Effects of Neem and Pyrethrum on the Biology and Abundance of *Liriomyza huidobrensis* (Diptera: Agromyzidae) and its Natural Enemy, *Phaedrotoma scabriventris* (Hymenoptera: Braconidae)

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University or for any other award.

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DEDICATION

This thesis is dedicated to my husband Philip, my children: Allan, Chris, Mitchel and Nicole. My parents: Samita and the late Eunice. My siblings: Masinde, Sue and Rossy. I dedicate this work in memory of the late Dr Chabi, who inspired me.

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ACRONYMS AND ABBREVIATION

ANOVA	-	Analysis of Variance
AZA	-	Azadirachtin
BPIA	-	Biopesticide Industry Alliance
CAN	-	Calcium Ammonium Nitrate
DAP	-	Diamonium Phosphate
FPEAK	-	Fresh Produce Exporters Association of Kenya
GLM	-	General Linear Model
HCDA	-	Horticultural Crop Development Authority
HDC	-	Horticultural Development Company
ICIPE	-	International Centre of Insect Physiology and Ecology
ICM	-	Intergrated Crop Management
IPM	-	Integrated Pest Management
I PC	-	International Potato Centre
KARI	-	Kenya Agricultural Research Institute
KEPHIS	-	Kenya Plant Health Inspectorate Service
LMF	-	Leafminer Flies
LSM	-	Least Square of Means
EPPO	-	European and Mediterrannean Plant Protection Organisation
SAS	-	Statistical Analysis System
WAR	-	Week after Release
WAG	-	Week after Germination
PBO	-	Piperonyl Butoxide

ABSTRACT

French bean crop (*Phaseolus vulgaris L.*) is a major export vegetable which is a source of revenue and income to the people of Kenya. Challenges facing French bean farmers are mainly pests. The pests includes the exotic leafminer of the genus *Liriomyza* (Diptera: Agromyzidae) which consists of *Liriomyza huidobrensis,* Blanchard, *L. sativae* Blanchard and *L. trifolii* Burgess. The most predominant of the three is *L. huidobrensis,* it is known to cause yield loss up to 70%. Control of *L. huidobrensis* is mainly by use of synthetic pesticides which is ineffective due to the quarantine status of the pest and its harmful effect on parasitoids. Botanical pesticides and use of parasitoids may offer a better alternative for the control of the *Liriomyza* flies. An exotic parasitoid, *Phaedrotoma scabriventris* has recently been introduced in Kenya from Peru where it successively controlled *Liriomyza* flies. Due to the scarcity of information on the use of botanical pesticides and parasitoids towards the control of *L. huidobrensis* in Kenya, this study is undertaken to investigate the effect of neem and Pyrethrum on *L. huidobrensis* and the just released parasitoid *P. scabriventris*. The effect of neem and Pyrethrum on mortality, survival of *L. huidobrensis* larvae and emergence of *P. scabriventris* from parasitized pupae were evaluated both in the greenhouse and the laboratory at ICIPE. Further field experiments were carried out at Sagana and Kabaru in Nyeri county of Kenya. Experiments on the effect of different concentration of Achook and Pyrethrum on feeding revealed a significant negative linear relationship between the number of feeding punctures and the concentration applied. The number of feeding punctures significantly reduced with increasing days before infestation in leaves treated with Achook and Pyrethrum while punctures in leaves treated with Neemroc increased significantly with days before infestation (DBI). The mean number of eggs oviposited when leaves were treated with different concentrations of Achook, Neemroc and Pyrethrum did not have any significant differences. Similarly, the mean number of eggs on plants infested shortly, one and two days after treatment with Achook and Neemroc did not differ significantly but the eggs increased with number of days before infestation when the leaves were treated with Pyrethrum. Egg-adult survival increased as the concentration of Achook, Neemroc and Pyrethrum reduced. It also increased with increase in the number of DBI. Achook and pyrethrum concentrations were highly toxic to the 1st larvae stages as % survival of the larvae reduced with increase in the concentration of Achook, Neemroc and Pyrethrum. Adult emergence from pupae of the 1st, 2nd and 3rd instars increased with reduction in the concentration of the biopesticide. The % emergence of adults from pupae of the 1st, 2nd and 3rd instars treated with Achook, Neemroc and Pyrethrum did not differ significantly.There was a significant increase in the mortality of larvae in soil sprayed with Neemroc and Pyrethrum and infested shortly there after and one day later as compared to larvae mortality in soil sprayed and infested two days later. Effect of different concentrations of Achook, Neemroc and Pyrethrum on emergence of *P. scabriventris* and the LMF showed a significant reduction in emerged parasitoids as the concentration of the pesticide increased. The parasitoids were more tolerant to the chemicals than the LMF and their longevity was not affected. Field experiments at Kabaru and Sagana showed a significant higher % mortality of LMF when they were exposed to parasitoids and treated with four applications (A4) of Neemroc & Pyrethrum as well as in Farmers practice where Dimethoate and Bulldock star was used.

The total yield, percentage of unmarketable yield, leaf infestation, *L. huidobrensis* and *P. scabriventris* abundance registered no significance difference among all the application schemes applied. This study therefore has clearly shown that low concentrations of Achook, Neemroc and Pyrethrum can be used to control *L. huidobrensis* especially in controlled environments without adverse effects on the parasitoids. However, field studies were only carried out for 8 and 10 weeks in Kabaru and Sagana respectively. Therefore increased parasitoids release and recovery should be carried out frequently to monitor the establishment of the parasitoid and their spread to the surrounding regions.

CHAPTER ONE

INTRODUCTION

1.1 Background

French beans (*Phaseolus vulgaris L.*) are a major export vegetable commodity in Kenya. Most of this crop is grown by small scale farmers in Central and Eastern provinces of Kenya. Recent data shows that almost 100,000 people earn a living from French bean production while 500,000 indirectly derive their income from the export of this crop (Seif *et al.,* 2001). French bean farmers however face challenges mainly from insect pests and diseases. Insect pests infesting French beans are the indegenous white flies *(Bemicia tabaci)* and bean flies *(Ophiomyia spp)*. The exotic spider mites *(Tetranychus spp),* and Leafminers (*Liriomyza huidobrensis*) (Prijono *et al.,* 2004) are also serious pests of French beans. *Liriomyza huidobrensis* is considered as one of the major pests of French beans. The pest has been reported to develop resistance to synthetic pesticides such as Abamectin very fast (Weintraub and Horowitz, 1998) due to the frequency of spraying. This kills increasing numbers of pests, but some resistant insects survive and multiply rapidly making their control difficult.

Leafminer species belonging to the genus *Liriomyza* (Diptera: Agromyzidae) are important pests of various vegetable crops worldwide (Murphy and LaSalle, 1999; Musundire *et al.,* 2010). *Liriomyza huidobrensis* is aquarantine species because products damaged by these pests are rejected by all European Union countries. It is therefore the most important species in Kenya, representing 80 % of the total *Liriomyza* species found in the highland areas (Chabi-Olaye *et al.,* 2008). *Liriomyza huidobrensis* is one of the leading quarantine pests that significantly affects vegetable production and fresh produce export in Kenya (Kedera and Kuria, 2003; Gitonga *et al.,* 2010). The pest attacks a

variety of crops of commercial value, which include snow pea (*Pisum sativum* L.), French bean (*Phaseolus vulgaris* L.), tomato (*Lycospersicon esculentum* Miller), runner bean (*Phaseolus coccineus*), potato (*Solanum tuberosum*) and a variety of cut flowers (Chabi-Olaye *et al.,* 2008).

The control of *Liriomyza* Leafminers species in Kenya poses serious difficulties due to their biology and quarantine status of the pests. Most of control measures used against *Liriomyza* Leafminers are pesticide-based and include a range of chemicals like: deltamethrin, dimethoate, tebuconazole and lambda-cyhalothrin (Gitonga *et al.,* 2010). However, many of these chemicals have been reported in several countries to be ineffective and uneconomical in controlling the pests, mainly because of the evolution of resistance (Vandeveire, 1991; Weintraub and Horowitz, 1998) and the poor quality of applications (Gitonga *et al.,* 2010). In addition, frequent pesticide applications undermine the potential of antagonistic effects of natural enemies on the pests (Helyer and Ledieu, 1986).

The introduction of the maximum residue levels (MRL) for export vegetables by the European Union (EU) is also a potential complication for the farmers, since the MRL is high, (CBI, 2005). Biological control methods of *L. huidobrensis* have been explored. In greenhouses in Europe, the LMF has been controlled successfully with releases of *Dacnusa sibirica* (Hymenoptera: Braconidae), *Opius paillipes* (Hymenoptera: Braconidae) and *Diglyphus isaea* (Hymenoptera: Eulophidae) (Van de Linden, 1993). Parasitoids of the leafminers have been widely investigated and evaluated in vegetables and ornamentals grown in commercial green houses in Europe and North America (Chen

et al., 2003). The parasitoid *D. isaea* has been encountered in several vegetable fields in Central and Eastern Provinces of Kenya (Varela *et al.*, 2003). The percentage parasitization of *Diglyphus isaea* was estimated to be 35.8% in cucumbers infested by *L. huidobrensis* (Yankova *et al.,* 2008). Introduction of other exotic parasitoids is being proposed at *icipe* for the control of *Liriomyza* Leafminer. One of these parasitoids is *Phaedrotoma scabriventris* (Hymenoptera: Braconidae) that has successfully controlled *L. huidobrensis* in potatoes and vegetable production in Central Peru (Cisneros and Mujica, 1997). *Phaedrotoma scabriventris* has been released in field crops infested by *L. huidobrensis* in Nyeri county of Central Kenya in January and March 2010 (ICIPE, 2010).

In recent years, alternative pesticides from natural sources such as plants and micro-organisms have been tested and are gradually replacing synthetic pesticides. Such biopesticides like azadirachtin from the neem tree *Azadirachta indica* A juss (Tedeschi *et al.*, 2001) have been reported to efficiently control different important pest species. Neem has further been found to be less harmful to the indigenous and exotic natural enemies of the insect pests (Mansour *et al.*, 1986, Lowery and Isman 1994). Neem has a large number of biologically active compounds such as nimbidin, salannin and Antihormones that counteract resistance among target pests (Lowery and Isman, 1994). It has been used to control various pests in Kenya and was found to be effective. For example, Diamondback moth in cabbages was successfully controlled by use of neem (Okoth, 1998). According to Babul and Poehling, 2005, neem has also been used in the control of *Liriomyza sativae* with positive results in Bangkok and Thailand. Unlike neem, Pyrethrum has no record in the control of LMF, although it is considered an excellent

choice as an agricultural pesticide since it has rapid degradation which discourages insect resistance Glynne (1999). There is currently scarcity of information on neem and Pyrethrum effects on *L. huidobrensis* and the parasitoid *P. scabriventris* in Kenya and elsewhere necessitating this study. No information is available on the efficacy of neem and Pyrethrum products on the invasive *Liriomyza huidobrensis*, *L.trifolii, L. sativae* and *P. scabriventris*. Therefore, this study explores the efficacy of using these botanicals together with the parasitoid *P. scabriventris* in the control of *L. huidobrensis*.

1.2 Problem statement and justification

Major challenges faced by French bean farmers are pests and diseases. In Kenya, French beans is among crops infested by the three invasive *Liriomyza* species of which *L. huidobrensis* makes up 80% (Chabi-Olaye *et al.*, 2008). It causes yield losses ranging between 10-100 % depending on the crop type, crop variety, cropping season and location (Cisneros and Mujica, 1997; Shepard and Barrion, 1998; Gitonga *et al.,* 2010). Recent export figures have also shown higher rejection rates of certain vegetable commodities; valued at US$ 10 million due to significant damage caused by *Liriomyza* leafminer flies (HCDA, 2006). In response to these challenges, a range of chemicals including broad-spectrum pyrethroids, organophosphates and translaminar compounds such as cyromazine and abamectin are used by farmers to manage these pests. However, the same chemicals result into multiple problems such as development of pesticide resistant strains (Williams and Dennehey, 1997), pesticide-induced resurgence of insect pests, residue effects of the pesticides, adverse effects on non target organisms, contamination of water sources and direct health hazards to both farmers and consumers (Raguraman and Singh, 1999).

The participation of small scale farmers in vegetable and fruit exports has been under threat since the introduction of maximum residue level (MRL) legislation in the European Union (EU). These constraints to trade represent the newest and potentially most challenging limitations to future development of the horticultural sector in Kenya. Indeed, violations of sanitary and phytosanitary compliance restrict market access and impact the socio-economic sustainability of many smallholder families that are involved in French bean, snow pea and sugar snap export. Therefore, more suitable management methods with reduced insecticide load, more safe, selective and environmentally friendly pesticides are urgently needed for the management of *Liriomyza* species. Such methods together with parasitoids should be used as the main alternative control against *Liriomyza* species (Kang, 1996; Chen *et al.*, 2003).

Neem and Pyrethrum are less toxic to mammals and are bio-degradable hence should be considered for the control of *L. huidobrensis* in Kenya. Whilst Pyrethrum is a contact pesticide, it has never been used in the control of LMF. Furthermore, the impact of neem on predators and natural enemies of LMF should be studied in more details (Lowery *et al.*, 1993; Schmutterer, 1997). In Kenya, very little information is available on the use of parasitoids in the control of *L. huidobrensis*. Since *P. scabriventris* is among parasitoids that have successfully parasitized LMF in Peru (Fenoglio and Salvo, 2009), its combination with neem and Pyrethrum provides a possible alternative IPM strategy for better management of *L. huidobrensis*. This study therefore investigates the effect of neem and Pyrethrum on the biology and abundance of *L. huidobrensis* and the newly introduced parasitoid *P. scabriventris*

1.3 Research questions

a) Is there any difference in the feeding, oviposition and egg to adult survival of *L. huidobrensis* exposed to various spray regimes of Achook Neemroc and Pyrethrum biopesticide?

b) What effect do Achook, Neemroc and Pyrethrum have on the survival of larval stages and emergence of adults from pupae of *L. huidobrensis*?

c) What effect do Achook, Neemroc and Pyrethrum have on the mortality of the late 3rd instar larvae and emergence of adults from pupae of *L. huidobrensis*?

d) Does Achook, Neemroc and Pyrethrum have similar effects on the emergence of adults from parasitized and non parasitized pupae of *L. huidobrensis*?

e) Does application of Neemroc and Pyrethrum have any effect on infestation and abundance of adult *L. huidobrensis* and *P. scabriventris* in relation to yield in the field?

1.4 Hypotheses

a) There is no difference on the feeding, oviposition and egg to adult survival of *L. huidobrensis* exposed to various spray regimes of Achook Neemroc and Pyrethrum biopesticide.

b) There is no difference in the effects of Achook, Neemroc and Pyrethrum on the survival of larval stages and emergence of adults from pupae of *L. huidobrensis.*

c) There is no difference in the effect of Achook, Neemroc and Pyrethrum on the mortality of the late 3rd instar larvae and emergence of adults from pupae of *L. huidobrensis*?

d) There is no difference in the effects of Achook, Neemroc and Pyrethrum on emergence of adults from parasitized and non parasitized pupae of *L. huidobrensis*.

e) There is no difference in the effects of Neemroc and Pyrethrum on infestation and abundance of adult *L. huidobrensis* and *P. scabriventris* in relation to yield in the field.

1.5 Objectives

1.5.1 General objectives

To evaluate the effect of neem and Pyrethrum biopesticides on the biology and abundance of *L. huidobrensis* and its natural enemy *P. scabriventris*.

1.5.2 Specific objectives

a) To asses the effects of various spray regimes of Achook, Neemroc and Pyrethrum biopesticide on feeding, oviposition and egg to adult survival of *L. huidobrensis*.

b) To establish the effects of Achook, Neemroc and Pyrethrum on survival of larval stages and emergence of adults from pupal stage of *L. huidobrensis*.

c) To determine the effect of Achook, Neemroc and Pyrethrum on the mortality of the late 3rd instar larvae and emergence of adults from pupae of *L. huidobrensis*?

d) To determine the effects of Achook, Neemroc and Pyrethrum on emergence of adults from parasitized and nonparasitized pupae of *L. huidobrensis*.

e) To investigate the effects of Neemroc and Pyrethrum on infestation and abundance of adult *L. huidobrensis* and *P. scabriventris* in relation to yield in the field.

CHAPTER TWO

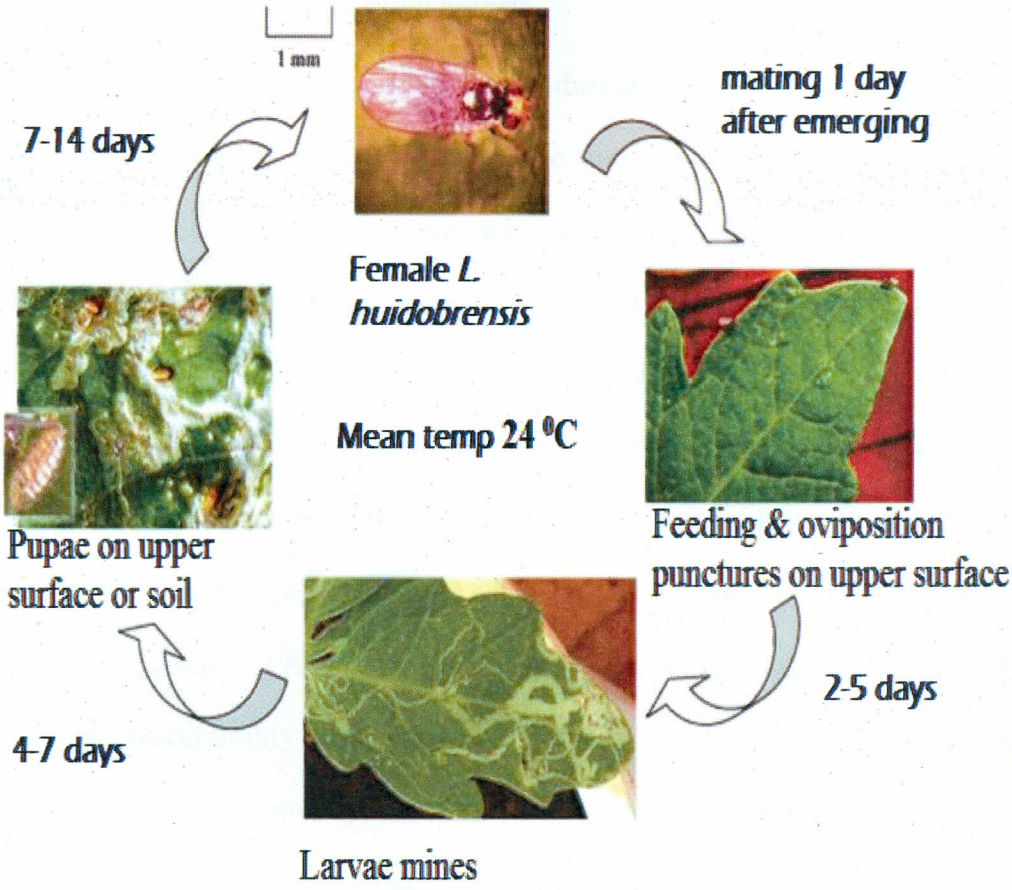
LITERATURE REVIEW

2.1 Pest status of *Liriomyza huidobrensis* Blanchard

Liriomyza huidobrensis (Diptera: Agromyzidae) is a phytophagous pest found abundantly in temperate and tropical areas. It appears to be normally limited to altitudes above the rain forest. It is a phytophagous pest that mines cultivated crops such as French beans, sugar snap peas, snow peas and other vegetable crops (Weintraub and Horowitz, 1995). It is also a serious pest of ornamental and vegetable crops in green houses and open fields. *Liriomyza huidobrensis* is extremely proliferous and difficult to control. It has been described together with *L. sativae* as being the major pest of vegetable crops in Indonesia (Shepard and Barrion, 1998). In Kenya, *L. huidobrensis* is found in all agro-ecosystems from lowland to highland areas. It is the predominant *Liriomyza* spp in peas and beans in the mid and highland of Kenya (Chabi-Olaye *et al.*, 2008). It has a history of causing serious economic damage to ornamental plants and vegetables crops worldwide (Spencer, 1981). In Kenya, the pest is reported from several horticultural crops with sometimes extremely heavy damage on snow peas, sugarsnap pea, french bean, tomatoes, runner beans, okra, aubergines, passion fruit and cut chrysanthemum flowers. Severe infestations have also been recorded on indigenous vegetables such as amaranth, nightshade, and agricultural crops such as local beans and potatoes (Chabi-Olaye *et al.*, 2008). A brief on the identification and management of (LMF) was developed and distributed to farmers and crop officers in the regions where icipe on-farm experiments were set up. An economic impact assessment study showed high awareness of LMF crop yield and economic damage amongst farmers in Kenya (Gitonga *et al.*, 2010).

2.2 Biology of *L. huidobrensis*

Adult *L. huidobrensis* females are slightly larger than males. Females live up to 18 days and males about 6 days (Charton and Allen, 1981). Females puncture predominantly on the upper leaf surface with their ovipositor and feed on these holes. Males also feed in the puncture sites. Female flies make feeding punctures and oviposition punctures, 87% of the eggs laid develop to first instar (Salas *et al.,* 1988). Larvae hatch from the eggs after 2-5 days depending on the temperature; it feeds in the spongy mesophyll of the leaf. Three larval instars develop in the leaf and the mines become progressively larger with each molt. Larval stage ranges between 3.6-10 days (Hincapie *et al.*, 1993). First instar larvae are colorless on hatching, turning pale yellow-orange, distinguishable from the later instars which are yellow-orange (Salas *et al.,* 1988). The pupal stage lasts 7.9-12.6 days and pupa varies in color from light brown to almost black (van de Linden, 1993). *Liriomyza huidobrensis* pupates within the leaf, and some times it pupates externally, either on the foliage or in the soil just beneath the surface. Adult emergence occurs 7-14 days after pupation, at temperatures between 20 and 30°C (Leibee, 1982). At low temperatures emergence is delayed. In the southern USA, the life-cycle is probably continuous throughout the year with a noticeable first generation which reaches a peak in April (Spencer, 1973). In California, *L. huidobrensis* completes its life-cycle in 17-30 days during the summer and in 50-65 days during the winter (Lange *et al.*, 1957).



Life cycle of *L. huidobrensis*
Adopted from Varella *et al.*, 2003

2.3 Control options of *L. huidobrensis*

Liriomyza huidobrensis is a phytophagus pest that is difficult to control through the use of one particular method, therefore the following ways have been used towards its Management;

2.3.1 Chemical Control of *L. huidobrensis*

It is the repetitive application of pesticides during a pest outbreak for the purpose of killing them. However, a few pests survive and develop resistance to the pesticide applied. Chemical control in Europe and Israel has proven very difficult. MacDonald (1999) documented that the pea Leafminer in the United Kingdom is insecticide resistant. Pesticides normally used against *L. trifolii* are not effective against *L. huidobrensis*. The most effective insecticide for control of the larval stages have systemic and translaminar properties. The following pesticides have been found to have good efficacy on *L. huidobrensis* larval stages: Oxamyl, Abamectin and Cyromazine (Masis, 1991; Van der Veire, 1991; Hurni, 1992; Van der Staay, 1992; Ochoa and Carballo, 1993). But insecticide susceptibility varies among populations and the level of susceptibility is directly related to frequency of insecticide application. Rotation among classes of insecticide is recommended to delay development of resistance. Reduction in dose level and frequency of insecticide application, as well as preservation of susceptible populations through non-treatment of some areas, are also suggested as means to preserve insecticide susceptibility among LMF populations (Mason *et al.*, 1989). However, increased use of pesticide cocktails in response to damage by LMF also constrains the impact of the pest's natural enemies and increases environmental contamination, health risks, pesticide residues and production costs (Isman, 2006). Most of control measures used against *Liriomyza* leafminers in Kenya are pesticide-based and include a range of chemicals like: deltamethrin, dimethoate, tebuconazole and lambda-cyhalothrin (Gitonga *et al.*, 2010).

2.3.2 Cultural control of *L. huidobrensis*

This is the deliberate alteration of the production system, either the cropping system itself or specific crop production practices, to reduce pest populations or avoid pest injury to crops. Field studies in Netherlands showed that polythene gauze (0.6 X 0.6mm) was effective against *L. huidobrensis* but this was more expensive than chemical control (Ester *et al.*, 1994). Sticky traps have also been used to reduce the density of Leafminers (Chavez and Raman, 1987). In the United Kingdom, long 150mm wide curtains of yellow polythene coated with sticky poly butane were developed and are commercially available for trapping large numbers of Leafminers (Bartlett, 1992). In China, intercropping of sugarcane and pepper reduced the population of *L. huidobrensis* as compared to a monoculture of sugarcane alone (Chen *et al.,* 2010). In Kenya, normal agricultural hygiene is in controlling Leafminer damage. This is acquired through hand-picking and destroying of mined leaves; destroying all infected leaves and other plant material after harvest; destroying pupae before planting a new crop; ploughing and hoeing helps reduce leafmining flies by exposing pupae, which then are killed by predators or by desiccation. Where new stock is obtained as seedlings rather than seed, it should be checked careful and infected plants destroyed before planting to prevent introduction of pests including leafminers (Varella *et al.*, 2003).

2.3.3 Biological control of *L. huidobrensis*

This is the use of living organisms such as predators, parasitoids and pathogens in the control of pest populations on agricultural crops. It can be through the conservation of the already existing natural enemies (conservational biological control) or breeding and

rearing of biological agents in mass numbers and releasing them on infested crops (augumentative biological control) to reduce pest population. Natural enemies periodically suppress Leafminer population (Spencer 1973). *Dacnusa sibirica* (Hymenoptera: Braconidae), *Opius pallipes* (Hymenoptera; Braconidae) and *Diglyphus isaea* (Hymenoptera: Eulophidae) are also under consideration for use as natural enemies of the pest in European glass houses (Van der veire, 1991; Leuprecht, 1992). Recently, an endoparasitoid *P. scabriventris* Nixon (Hymenoptera: Braconidae) has a significance importance as a parasitoid of Agromyzidae flies (Salvo, 1996 and Salvo *et al*., 2005). The parasitoid represents 50% of total parasitism in Peru, Chile, Brazil and Argentina (Salvo and Valladares, 1995). It is also being considered for biological control of the LMF and is still under study in the quarantine at ICIPE. *Phaedrotoma scabriventris* is an endoparasitoid whose females lay their eggs directly into the Leafminer larvae, the parasitized Leafminer larvae continue to cause damage and develop to pupae, but the adult parasitoid emerges instead of the Leafminer. *Liriomyza huidobrensis* offers the highest survival rate and shortest developmental time to the parasitoid *P. scabriventris*. Higher parasitism rates by *P. scabriventris* were found on plants from which smaller Leafminers were reared but the larger parasitoids that caused higher mortality rates of the leafminer were found on crops with large LMF (Vidella *et al.,* 2006). Studies on the reproductive potentials of *P. scabriventris* and its adaptability to different agro ecosystems have been done and the host range as well as risks posed to non-target and other trophic levels have been carried out. There's an indication that the endoparasitoid *P. scabriventris* is a very promising candidate and can substantially contribute to suppression of (LMF) in the vegetable production of Kenya (Chabi-Olaye, 2008).

2.3.4 Host plant resistance towards *L. huidobrensis*

Host plant resistance suppresses insect pest abundance or elevates the damage tolerance level in plants. Insect-resistant plants alter the relationship an insect pest has with its plant host by affecting its resistance through antibiosis, antixenosis, or tolerance. In potatoes, the mechanism of resistance is ascribed to the high density of glandular trichomes which physically reduce feeding and restrict oviposition rate of *L. huidobrensis* (Mujica *et al.,* 1993). Leaf tissue structure is influential in feeding and probing behavior of *L. huidobrensis* hence the thickness of the epidermis wall, densities of the palisade and spongy tissues act as female barrier to female oviposition (Wei *et al.,* 2000). *Liriomyza huidobrensis* females strongly prefer *Vicia faba* over *Beta vulgaris* var. *cicla* for feeding and egg laying. Only larval and adult experience modifies feeding behaviour of the leafminer, whereas oviposition preferences remain unaltered regardless of female previous experience. Offspring performance was higher on *V. faba* which was the preferred host indicating a preference–performance linkage for this leafminer (Vidella *et al*., 2010). Since *V. faba* is a soft tissue as compared to *B. vulgaris* it was preferred by *L. huidobrensis* for feeding and oviposition. Leafminers prefers to feed and deposit eggs on plants with high nitrogen content (Minkenberg and Fredrix, 1989; Minkenberg & Ottenheim, 1990). It was also reported that distribution, density and length of leaf trichome affect the host selection of leafminers, the mobility and its feeding activities; high trichome density acts as a physical deterrent to *L. trifolii* (Fagoonee and Toory, 1983 and KnodelMontz *et al*., 1985).

2.3.5 Integrated pest management of *L. huidobrensis*

This is a sustainable approach to managing crop pests which combines biological, cultural, physical and chemical tactics in away that minimises economic, health and environmental risks. Leafminers, mainly *Liriomyza* species cause havoc world wide in a variety of cropping systems (Shepard *et al.*, 1998). IPM practices are not only about preventing environmental and health problems but also about sustainable agriculture and improving farmer income through reduced production costs. There is a wide range of IPM component technologies that have been used in Guatemala for the control of *L. huidobrensis*. *Diglyphus isaea* in compatibility with abamectin has been used in the control of Leafminers in green houses (Kaspi and Parrella, 2005). There is also the use of certified seeds, adequate fertilizer application, using wheat straw mulch and weekly scouting of pest levels. Threshold based spraying of chemicals and the use of mobile yellow sticky traps was also used when infestation was high and adults were many. The above have reduced pesticide application for the typical Guatemalan snow pea farmer from 13 to 4 in each cropping cycle (Dix, 1995).

IPM management can also be done through general sanitation, exclusion, cultural practices, resistant cultivars and entomopathogenic nematodes such as *Steinernema feltiae* that has potential to control the leafminers (Head *et al.*, 2000). The potential of microbial control agents, especially the entomopathogenic fungi *Isaria fumosorosae, Metarhizium anisoplae* and *Beauveria bassiana* have been used in the laboratory and field trials to control *Liriomyza* species with success (Migiro *et al.*, 2010). The use of faba beans as a trap crop for *L. huidobrensis* has been used because the LMF prefers it to

snow pea. Neem has also been used due to its effectiveness in disrupting the life cycle of the LMF as it lowers the fecundity and longevity of the adults (Azam *et al.,* 2003). It also increases mortality of the larvae stages.

2.4 Bioactivity of botanical pesticides

Chemicals from plant sources, generally called phytochemicals, play an important role in the management of crop pests because plants contain a wide spectrum of secondary metabolites such as azadirachtin, pyrethroids and cocaine that may be exploited for different biological properties. The popularity of botanical pesticides in the world is increasing and some plant products are being used globally as green pesticides. A study on the bioactivity of plant derivatives to different crop pests continues to expand, yet only a handful of botanicals are currently used in agriculture. Recently there has been an attempt to replace the synthetic insecticides with less expensive, locally available, ecologically safe and socio-friendly options including botanicals (Talukder, 2006, Isman, 2006). In Kenya, pyrethroids and neem products are well established commercially as botanical pesticides.

2.5 Mode of action of Pyrethrum

Pyrethrin which is the active ingredient in Pyrethrum exerts its toxic effect by disrupting the sodium potassium ion exchange process in the insect's nerve fibres; this interrupts the normal transmission of nerve impulses. Pyrethrin is a contact pesticide that rapidly penetrates the nervous system and causes an immediate knock down paralysis in insects (Elliots and Janes 1973, Obrochta *et al.*, 1996). On the contrary, Pyrethrum pesticide has

some limiting factors such as metabolic degradation that occurs once the pesticide is inside the insect system. The pesticide is acted upon by enzymes and degradation occurs rapidly. This renders the insecticide inactive and the insect is able to recover after a short time. There is also rapid degradation of Pyrethrum from 1- 100% within five hours in the presence light and high temperatures (Gunasekara, 2004). Pyrethrum degrades rapidly when exposed to natural sunlight and does not persist in the environment beyond a few weeks (Todd *et al.*, 2003). Vapor phase compounds of pyrethrin are however more susceptible to rapid degradation through photolysis compared to particulate phase of Pyrethrin which degrades at a slower rate (Atkinson *et al.*, 2004). Some synergists with low mammalian toxicity are combined with Pyrethrum to inhibit certain detoxification enzymes in the insect system, but they are very expensive. Piperonyl butoxide (PBO) is a synergist found in Pyrethrum. Glynne (1999) noted that the synergistic effect of PBO was often greatest against resistant insects than against susceptible members of the same species. Raffa and Priester (1985) noted the potential role of synergists in managing insect resistance and stated that 'synergists are among the most straightforward tools for overcoming metabolic resistance because they can directly inhibit the resistance mechanism itself'. They also demonstrated that when mortality rate is plotted against dose, the effect of a synergist against a resistant insect increases the gradient of the regression line. According to the study by McGarry and Trees (1991), 70% reduction of *D. gallinae* mite was observed on pigeon treated with Pyrethrum. Field trials however led to the conclusion that ineffectiveness of PBO was due to its chemical instability in field conditions when it is broken down by sunlight (Herbach *et al.*, 2006). Over the last few decades, chemists have been able to improve on Mother Nature's pyrethrin insecticides in

better, odorless and more stable pesticide formulations. These products such as Cypermethrin, Permethrin and Deltamethrin work the same way as Pyrethrin (Thomas *et al.*, 1991, Scott *et al.*, 1990). The products above are known as pyrethroids, they also have various percentages of the synergist Piperonyl Butoxide (PBO).

2.6 Mode of action of Azadirachtin

Neem is a systemic pesticide that is absorbed through the roots or leaves of the plant. The insect gets the active ingredient when it feeds on the parts of the plant. The major study of neem materials has been its recognition as a source of valuable plant allelochemicals, specifically for the insecticidal insect repellent antifeedant (Mordue and Blackwell, 1993) which have attracted worldwide attention. Both primary and secondary antifeedant effects have been observed in the case of azadirachtin (Ascher, 1993). Primary effects include the process of chemoreception by the organism's sensory organs such as mouthparts which stimulate the organism to begin feeding. Secondary processes are effects such as gut motility disorders due to topical application only (Schmutterer, 1990; Ascher, 1993). Inhibition of feeding behavior by azadirachtin results from blockage of input receptors for phagostimulants or by the stimulation of deterrent receptor cells or both (Mordue and Blackwell, 1993). Neem can persist in the soil for a period of three weeks (Larew *et al.*, 1985) while it can persist for only 3 days when foliage application is used (Babul and Poehling, 2005). The key active ingredient is azadirachtin, a tetranotriterpenoid that exhibits classical insect growth regulatory (IGR) effects on the immature stages of insects for which the molecular mechanisms of action (Mordue and Blackwell, 1993) are still not well documented.

Schmutterer, 1990 reported that azadirachtin modifies the programs of insects by influencing hormonal systems, especially that of ecdysone. The effects of Azadirachtin are both dose and time dependent, prevent both ecdysis and apolysis and can cause death before or during molting or possibly inducing "permanent larvae" (Mordue and Blackwell 1993). The main action of azadirachtin appears to be at the release sites of Prothoracicotropic hormone (PTTH) (Isman *et al.,* 1990) from the corpora cardiaca. This affects the synthesis of PTTH by the brain neurosecretory cells (Mordue and Blackwell, 1993). However, neem has a major drawback in its ability to biodegrade fast, though simultaneously an attractive advantage in domestic areas, its shorter persistence lowers the efficacy in field applications (Johnson *et al.,* 2003).This is why in this study, the combined effect of both the biopesticides and the parasitoid is being explored.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study site

Greenhouse experiment were conducted at the International Center of Insect Physiology and Ecology (ICIPE), Nairobi (36°- 53 ° E, 1°- 13 ° S) with an altitude of 1617 m above the sea level (masl) Field experiments were conducted at Sagana (00 °S 037 °E) and Kabaru (00 °S 037 °E) in Nyeri county of Kenya at an altitude of 1833 masl and 2187 masl respectively.

3.2 Growing of experimental plants

French beans (*Phaseolus vulgaris*) of Pauliesta variety was used in all experiments. The plants were grown in a Leafminer-free screen house at ICIPE in Nairobi, Kenya. Four French bean seeds were planted directly in plastic pots (7 cm height × 7 cm diameter) filled with red soil mixed with farm yard manure at the ratio of 7:1. The pots were maintained in the greenhouse at 27 ± 2 °C and approximately 30 ± 10 % relative humidity (RH). Two Weeks after germination the plants were thinned to two plants per pot with four leaves per plant. The average leaf area per plant was 14.75 cm^2 prior to the setting of the experiments. The test plants were planted twice per week to ensure availability of plants throughout the experiment.

3.3 Mass rearing of *L. huidobrensis*

Liriomyza huidobrensis used in all experiments were obtained from stock colonies maintained at ICIPE for about 30 generations. The culture of *L. huidobrensis* was initiated from adult Leafminers that occurred naturally on vegetables in the proximity of the ICIPE campus. Adults were identified using Polymerase Chain Reaction-restriction fragment length polymorphism (PCR-RFLP) adapted from Scheffer and Lewis (2001), Kox *et al.* (2005), and Musundire *et al.* (2010).

3.4 Mass rearing of parasitoids

The parasitoid *P. scabriventris* was collected from a stock colony maintained at ICIPE quarantine facilities for about 12 generations. The parasitoid was maintained on faba beans infested with 2-3rd instar larvae *L. huidobrensis*.

3.5 Chemical sources

Two neem-based insecticides (Achook^{TM} and Neemroc^{TM}) and one Pyrethrum (PYAGRO^{TM} 4EC) were used for all experiments. Achook and Neemroc are extracted from seeds and seed kernels of the tree *Azadirachta indica* while Pyrethrum is extracted from flowers of the plant *Pyrethrum cinerariae folium*. They are among the most popular botanicals used by small scale farmers in Kenya for the control of horticultural pests. Azadirachtin concentration was 0.15 % w/w in Achook and 0.03 % w/w in Neemroc. The two Azadirachtin-based insecticides have low mammalian toxicity and can be applied as foliar spray or soil drench (Dhaliwal and Ramesh, 2000). The recommended dose for Achook was 0.5ml in 100ml of water and 0.5 ml in 100ml of water for Pyrethrum and Neemroc. Achook and Neemroc were bought at Kasarani in Nairobi in a neem product

shop. PYAGRO™ 4EC was obtained from the Pyrethrum Board of Kenya in Nakuru, Kenya. PYAGRO™ 4EC is the trade name for Pyrethrum and consists of Pyrethrum plant waxes, a synergist called Piperonyl Butoxide and emulsifiers. The active ingredient is natural pyrethrin 4 % w/w. It's applied as foliar spray early in the morning or late in the evening when the effect of UV radiation is low.

3.6 Preparation of formulations and application of the biopesticides

Different concentrations of neem-based pesticides were each mixed with 100ml of water. Concentrations of 0.5ml, 0.25ml, 0.1ml & 0.05 ml of Achook™ and 1ml, 0.75ml, 0.5ml & 0.25ml of Neemroc™ . Similarly, 0.75ml, 0.5ml, 0.25ml and 0.125ml concentrations of Pyrethrum were mixed with 100ml of water. A blank formulation containing distilled water was used as a control. All concentrations were prepared just before the setting of the experiment and stirred for about 5 minutes prior to use.

3.7 Assessing the effects of various spray regimes of Achook, Neemroc and Pyrethrum biopesticide on feeding, oviposition and egg to adult survival of *L. huidobrensis.*

French bean plants were sprayed with different concentrations of Achook, Neemroc and Pyrethrum as explained in 3.6. This experiment was conducted separately for each type of chemical. One sprayed potted plant was selected from each pesticide concentration and put in the oviposition cages (40 cm length × 40 cm width × 30 cm height). The Pots, each with two plants that had two leaves each were arranged in a three factor randomized block design. The five treatments were replicated four times and randomly put in five cages each with four pots. The whole procedure was repeated separately for potted plants

sprayed zero, one, and two days prior to the infestation of the plants by adult *L. huidobrensis*. At day Zero of the infestation (plants were sprayed and immediately infested by adult Leafminers), 36 couples of one-two day-old *L. huidobrensis* flies were released in each cage and allowed to mate and oviposit for a period of 24 hrs. The potted plants were then removed from the oviposition cages, and flies that were still on the plants were removed using an aspirator. The same procedure was repeated for plants sprayed and infested one and two days later.

Upon adult removal from the plants, the numbers of punctures per potted plant were estimated per leaf area (cm^2). This was done by randomly sampling four regions on every four leaves per replicate using a square constructed on the bottom of a petri-dish. In addition, one leaf was cut at random from each three replicates of the five treatments. The leaves were used to assess the egg load using the lacto-phenol acid fuchsin egg-staining technique, as described by Migiro *et al.*, 2010. The infested leaves were stained with a mixture solution of 135 ml phenol, 135 ml lactic acid, 135 ml distilled water, 270 ml glycerin and 0.675 gm fuchsin acid. The mixture solution was stirred for about 10 minutes and then boiled on a hot plate in the fume chamber. Infested leaves were dipped in the boiling mixture one at a time for 3-5 minutes. The leaf was then steeped in the solution for about 3 hours and washed in warm water. Thereafter, the leaves were observed under a microscope for egg load count. The development of mines and larval mortality were monitored on a daily basis on plants incubated in the holding cages. The larvae that successfully pupated were kept in ventilated vials (6.5 cm length × 2.7 cm

diameter), where they remained till adult emergence. Emerged adults were counted and recorded.

3.8 Establishing the effects of Achook, Neemroc and Pyrethrum on survival of larval stages and emergence of adults from pupal stage of *L. huidobrensis*

Different larvae stages of *L. huidobrensis* were obtained by exposing the potted plants to 36 couples of adult flies per cage for oviposition for 6 hrs. New plants were exposed to flies every day for about 7 days continuously. Three, five and seven days after infestation at 25 °C and 60-75% RH, the plants carrying the 1st 2nd and 3rd instar larvae were respectively selected and the number of larvae per replicate counted. Thereafter the plants were sprayed separately with concentrations of Achook, Neemroc and pyretrhrum as described in 3.6. The leaves were cut and incubated for pupation. Pupae was counted and incubated in ventilated vials for emergence of adults. Emerged adult flies were also counted.

3.9 Determining the effects of Achook, Neemroc and Pyrethrum on the mortality of the late 3rd instar larvae and emergence of adults from pupal stage of *L. huidobrensis*

French bean plants were infested with 72 one-two adults of *L. huidobrensis* for 6 hours. The plants were then transferred into the holding cages for mines development. When larvae were about to pupate, leaves with late third instar larvae were cut and kept on trays measuring (70 cm length × 40 cm width × 6cm height) where the late 3rd instar larvae dropped on paper towels. Petridishes filled with soil were drenched with different concentrations of the Achook, Neemroc and Pyrethrum. Zero, one, and two days after spraying, 20 late third instar larvae were dropped in each concentration as described in

3.6 Each treatment was replicated ten times. The experiment was repeated twice. Dead larvae were recorded and emerging adults were counted.

3.10 Determining the effects of Achook, Neemroc and Pyrethrum on emergence of adults from parasitized and nonparasitized pupae of *L. huidobrensis*

Two experiments were carried out by spraying the 3rd instar larvae on leaves and the late 3rd instar larvae in the soil.

3.10.1 Effects of foliar spray of different concentrations of Achook, Neemroc and Pyrethrum on emergence of adults from parasitized and nonparasitized pupae of *L. huidobrensis*.

Potted plants infested with 2nd and 3rd instar larvae of *L. huidobrensis* were obtained as described in 3.8.1. Prior to the experiment, the number of larvae per plant was counted. 36 couples of *P. scabriventris* aged two days were released on the infested plants for 6 hrs to ensure that parasitization took place. The larvae exposed to parasitoids were then sprayed with different concentrations of selected pesticides as described in 3.6 except for concentrations 0.5, 1 and 0.75 of Achook, Neemroc and Pyrethrum respectively. Four replications were performed for each pesticide and treatment level. Thereafter, infested plants were transferred into the holding cages and incubated till pupation. Pupae were collected, counted and incubated. At emergence, the numbers of leafminers and *P. scabriventris* that survived were counted. Emergence of the leafminer indicated non parasitized larvae while that of the parasitoid indicated parasitization.

3.10.2. Effects of soil spray of different concentrations of Achook, Neemroc and Pyrethrum on emergence of adults from parasitized and nonparasitized pupae of *L. huidobrensis*.

Plants were exposed daily to *Liriomyza huidobrensis* as described in 3.8.1. At the 2nd and 3rd instar larval stages, 36 couples of *P. scabriventris* were released on the plants to ovipost for 6 hours. Thereafter, the infested leaves were cut and put on a tray where the late 3rd instar larvae dropped. Twenty late third instar larvae were transferred to petridishes filled with soil drenched with different concentrations of Achook, Neemroc and Pyrethrum as described in 3.6. Each treatment was replicated ten times and the experiment repeated twice. At emergence, the number of adult leafminers and parasitoids emerged was recorded.

3.11 Effects of Neemroc and Pyrethrum on infestation and abundance of adult *L. huidobrensis* and *P. scabriventris* in relation to yield in the field

Two experimental trials were carried out at Kabaru and Sagana using Pyrethrum (PYAGRO™) and Neemroc™, respectively. Each trial involved application of one chemical with six different treatments: no insecticide (Co); one application at 2 Weeks after germination (A1); two applications at 1 and 3 WAG (A2); three applications at 1, 3 and 5 WAG (A3); four applications at 1, 3, 5 and 7 WAG (A4) and farmers practice (FP), where Dimethoate and Bulldock star were alternated on a weekly basis. Pyrethrum (PYAGRO™) was applied at a rate of 0.5 ml ml/100ml with a concentration of 400 litres/ha and Neemroc at a rate of 0.5ml /100ml of water with a concentration of 1000 litres/ha. Copper oxchloride was applied to control blight, Bulldock™ to control bean fly and Diazinon™ for controlling cutworms. The treatments were arranged in a completely

randomized block design with four replications. Plots were 2m × 3m each. The distance between plots within a block was 1m and a polythene paper was used as a screen during spraying to reduce interactions between treatments. In all trials, beans were planted at 50cm × 10cm distance. Each plot received 0.3 kg of fertilizer (DAP) at the rate of 250 kg/ha applied during planting and the same rate for CAN three Weeks after germination. Plots were weeded two-three times at 2, 4 and 6 Weeks after germination. Harvesting of the french bean produce started between 5 – 6 WAG. Field experiments were carried out in two seasons. The first season tested the effect of the botanicals on the pest while the second season was investigating the effect of the botanicals on both the pest and the parasitoid. During the second season, there were 6 treatments including the five used in season 1 in addition to farmers practice (FP) treatment.

3.12 Sampling of yield and leaves infested by *L. huidobrensis* and parasitized by *P. scabriventris*

The population of the leafminers and the levels of parasitism were assessed by sampling of leaves. Five to ten middle leaves were randomly selected for further investigations in the laboratory. The leaves were kept for 4 weeks to allow for the emergence of adult flies and parasitoids. The total number of infested plants was counted and the level of parasitism was estimated as the number of parasitoids divided by total number of flies and parasitoids that emerged. At harvest, crop damage was estimated on each plot from 10 predetermined plants (5 consecutive plants per row) left without being sampled. Level of plant damage was scored using the scales developed by Rahman *et al.*, (1994). 0 = no leaf damage; 1= leaf damage restricted to basal half of plant (1-25 % damage); 2 = leaf

damage restricted to middle and basal half of plant (26-50 % damage); 3= entire plant damaged except terminal leaves (51-75 % damage); 4 = most of the plant damaged (76-100 % damage). Thereafter bean pods were harvested, and yield assessed per plot by measuring the produce per treatment.

3.13 Data analysis

Difference in the mean total puncture per cm^2, eggs laid per cm^2, larval survival, egg-adult survival and pupal emergence, were analyzed by analysis of variance (ANOVA) using the general linear model (GLM) procedure of SAS. Regression was also further carried out between the pesticide concentration and the eggs/cm^2, egg adult survival, mortality and emergence of the LMF. ANOVA was used to test the significance of mean differences and least square mean values were computed. The significance level was set at $P = 0.05$. The means were separated using the Student-Newman-Keuls (SNK). Damage of leaves, mortality variables and variation in LMF and parasitoids abundance in the field were analyzed by analysis of variance (ANOVA) using the GLM procedure of SAS SAS, 2008. Thereafter means were separated using the Student-Newman-Keuls (SNK). Larval mortality in the field was analyzed by ANOVA, least square means were computed and a t- test was used to differentiate the means.

CHAPTER FOUR

RESULTS

4.1 Effects of various spray regimes of Achook, Neemroc and Pyrethrum biopesticide on feeding, oviposition and egg to adult survival of *L. huidobrensis*

The results for experiments carried out on effect of concentration of the three biopesticides and days before infestation (DBI) on feeding, oviposition and egg to adult survival were combiled under concentration effects and DBI effects.

4.1.1 Effects of different concentations of Achook, Neemroc and Pyrethrum on the feeding, oviposition and survival of *L. huidobrensis*

Feeding

Achook

There were significant differences among the mean number of feeding punctures on leaves treated with different concentrations of Achook. The highest mean number of punctures was recorded in the 0 concentration (control) and this was significantly higher than the mean number recorded in 0.1, 0.25 and 0.5 concentrations. The number of punctures in 0.05 and 0.1 concentrations did not vary significantly but were significantly higher than those in 0.25 and 0.5 concentrations which showed no significant difference. Regression analyses showed a significant negative relationship between the number of punctures and the concentration of Achook applied (Table 4.1).

Neemroc

The mean number of feeding punctures on leaves treated with different concentrations of neemroc had significant differences. The highest number of feeding punctures was recorded in 0.25 concentrations and this was significantly higher than the one in the

control, 0.5, 0.75 and 1 concentration. The mean number of feeding punctures in 0.5 and 0.75 concentrations did not vary significantly but they were significantly lower than those in 0 and 1 concentration which did not show any significant differences. Regression analyses showed a nonsignificant negative relationship between the number of punctures and the concentration of neemroc applied (Table 4.1).

Pyrethrum

The mean number of feeding punctures on leaves treated with different concentrations of pyrethrum had significant differences. The mean number of punctures recorded in leaves treated with 0.5 and 0.75 concentrations had no significant differences but they were significantly lower than those recorded in 0, 0.125 and 0.25 concentrations which did not show any significant difference. Regression analyses showed a significant negative relationship between the number of punctures and the concentration of Pyrethrum applied (Table 4.1).

Oviposition: The mean number of eggs after treatment with different concentrations of Achook, Neemroc and Pyrethrum showed no significant diffences (Table 4.1).

Survival of *L. huidobrensis*

Achook

There were significant differences in the survival of *L. huidobrensis* from egg to adults when leaves were treated with different concentrations of Achook. The highest

percentage survival was recorded in 0 concentration (control) followed by 0.05 and 0.1 which showed no significant differences but were significantly higher than survival in 0.25 concentration. The later recorded a significantly higher survival than 0.5 concentration (Table 4.1).

Neemroc

Percentage survival of *L. huidobrensis* recorded significant differences after treatment with different concentrations of Neemroc. The control (0) recorded a significantly higher % survival followed by 0.25 and 0.5 that did not have any significant differences but was significantly higher than 0.75 and 1 concentratons. However, survival in 0.75 and 1 concentration did not have any significant differences. Regression analyses showed a significant negative relationship between Neemroc concentrations and egg to adult survival (Table 4.1).

Pyrethrum

Percentage survival of *L. huidobrensis* recorded significant differences after treatment with different concentration of Pyrethrum. The highest % survival was recorded in 0 concentrations (control) and this was significantly higher than survival in 0.25, 0.5 and 0.75 concentration that did not vary significantly. The percentage survival in 0.125 did not vary significantly with that in 0 and 0.25 concentration but it was significantly higher than the one in 0.5 and 0.75. Regression analysis showed a significant negative relationship between Pyrethrum concentrations and egg to adult survival (Table 4.1)

Table 4.1 The mean number of punctures (±SE), egg/cm^2 (±SE) and % survival (±SE) of *L. huidobrensis* on plants treated with different concentrations of Achook, Neemroc and Pyrethrum

Botanical	Concentration v/v ml/100ml	Feeding Puncture/cm^2	oviposition eggs/cm^2	Egg-adult Survival (%)
Achook	0	78.7 ± 0.03a	1.4 ± 0.26	44.0 ± 2.21a
	0.05	73.4 ± 0.03ab	1.6 ± 0.27	26.3 ± 2.21b
	0.1	67.7 ± 0.04b	1.3 ± 0.30	19.2 ± 2.21b
	0.25	52.4 ± 0.04c	1.2 ± 0.31	9.6 ± 2.21c
	0.5	47.0 ± 0.04c	0.9 ± 0.34	0.6 ± 2.21d
P-val		<0.0001	0.7905	<0.0001
Y=α+βx		-63.9+75.34x	-	-
P-val(reg)		0.01	-	-
Neemroc	0	55.1 ± 0.04b	1.7 ± 0.25	31.1 ± 1.42a
	0.25	61.2 ± 0.04a	1.3 ± 0.29	24.2 ± 1.42b
	0.5	49.2 ± 0.04c	1.3 ± 0.29	20.1 ± 1.42b
	0.75	49.5 ± 0.04c	1.0 ± 0.34	12.7 ± 1.42c
	1	53.7 ± 0.04b	0.8 ± 0.37	9.7 ± 1.42c
P-val		<0.0001	0.4028	<0.0001
Y=α+βx		-5.8+56.64x	-	-21.64+30.36x
P-val(reg)		0.42	-	0.0007
Pyrethrum	0	48.7 ± 0.04a	1.7 ± 0.25	32.9 ± 2.33a
	0.125	47.5 ± 0.04a	1.6 ± 0.26	26.2 ± 2.33ab
	0.25	47.2 ± 0.04a	1.5 ± 0.27	19.9 ± 2.33bc
	0.5	19.4 ± 0.06b	1.2 ± 0.30	16.8 ± 2.33c
	0.75	21.2 ± 0.06b	1.0 ± 0.32	13.4 ± 2.33c
P-val		<0.0001	0.6635	<0.0001
Y=α+βx		-45.41+51.55x	-	-24.25+29.72x
P-val(reg)		0.03	-	0.01

Means within a column per row followed by the same letter are not significantly different ($P = 0.05$). (0) control- distilled water

4.1.2 Effect of days before infestation (DBI) on feeding, oviposition and survival of *L. huidobrensis* on plants sprayed with Achook, Neemroc and Pyrethrum

Feeding

Achook

There were significant differences in the mean number of punctures on leaves treated with Achook on the different days before infestation (DBI). Leaves infested one day after

spraying had significantly lower punctures compared to the leaves which were sprayed and immediately infested (0 DBI). However, the mean number of punctures in leaves infested immediately after spraying and those infested two days after spraying had no significant differences. Similarly, leaves infested one and two days after spraying did not differ significantly (Table 4.2).

Neemroc

There were significant differences in the mean number of punctures on leaves treated with Neemroc on the different days before infestation (DBI). The mean number of punctures was significantly higher on leaves infested two days after spraying followed by punctures in leaves infested one day after spraying. This was significantly higher than punctures in leaves sprayed and immediately infested (0 DBI) (Table 4.2).

Pyrethrum

There were significant differences in the mean number of feeding punctures on leaves treated with Pyrethrum on different days before infestation (DBI). Leaves sprayed with Pyrethrum and immediately infested (0 DBI) had significantly more punctures compared to leaves sprayed one and two days before infestation. Punctures in leaves infested and sprayed one and two days later had no significant differences (Table 4.2).

Oviposition

Achook and Neemroc

There was no significance differences in the mean number of eggs in leaves treated with Achook and Neemroc on different days before infestation (Table 4.2).

Pyrethrum

There were significant differences in the mean number of eggs in leaves treated with pyrethrum on different number of days before infestation.The mean number of eggs on leaves infested two days after spraying (2 DBI) was significantly higher than those on leaves sprayed and immediately infested (0 DBI) but this did not significantly differ with the mean number of eggs on leaves infested one day after spraying (1 DBI). Similarly, eggs on leaves infested immediately after spraying and those on leaves infested one day after spraying did not differ significantly (Table 4.2).

Survival of *L. huidobrensis*

There were significant differences in the % survival from egg to adult on leaves treated with Achook, Neemroc and Pyrethrum on the different days before infestation (DBI). Egg to adult (%) survival was significantly lower on leaves infested immediately after spraying (0,DBI) as compared to that in leaves infested one and two days (1 & 2 DBI) after spraying with the three biopesticides. Percentage survival in leaves infested one and two days after spraying with Achook, Neemroc and Pyrethrum had no signicant differences (Table 4.2).

Table 4.2 The mean number of punctures (±SE), egg/cm^2 (±SE) and % survival(±SE) of *L. huidobrensis* on plants treated with Achook, Neemroc and Pyrethrum on 0, 1 and 2 days before infestation

Botanical	DBI	Feeding Puncture/cm^2	oviposition Eggs/cm^2	Egg-adult Survival (%)
Achook	0	67.0 ± 0.03a	1.3 ± 0.22	10.1 ± 1.72b
	1	58.3 ± 0.03b	1.0 ± 0.26	22.8 ± 1.72a
	2	62.9 ± 0.03ab	1.5 ± 0.20	26.9 ± 1.72a
P-value		0.002	0.3628	<0.0001
Neemroc	0	45.9 ± 0.03c	1.2 ± 0.23	12.6 ± 1.10b
	1	52.4 ± 0.03b	1.3 ± 0.22	21.4 ± 1.10a
	2	63.9 ± 0.03a	1.1 ± 0.25	24.7 ± 1.10a
P-value		0.0003	0.8149	<0.0001
Pyrethrum	0	39.5 ± 0.03a	1.0 ± 0.26b	13.4 ± 1.80b
	1	32.5 ± 0.04b	1.3 ± 0.22ab	23.7 ± 1.80a
	2	30.4 ± 0.04b	2.0 ± 0.18a	28.4 ± 1.80a
P-value		<0.0001	0.0477	<0.0001

Means within a column per row followed by the same letter are not significantly different (P =0.05). (0) control- distilled water.

4.2 Effects of Achook, Neemroc and Pyrethrum on survival of larval stages and emergence of adults from pupal stage of *L. huidobrensis*.

The results for the experiment carried out on foliage spray of Achook, neemroc and pyrethrum on 1st, 2nd and 3rd larvar instars was combiled under % surving larvae and % emergence of adults from pupae of the larvae instars. Experiments on soil spray of the three biopesticides on 1st, 2nd and 3rd instars were combiled under % larvae mortality and % adult emergence.

4.2.1 Effects of foliar spray of different concentrations of Achook, Neemroc and Pyrethrum on survival on the 1st, 2nd and 3rd larval stages of *L. huidobrensis*

Achook

There were significant differences in the % survival of the 1st, 2nd and 3rd larval instars treated with different concentrations of Achook. The % survival of the 1st instar larvae in

the control recorded a significantly higher % survival followed by 0.05 concentration. This % survival was significantly lower than the one in 0.1, 0.25 and 0.5 concentrations which did not differ significantly. Percentage survival of the 2nd instar larvae was significantly higher in control and this did not differ with survival in 0.05 concentration. Survival in 0.1, 0.25 and 0.5 concentrations had no significant differences but was significantly lower than the one in 0 and 0.05 concentrations. Contrary to the 1st and 2nd instars, there was no significant difference in the survival of the 3rd instar larvae among the concentrations applied. Percentage survival in the 1st instars was significantly lower than the one in the 3rd instars but it was not significantly different from the one in the 2nd instars. Similarly % survival in the 2nd instars was not significantly different from the the one in 3rd instars (Table 4.3).

Neemroc

There were significant differences in the % survival of the 1st, 2nd and 3rd larval instars teated with different concentrations of neemroc. Percentage survival was significantly lower in 1st instar larvae treated with 1 concentration. However survival in 0.25, 0.5 and 0.75 concentrations did not differ significantly as well as survival in the control, 0.25 and 0.5 concentrations. Percentage survival in the 2nd and 3rd instar larval stages was significantly higher in the control as compared to that in 0.25-1 concentrations which did not vary significantly. There were no significant differences in the survival of the 1st, 2nd and 3rd instars when they were treated with neemroc (Table 4.3).

Pyrethrum

There were significant differences in the % survival of the 1st, 2nd and 3rd larval instars treated with different concentrations of Pyrethrum. Survival of the1st instar larvae was significantly higher in the control (0) followed by survival in 0.125 and 0.25 which differed significantly with survival in 0.5 and 0.75 concentrations. However 0.5 recorded a significantly lower survival although it did not differ with the one in 0.75. Percentage survival of the 2nd and 3rd larval instars was significantly higher in the control and significantly lower in 0.75 concentration. There was no significant difference in the survival of both the 2nd and 3rd larval instars treated with 0.125, 0.25 and 0.5 concentrations but this survival was significantly lower than the one in the control and higher than the one in 0.75. The 1st instars had a significantly lower % survival as compared to the 2nd and 3rd instars which did not vary significanty (Table 4.3).

Table 4.3 The mean % survival (±SE) of 1st , 2nd and 3rd larval stages of *L. huidobrensis* on leaves treated with different concentrations of Achook, Neemroc and Pyrethrum

Botanical	Concentration ml/100ml	% survival of 1st instar larvae	% survival of 2nd instar larvae	% survival 3rd Instar larvae
Achook	0	81.8 ± 4.1a	92.0 ± 6.6a	88.9 ± 4.8
	0.05	48.3 ± 5.1b	81.3 ± 4.8a	85.8 ± 5.3
	0.1	22.7 ± 4.2c (B)	44.6± 6.4b (AB)	84.4 ± 4.9 (A)
	0.25	17.4 ± 8.8c	37.4 ± 3.9b	81.2 ± 6.3
	0.5	20.5 ± 4.9c	36.7 ± 9.9b	80.2 ± 5.6
P-value		<0.0001	<0.0001	0.7860
Neemroc	0	90.0 ± 1.7a	91.5 ± 2.3a	93.3 ± 1.6a
	0.25	67.3 ± 11.9ab	65.4 ± 9.5b	68.3 ± 1.4b
	0.5	63.7 ± 4.2ab (A)	66.5 ± 4.4b (A)	66.2 ± 10.9b (A)
	0.75	60.4 ± 9.4b	64.4 ± 3.4b	63.2 ± 8.0b
	1	38.9 ± 2.6c	56.4 ± 3.6b	54.1 ± 2.8b
P-value		0.0031	0.0035	0.0065
Pyrethrum	0	82.1 ± 1.4a	89.2 ± 1.4a	85.1 ± 4.8a
	0.125	34.6 ± 4.5b	53.4 ± 4.5b	56.4 ± 2.4b
	0.25	31.9 ± 5.5b (B)	54.4 ± 6.3b (A)	60.6± 5.1b (A)
	0.5	27.3 ± 3.7bc	53.1 ± 4.5b	58.8 ± 4.8b
	0.75	16.3 ± 2.8c	21.4± 3.7c	38.9 ± 3.8c
P-value		<0.0001	<0.0001	<0.0001

Means (±) followed by the same letter are not significantly different: lower case letters within the column per row and upper case letters within a raw per column ($P = 0.05$). (0) control- distilled water

4.2.2 Effect of foliar spray of different concentrations of Achook, Neemroc and Pyrethrum on emergence of adult *L. huidobrensis* from pupae of the 1st, 2nd and 3rd larval stages

Achook

There were significant differences in the adult emergence of LMF from pupae of all the three larval instars that had been treated with different concentrations of Achook. Adult emergence in pupae that formed from the 1st and 2nd instars was significantly higher in the control as compared to emergence in 0.05-0.5 concentrations which did not vary significantly. The 3rd instar larvae recorded a significantly higher % emergence in the control followed by emergence in 0.05, 0.1 and 0.25 concentrations which did not differ

significantly but was lower than the one in 0.5. Emergence among all the three instars did not vary significantly after treatment with Achook (Table 4.4).

Neemroc

There were significant differences in the adult emergence of LMF from pupae of all the three larval instars that had been treated with different concentrations of Neemroc. Adult emergence from pupae of the 1st instar was significantly higher and lower in the control (0) and in 1 concentration respectively. Percentage emergence in 0.75 concentrations was significantly lower than emergence in 0.25 and 0.5 which did not vary significantly. The 2nd and 3rd larvae instars had similar trends with the control (0) recording significantly higher % emergence followed by the one in 0.25 and 0.5 that did not differ significantly. However, this emergence was significantly higher than the one in 0.75 and 1 concentrations which were not significantly different. Regression analyses indicated a significant negative linear relationship between the concentrations used and the emergence of adults from pupae of the 1st, 2nd and 3rd larvae instars. Emergence among all the three instars did not vary significantly after treatment with Neemroc (Table 4.4).

Pyrethrum

There were significant differences in the adult emergence of LMF from pupae of the all the three larval instars that had been treated with different concentrations of Pyrethrum. Adult emergence in pupae that formed from the 1st instar was significantly higher in the control (0) followed by emergence in 0.125 and 0.25 concentrations which did not varry significantly. This was significantly higher than emergence in 0.5 and 0.75 concentrations that had no significant differences. Emergence of adults in pupae surviving from 2nd

instar larval stage was significantly higher and lower in the control and 0.75 respectively. Adult emergence in 0.125, 0.25 and 0.5 concentrations did not vary significantly but was significantly lower than the control and higher than the one in 0.75. Emergence of adults in pupae surviving from 3rd instar larval stage was significantly higher in the control as 0.125 - 0.75 concentrations showed no significant differences. Emergence among all the three instars did not vary significantly after treatment with Pyrethrum (Table 4.4).

Table 4.4 The mean % emergence (±SE) of adults from pupae of the 1st , 2nd and 3rd larval instars of *L. huidobrensis* on plants treated with different concentrations of Achook, Neemroc and Pyrethrum

Botanical	Concentration ml/100ml	% adult emergence 1st instar larvae	% adult emergence 2nd instar larvae	% adult emergence 3rd Instar larvae
Achook	0	73.3± 6.4a	79.9± 4.9a	90.9 ± 2.8a
	0.05	13.6 ± 3.4b	3.3± 1.9b	14.9 ± 3.5b
	0.1	0.0 ± 0.0b (A)	6.6± 1.6b (A)	8.1 ± 1.1b (A)
	0.25	0.0 ± 0.0b	7.0 ± 0.5b	11.6 ± 2.8b
	0.5	0.0 ± 0.0b	5.6 ± 2.2b	0.0 ± 0.0c
P-value		<0.0001	<0.0001	<0.0001
Neemroc	0	93.6 ± 3.9a	93.2 ± 2.6a	93.8 ± 0.8a
	0.25	63.7 ± 4.1b	52.7 ± 2.9b	59.8 ± 3.0b
	0.5	57.5 ± 2.5b (A)	44.6± 6.5b(A)	51.1 ± 6.0b (A)
	0.75	42.4 ± 5.5c	18.6 ± 3.8c	28.7 ± 5.6c
	1	26.7 ± 2.8d	15.5 ± 2.1c	22.6 ± 8.2c
P-value		<0.0001	<0.0001	<0.0001
Y=α+βx		-62.04+87.80x	-75+82.82x	-69.40+85.90x
P-val(reg)		0.003	0.01	0.006
Pyrethrum	0	92.3 ± 1.9a	93.0 ± 1.9a	86.8 ± 3.0a
	0.125	54.9 ± 1.7b	39.6 ± 1.5b	24.9 ± 1.2b
	0.25	45.3 ± 9.7b (A)	34.9± 7.7b (A)	21.9± 3.0b (A)
	0.5	27.9 ± 4.7c	31.0 ± 0.9b	20.0 ± 1.3b
	0.75	18.9 ± 2.1c	9.8± 6.1c	18.8 ± 3.6b
P-value		<0.0001	<0.0001	<0.0001

Means (±) followed by the same letter are not significantly different: lower case letters within the column per row and upper case letters within a raw per column ($P \leq 0.05$). (0) control- distilled water

4.3 Effect Achook, Neemroc and Pyrethrum on the mortality of the late 3rd instar larvae and emergence of adults from pupal stage of *L. huidobrensis*

Results on this experiment were combiled under % mortality of the late 3rd instar larvae of *L. huidobrensis* and % adult emergence from pupae of the late 3rd instar larvae after treatment with the three biopesticides.

4.3.1 Effect of soil spray of different concentrations of Achook, Neemroc and Pyrethrum on mortality of the late 3rd instar larvae of *L. huidobrensis*

Achook

There were significant differences in the % mortality of the late 3rd instar larvae treated with different concentrations of Achook and infested immediately, one or two days later. Mortality of larvae in soil treated and immediately infested (0 DBI) was significantly lower in the control (0) as compared to the one in 0.05 - 0.5 that did not differ significantly. Larvae mortality in soil treated and infested one day later (1 DBI) was significantly lower in the control (0) as compared to the one in 0.25 and 0.5 concentrations. However mortality in 0.05 – 0.5 concentrations did not vary significantly as well as mortality in 0, 0.05 and 0.1 concentrations. Mortality of larvae in the soil treated and infested 2 days later showed a significantly higher mortality in 0.5 as compared to the one in 0 - 0.25 which did not differ significantly. Regression analyses indicated a significant positive linear relationship between the concentration used and mortality of larvae in soil treated and infested one and two days later. Mortality of the late 3rd instar larvae was significantly higher in soil treated and infested immediately (0 DBI) as compared to the one in soil treated and infested one and two days later 1&2 DBI. The later two did not differ significantly (Table 4.5).

Neemroc

There were significant differences in the % mortality of the late 3rd instar larvae treated with different concentrations of Neemroc and infested immediately (0 DBI) and one day later (1 DBI). The % mortality of larvae in soil treated with 0.75 & 1 concentrations and immediately infested did not vary significantly but was significantly higher than mortality in the control, in 0.25 and 0.5 which did not vary significantly. Mortality of larvae in the soil treated and infested one day later (1 DBI) was significantly lower in the control as compared to mortality in 0.25-0.75 concentrations that did not differ significantly. However, % mortality in 0.25, 0.5 and 0.75 concentrations did not vary significantly but was significantly lower than the one in 1 concentration. There were no significant differences in mortality of the 3rd instar larvae in soil treated and infested 2 days later. Regression analyses showed a significant positive linear relationship between the concentration used and mortality of larvae in soil sprayed and infested immediately and one day later. Mortality of the late 3rd instar larvae was significantly higher in soil treated and infested immediately (0 DBI) and one day later (1 DBI) as compared to the one in soil treated and infested two days later (Table 4.5).

Pyrethrum

There were significant differences in the % mortality of the late 3rd instar larvae treated with different concentrations of Pyrethrum and infested immediately (0 DBI) and one day (1 DBI) later. Mortality of larvae in soil treated and immediately infested was significantly higher in the control followed by mortality in 0.125 concentration. This differed significantly with mortality in 0.25-0.75 concentrations which did not vary

significantly but was significantly higher than emergence in 0 and 0.125. Mortality of larvae in soil treated and infested one day later did not vary significantly in 0.5 and 0.75 concentration but this was significantly higher than mortality in the control. Mortality in the control, 0.125 and 0.25 did not vary significantly as well as the one in 0.125-0.75 concentrations. There was no significant difference in mortality of the 3rd instar larvae when soil was treated and infested 2 days later. Regression analyses showed a significant positive relationship between the concentration used and mortality of larvae in soil treated and infested one day later. Mortality of the late 3rd instar larvae was significantly higher in soil treated and immediately infested (0 DBI) as compared to the one 2 DBI. However, mortality in soil treated and infested one day later (I DBI) was not significantly different from 0 DBI as well as 2 DBI. (Table 4. 5)

Table 4.5 The mean % mortality (±SE) of the 3rd larval instar of *L. huidobrensis* treated with different concentrations of Achook, Neemroc and Pyrethrum on 0, 1 and 2 days before infestation

Botanical	concentration ml/100ml	% mortality on 0 DBI	% mortality on 1 DBI	% mortality on 2 DBI
Achook	0	25.0± 2.7b	19.0± 2.9b	24.0 ± 2.3b
	0.05	49.5 ± 2.5a	20.5± 1.7ab	22.0 ± 1.7b
	0.1	50.5 ± 1.2a (A)	22.0± 1.1ab (B)	25.5 ± 1.6b (B)
	0.25	48.5 ± 1.7a	26.5 ± 2.2a	28.0 ± 3.4b
	0.5	52.0 ± 3.7a	27.5 ± 2.1a	35.5 ± 2.4a
P-value		<0.0001	0.0079	0.0025
Y=α+βx		31.04+39.5x	17.23+19.99x	25.15+22.47x
P-val(reg)		0.3	0.02	0.006
Neemroc	0	20.5 ± 1.6b	19.0 ± 1.6c	14.0 ± 3.8
	0.25	21.0± 2.9b	25.0 ± 1.5b	11.0 ± 1.8
	0.5	22.0± 1.5b (A)	27.0± 1.7b (A)	12.5 ± 1.1 (B)
	0.75	37.5 ± 3.7a	32.0 ± 2.7ab	14.0 ± 2.1
	1	37.0 ± 2.0a	36.5 ± 2.5a	14.5± 1.6
P-value		<0.0001	<0.0001	0.6033
Y=α+βx		19.8+17.7x	16.8+19.5x	1.6+12.4x
P-val(reg)		0.04	0.0008	0.45
Pyrethrum	0	26.5 ± 2.2c	27.5 ± 1.7b	24.5 ± 1.9
	0.125	56.5 ± 2.5b	38.0 ± 3.2ab	25.0 ± 1.3
	0.25	66.5 ± 3.3a (A)	37.5± 2.5 ab(AB)	26.5± 2.0 (B)
	0.5	65.0 ± 3.2a	50.0 ± 3.4a	28.0± 2.4
	0.75	68.5 ± 2.2a	51.0± 6.0a	26.0 ± 2.5
P-value		<0.0001	<0.0001	0.7718
Y=α+βx		43.0+42.62x	30.55+30.87x	2.75+25.10x
P-val(reg)		0.15	0.01	0.27

Means (±) followed by the same letter are not significantly different: lower case letters within the column per row and upper case letters within a raw per column ($P = 0.05$). (0) control- distilled water, (DBI)

4.3.2 Effects of soil spray of different concentrations of Achook, Neemroc and Pyrethrum on emergence of adults from pupae of the late 3rd instar larvae of *L. huidobrensis*

Achook

There were significant differences in the % emergence of adults from pupae of the late 3rd instar larvae treated with different concentrations of Achook and infested immediately (0 DBI), one (1 DBI) and two days (2 DBI) later. Emergence in soil that was treated and

immediately infested was significantly higher in the control (0) followed by emergence in 0.05 and 0.25 which did not vary significantly. This was however; significantly higher than emergence from 0.1 and 0.5 concentrations. Emergence of adults from soil treated and infested one day later showed a significantly higher emergence in the control followed by the one in 0.05 and 0.1 concentrations. This did not vary significantly but was significantly higher than emergence in 0.25 and 0.5 concentrations. Emergence of adults in soil sprayed and infested two days later was higher in the control followed by emergence in 0.05 which was significantly higher than emergence in 0.1 and 0.25 concentrations. Concentrations of 0.1 and 0.25 did not vary significantly as 0.5 significantly recorded the lowest % emergence. Emergence of adults did not significantly vary among 0 DBI, 1 DBI and 2 DBI. Percentage emergence from the late 3rd instar larvae among 0 DBI, 1 DBI and 2 DBI was not significantly different (Table 4. 6).

Neemroc

There were significant differences in the % emergence of adults from pupae of the late 3rd instar treated with different concentrations of Neemroc and infested immediately (0 DBI), one (1 DBI) and two days (2 DBI) later. Emergence in soil sprayed and infested immediately and two days later had a significantly higher emergence in the control as compared to emergence in 0.25, 0.5, 0.75 and 1 concentrations which did not differ significantly. Emergence of adults in soil sprayed and infested 1 day later was significantly higher in the control followed by emrgence in 0.25, 0.5 and 0.75 respectively. However, emergence in 0.75 and 1 did not have significance differences.

Percentage emergence among 0 DBI, 1 DBI and 2 DBI was not significantly different (Table 4.6).

Pyrethrum

There were significant differences in the % emergence of adult *L. huidobrensis* when soil was treated with different concentrations of pyrethrum and infested immediately, 1 and 2 days later. Emergence of LMF in soil sprayed and infested immediately was significantly higher in the control followed by emergence in 0.125 and 0.25 that did not significantly vary. This however was significantly higher than emergence in 0.5 concentration. Emergence of adults in 0.75 was significantly lower as compared to the later concentrations. Adult emergence in soil sprayed and infested 1 day later significantly reduced with increase in concentration from the control to 0.5 concentration. However, 0.5 and 0.75 did not vary significantly. Soil sprayed and infested two days later recorded a significantly higher and lower emergence in the control (0) and 0.75 respectively. Emergence of adults in 0.125, 0.25 and 0.5 concentrations did not vary significantly but was significantly higher than emergence in 0.75. Percentage emergence among 0 DBI, 1 DBI and 2 DBI was not significantly different (Table 4. 6).

Table 4. 6 The mean % emergence (±SE) of adults from pupae of the late 3rd larvae instar of *L. huidobrensis* treated with different concentrations of Achook, Neemroc and Pyrethrum on 0, 1 and 2 days before infestation

Botanical	concentration ml/100ml	% emergence on 0 DBI	% emergency on 1 DBI	% emergency on 2 DBI
Achook	0	47.7± 3.6a	55.0± 1.4a	60.7 ± 2.0a
	0.05	20.7± 1.5b	19.6± 1.8b	31.8 ± 2.1b
	0.1	16.3 ± 1.7bc (A)	20.6± 1.4b (A)	24.8 ± 1.8c (A)
	0.25	19.3 ± 1.9b	10.8 ± 1.0c	26.0± 1.0c
	0.5	12.9 ± 1.4c	8.55 ± 1.5c	13.2 ± 1.2d
P-value		<0.0001	<0.0001	<0.0001
Y=α+βx		-43.57+31.22x	-64.95+34.61x	-67.48+43.44x
P-val(reg)		0.2	0.18	0.13
Neemroc	0	56.0 ± 2.0a	57.7± 2.3a	54.5 ± 2.6a
	0.25	16.5± 1.2b	23.4 ± 2.0b	23.4± 2.4b
	0.5	13.5± 1.2b (A)	14.5± 1.3c (A)	20.6± 2.6b (A)
	0.75	12.0 ± 1.1b	4.5± 1.2d	20.4± 2.3b
	1	12.5 ± 1.1b	8.8± 1.5d	24.7± 1.8b
P-value		<0.0001	<0.0001	<0.0001
Y=α+βx		-36.06+40.40x	-46.68+45.12x	-25.04+42.12x
P-val(reg)		0.13	0.05	0.20
Pyrethrum	0	54.5 ± 1.4a	55.2 ± 1.8a	58.3 ± 1.4a
	0.125	47.0 ± 2.0b	41.9 ± 2.3b	38.8 ± 2.0b
	0.25	43.6 ± 2.7b (A)	34.3± 2.0c (A)	38.7± 2.1b (A)
	0.5	32.2± 2.9c	20.6± 1.4d	40.3± 2.0b
	0.75	21.9± 1.4d	18.3± 1.5d	29.8 ± 2.1c
P-value		<0.0001	<0.0001	<0.0001
Y=α+βx		-42.59+5368x	-48.30+49.79x	-27.28+58.04x
P-val(reg)		0.0001	0.01	0.11

Means (±) followed by the same letter are not significantly different: lower case letters within the column per row and upper case letters within a raw per column (P = 0.05). (0) control- distilled water

4.4 Effects of Achook, Neemroc and Pyrethrum on emergence of adults from parasitized and nonparasitized pupae of *L. huidobrensis*

These results were combiled under effects of foliage and soil treatment of Achook, Neemroc and Pyrethrum on the emergence of parasitoids and leafminers from the pupae of parasitized and nonparasitized larvae of *L. huidobrensis*.

4.4.1 Effect of foliar spray of different concentration of Achook, Neemroc and Pyrethrum on emergence of adults from parasitized and nonparasitized pupae of *L. huidobrensis*.

Achook: There were significant differences in emergence of the leafminers and parasitoids from pupae of *L. huidobrensis* larvae exposed to parasitoids and sprayed by different concentrations of Achook. Emergence of LMF and parasitoids was significantly higher in the control followed by the one in 0.05, 0.1 and 0.25 respectively. Parasitoids emerged from 0.25 concentration recorded the lowest emergence of 3.2 while no LMF emerged from the same concentration (Table 4. 7).

Neemroc: There were significant differences in emergence of the leafminers and parasitoids from pupae of *L. huidobrensis* larvae exposed to parasitoids and sprayed by different concentrations of Neemroc. Emergence of parasitoids and LMF was significantly higher in the control as compared to 0.25, 0.5 and 0.75 concentrations which did not vary significantly (Table 4.7).

Pyrethrum: There were significant differences in emergence of the leafminers and parasitoids from pupae of *L. huidobrensis* larvae exposed to parasitoids and sprayed by different concentrations of Pyrethrum. The control had a significantly higher emergence of parasitoids followed by the one in 0.125, 0.25 and 0.75 which significantly differed from one other. Emergence of LMF was significantly higher in the control as compared to emergence in 0.125, 0.25 and 0.75 concentrations that did not vary significantly (Table 4.7).

Table 4.7 The mean % emergence (±SE) of adults from pupae of the 3rd larvae instar of *L. huidobrensis* exposed to parasitoids and treated with different concentrations of Achook, Neemroc and Pyrethrum

Botanical	concentration ml/100ml	(% parasitoid emerged)	(% LMF emerged)
Achook	0	51.4 ± 2.4a	22.9± 1.7a
	0.05	31.6 ±1.9b	13.0± 1.7b
	0.1	9.8 ± 1.2c	5.2 ± 1.8c
	0.25	3.2 ± 1.1d	0 ± 0 d
P-value		<0.0001	<0.0001
Neemroc	0	54.8 ± 1.7a	21.1 ± 1.5a
	0.25	42.8 ± 1.9b	5.2 ± 2.6b
	0.5	41.5 ± 1.7b	5.0 ± 1.7b
	0.75	38.2 ± 3.4b	3.5±1.2b
P-value		<0.0015	<0.0001
Pyrethrum	0	54.3 ± 3.05a	19.3± 1.6a
	0.125	39.3 ± 1.37b	9.23 ± 1.6b
	0.25	27.3± 2.1c	10.6 ± 1.5b
	0.5	18.9 ±2.98d	5.9 ± 2.1b
P-value		<0.0009	<0.0001

Means within column followed by the same letter are not significantly different ($P = 0.05$). (0) control-distilled water

4.4.2 Effect of soil spray of different concentrations of Achook, Neemroc and Pyrethrum on emergence of adults from parasitized and nonparasitized pupae of *L. huidobrensis*.

Achook: There were significant differences in emergence of the leafminers and parasitoids from pupae of *L. huidobrensis* larvae that had been exposed to parasitoid and treated with Achook. Similarly, adult emergence from pupae of the LMF larvae that was not exposed to parasitoids and treated with Achook registered a significance difference. Percentage emergence of *L. huidobrensis* from pupae of LMF larvae that was not exposed to parasitoids was significantly higher in the control followed by emergence in 0.05 which was significantly higher than emergence in 0.1 and 0.25 concentrations. However, emergence of LMF from 0.1 and 0.25 concentrations had no significant difference. The control recorded a significantly higher emergence of *P. scabriventris*

from pupae of larvae that was exposed to parasitoids. This was followed by emergence in 0.05 that was significantly higher than emergence in 0.1 and 0.25 concentrations which did not vary significantly. The LMF that emerged from larvae exposed to parasitoids was significantly higher in the control and 0.05 as compared to emergence in 0.1 and 0.25 concentrations that did not vary significantly. There were no significant differences in the longevity of emerged adult parasitoids under the concentrations applied (Table 4.8).

Neemroc There were significant differences in emergence of the leafminers and parasitoids from pupae of *L. huidobrensis* larvae that had been exposed to parasitoid and treated with Neemroc. Similarly, adult emergence from pupae of the LMF larvae that was not exposed to parasitoids and treated with Neemroc registered a significance difference. Adult emergence of *L. huidobrensis* from pupae of unexposed larvae was significantly higher in the control followed by 0.25. Emergence in 0.5 and 0.75 was not significantly different but was significantly lower than emergence in 0.25 concentrations. Emergence of parasitoids and LMF from pupae of larvae exposed to parasitoids was significantly higher in the control as compared to 0.25, 0.5 and 0.75 that did not vary significantly. There were no significant differences in the longevity of emerged adult parasitoids under the concentrations applied (Table 4.8).

Pyrethrum There were significant differences in emergence of the leafminers and parasitoids from pupae of *L. huidobrensis* larvae that had been exposed to parasitoid and treated with Pyrethrum. Similarly, adult emergence from pupae of the LMF larvae that was not exposed to parasitoids and treated with Pyrethrum registered a significance

difference. Adult emergence of *L. huidobrensis* from pupae of unexposed larvae was significantly higher in the control followed by the one in 0.25. The emergence of LMF in 0.125 and 0.5 concentrations did not vary significantly but was significantly higher than the one in 0.125. When *L. huidobrensis* larvae were exposed to parasitoids, the emergence of *P. scabriventris* was significantly higher in the control as compared to that in 0.125, 0.25 and 0.5 that did not differ significantly. A similar trend was observed in the emergence of *L. huidobrensis* from pupae of larvae exposed to parasitoids although emergence in the control and 0.25 were not significantly different There were no significant differences in the longevity of emerged adult parasitoids under the concentrations applied (Table 4.8).

Table 4.8 The mean % emergence (±SE) of *P. scabriventris*, *L. huidobrensis* and longevity of parasitoids after treatment with different concentrations of Achook, Neemroc and Pyrethrum

Botanical	conc ml/100 ml	Un exposed to parasitoids	Exposed to parasitoids		
		%Emergence *L. huidobrensis*	%Emergence *L. huidobrensis*	%Emergence *P. scabriventris*	Parasitoid Long (d)
Achook	0.000	68.9±0.7a	12.8±2.0a	55.4±2.4a	7.4±0.5
	0.050	21.2±1.0b	11.8±1.2a	35.2±4.2b	7.5±0.4
	0.100	9.5±0.6c	4.7±1.3b	14.2±4.5c	7.4±0.4
	0.250	8.0±0.6c	3.1±0.6b	8.0±1.5c	7.3±0.5
P- value		<0.0001	0.0041	0.0001	0.6452
Neemroc	0.000	73.9±1.6a	11.8±1.2a	58.3±3.6a	7.2±0.5
	0.250	25.1±1.3b	2.3±0.4b	35.4±0.7b	7.2±0.6
	0.500	13.8±0.6c	2.8±0.5b	32.5±2.4b	7.5±0.5
	0.750	12.5±1.1c	3.0±1.0b	28.8±3.8b	7.3±0.5
P- value		<0.0001	0.0023	0.0101	0.7020
Pyrethrum	0.000	72.6±1.0a	14.4±1.3a	56.0±2.0a	7.3±0.8
	0.125	30.4±2.0b	10.2±0.8b	28.6±2.3b	7.2±0.6
	0.250	18.4±0.6c	11.2±1.5ab	28.1±2.0b	7.4±0.6
	0.500	15.5±1.0c	7.8±0.7b	28.9±1.5b	7.5±0.5
P- value		<0.0001	0.0062	0.0082	0.7602

Means within column followed by the same letter are not significantly different ($P = 0.05$). (Long)- Parasitoid longevity in days. (0) control- distilled water

4.5 Effects of Neemroc and Pyrethrum on infestation and abundance of adult *L. huidobrensis* and *P. scabriventris* in relation to yield in the field

Results on experiments carried out were combiled under infestation and abundance of *L. huidobrensis* when the french beans were treated with Neem in Sagana and Pyrethrum in Kabaru. Similarly, infestation, abundance of the LMF and parasitoid was also recorded when parasitoids were released in the french bean fields and there after treated with Neemroc and Pyrethrum. Abundance results were combiled under pooled graphs, mean number of parasitized larvae among different application schemes and % mortality of LMF larvae after exposure to parasitoids and application of the two biopesticides. Yield results were also combiled at the two places when the biopesticides were used alone and when they were combined with parasitoids.

4.5.1 Abundance of *L. huidobrensis* in the french bean field treated with different application schemes of Neemroc and Pyrethrum

Kabaru Field Site

There were no significant differences on infestation and abundance of *L. huidobrensis* when french beans were sprayed with AI, A2, A3, A4 and CO applications schemes of Pyrethrum. Pooled data for all the application schemes was therefore used. At two Weeks after germination (WAG), leaf infestation was at 45% and reduced to 30% after five weeks. Thereafter, the leaf infestation increased progressively for the next two weeks and reached 44%. However, there was no significant relationship between the infestation and time in WAG ($Y= 0.47*week+ 35.39$, $r^2 = 0.02$, $P= 0.76$, $t = 0.32$). There was a significant positive relationship between the number of *L. huidobrensis* per square metre and the number of weeks after germination ($Y= 0.44*week+ 0.22$, $r^2 = 0.8$, $P= 0.004$, $t = 0.88$), (Fig 4.1).

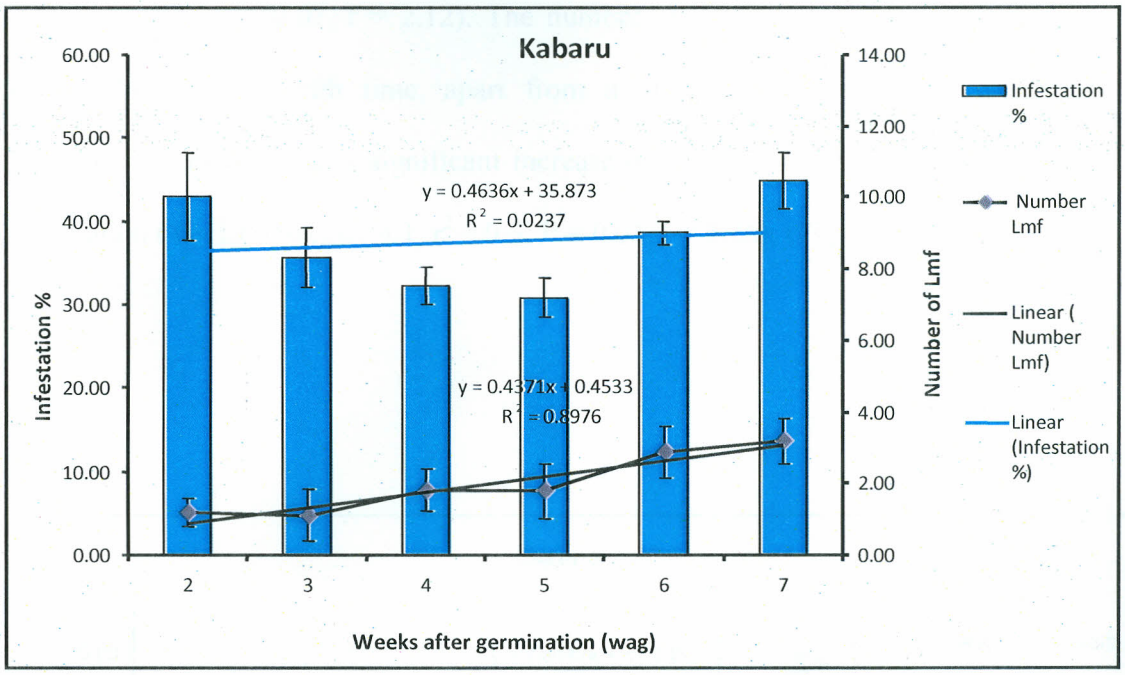


Fig 4.1 The mean % leaf infestation (±SE) and mean number/m^2 of *L. huidobrensis* (±SE) (Lmf) after treatment with different applications of Pyrethrum at Kabaru over Weeks after germination

Sagana Field Site

There were no significant differences on infestation and abundance of *L. huidobrensis* when french beans were sprayed with AI, A2, A3, A4 and CO applications schemes of Neemroc. Pooled data along all the Neemroc application schemes indicated fluctuations of percentage leaf infestation along time in WAG. Leaf infestation started at 26% and rose steadily to 65% at week four after germination. A sharp drop in percentage leaf infestation was registered at weeks 5 and 7 after germination. However, weeks 6, 8 and 9 posted high infestations of 63, 64 and 69% respectively. There was no regular pattern in infestation and this was supported by regression data that did not reveal any significant

relationship between infestation and time in Weeks after germination (Y= 3.84*week+ 37.10, r^2 = 0.4, *P* = 0.07, t = 2.12). The number of adult *L. huidobrensis* increased steadily from 4- 13 with time, apart from a drop which was at six weeks after germination. This showed a significant increase of infestation with time in weeks after germination (Y= 1.05*week+ 4.1, r^2 = 0.6, *P* = 0.01, t = 3.26), (Fig 4.2).

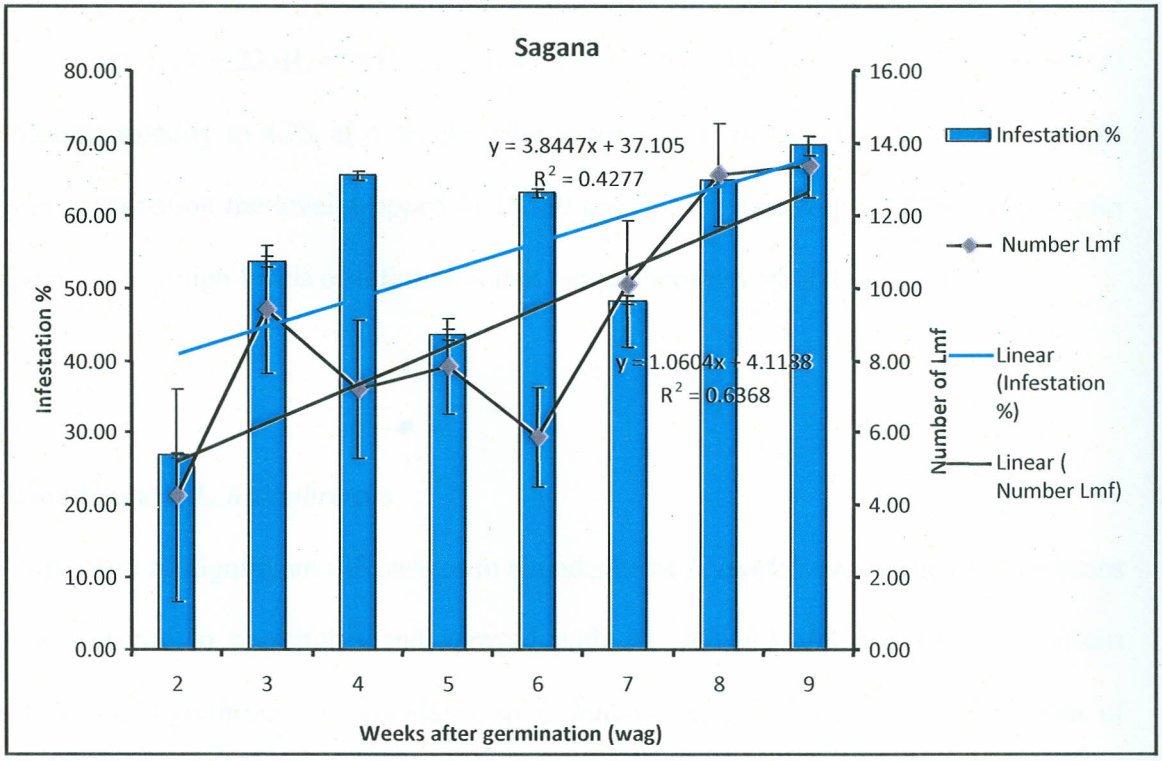


Fig 4.2 The mean % leaf infestation (±SE) and mean number/m^2 of *L. huidobrensis* (±SE) (Lmf) after treatment with different applications of Neemroc at Sagana over Weeks after germination

4.5.2 Infestation and Abundance of *L. huidobrensis* and *P. scabriventris* in french beans treated with different applications schemes of Neemroc and Pyrethrum

Kabaru Field Site

Leaf infestation rates

There were no significant differences on infestation when french beans were exposed to parasitoids and sprayed with AI, A2, A3, A4 and CO applications schemes of Pyrethrum. Percentage leaf infestation from pooled data across all Pyrethrum application schemes showed fluctuations throughout the Weeks after germination. There was a significant positive relationship between Weeks after germination (WAG) and percentage infestation ($Y = 2.30*week + 23.01$, $r^2 = 0.4$, $P = 0.02$, $t = 2.7$). Infestation started at 15% at 2 WAG and rose steadily to 42% at 4 Weeks after germination. However, 5, 11 and 12 Weeks after germination the level dropped to 15, 39 and 36% respectively. All the other weeks had relatively high levels of infestation that ranged between 54 and 59% (Fig 4.3).

Abundance of *L. huidobrensis*

There were no significant differences in abundance of *L. huidobrensis* when french beans were exposed to parasitoids and sprayed with AI, A2, A3, A4 and CO applications schemes of Pyrethrum.The abundance of *L. huidobrensis* increased with the number of Weeks after germination up to the 4^{th} WAG. A drop to two *L. huidobrensis* /m^2 was observed at 5 WAG. The number of leafminers/m^2 thereafter showed an increase from 5 flies to 8 flies at week 6-9 after germination. From the 10^{th}-14^{th} WAG, there was a tremendous decrease in the number of *L. huidobrensis* /m^2. There was no significant

relationship between the number flies and time in WAG (Y = 1.8*week + -0.17, r^2 = 0.19, P = 0.13, t = -1.62), (Fig 4.3)

Abundance of *P. scabriventris*

There were no significant differences on abundance *P. scabriventris* when french beans were sprayed with AI, A2, A3, A4 and CO applications schemes of Pyrethrum. There was a significant positive relationship in the number of parasitoids and weeks after germination (Y = 0.07*week + -0.24, r^2 = 0.7, P < 0.0001, t = 5.96) when pooled data was used. The parasitoids were introduced at six Weeks after germination and one week later the mean number of parasitoids was at 0.4/m^2, the number increased slightly to 0.7 at the 9th and 10th week after germination. By the 14th week after germination the parasitoids had increased from 0.5 to 1/m^2. It was also noted that as the number of *P. scabriventris* increased, the number of *L. huidobrensis* /m^2 decreased as well as percentage leaf infestation. This was clearly noted between the 7th and 12th Weeks after germination. However, the last two weeks showed a rise in the percentage leaf infestation (Fig 4.3).

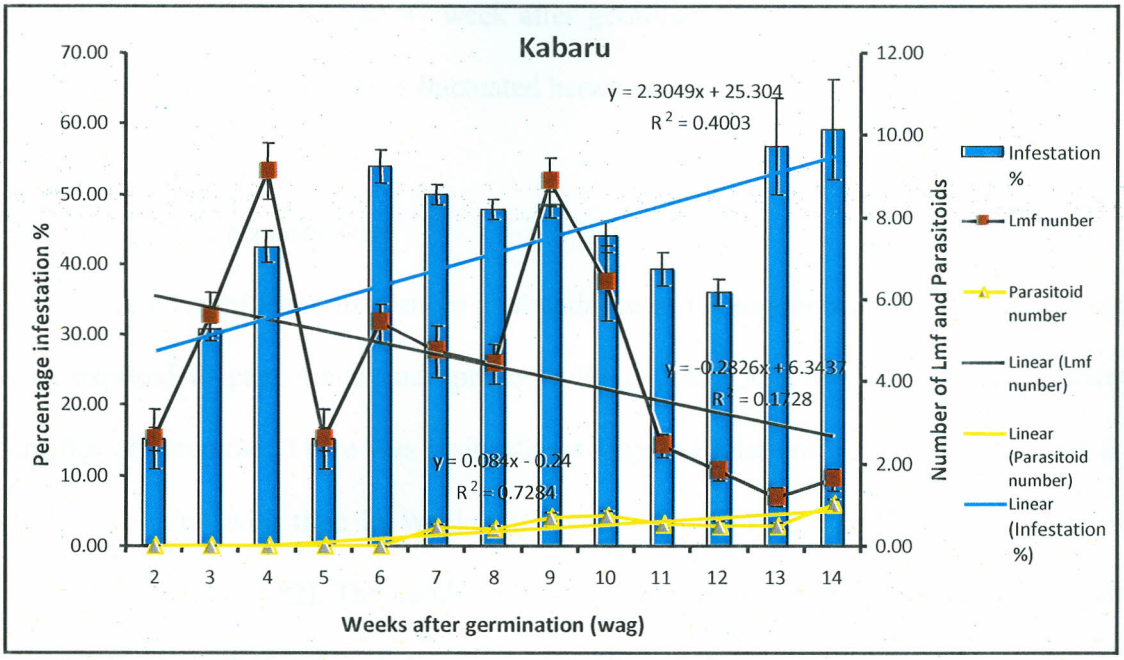


Fig 4.3 The mean % leaf infestation (±SE), mean number/m^2 of *L. huidobrensis* (±SE) (Lmf) and mean number/m^2 of Parasitoids (±SE) (*P. scabriventris)* after treatment with different applications of Pyrethrum over Weeks after germination

Sagana Field Site

Leaf infestation rates

There were no significant differences on infestation when french beans were exposed to parasitoids and sprayed with AI, A2, A3, A4 and CO applications schemes of Neemroc. Therefore, pooled data from all the Neemroc application schemes was used. The percentage leaf infestation had no significant relationship with time in Weeks after germination (Y = -2.32*week + 58.23, r^2 = 0.23, P = 0.13, t = -1.63). This is clearly shown by fluctuations throughout the period without any regular trend. However, this infestation was kept below 59% at 5 WAG as the least infestation (19% and 15%) was

registered between the 8th and 9th week after germination. From the 10th to 12th Weeks after germination, the infestation fluctuated between 48% and 45% (Fig 4.4).

Abundance of *L. huidobrensis*

There were no significant differences in abundance of *L. huidobrensis* when french beans were exposed to parasitoids and sprayed with AI, A2, A3, A4 and CO applications schemes of Neemroc. There was a significant negative relationship in the number of *L. huidobrensis* /m^2 with time in Weeks after germination (Y = -1.55*week + 21.7, r^2 = 0.65, P = 0.002, t = 7.52). The number of *L. huidobrensis* /m^2 was as high as 20-18 from 2nd -5th WAG and it reduced drastically by 6th -9th WAG to 4 *L. huidobrensis* /m^2. Thereafter their number increased slightly to 7 by the 12th Weeks after germination (Fig 4.4).

Abundance of *P. scabriventris*

There were no significant differences on abundance *P. scabriventris* when french beans were sprayed with AI, A2, A3, A4 and CO applications schemes of Pyrethrum. *P. scabriventris* parasitoid showed a slight increase per square metre from the time they were introduced at 2 Weeks after germination (WAG). The number of parasitoids increased significantly along time in WAG (Y = 0.15*week + -0.01, r^2 = 0.75, P = 0.0006, t = 5.25) as the percentage leaf infestation and the number of *L. huidobrensi*/m^2 decreased from the 6th - 9th WAG. The number of *P. scabriventris* gradually increased from 0.5 at the time it was introduced to 1.6 by the 12th week after germinsation (Fig 4.4).

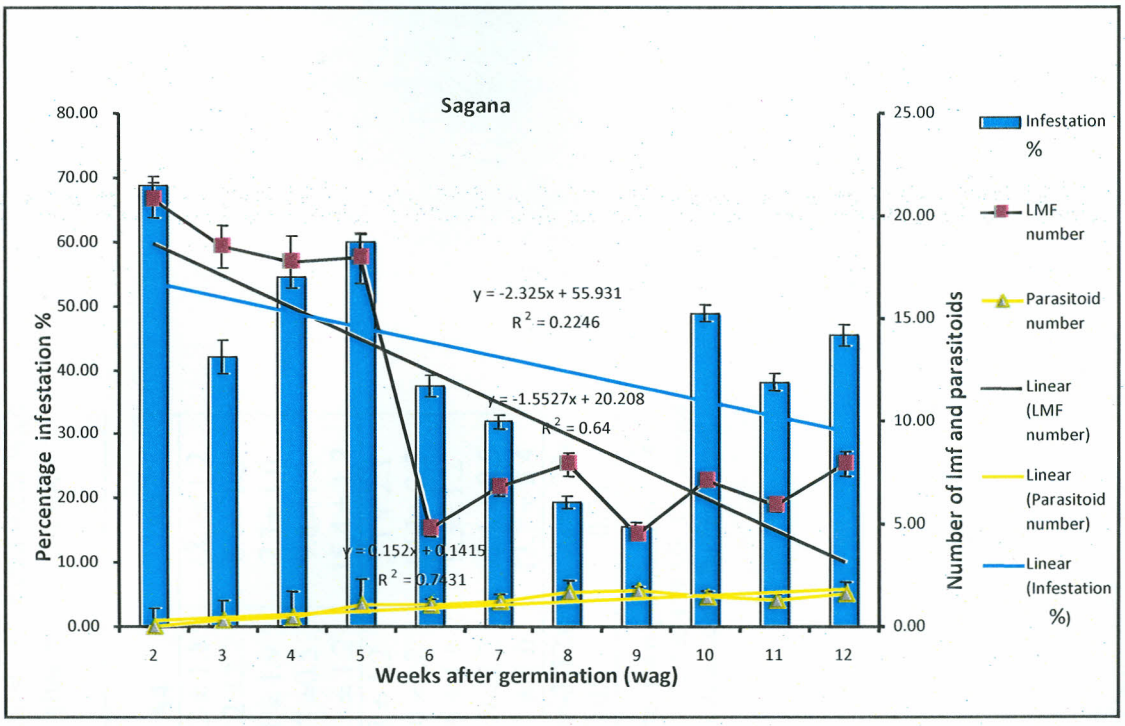


Fig 4.4 The mean % leaf infestation (±SE), mean number/m^2 of *L. huidobrensis* (±SE) (Lmf) and mean number/m^2 of Parasitoids (±SE) (*P. scabriventris)* after treatment with different applications of Neemroc over Weeks after germination

4.5.3 Larval parasitism in french beans after treatment with different application schemes of Pyrethrum at Kabaru and Neemroc at Sagana

There were no significance difference in larval parasitism by *P. scabriventris* in french bean fields treated with different Pyrethrum application schemes at Kabaru. Similarly parasitism in the LMF larvae had no significance differences when the beans were treated with different application rates of Neemroc at Sagana. However, there was a significant increase of parasitism with increase in time (weeks after germination) in all the application schemes except in two applications (A2) (Table 4.9).

Table 4.9 The mean number of LMF larvae (±SE) parasitized by *P. scabriventris* after treatment with different application schemes of Neemroc and Pyrethrum over time in weeks after release (WAR

		Application schemes					
Location	WAR	CO	A1	A2	A3	A4	FP
Kabaru	2	22.9 ± 2.1	21.6± 1.2	21.3 ± 1.3	22.8 ± 2.5	21.3 ± 1.8	18.1± 1.2
(Pyrethrum)	3	16.5 ±1.9	17.1± 1.1	15.0 ±1.2	18.1 ±2.6	12.2 ±2.2	8.0± 1.2
	4	16.1 ± 1.3	16.3 ± 1.8	14.0 ± 1.7	12.4 ± 1.2	9.1 ± 1.9	7.7± 1.0
	5	25.5 ± 1.5	17.9 ± 0.9	19.1 ±0.9	15.9 ±1.4	9.7 ±0.5	9.8± 1.3
	6	26.2 ±2.2	21.5 ±1.7	24.1 ± 1.7	22.4 ± 1.7	18.1 ± 1.2	15.4 ± 1.7
Sagana	2	18.4 ± 2.4	15.6 ± 0.7	21.3 ± 1.2	8.7 ± 0.8	4.8 ± 1.2	5.1 ± 2.1
(Neemroc)	3	21.5 ± 0.9	12.9± 0.9	11.6 ±1.1	4.9 ±1.0	3.0 ±1.2	10.1± 2.5
	4	25.8 ± 1.5	19.4± 1.1	23.2 ± 2.6	14.7 ± 1.0	14.6 ± 1.6	9.3± 1.2
	5	30.1 ± 0.7	30.5 ± 1.1	28.0 ±2.8	20.3 ±1.7	15.9 ±2.2	15.1 ± 0.5
	6	33.2 ± 1.0	30.5 ± 1.2	30.1 ± 0.6	21.9 ± 3.1	16.1 ± 1.0	13.7 ± 2.4
Y=α+βx		3.82+10.52x	4.74.2.82x	3.38+9.30x	4.18+-2.62x	3.55+-3.32x	2.22+1.78x
P-val(reg)		0.01	0.03	0.15	0.03	0.05	0.04

WAR, week after release of the parasitoids; A1, one application of pesticide first week after plant germination (WAG); A2 two applications 1 & 2 WAG, A3, three applications 1, 2 & 3 WAG; A4, 4 applications 1, 2, 3 & 4 WAG; Co, control without pesticide application; FP, farmer practice (weekly alternating and application of Dimethioate and Bulldock star).

4.5.4 Mortality of *L. huidobrensis* larvae in french beans exposed to parasitoids and treated with different application schemes of Neemroc at Sagana and Pyrethrum at Kabaru

There were significant differences in the mortality of *L. huidobrensis* larvae when french beans at Sagana were exposed to parasitoids and treated with different Neemroc application schemes ($P<0.5$). Larvae mortality was significantly lower in the control and this was not significantly different from one application (A1). Mortality in CO was significantly lower than that in A2 and A3 which did not vary significantly. Larvae mortality was significantly higher in FP (farmers practice) and this did not differ significantly with A4 (Table 4.10).

There were significant differences in the mortality of *L. huidobrensis* larvae when french beans at Kabaru were exposed to parasitoids and treated with different Pyrethrum application schemes ($P<0.5$). Larval mortality was significantly lower in the control followed by mortality in A1 and A2 that did not vary significantly. Larval mortality in FP was significantly lower than mortality in A3 but did not vary significantly with A4 (Table 4.10).

Table 4.10 The mean (%) Mortality (±SE) of *Liriomyza huidobrensis* larvae exposed to parasitoids and treated with different application schemes of Neemroc and Pyrethrum

Treatment	Larval mortality (%)	
	Neemroc	Pyrethrum
C0	18.57c	19.40d
A1	23.82bc	26.31c
A2	26.44b	25.64c
A3	29.98b	33.95b
A4	39.66a	37.47ab
FP	45.42a	40.78a
S.E.	1.62	1.01
F-value	38.97	66.09
P-value	<0.0001	<0.0001

A1, one application of pesticide first after plant germination (WAG); A2 two applications 1 & 2 WAG, A3, three applications 1, 2 & 3 WAG; A4, 4 applications 1, 2, 3 & 4 WAG; Co, control without pesticide application; FP, farmer practice ((weekly alternating and application of Dimethioate and Bulldock star). Within column, LSM followed by the same lower case letter are not significantly different at $P = 0.5$ (t-test).

4.5.5 Green pod yield and unmarketable pods of french beans exposed to parasitoids and treated with different application schemes of Neemroc and Pyrethrum

Yield when french beans were treated with biopesticides only

There were no significant differences in the yield per hectare (F =0.15, P =0.9581) when french beans were treated with different applications schemes of Neemroc at Sagana. Similarly, there was no significant difference in the % pods that were unmarketable among all applications schemes (Table 4.11). There were no significant differences on the yield per hectare and % pods that were unmarketable when french beans were treated with different applications schemes of Pyrethrum at kabaru (F = 0.4, P = 0.806, F =1.5, P = 0.251) (table 4.11). FP (Farmers Practice) and CO (control) did not vary significantly when both Neemroc and Pyrethrum applications used (Table 4.11).

Table 4.11 The mean yield of green pods (±SE) (t/ha) of french beans treated with different application schemes of Neemroc and Pyrethrum during the first season

Treatment	Neemroc		Pyrethrum	
	Green pod yield (t/ha)	%Pods unmarketable	Green pod Yield (t/ha)	%Pods Unmarketable
C0	4.73±0.42	22.7±4.8	6.35±0.28	30.2±2.6
A1	4.60±0.53	19.9±5.5	6.35±0.43	24.2±3.7
A2	5.10±0.41	21.5±4.8	6.90±0.28	21.4±3.1
A3	4.88±0.55	17.1±5.2	6.55±0.64	23.8±2.5
A4	4.53±0.89	19.4±4.9	6.80±0.25	20.2±3.6
F-value	0.15	0.18	0.4	1.5
P-value	0.9581	0.9459	0.806	0.251

A1, one application of pesticide first after plant germination (WAG); A2 two applications 1 & 2 WAG, A3, three applications 1, 2 & 3 WAG; A4, 4 applications 1, 2, 3 & 4 WAG; Co, control without pesticide application

Yield when french beans were exposed to parasitoids and treated with biopesticides

There were no significant differences on the yield per hectare and % pods that were unmarketable when french beans were exposed to *P. scabriventris* and treated with different application schemes of Neemroc ($F = 0.81$, $P = 0.9581$; $F = 0.2$, $P = 0.962$) (Table 4.12). Similarly, when Pyrethrum application schemes were applied in conjunction with parasitoid ($F = 0.5$, $P = 0.77$; $F = 1.73$, $P = 0.1784$) no significance difference was recorded in the yield and in the % pods that were unmarketable. FP application schemes did not have any significance difference in yield and unmarketable pods as compared to the control (Table 4.12).

Table 4.12 The mean yield of green pods (±SE) (t/ha) of french bean exposed to parasitoids and treated with different application schemes of Neemroc and Pyrethrum during the second season.

Treatment	Neemroc		Pyrethrum	
	Green pod yield (t/ha)	% Pods unmarketable	Green pod yield (t/ha)	% Pods Unmarketable
C0	6.05±0.16	25.2±4.4	3.60±0.02	35.7±4.2
A1	5.85±0.07	27.3±3.4	3.59±0.15	31.0±1.2
A2	5.86±0.32	28.0±3.3	3.58±0.07	28.4±1.5
A3	5.64±0.18	26.7±2.8	3.50±0.07	31.7±3.8
A4	5.40±0.60	27.8±5.8	3.64±0.06	28.1±1.7
FP	5.35±0.23	23.5±2.4	3.65±0.04	26.1±1.2
F-value	0.81	0.2	0.5	1.73
P-value	0.5581	0.9562	0.77	0.1784

A1, one application of pesticide first week after plant germination (WAG); A2 two applications 1 & 2 WAG, A3, three applications 1, 2 & 3 WAG; A4, 4 applications 1, 2, 3 & 4 WAG; Co, control without pesticide application; FP, farmer practice (weekly alternating and application of Dimethioate and Bulldock star).

CHAPTER FIVE

DISCUSSIONS

5.1 Effects of various spray regimes of Achook, Neemroc and Pyrethrum biopesticide on feeding, oviposition and egg to adult survival of *L. huidobrensis*

Results on effect of different concentrations of achook and pyrethrum on feeding punctures made by *L. huidobrensis* had a significant negative linear relationship with increase in the concentration of the Achook and Pyrethrum. These results corroborate the findings by Mordue & Blackwell (1993) and Gonzales *et al.* (1999) who reported that feeding punctures reduced in insects due to the presence of anti-feedant deterring allelochemical in neem. Azadirachtin in Achook increased along the dose concentration and this might have reduced the feeding behavior by blocking input receptors for phagostimulants or by the stimulation of deterrent receptor cells in the LMF. Pyrethrum is a contact pesticide that might have caused nervous paralysis in adult flies which interfered with the feeding process Obrochta *et al.* (1996). Neemroc had no regular trend in the feeding punctures and this could be propably due to the low % of azadirachtin (0.03) combined with the unique behaviour of the leafminer and its poliphagy nature of feeding on most field crops and weeds.

Results on effects of days before infestation on leaves sprayed by Neemroc showed a significant increase in feeding punctures with increasing days before infestation. Low number of feeding punctures on plants sprayed and immediately infested by LMF indicated that Neemroc has strong feeding deterrent effects immediately it is applied. These results conform to the findings of Banchio *et al.* (2005) who reported that leaves treated with Melia azadirachtin and immediately exposed to *L. huidobrensis* received up

to 90% fewer punctures than un treated leaves. However, it is not clearly established why Achook (0.15%) with a high concentration of azadirachtin than Neemroc (0.03%) had a higher percentage of feeding punctures immediately it was sprayed and infested. The same trend is exhibited in leaves sprayed by Pyrethrum and therefore, it is possible to presume that Achook and Pyrethrum are unique and do not display their translaminar properties immediately they are sprayed. It is also presumed that the active ingredient takes time to be absorbed in the leaf tissues hence becoming more effective with increasing time span between spraying and infestation. This may explain the lower number of feeding punctures in leaves sprayed by achook or pyrethrum and infested one and two days later.

Results on effect of different concentrations of achook, neemroc and pyrethrum on oviposition by *L. huidobrensis* showed no effect. Adult females laid the same number of eggs even when high concentrations of the pesticides were applied. Similarly, time span between application of Achook or Neemroc and exposure to leafminers had no effect on oviposition. This may be due to eggs being strongly protected by their impermeable membrane or egg chorion which may inhibit the penetration of azadirachtin into the egg. These findings on oviposition agree with results from Larew *et al.* (1985) who reported that high concentration of neem (0.4%) applied on chrysanthemum plants had no effect on oviposition by *L. trifolii*. Webb *et al.* (1983) however, reported that *L. trifolii* laid fewer eggs when bean leaves were immersed in neem seed solution. This differs with findings of this study. Oviposition increased with the time span between Pyrethrum spray and exposure to leafminers and this may be due to Pyrethrum biodegrading rapidly when

exposed to natural light leading lack of persistence in the environment as reported by Todd *et al.* 2003. It is therefore possible to assume that Pyrethrum dissipated rapidly with time as reported by William and Markowitz (2007) and this led to increased oviposition on plants sprayed and infested two days later.

Percentage survival from egg to adult significantly reduced with increase in the concentration of Achook, Neemroc and Pyrethrum. This is probably due to the increasing concentration of the active ingredient along the concentration of the pesticides applied. The low % survival in plants treated with high concentration of Achook and Neemroc can also be explained by Banchio *et al.* (2005), who reported that translaminer action of Melia azedarach (has azadirachtin as an active ingridient) negatively affected *L. huidobrensis* survival. In adittion, a report by Yoshida and Toscano (1994) agrees with findings of this study when survival of the tobacco budworm recorded 56.7% on leaves treated with pyrethrum as compared to 83.3% on untreated leaves. Its therefore assumed that as the egg hatches into larval stages, the larvae feeds on the leaf tissues that are loaded with azadirachtin and pyrethrin and this eventually affects their survival depending on quantity of the active ingredient consumed.

Percentage survival from egg to adult was significantly lower when plants were sprayed and immediately exposed to LMF. This conforms to findings by Prabhat and Poehling (2006) who reported a mortality range of 32-42% when plants were sprayed with neem and infested immediately with white flies immature nymphs. The mortality drastically reduced to 5-7% when plants were sprayed and infested 7 days later. It is therefore

evident to conclude that rapid dissipation of the active ingredient due to long exposure to high temperatures in the greenhouse led to increased % survival in leaves sprayed and infested 2 days later. Neem has a major drawback in its ability to biodegrade fast and has a shorter persistence which lowers its efficacy as stated by Johnson *et al.* (2003) when plants are sprayed and infested two days later. This is further corroborated by Hossain *et al.* (2008) who reported that mortality of larvae on neem application declined steadily with time span between application and infestation.

5.2 Effects of Achook, Neemroc and Pyrethrum on survival of larval stages and emergence of adults from pupal stage of *L. huidobrensis*

Results on effect of biopesticides on % larval survival reduced with increase in the concentration of Neemroc and Pyrethrum in all the three larval instars. Similarly, % survival in 1st and 2nd instars reduced with increase in Achook concentrations but the 3rd instar was not afected. These results conforms to those of Facknath (2005) who reported that azadirachtin at higher concentrations of 1.0% and 1.5% is as effective as cyromazine in reducing % survival of *L. trifolii* larvae. The 1st and 2nd instar stages had a significantly higher mortality when Achook was applied. This means that mortality and survival are age specific with young larval stages being more susceptible to neem than older stages. Similarly, studies by Weintraub and Horowitz (1997) indicated that 2nd instar larvae of *L. huidobrensis* had higher mortalities compared to 3rd instars on treatment with neem. Young larvae showed a significantly higher sensitivity to neem compared to older larvae. Similar results were reported by Hossain *et al.* (2008) when they used neem on different larval stages of *L. sativae*. The possible reason for the high mortality in 1st instar larvae could be that, young larvae receive the highest amount of active ingredient per body mass

if in contact with azadirachtin loaded tissues in mines. They also tend to feed more than the older larvae as reported by Hossain and Poehling (2009). Lower percentage survival in the 1st instar larvae after treatment with pyrethrum may be as a result of the strong contact effect of Pyrethrin to the larvae due to their susceptibility as suggested by Weintraub and Horowitz (1997). The sensitivity of the young instars to Pyrethrum could also be due to easy penetration of the active ingredient through the incompletely or partly sclerotisized cuticula.

Adult emergence was absent in pupae of the first instar larvae when they were treated with achook concentrations of 0.1, 0.25 and 0.5v/v. These results agree with those of Weintraub and Horowitz, (1997) and Larew *et al.* (1985) who reported that no adults emerged from pupae of *L. huidobrensis* developed from bean plants drenched with neem extracts with 4.5% of azadirachtin. Emergence of *L. huidobrensis* from pupae whose larvae was treated with Neemroc significantly reduced as the concentration of the pesticide increased in all the three instars. These results agrees with those of Hossain *et al.* (2008) who conducted a direct toxicity study and reported that pupae eclosion decreased from 30.29% to 0% when the concentration of neem was increased from 1-10ml/1w. Emergence of adults from pupae of the 1st, 2nd and 3rd instars treated with achook and neemroc did not vary significantly and this may be possibly due to the short persistence of azadirachtin that takes three days in the foliage as stated stated by Babul and Poehling (2005).

Adult emergence in pupae from the three larval stages was significantly reduced when they were treated with Pyrethrum concentrations. The activity of pyrethrin being enhanced by additive compounds such as Piperonyl Butoxide (PBO) which suppress detoxification within the insect as stated by Elliot and Janes, (1973) may be used to explain lower emergence in pupae from larvae treated with higher concentrations of pyrethrum that had equally higher concentration of PBO. Emergence of adults from pupae of the 1st, 2nd and 3rd instars was was not significantly different due to the length of time between pupation and emergence. The larval stage takes 11- days to pupate and eclose while five to ten days is enough for the PBO in pyrethrum to biodegrade. Herbach *et al.* (2006) affirms this in their report that the ineffectiveness of PBO is due to its chemical instability in field conditions when it is broken down by sunlight.

5.3 Effect of Achook, Neemroc and Pyrethrum on the mortality of the late 3rd instar larvae and emergence of adults from pupal stage of *L. huidobrensis*

Results on the effect of different concentrations of Achook, Neemroc and Pyrethrum on the late 3rd instar larvae had an effect on mortality of the 3rd larvae instar. These results are in agreement with findings of Hossain *et al.* 2008 who recorded a mortality of 9.4% to 100% when 0.75 – 3.0g/lw of NeemAzal was used in treatment of immature stages of *L. sativae* from tomatoes. The mortality of the late 3rd instars could only be as a result of external contact with Azadirachtin which diffuses into the tissues of the larvae and interfears with its development to pupae. The findings from this study showed that, Achook and Neemroc had low mortality of the late 3rd instar larvae when soil was sprayed and infested one and two days later as compared to soil sprayed and infested immediately. Low mortality of the larval instars was posted in soil sprayed and infested

two days later. Babul and Hossin, (2005) found out similar results when they applied neemazal to the soil about 36 hrs before 3rd instar of *L. sativae* started to fall in the soil for pupation. Their results showed lower mortality even with the deployment of higher neem concentration as compared to when soil was sprayed and infested immediately with late 3rd instar larvae of *L. sativae*. This could explain the lack of effect of Neemroc on the larvae when soil was sprayed and infested two days later as well as high mortality in soil sprayed and immediately infested. Pyrethrum recorded high mortality of larvae in soil sprayed and infested immediately but no effect in mortality when soil was sprayed and infested two days later. McGarry and Trees, (1991) reported that when *D. gallinae* mite was treated by Pyrethrum between 24 and 48 hours it showed an 87 and 70 % reduction respectively. A similar study, using 0.6% pyrethrin and 1.4 % piperonyl butoxide as an aerosol at a rate of 9.4 and 7.6 g/100 m^3 by Sullivan *et al.* (1976) against *A. albimanus* in Panama showed 100% effectiveness within 24 hours. The effect of both Neem and Pyrethrum on the late 3rd instar larvae is due external contact with the active ingredient since the larval stages are no longer feeding.

Adult emergence in pupae from the 3rd instar larvae in soil treated with different concentrations of Achook, Neemroc and Pyrethrum on 0, 1 & 2 days before infestation was significantly affected. Hossain and Poehling, (2009) corroborate these results when they found out that adult eclosion of pupae from larvae treated with neem showed a significant difference in respect with the concentrations used. Results in pyrethrum agree with those of Gunasekara, (2004) who reported that *A. albimanus* females were knocked-down in percentages that ranged from 81.5 to 100 after 1 hour following ULV pyrethrins

spraying hence reducing the number of vectors for the mosquito vectors. This % emergence may be attributed to the fact that *L. huidobrensis* is susceptible to neem and pyrethrum during pupal development if the soil is sprayed and immediately infested with the late third instar larvae as compared to soil sprayed and infested one and two days later. Percentage emergence after application of neemroc and achook was not significantly different among the days before application and this may be probably due to rapid biodegradation of neem with time as stated by Pavela *et al.* (2004)

5.4 Effects of Achook, Neemroc and Pyrethrum on emergence of adults from parasitized and nonparasitized pupae of *L. huidobrensis*

Results obtained from foliage spray showed that percentage emergence of parasitoids after Achook and Neemroc treatment increased with decrease in the biopesitcide concentrations. These findings are in agreement with that of Stark *et al.* (1990a) who found that low concentration of the active ingredient in neem seed extracts (10 ppm) allowed hymenopteran parasitoids to emerge unharmed and they were able to mate and seek new fruit fly hosts. Beckage *et al.* (1988) reported a suppression of ecdysis and 100% mortality of *Cotesia congregata*, a hymenopteran's parasitoid of *Manduca sexta* on an injection of 10µg of azadirachtin into newly ecdysed fourth or fifth instar host larvae. This could explain why Achook recorded the lowest adult emergence of parasitoids (3.2-31.6%) as its concentration increased. Achook has a high percentage of azadirachtin (0.15%) as compared to Neemroc with 0.03% azadirachtin, this factor is the most likely to have caused high adult emergence of parasitoids in Neemroc than in Achook. Achook with a very high concentration of azadirachtin suppressed most parasitoids from

emerging.These results are also similar to those of Anibal, (2007) who reported that there is minimal impact of neem on parasitoids if applied in low doses.

Adult emergence of *P. scabriventris* from the parasitized pupae whose larvae was treated with Pyrethrum had higher emergence in the concentration of 0.125 as compared to 0.5 concentrations. Anibal (2007) studied the effect of synthetic pyrethroids (karate) on *Diadegma mollipla* a parasitoid of *Plutella xylostella* which recorded 100% mortality of the parasitoid in higher doses. This is a different scenario compared the observations on *P. scabriventris* in the present study probably because Pyrethrum is a natural botanical pesticide whose toxicity is less than synthetic pyrethroids. Similarly, Pyrethrum has been used on the glasshouse whitefly *Trialeurodes vaporariorum* and it's parasitoid *Encarsia formosa* and it proved the most toxic to both the whitefly and the parasitoid as compared to other selected pesticides such as *Azadirachta indica* (Simmonds *et al.*, 2002). This may be the reason for lower parasitoid emergence in Pyrethrum compared to that of Neemroc. The findings of Simmonds *et al.* (2002) agree with these results because they found out that *A. indica* derived products had the highest potential for use in the IPM system and *E. formosa* for the biocontrol of whitefly.

The results obtained from the soil spray showed that, higher concentrations Achook, Neemroc and Pyrethrum significantly reduced the emergence of the parasitoid *P. scabriventris*. Same concentrations of the pesticides had more parasitoids emerging than that of the LMF from larvae exposed to parasitoids respectively. Similarly, the percentage emergence of *L. huidobrensis* from the parasitized larvae was relatively low compared to

the parasitoids across all the three pesticides. Therefore the pesticides had more effect on the leafminer as compared to the parasitoids which is an indication that the parasitoids wer more tolerant to the biopesticides as compared to the leafminer. Similar results were obtained by Lowery and Isman, (1994) who reported that while azadirachtin had an effect on many important horticultural and agricultural pests, it had a lower impact on non target organisms especially beneficial insects. Azadirachtin may have less effect on the parasitoid since the parasitoid feeds on the LMF larvae and this reduces its systemic effect unlike the LMF which directly feeds in the mesophyll cells of the leaf. Emergence of parasitoids in Achook treatment was lower compared to that in Neemroc and it decreased with increase in the concentration of Achook. This is in agreement with Saber *et al.* (2004) findings which reported adverse effects of neemazal 1% on *Trichogramma cacoeciae* when the parasitized eggs of *Cydia pomonella* were exposed to insecticide at pupal stages leading to the lowest percentage emergence. It is then true to say that high concentration of Achook, Neemroc and Pyrethrum insecticide used on parasitoids can be harmful. A report by Feldhege and Schmutterer (1993) who used a dose of 20 ppm azadirachtin that reduced survival of female parasitoids of *T. minutum* concurs with findings of this study. Similarly, Ahmad *et al.* (2003) found out that neem kernel water extract applied in the soil at 30, 60, 125 and 250mg azadirachtin/litre on the parasitoid *Diaeretiella rapae* had low emergence rate of adults of F1 and F2.

Pyrethrum had an effect on the parasitoid since their emergence increased with decrease in the concentration of pyrethrum. However it suppressed more LMF from emerging compared to the parasitoids. This could be attributed to its direct contact mode of action.

The LMF larvae absorb more pyrethrin and only a small quantity is taken up by the parasitoid. Results obtained from this study showed that Achook, Neemroc and Pyrethrum did not affect longevity of the adults and this concurs with Mitchell *et al.* (2004) who found that the longevity of males and females of *Gryon fulviventre* on eggs parasitoid of *clavigralla scutellaris* (Hymenoptera: Coreidae) were not affected by pre-imaginal exposure of larval or prepupal stage to 5% neem suspension with different doses of neem. In this study the time span between spraying and emergence of parasitoids is approximately three weeks which is enough for the azadirachtin and Pyrethrin to have lost its persistence. Therefore, the longevity of the parasitoids would hardly be affected.

5.5 Effects of Neemroc and Pyrethrum on infestation and abundance of adult *L. huidobrensis* and *P. scabriventris* in relation to yield in the field

Results on effects of different neem and Pyrethrum application schemes with introduction of *P. scabriventris* had no effective protection to french beans infested by *L. huidobrensis* in the field. This was evident in the lack of effect by all treatment applications used during the first and second season on abundance of the leafminers and that of parasitoids. Similar results were also reported by Abou-Fakhr *et al.* (2000) who observed that field assays indicated no effect of aqueous neem extract against *L. sativae* and *L. trifolii*. Chaney (1995) also reported that azadirachtin was not efficacious in controlling leafminer populations but was successful in interfering with the pupation thereby reducing the adult emergence in the subsequent crop. Weekly applications of neem and Pyrethrum had no effect on infestation and *L. huidobrensis* abundance among the application schemes applied. However, a significant positive relationship was recorded between the number of LMF and parasitoids with increase in weeks after germination

when parasitoids were introduced at Kabaru and Sagana. Since Pyrethrum has a negative temperature coefficient, it is usually more toxic at lower temperatures as stated by Satpute *et al.* (2007). Similarly Hartzell, (1932) and Harries *et al.* (1945) reported that natural pyrethrins and synthetic pyrethroids have higher toxicity at lower temperatures.This may explain why pyrethrum is not effective in the field where temperatures were high. Neemroc results are different from those of Ostermann and Dreyer (1995) findings who reported that weekly application of aqueous neem seed extract at 60g/l and neem oil 2.5-3% reduced leafminer damage on tomatoes. Similarly, a report by Varela *et al.* (2003) noted that weekly foliar sprays of commercial neem products at the rate of 25-50g/l water and a spray volume of 900l/ha controlled leaf mining flies on experimental tomato fields in Kenya. However, given enough time for the parasitoid to establish followed by other subsequent releases of *P. scabriventris*, perhaps Neem and pyrethrum might be able to reduce infestation and abundance of *L. huidobrensis* in the field.

Yield and unmarketable pods in Kabaru and Sagana showed no significant difference between the farmers practice and the control. This is an important aspect since Bulldock star was alternated with Dimethoate in farmers practice treatment. Therefore the use of these two synthetic chemicals does not add value to the crop yield and yet they are harmful not only to the beneficial nontargets but also result into high Maximum Residue level in the produce.

Larval mortality in the french bean fields exposed to parasitoids and treated with different applications of neem and Pyrethrum increased with the frequency of application. The highest mortality was recorded in fields sprayed with bulldock and dimethiote (FP) and treatment that received four applications (A4). Therefore if the farmers were to adapt the combined method of biopesticides and parasitoids, then three applications (A3) at 1, 3 and 5 weeks after application of Neemroc or Pyrethrum would be appropriate since they would cause mortality of some larvae as others remain for playing host to *P. scabriventris*. Four applications (A4) of neem and FP (Farmers practice) are likely to cause more mortality to *L. huidobrensis* larvae and this may reduce the host for the parasitoid.

Field experiments in this study showed no effect of neem and Pyrethrum on parasitization and *P. scabriventris* abundance. This is due the short period between the releases of the parasitoid to the end of the investigation. The parasitoids were monitored for only 8 and 10 weeks at Kabaru and Sagana respectively. This period was not enough for the parasitoid to establish. Furthermore the number of parasitoids released per treatment was only 14, which was quite small owing to the fact that some parasitoids died before even ovipositing. Therefore field studies should be repeated with more releases and recovery to monitor the establishment of the parasitoid around the released areas.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

1. Egg to adult survival of *L. huidobrensis* pest reduced significantly with increase in concentrations of Achook, Neemroc and Pyrethrum as well as with the time frame between treatment of foliage and infestation by the LMF (DBI).

2. Oviposition was not affected among the different concentration of Achook, Neemroc and Pyrethrum applied. Similarly Achook and Neemroc did not affect oviposition in all the time frame between treatment and infestation (DBI).

3. Survival of larval stages and emergence of adults from pupae of *L. huidobrensis* reduces with increase in the concentrations of Achook, Neemroc and Pyrethrum.

4. Larval stages of *L. huidobrensis* have differences in their susceptibility to Achook and Pyrethrum and this may be of interest to the timing of application of pesticides. The first instar larval stage is the most susceptible to these biopesticide as compared to 2^{nd} and 3^{rd} larval instars.

5. Mortality of the late 3^{rd} instar larvae stage increases with increase in the concentrations of Achook, Neemroc and Pyrethrum as well as the time frame between treatment of the soil and infestation by the larvae (DBI).

6. *P. scabriventris* is more tolerant to different concentrations of Achook, Neemroc and Pyrethrum as compared to *L. huidobrensis*. This is seen in the way more parasitoids than the leafminers emerge from the same concentration of the biopesticides. However, higher concentrations of the biopesticides suppress parasitism while low concentrations favour it.

7. Exposure of french beans to parasitoids and treatment with different application schemes of Neemroc and Pyrethrum had no effect on infestation, yield, and abundance of both the leafminer and the parasitoid. However, larval mortality of the LMF due to the combined effect of parasitoids and biopesticides had an effect with the control having the lowest mortality. Highest mortality was found in the farmers' practice which was not significantly different from four applications (A4).

6.2 Recommendations to Farmers

1. In the management of *L. huidobrensis* both foliar and soil sprays of Achook, neemroc and Pyrethrum can be carried out and can successfully control the leafminer.

2. The susceptibility of 1st instar larval stage may be of interest to the timing of application of pesticides. Therefore the highest efficacy of managing the population development of the pest could be expected if Achook, Neemroc and Pyrethrum is sprayed and distributed into the plant before the 1st instar larvae of *L. huidobrensis* starts to feed on the leaf. (Immediately the crop is infested or when the adult flies are spotted).

3. In cases where crop infestation by the leafminer has reached the mining stage, farmers should spray both the foliage and the soil with Achook, Neemroc and Pyrethrum to ensure that the young larvae stages are killed by the foliar spray while the late instars are killed by the soil spray.

4. Farmers should limit the use of synthetic pesticides such Bulldock star and Dimethoate in areas where parasitoids have been released to encourage their establishment. This is an advantage to the farmer who will be able to combine both methods of using botanical pesticide and the parasitoid in the control of *L. huidobrensis*.

5. The best biopesticide to be used in combination with the parasitoid in the management of *L. huidobrensis* would be Neemroc. This is because Neemroc favoured the increase of the parasitoid with time in weeks after germination.

6.3 Suggestions for further studies

1. Further field studies should be carried out with increased parasitoid release and recovery to monitor the establishment *P. scabriventris*. This is because of the limited time in this study where release of the parasitoid was only done once and studied for 9 and 11 weeks at Sagana and Kabaru respectively.

2. Synergy or combination of *P. scabriventris*, the local parasitoids and biopesitcides should be studied in greater details in the laboratory and the field.

3. Further studies on effect of different concentrations of Achook, Neemroc and Pyrethrum biopesticides should be used in field conditions for more efficient control of the leafminer *L. huidobrensis*.

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