 hours. They were then removed using a blunt pair of forceps and placed on clean petri dishes containing sterilized (in an oven at 75°C for 24 hours) maize flour. There were three replicates with ten larvae each, for each fungal culture. The set up was done in a completely randomized design in the Medicinal Plant Laboratory.

The larvae used as control were not exposed to fungi. This was observed daily for larval mortality. Dead larvae were picked out, sterilized as described previously and placed on moist tissue papers to observe for fungal growth.

3.5.4 Myco-insecticidal effect of entomopathogenic fungi on *H. armigera* larvae

To determine fungal entomopathogenicity, the tomato fruit worms, 3rd instar larvae were used. There were three replicates with ten larvae each for all the fungal cultures. Larvae not exposed to fungi were set up as control. The set up was in a completely randomized design. The larvae were tested by emersion directly on fungal plates for about two minutes and then placed inside the plastic bowls containing food materials. The set up was observed daily for larval mortality.

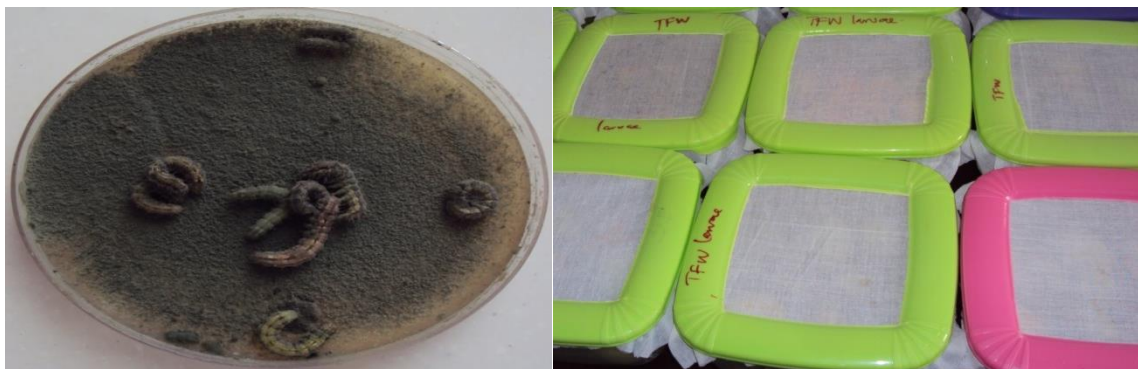


Plate 11: Fungal treatment of tomato fruit worm.

Fungal cultures that were entomopathogenic to the tomato fruit worm larvae were re-tested on the larvae. The dead larvae from some fungal cultures were surface-sterilized using the procedure mentioned above and placed on moist tissue paper in Petri dishes. Fungal culture recovered from dead tomato fruit worm larvae was considered an important entomopathogenic fungus and it was further used for compatibility test and identified morphologically as previously described.

3.6 Morphological identification of entomopathogenic fungus

The morphological identification was carried out at the Pathology Division of the Department of Crop Science, at the University of Ghana, Legon.

Identification of the isolates was done as described by Barnett and Hunter (1998). A drop of lactophenol was put on a clean slide and a portion of mycelia and spores of the fungal culture growing on the SDA medium was transferred into the lactophenol on the slide using an inoculating pin. The slide was covered with a cover slip and tapped gently with the wooden end of the inoculating pin to spread the fungus. The slide was observed under a hund Wetzer H500 compound microscope and the isolate identified with the aid of an identification key described by Barnett and Hunter (1998).

3.7 *Piper guineense* seed methanol extract preparation and testing

3.7.1 Collection and preparation

The *P. guineense* seeds were obtained from Amedzofe village in the Volta Region of Ghana and taken to the Medicinal Plant Laboratory of the Department of Biochemistry and Molecular Biology, University of Ghana.

Stalks were cut off and the seeds were hot air oven dried (40°C) in the laboratory for about 5 days. The dry seeds were ground into fine powder using SIZE 8 INCH® laboratory mill (Christy and Norris Ltd, Chelmsford, England). The seed powder was transferred into plastic bags and stored in a desiccator.



A



B

Plate 12: A. Fresh *P. guineense* seeds and B. Powdered *P. guineense* seed

3.7.2 Methanol Extraction of *P. guineense* seeds

Seed powder (20 g) was transferred into a clean 250 ml conical flask, 200 ml methanol was added and the content stirred using Stuart heat-stir (CB162) for 24 hours. The solution was filtered using filter funnel. The filtrate was concentrated using BUCHI Rotavapor (R-210, BUCHI, Switzerland). The concentrated filtrates (1.1 ml portions) were dried in pre-weighed vials by evaporation using the LABCONCO vacuum. The weights of the dried extracts were obtained. The extracts were taken up in acetone to give 100 mg/ml. These solutions were used for bioassays.



A

B

C

Plate 13: A. Extraction on a stirrer plate B. Concentration of extract using a rotavapor and C. Drying of samples by vacuum.

3.7.3 Insecticidal effect of plant extract

The seed extract (20 μ l) of the stock solution was topically applied to each tomato fruit worm, in clean petri dishes using a Burkard hand microapplicator. Cypermethrin (20 μ l, 100 mg/ml) was also applied to tomato fruit worms as a positive control. Acetone was used as negative control. Each treatment was replicated three times with ten larvae per replicate. The set up was in a completely randomized design. After treatment, the larvae were placed inside plastic bowls containing their food materials and the set up was monitored daily for larval mortality.



Plate 14: Microapplicator used for topical application

3.7.4 Compatibility of *P. guineense* seed extract with entomopathogenic fungus

A compatibility test was carried out between isolated entomopathogenic fungus and plant seed extract to determine if they could be used in combination against the target pests.

Sterile water (50 ml) in a 250 ml conical flask was prepared by autoclaving at 121°C for an hour and then adding tetracycline anti-biotic to make a sterile aqueous solution containing the anti-biotic at 30 μ g/ml. 20 ml of the sterile water was poured onto the fungal plate to make spore suspension.

The presence of fungal spores in the suspension was confirmed by observing the spores under LEICA DM500 light microscope. Spore suspension (2ml each) was added to vials containing 2 ml seed extract at concentrations of 100 mg/ml and 50 mg/ml.

The mixtures were spread on SDA media plates by first adding two drops of the sterile water and then one drop of the mixtures on the media plates. Aqueous plant extract was also used for the compatibility test. The inoculated plates were placed in a dark cupboard.

The treatments used were:

- A. Spore suspension (2 ml)
- B. Spore suspension in 50 mg/ml acetone seed extract (2 ml)
- C. Spore suspension in 25 mg/ml acetone seed extract (2 ml)
- D. Spore suspension in acetone (2ml)
- E. Spore suspension in 50 mg/ml aqueous seed extract (2 ml)
- F. Spore suspension in 25 mg/ml aqueous seed extract (2 ml)

Each treatment was plated on SDA media plates in three replicates. The set up was arranged in a completely randomized design. Number of fungal colonies on each plate was counted after 48 hours of plating. This counting was done daily, for five days.

3.9 Data Analyses

Data collected were summarized and subjected to multivariate analysis of general linear model (GLM) using SPSS 16.0 software. Where significant differences were found, means were separated by the post hoc test of Student Newman-Keuls Tests (SNK), at 0.05 level of significance.

CHAPTER FOUR

4.0 RESULTS

4.1 Tomato fruit worm population dynamics

4.1.1 Population of larvae on tomato field in two planting seasons in 2014/2015

The tomato fruit worm population on tomato field was more during the major cultivation season than during the minor season (Figure 3), but the difference was not statistically significant.

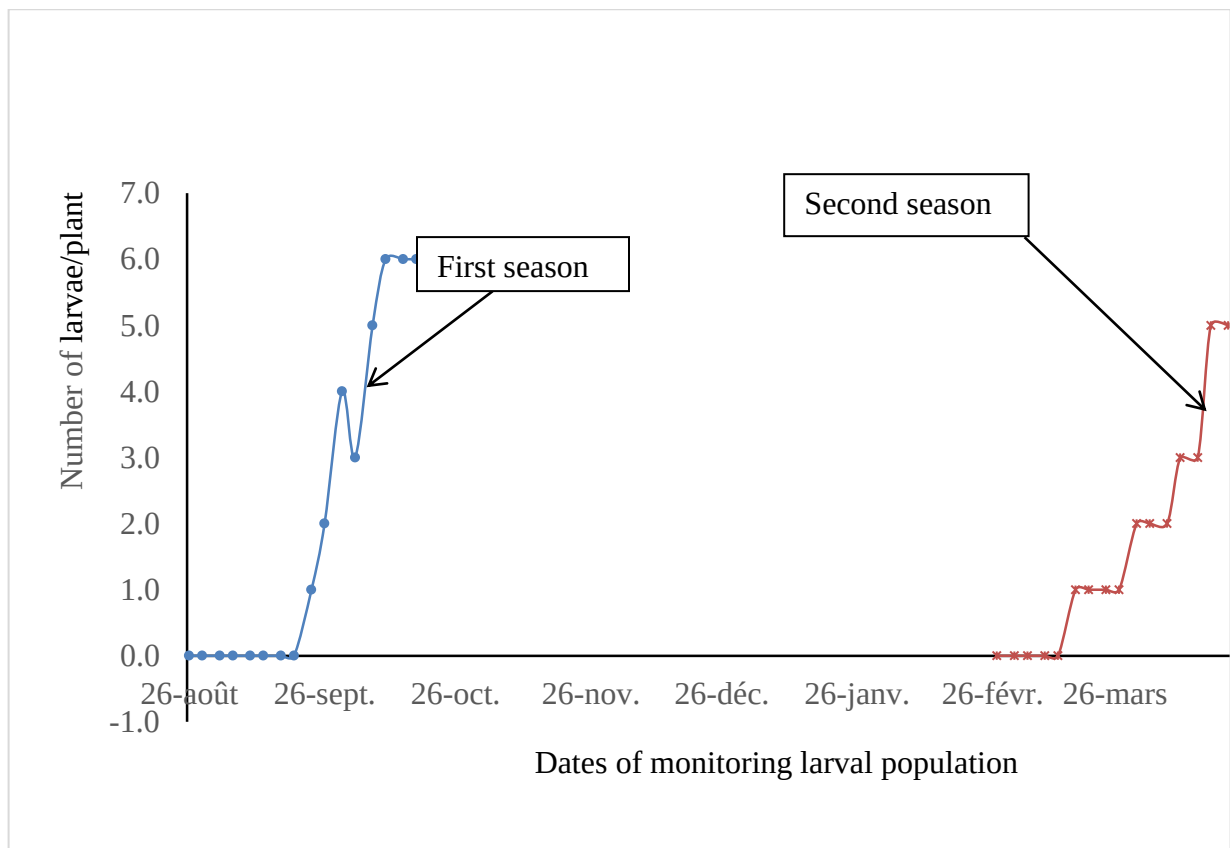


Figure 3: Population of tomato fruit worm per tomato plant during major and minor cropping seasons

4.1.2 Relationship between tomato fruit worm population and some environmental factors

Daily environmental temperature and rainfall data were collected from Ghana meteorological agency at Mempehuasem.

In the first season, there was a positive correlation between the number to larvae per plant and temperature (Figure 4). The relationship was significant at 95% probability level ($R=0.764$, $P = 0.00057$). The correlation between larval population and rainfall however was weak and insignificant (Figure 5). Furthermore, in the minor season, the relationship between larval population and temperature was weak and insignificant (Figure 6); so also was the relationship between larval population and rainfall (Figure 7).

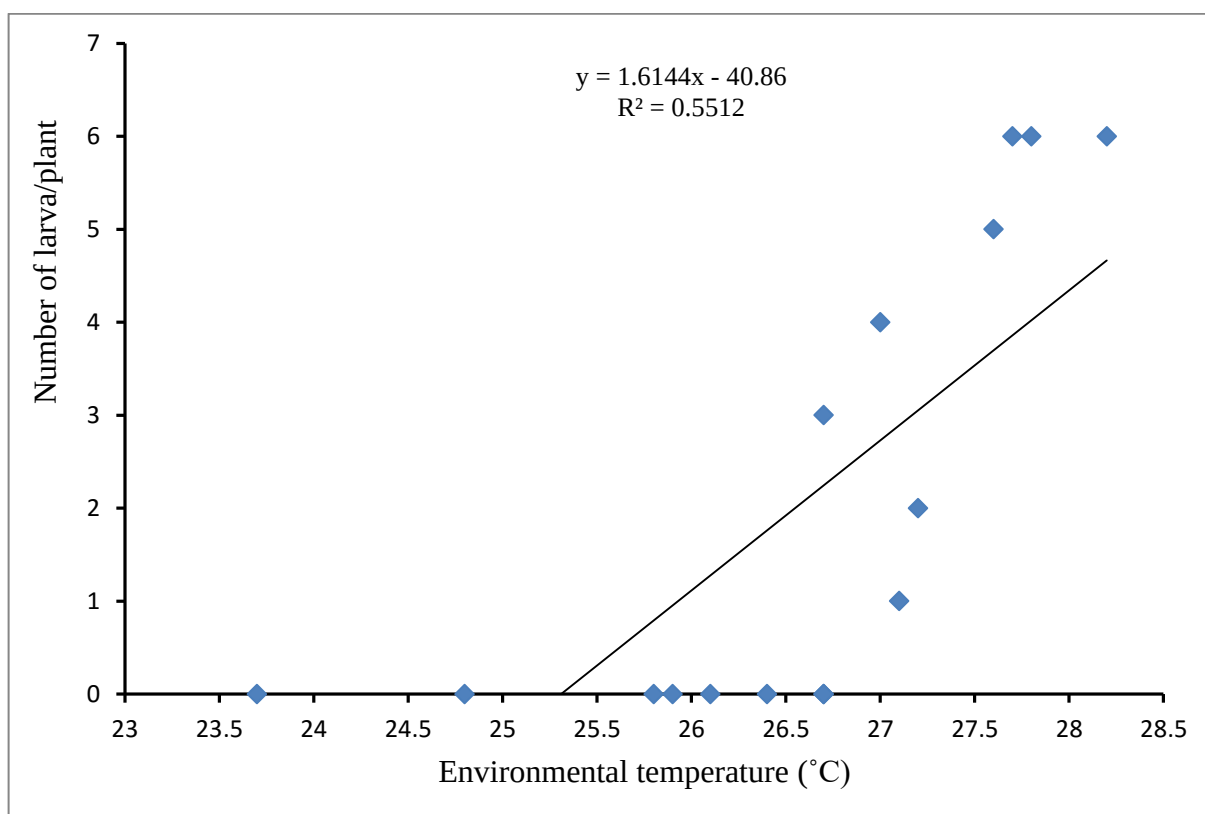


Figure 4: Relationship between larval population and environmental temperature in the major cropping season

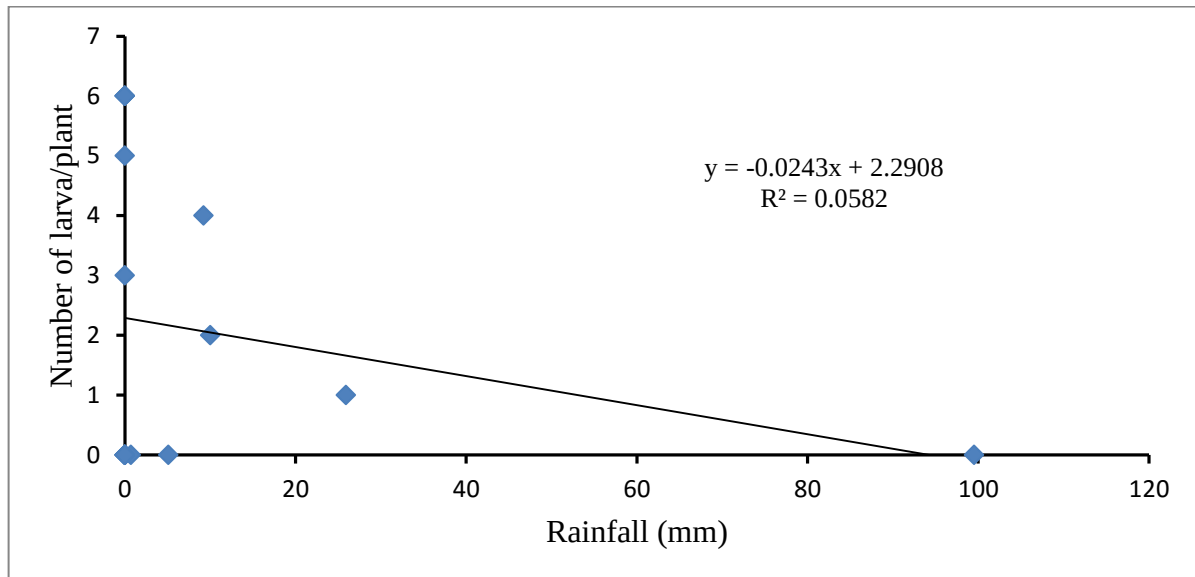


Figure 5: Relationship between larval population and rainfall in the major cropping season

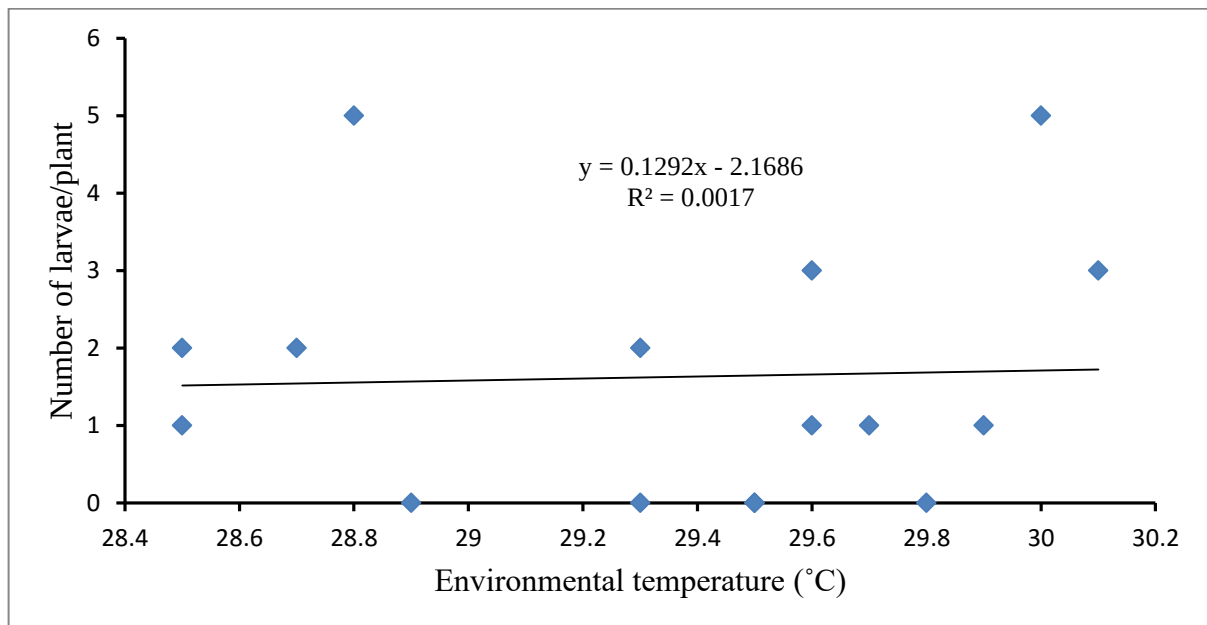


Figure 6: Relationship between larval population and environmental temperature in the minor cropping season

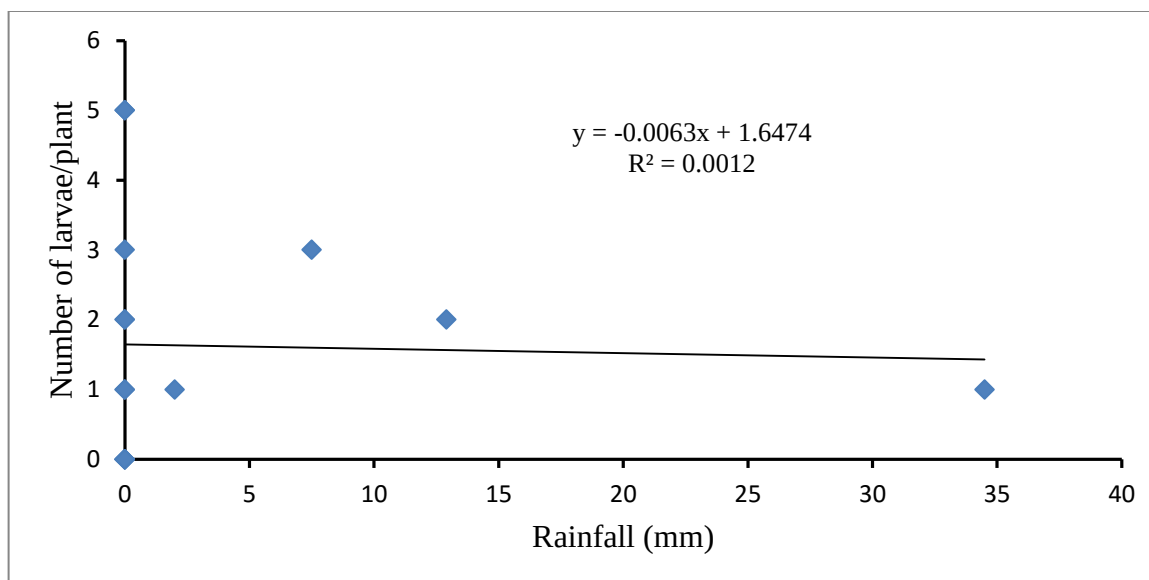


Figure 7: Relationship between larval population and rainfall in the minor cropping season

4.2 Isolation and confirmation of entomopathogenic fungi

4.2.1 Fungal isolation

Of the 50 soil samples collected from ten sites, four fungal isolates, putative entomopathogens were isolated and maintained in pure cultures. Fungal isolates obtained from cadavers retrieved from most of the samples (92%) were casually identified to be characteristic saprophytes based on their morphology on Sabouraud Dextrose agar plates hence were subsequently discarded. The four putative entomopathogenic fungi (Table 2), isolated from soil samples collected from Staff Village (SV), Assow Garden (AG) and Bush Canteen (BC) area together with two other fungi, isolated from the cadavers of tomato fruit worm (TW) and a green grasshopper (common katydid) (GG) respectively, were assayed against the larvae of *Tribolium* spp. to confirm pathogenicity.

Table 2: Geographic location of sample collection sites in isolated putative entomopathogenic fungi

Location	Site	GPS Coordinate	Putative Entomopathogen
	Codes		
Assow Garden	AG1 –	5°39'17.8"N	AG3
	AG5	0°11'16.4"W	
Bush Canteen	BC1 –	5°38'57.8"N	BC2
	BC5	0°11'04.8"W	
Staff Village	SV1 –	5°39'00.1"N	SV1 and SV3
	SV5	0°10'39.2"W	

4.2.2 Confirmation of entomopathogenicity of isolated fungi on larvae of *Tribolium* spp.

Larvae of *Tribolium* spp. are universally susceptible to broad range of entomopathogenic fungi. To confirm pathogenicity of the isolated fungi, the six isolates were assayed against the highly susceptible larvae of *Tribolium* spp. (Koch postulate test). All the six fungi caused some level of mortality to the larvae (Figure 8). This confirmed that the fungi isolated from the soil were entomopathogenic to the larvae of *Tribolium* spp. Compared to the control, there were significant ($P < 0.001$) differences in the entomopathogenicity of all the fungi to the larvae of *Tribolium* spp. The estimated time for the fungi to cause 50% mortality was 7 and 8 days, respectively for SV1 and AG3 while treatment with BC2 and SV3 resulted in 50% mortality in 12 and 13 days, respectively (Figure 8).

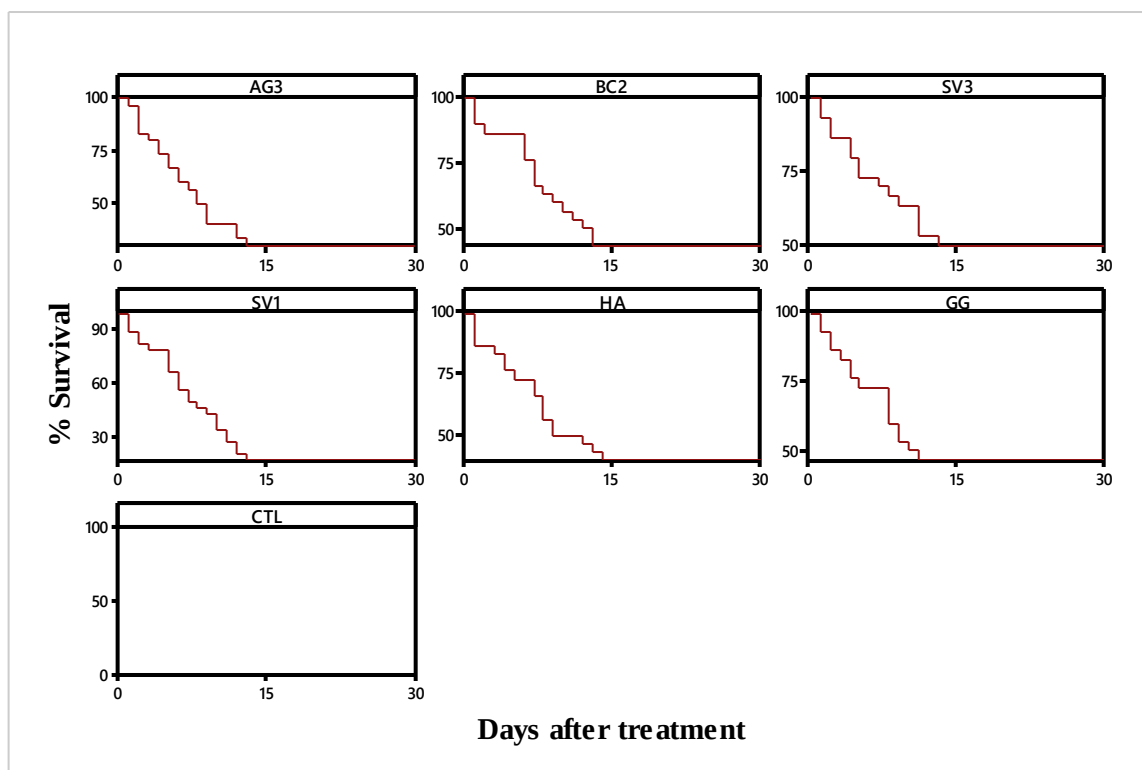


Figure 8: Survival of larvae of *Tribolium* spp. inoculated with entomopathogenic fungi.

4.2.3 Myco-insecticidal effect of isolated fungi on *H. armigera* larvae

To determine the myco-insecticidal effect of the fungi on tomato fruit worm, thirty (30) *H. armigera* larvae were inoculated with spores of each fungal isolate. The common meadow katydid (GG) and TW fungal isolates showed no myco-insecticidal effect on the tomato fruit worm (100% survival) although these isolates were myco-insecticidal to the larvae of *Tribolium* spp. (Figure 8). However, treatment of the tomato fruit worm with fungal isolates SV3, SV1, BC2 and AG3 resulted in mortality to tomato fruit worms with AG3 induced mycosis being significantly ($p < 0.001$) different from SV3, SV1 and BC2 induced mycosis. Compared with the 90% larvae survival beyond 10 days following AG3 treatment, SV3, SV1 and BC2 treatments resulted in 30% to 46% survival beyond 10 days after treatment. SV3 seemed to be the most entomopathogenic among the isolates

although, there were no significant differences in the mortalities caused by SV1, SV3 and BC2 isolates (Figure 9).

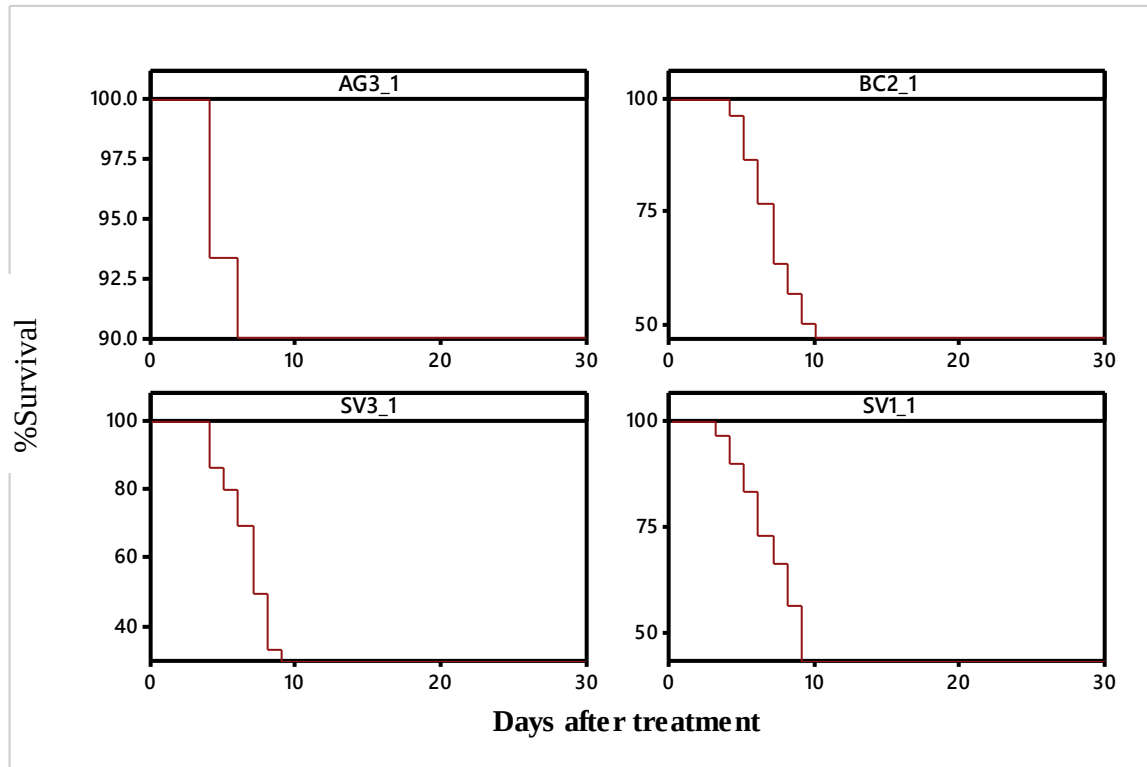


Figure 9: Percent survival of *H. armigera* after exposure to fungi for 11 days.

To determine whether the death of the tomato fruit worm was due to mycosis, the fungi were re-isolated from the cadavers and identity confirmed. Fungus was recovered from cadavers of SV3 isolate treated tomato fruit worms and further assayed against the tomato fruit worm. Plate 15 shows dead larvae covered with fungal growth.



Larva with body covered with fungi

Plate 15: Fungal growth on dead tomato fruit worm larvae

4.3 Insecticidal Effect of seed extract of *Piper guineense* seed on *H. armigera*

There were significant ($P < 0.001$) differences in mortality between the treatments. The seed extract demonstrated some lethal activity against the larvae but acetone did not (Table 3). Cypermethrin treatment gave a significantly higher mortality (80% within 48 hours) followed by *P. guineense* seed extract which resulted in 50% mortality in six days with a maximum of 60% mortality. There was no death in the acetone treatment. Mortality caused by seed extract was increased with time (Figure 10).

Table 3: Effect of seed extract on *H. armigera* larvae after 6 days of treatment

Treatments	Percent Mortality
Cypermethrin	90a
Seed extract	60b
Acetone	0.00c

NB: Mortalities are significantly different.

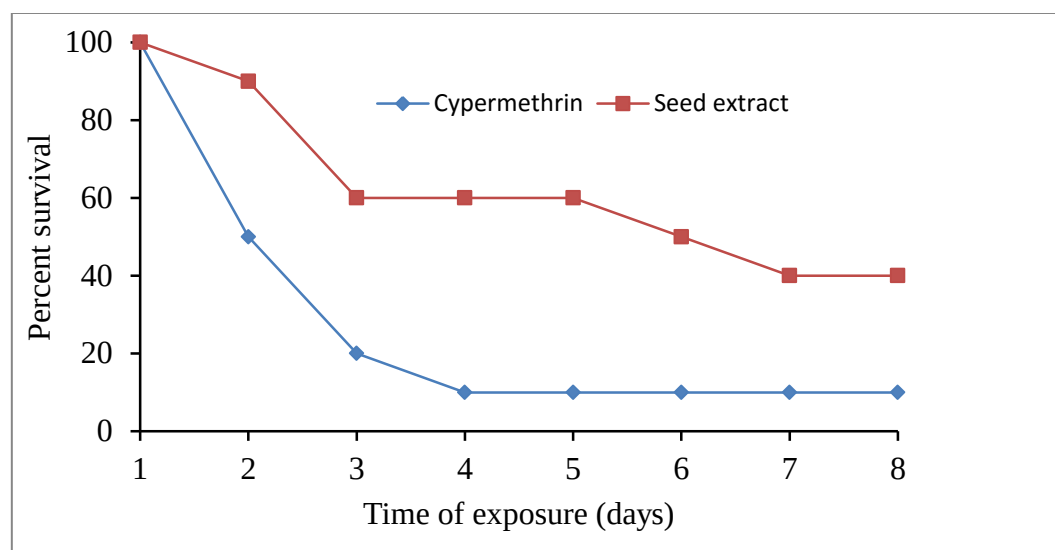


Figure 10: Percent survival of *H. armigera* larvae treated with seed extract over 8 days period

4.4 Insecticidal effect of seed extract and entomopathogenic fungus

The re-isolated SV3 fungal isolate and *P. guineense* seed extract were again assayed against the tomato fruit worm and monitored for eight days to compare their insecticidal effects. There were significant ($p < 0.001$) differences in the average number of deaths recorded for the different treatments on tomato fruit worms. Cypermethrin used as positive control significantly recorded the highest number of deaths (90%). The plant extracts recorded 50% compared to 20% by SV3 fungus on the sixth day. There were no deaths in the acetone control.

Thus, the plant extract appeared to be significantly ($p = 0.008$) more effective in killing the tomato fruit worms than the entomopathogenic fungus within the test duration. The seed extract acted faster but had a poor long term effect (40% larval survival after 8 days) (Figure 11) while the SV3 isolate acted more slowly but with better long term effect (30% larval survival after 8 days).

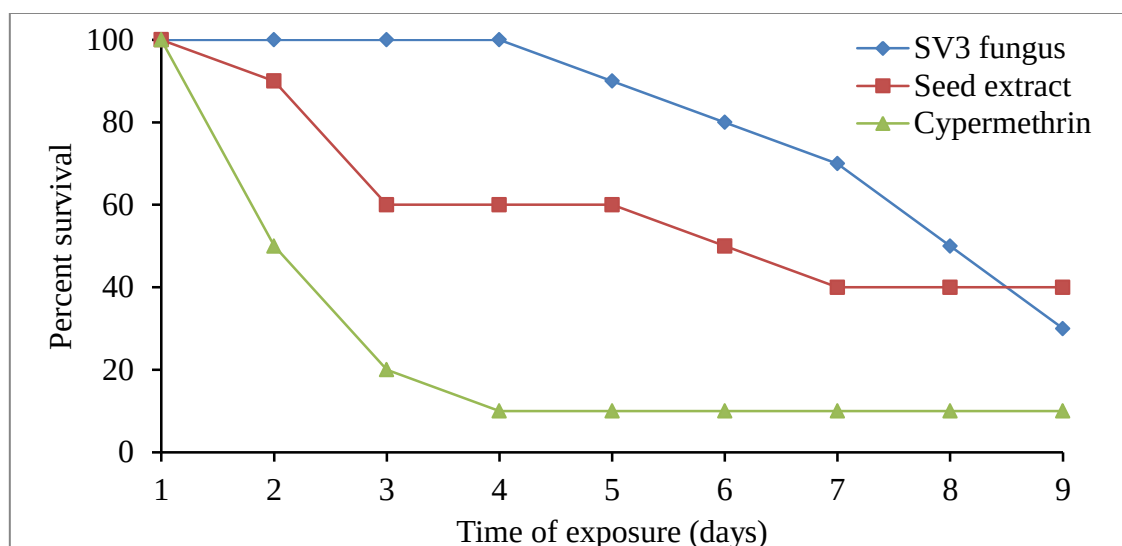


Figure 11: Percent survival of tomato fruit worm after exposure to fungus and extract for 9 days.

4.5 LT_{50} of seed extract and SV3 fungus

While the seed extract took about 4 days to cause 50% mortality (Table 4), the fungus SV3 took longer (6 days) to cause 50% mortality. The positive control, cypermethrin on the other hand took less than 24 hours to cause 50% mortality.

Table 4: Lethal time (days) for cypermethrin, seed extract and SV3 fungus on *H. armigera*.

Treatment	LT_{50} (days)
Cypermethrin	0.801(1)
Seed extract	3.739(4)
SV3 fungus	6.318(6)

4.6 Compatibility of entomopathogenic fungus with seed extracts

The number of fungal colonies on media plate with spore suspension alone (42 colonies) was significantly ($P < 0.001$) more than the number of fungal colonies on the media plates with the combined treatments (Table 5). The combinations of spore suspension with seed

extract and spore suspension with acetone had an average maximum number of only one fungal colony. The number of fungal colonies in plate containing spore suspension with acetone was not statistically different from the number of fungal colonies in plate containing spore suspension with seed extracts. The seed extract and acetone therefore appear to inhibit the growth of the fungus.

Table 5: Compatibility of entomopathogenic fungus with acetone and aqueous solutions of seed extract

Treatments	Mean number of fungal colonies
Spore suspension only	41.6 (42)a
Spore suspension in Acetone	1.0b
Spore suspension in 25 mg/ml acetone seed extract	1.4 (1)b
Spore suspension in 50 mg/ml acetone seed extract	1.0b
Spore suspension in 25 mg/ml aqueous seed extract	0.6 (1)b
Spore suspension in 50 mg/ml aqueous seed extract	1.2 (1)b

NB: Means with the same letter are not significantly different.

4.7 Morphological identification of the SV3 fungus

Colonies were yellow to green and turning into dark green over time. The conidiophores were upright, simple and terminating in globose swellings. Conidial heads with radiating phialides to the entire surface (Plate 16) and this fungus was therefore identified as an *Aspergillus* sp. (Barnett and Hunter, 1998).



Plate 16: Morphological structure of *Aspergillus* sp. under compound microscope

CHAPTER FIVE

DISCUSSION

Piper guineense is a tropical plant that has been in use for a long time especially for its medicinal value. Its seed has also been used as a spice in most African dishes, implying that it is safe for human consumption. Extracts of leaves and seeds of this plant are also reported to have insecticidal effects on some insect pests that damage food crops (Lale and Alaga, 2001; Asawalam and Emosairue, 2006; Oparaeke and Bunmi, 2006 and Ihemanma *et al.*, 2014). This makes it very important because most insecticides in use are toxic to humans and the environment but *P. guineense* is safe. Fungal entomopathogens have also been studied and some like *B. bassiana* and *M. anisopliae* have been found to be entomopathogenic to some insect pests. Combined treatments of plant extracts and fungal entomopathogens have also been reported to be effective in the control of some insect pests. But the insecticidal property of *P. guineense* plant extracts and its combination with fungal entomopathogens have not been tested against the tomato fruit worm, *H. armigera* which is a polyphagous and a very important insect pest of tomato.

The seed extracts of the plant being insecticidal against the tomato fruit worm could subsequently be used as end product on tomato fields to manage the pest. It is therefore necessary to study the population dynamics of the pest on the crop field for timely and accurate application. In the present study, a higher number of tomato fruit worm was observed in the first planting season than the second season. This could be due to availability of more tomato farms as hosts to the tomato fruit worms in the first planting season. This finding is similar to the findings of Boukhris-Bouhachem (2007) who recorded higher number of moths, eggs and larvae on tomato fields in major season in

Tunisia. The positive significant relationship between temperature and larval population in the major season corroborates the finding of Nadaf and Kulkarni (2006) who found that maximum temperature indicated positive significant relation with incidence of *H. armigera* larvae. This shows that the insects develop well as temperatures increase within the optimum range. Intensive control of this pest will therefore be very important in the major tomato cropping season than in the minor season.

Because *H. armigera* is a serious insect pest of tomato plants, its control has relied mainly on imported synthetic insecticides, most of which are toxic to the environment. Naturally-occurring fungal entomopathogens in the soil could be safer biological agents for the control of this pest. It therefore will be necessary to isolate some putative fungal entomopathogens and test their potency on the pest.

The use of *Tribolium* spp. larvae as bait for the isolation of fungi was because they are readily susceptible to fungal infection (Ortiz-Urquiza and Keyhani, 2013), supporting the fact that insects can be used to isolate entomopathogenic fungi. Similar studies have been done by Mythili *et al.* (2010) though they used the larvae of houseflies and ticks to isolate fungi like *B. bassiana*. Other entomopathogenic fungi including *Aspergillus flavus* have also been recently isolated from *T. castaneum* adults (Bosly and El-Banna, 2015).

The isolated entomopathogenic fungi, including those isolated from *H. armigera* and green grasshopper (common meadow katydid) cadavers were lethal to larvae of *Tribolium* spp. during the entomopathogenicity confirmatory test. This shows that larvae of *Tribolium* spp. are susceptible to fungal infections, hence are good baits for fungal isolation.

The entomopathogenic fungi were the slowest acting insecticidal agents used in this study. The fungi took the longest time to produce 50% and 90% mortality compared to *P. guineense* seed extract and the positive control, cypermethrin. This result is as expected because fungal insecticides are slow acting since the spores have to adhere, germinate and penetrate through the pest's cuticle, proliferate within the pest while distorting the pest's internal organs, and subsequently causing the death of the pest (Prasad *et al.*, 2010). This process takes a long time and usually is the reason for the slow lethal action of the fungi. Entomopathogenic fungi like *M. anisopliae* have been previously proven to be lethal against larvae of *H. armigera* (Ritu *et al.*, 2012). In this study, we have demonstrated that *H. armigera* is susceptible to an *Aspergillus* sp. isolated from the Legon Staff Village (SV3). *Aspergillus* sp. are known entomopathogens that cause mortality to diamondback moth (Anaisie *et al.*, 2011). This finding that *Aspergillus* sp. is entomopathogenic against the tomato fruit worms also supports the findings of Sahayaraj *et al.* (2011) and Bhan *et al.* (2013) who found it to be pathogenic against *Rhynocoris marginatus* and mosquito species respectively. This Genus, *Aspergillus* could therefore be potential source of important entomopathogenic fungi. But unfortunately, this can only be in the laboratory where the environment is controlled and cannot be sprayed on the field because this fungus produces a very potent carcinogen (aflatoxin). Therefore it might not be healthy to commercially produce a formulation of this fungus for insect pest control.

Fungi isolated from *H. armigera* and common meadow katydid cadavers (TW and GG isolates) did not cause any mortality to the tomato fruit worms although these fungi were entomopathogenic against the larvae of *Tribolium* spp. This could be because the tomato fruit worm was not susceptible to these fungi. For example, while entomopathogens such

as *M. anisopliae* are effective against a broad range of insect host, there are others such as *Metarhizium acridum* that are selective and have activity against only a narrow range of insect species (Wang et al., 2011). Further work will be required to determine the host range of the TW and GG isolates and confirm their suitability for use as entomopathogens against other pests of economic importance. It may also be that these fungi are saprophytic fungi that colonized the cadavers of the tomato fruit worm and the common meadow katydid but showed activity against the highly susceptible larvae of *Tribolium* spp.

The potential of combining *P. guineense* seed extract with SV1, BC2 and AG3 fungal isolates which also caused some level of mortality to the tomato fruit worm in this study were not pursued but will be a useful follow-up study.

To determine the amount of mortality caused by any combined treatments, knowing the mortality caused by the individual treatments on the target pest will help to appreciate the difference in mortality caused when the two treatments are used together. It was therefore necessary to determine the insecticidal effect of *P. guineense* seed extract alone on the tomato fruit worm so that the results can be compared to that obtained when it is combined with the fungus. *P. guineense* seed extract has not been tested against the tomato fruit worm. This study also serves as a basis for further study into the insecticidal effect of the seed extract on the pest. In this present study, the seed extract recorded up to 50% mortality though it took about 3 days. This finding could suggest that the insecticidal effect of *P. guineense* seeds on the tomato fruit worm is slow.

This shows that *P. guineense* seed extract is insecticidal to the tomato fruit worm and therefore may be a potentially good botanical insecticide for insect pest management.

The present study has found *P. guineense* seed extract and the *Aspergillus* sp. fungus to cause some mortality to the tomato fruit worm. But it took much time for the fungus especially to cause significant mortality to the pest. A combination test may therefore improve on their insecticidal effect. Similar studies have been carried out on other insect. Sajjan (2006) was able to increase the mortality of *P. xylostella* when treated with products of combinations of neem seed extract with *B. bassiana* and *M. anisopliae*.

Sahayaraj *et al.* (2011) also found that phytophosphate and biofertilizers were compatible with *B. bassiana*. From these previous findings therefore, a study on the compatibility of *Piper guineense* seed extract with the isolated fungus was undertaken. In the study, the aqueous seed extract of *P. guineense* was found to inhibit growth of the fungus. Similar results have been obtained by Cardwell and Dongo (1994) who found that aqueous extracts from the combination of dry seeds of *P. guineense* and dry fruits of *Xylocarpus aethiopicus* completely inhibited the mycelial growth of *A. flavus*. The finding in the present study is contrary to most studies that have been able to combine fungi and botanicals and even synthetic insecticides against insect pest.

Incompatibility of insecticidal agents is not uncommon, since in a similar study, Sahayaraj *et al.* (2011) also found *Annona squamosa* extract to be incompatible with *B. bassiana*.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

In the present study, fungal entomopathogens and *P. guineense* seed extract were assayed against *H. armigera* and their compatibility tested.

Fungal entomopathogens isolated from soils and the extracts from seeds of West African black pepper have shown some level of insecticidal action against *H. armigera*.

The results obtained from this study seem to support previous findings that show that *P. guineense* and some pathogens have some insecticidal properties. Therefore the use of seed extract of *P. guineense* is a potentially safe and sustainable way of management of the tomato fruit worm.

However, *P. guineense* seed extracts inhibited the growth of *Aspergillus* sp. and therefore may not be used in combination for pest control.

Although *Aspergillus* sp. was found to cause some level of entomopathogenicity to the tomato fruit worm, it cannot be of practical use for the control of the insect. This is because *Aspergillus* sp. are known to produce aflatoxins which are potent carcinogens and cannot be applied to food crops.

The following recommendations are with regard to this study:

The study on the insecticidal effect of the *P. guineense* seed extract on *H. armigera* should be repeated with higher doses of the extract to confirm the findings of this study.

More soil samples around the University of Ghana should be collected for isolation of potential entomopathogenic fungi so that they could be tested on other insect pests.

The SV1, SV3, AG3 and BC2 fungal isolates should further be identified using molecular tools.

These fungal isolates should also be assayed against other insect pest to determine their host range.

REFERENCES

- Abel, P.P., Nelson, R.S., Hoffmann, B.D.N., Rogers, S.G., Fraley, R.T. and Beachy, R.N. (1986). Delay of disease development in transgenic plants that express the tobacco mosaic virus coat protein gene. *Science* **232**:738-743.
- Adimabuno, A.M. (2010). Marketing and market queens: a case of tomato farmers in the Upper East Region of Ghana. A Dissertation for Master Degree. Bonn University, Germany.
- Adosraku, R.K., Kyekyeku, J.O. and Attah, I.Y. (2013). Characterization and HPLC quantification of piperine isolated from *Piper guineense* (Fam. Piperaceae). *International Journal of Pharmacy and Pharmaceutical Sciences* **5**(1): 0975-1491.
- Adu-Dapaah, H.K. and Oppong-Konadu, E.Y. (2002). Tomato production in four major tomato-growing districts in Ghana: Farming practices and production constraint. *Ghana Journal of Agricultural Science* **35**: 11-22.
- Akhurst, R.J. (1990). Safety to non-target invertebrates of nematodes of economically important pests. In: Safety of Microbial insecticides. 234-238. Laird, M., Lacey, L.A. and Davidson, E.W. (Eds), CRC, Boca Raton, FL, USA.
- Akinwumi, F.O. (2011). Evaluation of some plant materials for the control of smoked fish pest, *Dermestes maculatus* degeer (Coleoptera: Dermestidae) in *Clarias gariepinus* Burchell (Pisces: Clariidae). *ARPJ Journal of Agricultural and Biological Science* **6**(7): 65-69.

- Ali, A., Choudhury, R.A., Ahmad, Z., Rahman, F., Khan, F.R. and Ahmad, S.K. (2009). Some biological characteristics of *Helicoverpa armigera* on chickpea. *Tunisian Journal of Plant Protection* **4**: 99-106.
- Al-shannaf, H.M., Mead, H.M. and Hassan S.A.K. (2012) Toxic and Biochemical Effects of Some Bioinsecticides and Igrs on American Bollworm, *Helicoverpa armigera* (hüb.) (noctuidae: lepidoptera) in Cotton Fields. *Journal of Biofertilizer and Biopesticide* **3**:118. doi:10.4172/2155-6202.1000118
- Anaisie, P.E., Eziah, V.Y and Owusu, E.O. (2011). The potential of indigenous entomopathogenic fungi for the management of the diamondback moth, *Plutella xylostella* L. (Lepidoptera: Yponomeutidae) in Ghana. *International Research Journal of Biochemistry and Bioinformatics*. **1**(10): 275-281.
- Armes, N.J., Jadhav, D.R. and DeSouza, K.R. (1996). A survey of insecticide resistance in *Helicoverpa armigera* in the Indian subcontinent. *Bulletin of Entomological Research*. **86**: 499-514.
- Arnó, J., Gabarra, R., Roig, J. and Fosch, T. (1999). Integrated pest management for processing tomatoes in the Ebro Delta (Spain). *Acta Horticulturae*, **487**(1): 207-212.
- Arno, J., Gabarra, R., Roig, J. and Fosch, T. (1999). Integrated pest management for processing tomatoes in the Ebro Delta (Spain). *ISHS Acta Horticulturae* **487**: 207-212.

- Asawalam, E.F. and Emosairue, S.O. (2006). Comparative efficacy of *Piper guineense* (Schum and Thonn) and pirimiphos methyl on *Sitophilus zeamais* (motsch). *Tropical and Subtropical Agroecosystems* **6**(3): 143-148.
- Bantewad, S.D. and S.V. Sarode. (2000). Influence of different hosts on the biology of *Helicoverpa armigera* (Hübner). *Shashpa* **7**(2): 133-136.
- Barnett, H.L. and Hunter, B.B. (1998). *Illustrated Genera of Imperfect Fungi*. (4th Ed.). The American Phytopathological Society Press, St Paul, Minnesota, USA. 218p.
- Bessin, R. (2003). Tomato Insect IPM guidelines. Extension Entomologist, U.K. Cooperative Extension Service. ENTFACT-313.
- Bhan, S., Shrankhla, Mohan, L. and Srivastava, C.N. (2013). Larvicidal toxicity of temephos and entomopathogenic fungus, *Aspergillus flavus* and their synergistic activity against malaria vector, *Anopheles stephensi*. *Journal of Entomology and Zoology Studies*. **1**(6): 55-60.
- Borchert, D.M., Magarey, R.D. and Fowler, G.A. (2003). Pest Assessment: Old World Bollworm, *Helicoverpa armigera* (Hubner), (Lepidoptera: Noctuidae). USDA-APHIS-PPQ-CPHST-PERAL/NCSU.
- Bosly, H.A. and El-Banna, O.M. (2015). Isolation and identification of fungal growth on *Tribolium castaneum* in stored wheat flour. *Journal of Entomology and Nematology*. **7**(2): 11-17.

- Boukhris-Bouhachem, S., Hdider, C., Souissi, R., and Ghazel Pizzol, I.J. (2007). Seasonal activity of *Helicoverpa armigera* (Lepidoptera Noctuidae) for improved control management strategies in processing tomatoes. *Acta Horticulturae* **758**: 89-94.
- CABI, (2007). Crop Protection Compendium. Commonwealth Agricultural Bureau, International. <http://www.cabicompendium.org/>
- Cardwell, K.F. and Dongo, L. (1994). Effect of extracts from nine plant species found in Africa on the mycelial growth of *Aspergillus flavus*. Link. Proc. of the 6th IWCSPP, Canberra, Australia. 978-980.
- Chabbouh, N. and Cherif, C. (1990). Cucumber mosaic virus in artichoke. *FAO Plant Production Bulletin* **38**:52-53.
- Chahal, J., Ohlyan, R., Kandale, A., Walia, A. and Puri, S. (2011). Introduction, phytochemistry, traditional uses and biological activity of genus Piper: A review. *International Journal of Current Pharmaceutical Review and Research*. **2**(2): 131-144.
- Cherry, A., Cock, M, van den Berg, and Kfir, R (2003). Biological control of *Helicoverpa armigera* in Africa. In Biological Control in IPM Systems in Africa. Neuenschwander, P., Borgemeister, C. and Langewald. J. (Editors). CABI Publishing in association with the ACP-EU Technical Centre for Agricultural and Rural Cooperation (CTA) and the Swiss Agency for Development and Cooperation (SDC). pp. 329-346. ISBN: 0-85199-639-6.

- Corbett, B.P., Jia, L., Sayler, R.J., Arevalo-Soliz, L.M., Goggin, F. (2011). The effects of root-knot nematode infection and mi-mediated nematode resistance in tomato on plant fitness. *Journal of Nematology* **43**(2):82-9.
- Del Valle, M., Cámara, M. and Torija, M.E. (2007). The nutritional and functional potential of tomato by-products. *Acta Horticulturae* **758**: 165-172
- El Shafie, H.A.F. and Abdelraheem, B.A. (2012). Field evaluation of three biopesticides for integrated management of major pests of tomato, *Solanum lycopersicum* L. in Sudan. *Agriculture and Biology Journal of North America* **3**(9): 340-344.
- Elizabeth, J.Z.R. and Shashi, L.K. (2010). The case of tomato in Ghana: Productivity. Development and Strategy Governance Division, IFPRI, Ghana. Paper No. 19.
- Enkerli, J., Reckenholz-Tänikon, A.R.T., Harer, Z.P.N. and Rahuri, M.P.K. (2008). Development of a Mycoinsecticide against *Helicoverpa armigera* in Pulses: From the Laboratory to the Market. ISCB Programme Report 2004-2007.
- EPPO, (2006). Distribution maps of Quarantine Pests. *Helicoverpa armigera*. On-line available at www.eppo.org/QUARANTINE/insects/Helicoverpa_armigera/HELIAR_map.htm.
- Evenson J. P and Basinski, J. J. (1973). Bibliography of cotton pests and disease in Australia. *Cotton Grow Review* **50**:79–86.
- Fern, K. (2014). Useful tropical plants data base. tropical.theferns.info. Accessed on 21st May 2015 at 10:30 GMT.

- Firempong, S. and Zalucki, M. 1990. Host plant selection by *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae); role of certain plant attributes. *Australian Journal of Zoology* **37**: 675-683.
- Fitt, G. P. (1989). The ecology of *Heliothis* species in relation to agroecosystems. *Annual Review of Entomology*, **34**(1): 17-52.
- Fitt, G. P. (1989). The ecology of *Heliothis* species in relation to agroecosystem. *Annual Review of Entomology* **34**:17–52.
- Gbewonyo, W.S.K. (1991). PhD Thesis, University of Birmingham, UK. pp191.
- Gbewonyo, W.S.K. and Candy, D.J. (1992). Chromatographic isolation of insecticidal amides from *Piper guineense* root. *UK Journal of Chromatography* **607**(1):105–111.
- Gbewonyo, W.S.K., Candy, D.J., Anderson, M. (1993). Structure-activity relationships of insecticidal amides from *Piper guineense* root. *Pesticide Science* **37**(1): 57-66.
- Gianessi, L. (2009). The Benefits of insecticide use: Tomatoes. Crop Life Foundation, Washington, USA. pp18.
- Glazer, I. and Navon, A. (1990). Activity and persistence of entomoparasitic nematodes tested against *Heliothis armigera* (Lepidoptera: Noctuidae). *J. Econ. Entomol.* **83**: 1795-1800.
- Guangyu, Z. (1989). The first commercial viral pesticide in China. *Integrated Pest Management practice*. **11**(13). Accessed on April 14th 2015 at 14: 45 GMT.

- Hasan, W.A., Assaf, L.H. and Abdullah, S.K. (2012). Occurrence of entomopathogenic and other opportunistic fungi in soil collected from insect hibernation sites and evaluation of their entomopathogenic potential. *Bulletin of the iraq natural history museum* **12(1): 19-27**.
- Hatting, J.L. (2012). Comparison of three entomopathogenic fungi against the bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae), employing topical vs per os inoculation techniques. *African Entomology* **20(1): 85-90**.
- Hebert, S., Jia L. and Goggin, L. (2007). Quantitative Differences in Aphid Virulence and Foliar Symptom Development on Tomato Plants Carrying the Mi Resistance Gene. *Environmental Entomology* **36(2):458-67**.
- Heuvelink, E. (2005). Tomatoes. Crop Production in horticulture series 13. CABI Publishing, The Netherlands. 232 pp.
- Hunt, D.W.A. and Whitfield G. (1986). Potato trap crop for control of Colorado potato beetle (Coleoptera: Chrysomelidae) in tomatoes. *Canadian Entomologist* **128: 407-412**.
- Ibarra, J.E. and Del Rincon-Castru, M.C. (2008). Insect viruses diversity, biology, and use as bioinsecticides. In: *International Commission on Tropical Biology and Natural Resources*. (Eds. Kleber Del Claro, Paulo S. Oliveira, Victor Rico-Gray, Alonso Ramirez, Ana Angelica Almeida Barbosa, Arturo Ronet, Fabio Rubio Scarano. Fernando Louis Consoli, Francisco Jose Morales Garzon, Jimi Naoki Nakajima, Julio Alberto Costello, Marcus Vinicius Sanpaio, Mauricio Quesada Molly R. Morris, Monica Palacios Rios, Nelson Ramirez Oswaldo Marcal Junior, Regina Helena

- Ferraz Macedo, Robert J. Marquis, Rogerio Parentoni Martins, Silvio Carlos Rodrigues, Ulrich Luttge). In Encyclopedia of Life Support Systems (EOLXS). Eolss Publishers, Oxford, UK.
- Ihemanna, C.A., Adindu, R.U., Kalu, M.K. and Kalu, E.J. (2014). Laboratory evaluation of ethanolic extracts of *Citrus sinensis* peels and *Piper guineense* (seeds and leaves) on mosquito larvae. *Journal of Environment and Human*. **1**(1): 19-24.
- Iwu, M.M. (Ed.) (1993). Handbook of African medicinal plants. CRC Press, Boca Raton, FL, pp435.
- Johnson, R., Narvaez, J., Am, G. and Ryan, C.A. (1989). Expression of proteinase inhibitors 1 and 11 in transgenic tomato plants: effects on natural defense against *Manduca sexta* larvae. *Proceeding of National Academic Sciences, USA*. **88**: 9871-9875.
- Jones, J.B., Lacy, G.H., Bouzar, H., Stall, R.E. and Schaad, N.W. (2004). Reclassification of *Xanthomonas* associated with Bacterial spot diseases of tomato and pepper. *Systematic and Applied Microbial* **27**(6):755-62.
- Karim, S. (2000). Management of *Helicoverpa armigera*: A review and prospectus for Pakistan. *Pakistan Journal of Biological Sciences*. **3**(8): 1213-1222.
- Kaushik, C., (2011). Incidence of aphid *Aphis gossypii* Glover (Hemiptera: Aphididae) on tomato crop in the agro climatic conditions of the Northern parts of West Bangal, India. *World Journal of Zoology*, **6** (2): 187-191

- Kennedy, G.G. (2003). Tomato, pests, parasitoids, and predators: Tritrophic interactions involving the genus *Lycopersicon*. *Annual Review of Entomology* **48**:51-72
- King, A.B.S. (1994). Heliothis/Helicoverpa (Lepidoptera: Noctuidae) In: G. A. Matthews & J. P. Tunstall (Eds), Insect Pests of Cotton. Wallingford, UK: CAB International, Wallingford, 39-106.
- Kollie, M.H., Osekre, E.A., and Mochiah, M.B. (2014). Management of Insect Pests using Chlorpyrifos Applications at different growth stages of Tomato, *Solanum lycopersicum* L. *Journal of Biology, Agriculture and Healthcare*, **4**(14): 2224-3208.
- Kumar, S., Saini, S. K. and RAM, P. (2009). Natural mortality of *Helicoverpa armigera* (Hübner) eggs in the cotton ecosystem. *Journal of Agricultural Science and Technology, Libertyville* **11** (1): 17-25.
- Lale, N.E.S. and Alaga, K.A. (2001). Exploring the insecticidal, larvicidal and repellent properties of *Piper guineense* Schum. et Thonn. seed oil for the control of rust-red flour beetle *Tribolium castaneum* (Herbst) in stored pearl millet *Pennisetum glaucum* (L.) R. Br. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz*. **108**(3): 305-313.
- Lammers, J.W. and Macleod, A. (2007). Report of a pest risk analysis: *Helicoverpa armigera* (Hübner, 1808). Available at: Access online Jan. 10, 2013.
- Lulie, N. and Raja, N. (2012). Evaluation of certain botanical preparations against African bollworm, *Helicoverpa armigera*, Hubner (Lepidoptera: noctuidae) and non-target organisms in chickpea, *Cicer arietinum* L. **3**(5): 130.

- Malarvannan, S., Murali, P.D., Shanthakumar, S.P., Prabavathy, V.R. and Nair, S. (2010). Laboratory evaluation of the entomopathogenic fungi, *Beauveria bassiana* against the tobacco caterpillar, *Spodoptera litura* Fabricius L. (Noctuidae: Lepidoptera). *Journal of Biopesticides* **3**(1): 126 – 131.
- Miller, E.C., Hadley, C.W., Schwartz, S.J., Erdman, J.W. Jr., Boileau, T.W-M. and Clinton, S.K. (2002). Lycopene, tomato products, and prostate cancer prevention. Have we established causality? *Pure and Applied Chemistry* **74** (8): 1435–1441.
- Miyakado M, Nakayama I, and Yoshioka H. (1980) Insecticidal joint action of piperidine and co-occurring compounds isolated from *Piper nigrum* L. *Agricultural and Biological Chemistry* **44**:1701–1703.
- Mueller, K. (2014). The tomato and its relatives. kdcomm.net/Tomato/tax.html. Accessed on March 21st 2015 at 5:17 GMT.
- Mythili, P., Gomathinayagam, S., Balachandran, C., Raj, D. and John, L. (2010). Isolation and mass propagation of entomopathogenic fungi for biocontrol assays. *Tamilnadu J. Veterinary & Animal Sciences*. **6** (6): 271-274.
- Nadaf, A.M. and Kulkarni, K.A. (2006). Seasonal incidence of the fruit borers, *Helicoverpa armigera* (Hubner) and *Spodoptera litura* Fabricius on chilli in Dharwad. *Karnataka Journal of Agricultural Science* **19**(3): (549-552).
- Naika, S., de Jeude, J.L., de Goffau, M., Hilmi, M. and van Dam, B. (2005). *Cultivation of tomato. production, processing and marketing*. Digigrafi, Wageningen, Netherlands. 92pp.

- Namba, K., Stubbs, G. (1986). "Structure of tobacco mosaic virus at 3.6 Å resolution: implications for assembly". *Science* **231** (4744): 1401– 1406
- Navickiene, H.M.D., AleÂcio, A.C., Kato, M.J., Bolzani, V.S., Young, M.C.M., Cavalheiro, A.J. and Furlan, M. (2000). Antifungal amides from *Piper hispidum* and *Piper tuberculatum*. *Phytochemistry*. **55**: 621-626.
- Obeng-Ofori, D., Danquah, E.Y. and Ofori-Anim, J. (2007). In: Ofori, K. *Vegetable and spice crop production in West-Africa*. The City Publisher, Ltd. Accra. Ghana. pp162.
- Okwute, S.K. (2012). Plants as potential Sources of pesticidal agents: a Review, In: Pesticides. *Advances in Chemical and Botanical Pesticides*, chapter 9. 207-232. In Tech, Rijeka, Croatia.
- Oparaeke, A.M. and Bunmi, J.O. (2006). Bioactivity of two powdered spices [*Piper guineense* Thonn & Schum and *Xylopia aethiopica* (Dunal) A. Richard] as homemade insecticides against *Callosobruchus subinnotatus* on stored Bambara groundnut. *Agricultura Tropica et Subtropica*. **39**(2): 132-135.
- Ortiz-Urquiza, A. and Keyhani, N.O. (2013). Action on the Surface: Entomopathogenic fungi versus the insect cuticle. *Insects*. **4**: 357-374.
- Planet Natural Since 1991. History of Tomatoes. <https://www.planetnatural.com/tomato-gardening-guru/history/>. Accessed on March 21st 2014 at 9:15 GMT.
- Ploetz, R. C., and Pegg, K. G. (1997). Fusarium wilt of banana and Wallace's line: Was the disease originally restricted to his Indo-Malayan region? *Australas Plant Pathology* **26**:239-249.

- Prasad, A., Syed, N. and Purohit, S. (2010). *Beauveria bassiana* (Balsamo) Vuillemin: A successful biopesticide against key pest *Helicoverpa armigera* (Hubner). *International Journal of Pharma and Bio Sciences*. **1**(2): 407-414.
- Pscheidt, J.W. and Stevenson, W.R (1988). The critical period for control of early blight (*Alternaria solani*) of potato. *American Journal of Potato Research* **65**: 425-438.
- Pscheidt, J.W. and Stevenson, W.R. (1986). Early blight of potato and tomato: A literature review. University of Wisconsin, Cooperative Extension Service Publication No. R3376.
- Rajapake, R.H.S. and Ratnaseka, D. (2008). Pesticidal potential of some selected tropical plant extracts against *Callosobruchus maculatus* F. and *Callosobruchus chinensis* L. *Tropical Agricultural Research and Extension*. **11**: 69-71.
- Reddy, G.V.P. and Miller, R.H. (2014). Biorational versus conventional insecticides- Comparative field study for managing red spider mite and fruit borer on tomato. *Crop Protection* **64**: 88-92.
- Rijal, J.P., Yubak Dhoj, G.C., Thapa, R.B. and Kafle, L. (2008). Efficacy of *Metarhizium anisopliae* and *Beauveria bassiana* against *Helicoverpa armigera* in Chickpea, under field conditions in Nepal. *Formosan Entomologist* **28**: 249-258.
- Ritu, A., Anjali, C., Nidhi, T., Sheetal, P. and Deepak, B. (2012). Biopesticidal Formulation of *Beauveria bassiana* effective against larvae of *Helicoverpa Armigera*. *Journal of Biofertilizers and Biopesticides* **3**(3): 120. doi:10.4172/2155-6202.1000120.

- Roh, Yul, J., Choi, J.Y., Li, M.S., Jin, B.R. and Je, Y.H. (2007). *Bacillus thuringiensis* as a specific, safe, and effective tool for insect pest control. *Journal of Microbiology and Biotechnology* **17**(4), 547-559.
- Sahayaraj, K., Namasivayam, S.K.R. and Rathi, J.M. (2011). Compatibility of entomopathogenic fungi with extracts of plants and commercial botanicals. *African Journal of Biotechnology*. **10**(6): 933-938.
- Sajjan, N.I. (2006). Role of biorationals in the management of diamondback moth, *Plutella xylostella* L. in cabbage ecosystem. Thesis submitted to the University of Agricultural Sciences, Dharwad In partial fulfillment of the requirements for the Degree of Master of Science (Agriculture)
- Scott, I.M., Jensen, H.R., Philogene, B.J.R. and Arnason, J.T. (2008). A review of *Piper* spp. (Piperaceae) phytochemistry, insecticidal activity and mode of action. *Phytochemistry Review* **7**:65–75.
- Shakeel, M., Akram, W., Hamza, A., Waqar M. and Ali A. (2014). Population Dynamics of Aphid (*Aphis Gossypii* G.) on Tomato Agro-Ecosystem in Faisalabad Region. *International Journal of Research in Agricultural Sciences* **1**(3): 2348 – 3997.
- Shanower, T.G. and Romeis. J. (1999). Insect pests of pigeon pea and their management. *Annals Review of Entomology*. **44**: 77-96.
- Sharma, H.C. (2001). Cotton bollworm/legume pod borer, *Helicoverpa armigera* (Hubner) (Noctuidae: Lepidoptera). Biology and Management. *Crop protection compendium*. Walingford, UK: Commonwealth Agricultural Bureau International. pp77.

- Sharma, H.C. (2001). Cotton Bollworm/Legume Pod Borer, *Helicoverpa armigera* (Hubner) (Noctuidae: Lepidoptera): Biology and Management. Crop Protec. Compend. CAB Int'l., Wallingford, 70 p.
- Sharma, H.C. (2006). Integrated Pest Management Research at ICRISAT: Present Status and Future Priorities. Andhra Pradesh: International Crops Research Institute for the Semi-Arid Tropics.
- Sharma, K.C., Bhardwaj, S.C. and Sharma, G. (2011). Systematic studies, life history and binfestation by *Helicoverpa armigera* (Hubner) (Lepidoptera: Noctuidae) on tomato in Semi-Arid Region of Rajasthan. *An International Journal*. **3**(1): 52-56.
- Sherwood, J.L., German, T.L., Moyer, J.W. and Ullman, D.E. (2003). Tomato spotted wilt. The Plant Health Instructor. DOI:10.1094/PHI-I-2003-0613-02
- Shivanna, B.K., Girish, M. R., Shruthi, H., Shilpa, M. E., Vikas, H.M., Mallikarjuna, G.B., Harishbabu, S., Shivanna, S. and Basavaraj, M.K. (2012). Bioecology and management of bud worm, *Helicoverpa armigera* (Hubner) on FCV tobacco. *I.J.S.N*. **3**(4): 892-899.
- Simpson, B.B. and Ogorzaly, M.O. (1995). Economic Botany: plants in our world. Simpson, B.B., and Ogorzaly, M.O. (Eds.) 2nd Ed. McGraw-Hill Inc., New York. pp742.
- Smith-Pardo., A.H. (2014). The Old World Bollworm *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae: Heliothinae): its biology, economic importance and its

- recent introduction into the Western hemisphere. *Boletin Del Museo Entomológico*. 6(1). USDA-APHIS-PPQ, PhD. (2014). Accessed on April 20th 2015 at 11:25 GMT.
- Srininivasan, R. (Ed.) (2010). Safer tomato production methods: A field guide for soil fertility and pest management. AVRDC- The World Vegetable Center, Shanhua, Taiwan. AVRDC Publication No. 10-740. pp97.
- Sullivan, M. and Molet, T (2007). CPHST Pest Datasheet for *Helicoverpa armigera*. USDA-APHIS-PPQ-CPHST. Revised April 2014.
- Talekar, N.S., Opena, R.T. and Hanson, P. (2006) H. armigera Management: A Review of AVRDC's Research on Host Plant Resistance in Tomato. *Crop Protection* 5: 461-467.
- Talekar, N.S., Su, F.C. and Lin, M. Y. (2003). How to produce safer leafy vegetables in nethouses and net tunnels. Asian Vegetable Research and development Center, Shadhua, Tainan, Taiwan, 18p.
- Tay, W.T., Soria, M.F., Walsh, T., Thomazoni, D., Silvie, P. and Behere, G.T. (2013). A brave New World for an Old World pest: *Helicoverpa armigera* (Lepidoptera: Noctuidae) in Brazil. *PLoS ONE*. 8(11): e80134.
- Thomas, C.S., Yoshida, M. and Peter, J. (1997). Survival, growth, fecundity, and behavior of *Helicoverpa armigera* (Lepidoptera:Noctuidae) on pigeon pea and two wild Cajanus species. *Journal of Economic Entomology* 90(3): 837-841.

- Ting, Y.C. (1986). An analysis on population fluctuation and damage characterization of cotton bollworm in cotton areas of North China. *Acta Entomologica Sinica*. **29**: 272-282.
- Wang, N.C. and LI, Z.H. (1984). Studies on the biology of cotton bollworm (*Heliothis armigera* Hübner) and tobacco budworm (*Heliothis assulta* Quenee). *Journal of the Shandong Agricultural University*, **12**(1): 13-25.
- Wang, S., Fang, W., Wang, C. and Leger, R.J. (2011). Insertion of an esterase gene into a specific locust pathogen (*Metarhizium acridum*) enables it to infect caterpillars. *PLoS Pathog.* **7**(6): e1002097.
- Warren, A. (2013). Mega-pest worms its way into Brazil. CSIRO Blog at World Press. Accessed on April 25th 2015 at 11:35 GMT.
- Xie, T.J., Jung, B.G., Zhong, L.S. and Wu, G.X. (1990). Commercial production and application of Bt insecticide in China. In: Proceeding 5th International Colloq. Invert. Pathol. Adelaide, Australia.
- Yousif, F., Mohamed, G., Dafalla, A. and Sadig, K., O. (1994). Races of *Fusarium oxysporum* f. sp. *melonis* Causing Wilt of Melons in Central Sudan. *Cucurbit Genetics Cooperative Report* **17**:66-68.
- Zalom, F.G., Van Steenwyk, R.A., Burrack, H.J., and Johnson, M.W. (2009) Pest notes: Olive fruit fly. University of California Agriculture and Natural Resources Publication 7411.

Zalucki, M. P., Daglish, G., Firempong, S. and Twine P. (1986). The biology and ecology of *Helicoverpa armigera* (Hübner) and *H. punctigera* Wallengren (Lepidoptera: Noctuidae) in Australia: what do we know? *Australian Journal of Zoology*, *Melbourne*, **34** (6): 779-814, 1986.

APPENDICES

Appendix 1. T-test for larval population during the major and minor tomato cropping season

Sample	Size	Mean	Variance	Sd	SE
First season	16	0.706	0.2660	0.5157	0.1289
Second season	16	0.643	0.4680	0.6841	0.1710

Difference of means: 0.063

Standard error of difference: 0.214

95% confidence interval for difference in means: (-0.3749, 0.4999)

Probability=0.772

Appendix 2. Comparison of Survival Curves for larvae of *Tribolium* spp. when treated with fungi

Test Statistics

Method	Chi-Square	DF	P-Value
Log-Rank	40.8919	6	0.000
Wilcoxon	35.9976	6	0.000

Appendix 3. Comparison of Survival Curves of *H. armigera* when treated with fungi

Test Statistics

Method	Chi-Square	DF	P-Value
Log-Rank	20.9242		3
	0.000		
Wilcoxon	18.2653		3
	0.000		

Appendix 4 ANOVA for insecticidal effect of *Piper guineense* seed extract on *H. armigera*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatment	2	240.381	120.190	70.11	<.001
Residual	18	30.857	1.714		
Total	20	271.238			

Appendix 5. ANOVA for comparing effects of fungus and plant extract on *H. armigera*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Treatments	4	397.600	99.400	42.75	<.001
Residual	35	81.375	2.325		
Total	39	478.975			

Appendix 6. ANOVA for compatibility of acetone seed extract and fungus

Source of	d.f.	s.s.	m.s.	v.r.	F pr.
-----------	------	------	------	------	-------

variation					
Treatments	3	6141.35	2047.12	64.94	<.001
Residual	16	504.40	31.53		
Total	19	6645.75			

Appendix 7. ANOVA for compatibility of aqueous seed extract and fungus

Source of variation	d. f.	s.s.	m.s.	v.r.	F pr.
Treatment_mixture	3	6068.55	2022.85	64.42	<.001
Residual	16	502.40	31.40		
Total	19	6570.95			