EFFECTS OF PESTICIDE APPLICATION ON THE POPULATION DYNAMICS OF *Liriomyza huidobrensis* (DIPTERA: AGROMYZIDAE) AND ITS PARASITOIDS ON PEAS (*Pisum sativum*) IN CENTRAL KENYA

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DECLARATIONS

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

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DEDICATION

I dedicate this thesis to my husband, Gerald Musyoki; My sons, Francis and Geoffrey and to Mum and Dad.

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ACRONYMS AND ABBREVIATIONS

ANOVA: Analysis of Variance

ANSE: Aqueous Neem Seed Extracts

C0: Control

CIP: International Potato Centre

EPPO: European Plant Protection Organization

EU: European Union

FF: Farmers field

FPEAK: Fresh Produce Exporters Association of Kenya

GDP: Gross Domestic produce

HCDA: Horticultural Crops Development Authority

icipe: International Centre for Insect Physiology and Ecology

IPM: Integrated Pest Management

KARI: Kenya Agricultural Research Institute

LC_{50} : Lethal Concentration that kills 50% of the population

LC_{90} : Lethal Concentration that kills 90% of the population

LMF: *Liriomyza* leaf miner flies

RP: Reduced Pesticide

SNK: Students Neumans Keuls

USD: United States Dollar

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ABSTRACT

Snow Peas (*Pisum sativum* var *saccharatum*) and Sugar Snap (*Pisum sativum* var *macrocarpon*) are two of the most important legume-vegetables cultivated by small scale farmers in Kenya for both consumption and sale. The major production constraints are insect pests, diseases, nematode, insufficient rainfall and poor agronomic practices. The *Liriomyza* leaf mining flies (LMF) are among the most important pests causing a significant damage to these crops. For this reason, effective control of this pest is of utmost importance. Some of the methods that have been used in the control of *L. huidobrensis* include cultural practices such as crop rotation, as well as the use of synthetic pesticides. These pesticides have been used indiscriminately against these pests without considering their effects on the non-target pests. This study aimed at investigating the effects of pesticide application on the population dynamics of LMF and their indigenous natural enemies in central Kenya, one of the main production areas of peas in Kenya. On-farm field trials were set up at three different locations (Sagana, Kabaru and Naromoru) in Central Kenya. The experimental set up was a Randomized Complete Block Design. The treatments included: farmer practice where farmers were allowed to manage their plot following their routine and without restrictions on the pesticides use (FF), controlled pesticide where monitoring was done before pesticides could be applied (RP) and a control where no pesticides were used. In addition, laboratory experiments were designed to test the effect of commonly used pesticides (Dimethoate, Dynamec, Thunder, Cyclone, Bestox, Folicur, Milraz and Bulldock) on *L. huidobrensis* and its two parasitoids *Diglyphus isaea* and *Phaedrotoma scabriventris*. The mean numbers of leafminer were significantly higher in the farmers' field (60.9 and 79.6) than the control (30.6 and 18.8) and reduced pesticide application plots (25.4 and 29.8) in the first and second cropping seasons respectively. Local parasitoids were rare (0-25) in the field and despite the relative constant presence of the pest; the parasitoids were only present in a sporadic manner. However, the control plot recorded significantly higher numbers of parasitoids (4.5 and 5.8) compared to the reduced pesticide (1.72 and 2.36) and Farmers' field (1.63 and 1.83) during the first and the second cropping periods respectively. Bestox, Cyclone and Dynamec were efficient in the laboratory against adult leafminers and their estimated LC_{50} (0.32, 0.5 and 0.24 ml/l respectively) were below the recommended doses (0.5, 0.75 and 0.5 ml/l respectively). None of the pesticides was successful in controlling the larva of leafminers and the LC_{50} for Bestox, Bulldock, Dimethoate, Dynamec and Thunder were 1.41, 1.64,.2.91, 1.16 and 2.94 ml/l respectively yet their recommended dosages were 0.5, 0.75, 1.5, 0.5 and 0.5ml/l respectively. Apart from Thunder which required 0.80 and 0.77 ml/l as the LC_{50} for *D. isaea* and *P. scabriventris* respectively against a recommended dosage of 0.5ml/l, all the other pesticides were harmful to the parasitoids at dosages below the recommended. For example, the LC_{50} for Bestox, Bulldock and Dynamec was 0.02, 0.01 and 0.02ml/l respectively. This study demonstrated that pesticide use in farmer fields had a role to play in decreased effectiveness of biological control by natural enemies. However, since the parasitoids already present in the field are few for effective control, there is need to increase the numbers through mass release. In addition, a study is needed on pesticides more friendly to the parasitoids such as botanicals.

CHAPTER ONE

1 INTRODUCTION

1.1 Background information

Pea crop is an annual legume with slender stems. The leaves end in one or more tendrils and have one to three pairs of leaflets. The pod is 5-10 cm long and may be straight or curved containing up to 10 globular smooth or wrinkled seeds. In Kenya, pea is grown in Central, eastern and some parts of rift-valley provinces (HCDA, 2011). There are various varieties of pea and the most common ones are Snow peas and Sugar snaps. Snow Peas (*Pisum sativum* var *saccharatum*), (Plate 1) and Sugar Snap (*Pisum sativum* var *macrocarpon*), (Plate 2) are two of the most important legume-vegetables cultivated by small scale farmers as a source of income. These two crops are usually sold as pods which serve as a vegetable. They are mainly produced for export market, though part is consumed locally. These two crops contribute significantly in the horticultural industry hence development of Kenyan economy. Production of high value horticultural crops such as peas has been identified as a key pathway out of poverty and a prospect for development, compared to the production of staple crops (McCulloch and Ota, 2002; Asfaw *et al.,* 2009).

Horticultural exports in Kenya in 2006 accounted for Ksh 52.5 billion, making it the second highest income earner after tourism. In 2007 the sub-sector earned Ksh. 45.7 billion with Ksh.1.5 billion contributed by peas (FPEAK, 2007). Currently, the sector provides livelihood to 500,000 farmers, 80% of whom are small and medium-scale. Kenyan horticultural industry also employs over 4 million people contributes close to 13% of Kenya's Gross Domestic Product, GDP (FPEAK, 2007). Hence, Kenya's horticulture industry is very important in meeting the country employment needs and improving livelihoods (McCulloch and Ota, 2002). Peas also serve as a direct source of proteins (when dry) and vitamins (when green) to a wide majority of the population in Kenya. The major production constraints includes insufficient rainfall, poor agronomic practices, soil infertility, diseases such as powdery mildew and blight as well pests such as leafminer flies, thrips, aphids, mites and the African bollworm. The leafminer flies some of which includes *Liriomyza huidobrensis* and African bollworm (*Helicoverpa* armigera Hubn.) are some of the pests in the EU list of quarantine pests (Kedera and Kuria, 2003), and very serious pests of Pea.

The pea leafminer, *L. huidobrensis* is the most important pest of snow peas and sugar snaps in Kenya (Njuguna *et al.*, 2001; Kedera and Kuria, 2003). Three factors make *Liriomyza* leafminer a pest of unique economic importance in snow peas production: it attacks the marketed part of the crop; the damage created by the larvae is often unnoticeable until the produce has reached the destination market and the pest has high ability to develop resistance to pesticides (Gitonga *et al.*, 2010). *Liriomyza* leafminers belong to the order Diptera, family Agromyzidae and are small and usually dark flies (Koppert, 2003).

Liriomyza leafminers inflict damage to leaves through punctures made by females to feed and lay eggs, and by mining in the mesophyll by larvae (Weintraub and Horowitz, 1997). Both forms of damage reduce the photosynthetic area of the leaf, hence lowering the ability of the plant to provide food to developing parts. In severe cases, leaves wilt and plants die. Yield losses ranging between 50-100% and also serious damage in cut flowers and passion fruits have been reported (Weintraub and Horowitz, 1997). Some insecticides such as organophosphates, pyrethroids and botanicals have been used in the control of leafminers but the pest resistance can sometimes make control difficult (Parrella and Keil, 1984). Natural enemies periodically suppress leafminer populations and also foliar applications of entomophagous nematodes have been reported to significantly reduce adult development of *L. trifolii* (Harris *et al.,* 1990). A few species of these entomopathogenic nematodes have been found infecting *Liriomyza* species. These are; *Heterohabditis heliothidis, Heterohabditis megidis, Heterohabditis* sp. (strain UK 211), *Steinernema carpocapsae* (Weiser) and *Steinernema feltiae* (Filipjev) (Hara *et al.,* 1993; Williams and Walters, 2000).

Recent field studies in Nyeri District, Kenya indicated that a range of pesticides are being routinely used by farmers against peas pests (Gitonga *et al.,* 2010). The authors gave a list of the most frequently used pesticides some of which were used in this study (Table 1).

1.2 Statement of the problem

Liriomyza huidobrensis is regarded as one of the most important pests of horticultural field crops in the tropics and sub-tropics. It is believed to be of Neotropic origin and currently is a big threat to pea production in Kenya. It is characterized by high degree of polyphagy and the extent to which it invades new geographical regions (Anderson *et al.*, 2002). Several pesticides including organophosphates (Methyl parathion), carbamates (oxamyl), pyrethroids (permethrin) and triazines (cryomazine) were identified for *Liriomyza huidobrensis* control during the outbreak period. However, resistance of the leafminer to these pesticides developed with time and they could no longer be relied on for control (Price and Nagle, 2002). Synthetic and natural insecticides for leafminer control have been extensively researched and are commonly used by farmers and producers regardless of production scale and crop (Tong-Xian *et al.,* 2009). The specific efficacy of these insecticides against *L.huidobrensis* in Kenya has however not been well understood. It is also not clear whether there are leafminer parasitoids in the field parasitizing the pest, their efficacy and the effect of the commonly used pesticides against them.

1.3 Justification of the study

Small scale farmers in Central Kenya depend on cultivation of pea crop as a major source of income. *Liriomyza huidobrensis* have been a major obstacle to pea production in this region. Developing a successful approach in the control of the pest would not only reduce the costs involved in the control, but also would result in increased yields, hence better living standards. Therefore, this study focused on this part of Kenya in order to understand the best method for pests' control which will help maximize profits from the crop. A survey carried out in the area earlier showed that these farmers use insecticides as a major control measure for leafminer flies which is rampant in the area (Gitonga *et al.*, 2010). In order to reduce the amount of insecticide application, there is need to test and develop specific Integrated Pest Management (IPM) system based on the use of selective insecticides in order to keep the level of leafminers at sub-economic levels. Parasitoids have been known to be effective in the control of LMF (Bjorksten, 2005). However, insecticides use has harmful effects on the parasitoids, making biological control difficult (Tong-Xian *et al*., 2009). There is a need to determine the population of the parasitoids in the field and their effectiveness in the control of *L. huidobrensis* as well as the possible effect of the pesticides used by farmers on these parasitoids.

1.4 Research questions

i. Do the parasitoids, temperature and rainfall affect the population dynamics of *L. huidobrensis*?

ii. What are the effects of pesticides application on the mortality of *L. huidobrensis* and its parasitoids?

iii. Which dosage of pesticides would control *L. huidobrensis* without harming its parasitoids?

1.5 Hypotheses

i. Parasitoids, temperature and rainfall do not affect the population dynamics of *L. huidobrensis* and its parasitoids.

ii. Pesticide application has no effect on the mortality of *L. huidobrensis* and its parasitoids.

iii. The recommended dose of pesticides does not affect *L. huidobrensis* or its parasitoids.

1.6 Objectives of the study

1.6.1 General objective

To investigate the effects of pesticide application on the population dynamics of *Liriomyza huidobrensis* and its parasitoids on Snow peas and Sugar snaps.

1.6.2 Specific objectives

i. To determine the effects of parasitoids, temperature and rainfall on the population dynamics of *Liriomyza huidobrensis*.

ii. To invesitigate the effects of pesticide applications on mortality of LMF and their parasitoids in the field and in the greenhouse.

iii. To evaluate the optimum dosage for pesticide application that would control *L. huidobrensis* and protect its natural enemies.

CHAPTER TWO

2 LITERATURE REVIEW

2.1 Snow peas and sugar snap production systems and constraints

Snow peas (*Pisum sativum* var *saccharatum)* and sugar snap (*Pisum sativum* var *macrocarpon*) are cultivated for the fresh green seeds and tender green pods, which is a major source of income for small scale growers in many parts of Kenya. Snow peas and originated in Central or South East Asia. In Kenya, Snow peas production is growing in importance, stimulated by the increasing demand in Europe (HCDA, 2011). It is grown in various parts of Kenya such as Nyeri (Central province), Timau (Eastern province) and Naivasha (Rift-valley province) among others.

Pea is an annual legume with slender stems. The leaves end in one or more tendrils and have one to three pairs of leaflets. The pod is 5-10 cm long and may be straight or curved containing up to 10 globular smooth or wrinkled seeds. Sugar snap are edible pods but they have larger sweeter seeds and thicker pods than snow peas (Plate 1 and 2), HCDA, (2011). The major importing countries in Europe are the United Kingdom, Holland, France, and Germany. With increasing demand in the international markets, there is ample room for expansion of production in Kenya. These crops contribute significantly in horticultural industry. However, yield losses of up to 30% have been reported due to pests (HCDA, 2011). Other pests includes African bollworm (Helicoverpa spp.), Thrips, Aphids, etc. Diseases, nematodes, insufficient rainfall, poor agronomic practices, soil infertility are among other constraints to pea production in Kenya.

Plate 1 Snow Peas pods ready for export

Plate 2 Sugar Snaps harvestable pods

2.2 Species composition and abundance of *Liriomyza* leafminers

Liriomyza leafminers are widely distributed in many parts of the world, including Kenya. There are 376 species recognized in the genus *Liriomyza* of which more than 20 have been reported as being economically important and at least six species are polyphagous: *Liriomyza sativae* (Blanchard), *Liriomyza trifolii* (burgress), *Liriomyza huidobrensis* (Blanchard), *Liriomyza bryoniae* (Kaltenbach), *Liriomyza strigata* (Meigen) and *Liriomyza langei* (Frick) (Tong-Xian *et al.*, 2009). The most important *Liriomyza* leafminers in crops in Africa are *L. huidobrensis, L. sativa* and *L. trifolii* (Chabi-Olaye *et al.,* 2008). These three *Liriomyza* species have been reported to pose a worldwide threat to horticultural field crops (Murphy and La Salle, 1999). The adult flies of this family look very similar making separation of these species difficult. Close examination reveals small external differences that can be used to separate the species, such as the relative lengths of sections along particular wing veins, the presence, position and size of certain setae or the colour of the cuticle at the point where particular setae arise (OEPP/EPPO, 2005). The pea leafminer, *L. huidobrensis* is the most important pest of snow peas and sugar snap, two crops of high horticultural value in Kenya (Njuguna *et al.*, 2001; Kedera and Kuria, 2003).

2.3 *Liriomyza huidobrensis*

Liriomyza huidobrensis originated from South and Middle America and since 1985 has spread to many parts of the world including Africa (Weintraub and Horowitz, 1997). The adult leafminer is slightly larger (1-2mm) than other *Liriomyza* species, darker in colour, and has a bright yellow thoracic shield (Plate 3). Larvae are slightly whiter than those of *L. trifolii* (milky to yellowish white) and are at most 3.5 mm in length. However a binocular microscope is usually necessary to distinguish the larvae of *L.trifolii* and *L.huidobrensis*. Pupae are about 2.2 mm long. Pupae in a single crop and at the same age can nevertheless vary enormously in colour, from pale yellow to black (Koppert, 2003).

2.4 Bionomics of *Liriomyza huidobrensis*

Under favourable conditions, a population of *L.huidobrensis* can build up fast. According to Weintraub and Horowitz (1997), the leafminer undergoes complete metamorphosis i.e. egg, larva, pupa and adult (Fig. 1). Eggs are inserted inside the upper epidermis of the leaf and mining begins on this upper part, after which larvae tunnel towards the underside. The mines usually run along mid-veins and side veins but they can also run irregularly over the leaf (Koppert, 2003). Larvae do not come into contact with the outside air. Apart from their increasing size and the size of the mouthparts, the three larval instars are difficult to distinguish (Koppert, 2003).

In all *Liriomyza* species, before pupating the full grown larvae cut sickle-shaped exit holes in the leaf with their mouthparts. After about one hour, the larva crawls out of the leaf and falls to the ground *(L. sativa* and *L. trifolii*) or remains on the surface of the leaf (*L. huidobrensis*) where it pupates (Weintraub and Horowitz, 1997). Sometimes pupation may occur inside the petiole (Plate 4). At a temperature of about 19°C, the egg stage of *L. huidobrensis* lasts 2 days, the larval period 6 days, pupa 8 days, with a pre-oviposition period of 1-2 days. At this and slightly warmer temperatures, the life cycle lasts about 2.5 weeks (Varella *et al*., 2003). Adults are approximately 2 mm long. Females live up to 18 days while the males live for about 6 days. Mating begins at about one day of age and the oviposition rate peaks at 4 to 8 days after emergence (Steck, 1996). *Liriomyza huidobrensis* does well in cooler temperatures; Lanzoni *et al*., (2002) reported that the flies can undergo complete development at a temperature between 8.1°C (min) to 29.5°C (max) and an optimum temperature of 25.0°C.

Plate 3 Adult *Liriomyza huidobrensis*

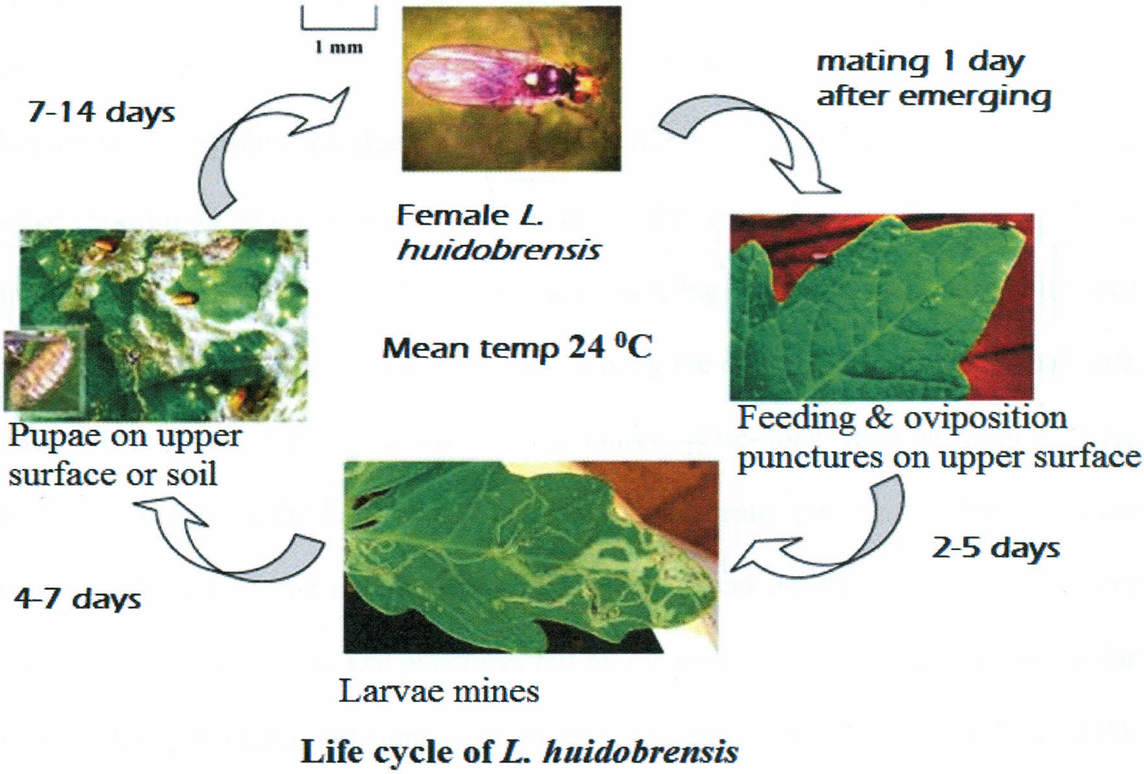

Fig. 2.1 Life cycle of a leafminer fly (Varella *et al.*, 2003).

Plate 4 Pupae of *L. huidobrensis* in the leaf petiole

2.5 Damage due to *Liriomyza huidobrensis* in vegetable production systems

The adult female bores a hole using its sharp ovipositor on the upper and lower parts of the leaf surfaces, for feeding or oviposition. This reduces plant vigor and its photosynthetic capacity, resulting in yield losses due to cosmetic damage to leaves and stems (Weintraub and Horowitz, 1997). Once the larva hatches from the egg, it immediately begins to eat into the leaf tissues, tunneling down into the mesophyll tissue where damage is caused by extensive mines, leaving the outer layers of the leaf and stalk intact. When more mines appear on the leaf, a large 'plate-mine' may be formed. These are mostly located at the base of the leaf. Because a greater part of the mine is on the underside of the leaf, the damage is less conspicuous and its effect is discovered later than with other leafminers. The punctures left by the female can serve as entry points for disease-causing bacteria and fungi such as *Alternata alternate* (Bjorksten *et al*., 2005), though in most cases they are not of economic importance (Koppert, 2003).

Crops such as lettuce and spinach, where the *Liriomyza* leafminer-damaged portions of the plant is harvested, are most likely to benefit from the biological control efforts implemented early in a certain growing period before the harvestable portion develops (Tong-Xian *et al*, 2009). Plants that have 25 % or more of the leaves mined late in the growing period, often suffer significant loss of photosynthetic surface and this extends the time required for crop maturation (Tong-Xian *et al*, 2009; Trumble *et al.,* 1985). Being a quarantine pest, *L. huidobrensis* spreads quickly in greenhouses. It is also known to spread outdoors in the field around infested greenhouses during hot weather. If not controlled, it might result to economically important losses in greenhouses (Anderson and Hofsvang, 2010).

2.6 Control of *Liriomyza huidobrensis*

Various methods have been widely used in efforts to control *L. huidobrensis*. These include; Use of insecticides, cultural practices, Use of biological control methods and integrated pest management.

2.6.1 Use of insecticides

Liriomyza huidobrensis has been a problem in production of vegetables and ornamental field crops in many countries. For most farmers, use of pesticides was the major pest control method. Several pesticides including organophosphates (Methyl parathion), carbamates (oxamyl), pyrethroids (permethrin) and triazines (cryomazine) were identified for *L. huidobrensis* control during the outbreak period. Gitonga *et al.* (2010) carried out a survey in Mount Kenya region in Nyeri district-Kenya and observed that, overall, 74% of respondents applied pesticides more often than usual, 61% increased pesticide concentration above recommended level while 58% used broad spectrum insecticides to avert leafminer damage. When asked whether the pesticides were effective in controlling leafminer, 65% of farmers reported that they were not effective while 35% stated that they were. The actual pesticide application rates were compared to the recommended rate, which showed that across all most commonly, used pesticides farmers tended to overdose. For example, 58%, 67% and 55%t of farmers who were interviewed exceeded the recommended application rates of Dimethoate, Dynamic and Cyclone respectively. These were the top three most commonly used pesticides.

In another survey by Sivapragasam and Syed (1999), it was observed that for the most popular insecticides used by farmers in Malaysia against *Liriomyza* leafminers, Abamectin, Cyromazine, Diazinon, Methamidophos, Chlorpyrifos, Prothiofos (organophosphates); Endosulfan (organochlorine), Deltamethrin, Cypermethrin (Pyrethroids), Cyfluthrin and Lamdacyhalothrin (pyrethrins) were the most commonly used. Among the farmers using insecticides, only 18% reported that insecticides were very effective, 72% reported that they were moderate and 5% reported that they were not effective at all. In terms of pesticide application, 71% of the farmers followed routine spraying while 27% sprayed after the onset of symptoms of attack. Most of the farmers (46%) sprayed on a weekly basis, 25% sprayed after every 5 days, 17% sprayed after every 3-4 days and 5% sprayed after every 10-12 days. According to the farmers, the main reasons for the indiscriminate use of pesticide use were mainly because of the following factors: Inaccurate diagnosis of the problem; Ignorance of the pesticide property, specificity and formulation; Use of wrong dosage rates; Poor spraying techniques; frequent irrigation; Pesticides resistance suspected and incompatibility of pesticide mixtures.

Botanical insecticides derived from seeds of neem tree, *Azadirachta indica (*Meliaceae) have shown promise in LMF control due to their physiological (insect growth regulating) and anti-feedant effects on a diversity of phytophagous insects (Weintraub and Horowitz, 1997). Neem products reduce fecundity and longevity of flies and disrupt the development of the maggots. They can be applied as drench or as foliar sprays. Weekly applications of aqueous neem seed extracts (ANSE) at 60 g/l and neem oil (2.5 - 3%) reduced leafminer damage on tomato (Varella *et al.,* 2003). Weekly foliar sprays with a commercial neem product (Neemros®) at rates of 25 and 50 g/l water and a spray volume of about 900 l/ha controlled leafmining flies on experime5rntal tomato fields in Kenya. Emergence of leafmining flies decreased with increased dosage (Varella *et al.,* 2003).

2.6.2 Cultural practices

Cultural practices are a broad set of management techniques or options which may be manipulated for agricultural producers to achieve their crop production goals. Cultural control on the other hand, is the deliberate alteration of the production system either the cropping system itself or specific production practices to reduce pest populations or avoid injury to crops (Graglia *et al*., 2006).

2.6.2.1 Detection and inspection methods

Small black and yellow flies may be detected flying closely around the crop or on the leaves. Inspection of the leaf surface will reveal punctures of the epidermis and the greenish-white mines in the leaves. Feeding maggots will be found at the end of the mine. When the maggots have already left the mine to pupate, the mine will end with a small convex slit in the epidermis. Occasionally the puparium may be found on the leaf surface, although in most cases they pupate in the soil (Varella *et al.*, 2003).

2.6.2.2 Trapping

Leafminers can also be monitored by foliage examination for the presence of mines and larvae and by trapping adult flies with yellow sticky traps. Yellow sticky traps used for mass trapping can effectively control the pest at low densities. Visual rating systems to assess the total number of leafminers on tomato have been developed in the USA (Varella *et al.,* 2003; Koppert, 2003).

2.6.2.3 Agricultural hygiene

Normal agricultural hygiene can play an important part in controlling leafminer damage: Varella *et al.,* (2003) advocated the following methods; Hand-picking and destroying of mined leaves, destroying all infected leaves and other plant material after harvest, ploughing and hoeing the soil before planting a new crop. This can help to reduce leaf mining flies by exposing pupae, which then would be killed by predators or by dehydration. Flooding the soil followed by hoeing could kill or release much of the buried pupae, which are then killed by exposure to natural enemies. Where new stock is obtained as seedlings rather than seed, careful examination for infected plants before planting will prevent introduction of pests.

Braun and Merle (1997) also advocated the following cultural control methods for *Liriomyza* leafminers: Destruction of plant residues from previous crops that were attacked. These can be burned or buried under the soil; Flowering weeds around the borders of the fields act as a reservoir for parasites, providing them with nectar needed for oviposition and longevity. These weeds should not be eliminated in order to maintain these natural enemies; Some host crops such as carrots and beets which are only slightly affected by LMF tend to harbour more parasites than other crops, probably because they are less sprayed frequently. These can be used in rotations or intercropped with susceptible crops such as potato; Use of sticky traps-Management of LMF with traps has given results comparable to those obtained with pesticides. They should be placed in and around the borders of the fields at about 10 cm above the foliage; Use of plant ash- Ashes prepared from dried plants of *Lantana camara* or from wood shavings may be applied as dusts from plastic talcum powder bottle.

2.6.3 Biological control

Biological control can be described as the reduction of pest populations by natural enemies and typically involves an active human role. Natural enemies of insect pest include predators, parasitoids and pathogens (Perdikis and Alomar, 2011). Biological control efforts have developed in two major agricultural areas: horticultural industries, under protected environments and in commercial vegetable production (Van der, 2004 and Trumble, 1990). Noyes (2004), listed over 300 species of Agromyzid parasitoids and over 80 species that are known to attack *Liriomyza* species. The most important parasitic wasps that parasitized on *Liriomyza* leafminers in greenhouses were *Dacnusa sibirica* (Telenga), *Diglyphus isaea* (Walker) (Hymenoptera: Eulophidae) and *Opius pallipes* (Koppert, 2003). *Phaedrotoma scabriventris* (Nixon) has recently been reported to parasitize leafminers. These wasps belong to the order Hymenoptera, family Braconidae and parasitize the larval stage of their hosts (Salvo and Valladares, 1995). Chabi *et al.*

(2008) observed a higher fecundity of *P. scabriventris* at lower temperatures and speed of mortality higher compared to codling moth

.

In central Argentina, Colombia, Mexico and Peru, *Chrysocharis caribea* (Hymenoptera: Braconidae) are an extremely important source of mortality on agromyzid leafminers with an average of 30 – 55% parasitism (Salvo and Valladares, 2001). *Diglyphus begini is* also used in United States for augmentative biological leafminer control (Sher *et al.*, 2000). *D sibirica* (Leuprecht, 1993), *O. pallipes* and *D. isaea* (Van der, 2004) are under consideration for use as natural enemies of the pest in European glasshouses. Amongst these parasitoids, *D. isaea* has been shown to be effective at higher temperatures (Minkenberg, 1989) and thus, could be effectively used in controlling leafminers in tropical environments. Neuenschwander *et al.* (1987) reported that invading leafminer populations declined naturally after a few years in Senegal due to the action of local natural enemies. Davies (1998) reported a significant control effect of locally occurring *Eucoilidea nitida* (Benoit) (Hymenoptera: Braconidae) and *Opius melanagromyzae* (Fischer) on *Ophiomyia spencerella* (Greathead), *O. phaseoli* Tyron and *Ophiomyia centrocematis* de Meijere in Mozambique. Integrated pest management approaches based on conservation of existing natural enemies and introduction of additional species, offer viable alternatives to the application of insecticides (Kang *et al.*, 2009). The need to introduce new biological control agents should, however, take into account the competitive risks of any potential candidate to extant species, as well as any potential non-target impacts to endemic non-insect pests and other beneficial species (Bokonon-Ganta *et al.*, 2005).

In general, biological control efforts under greenhouse conditions have focused on inoculation and augmentation strategies. Augmentative releases of natural enemies have been successfully applied in environments for control of many vegetable pests (Tong-Xian *et al.,* 2009). In field crops, conservation biological control has been the most successful approach (Reitz *et al.,* 1999). Interestingly, invading *Liriomyza* leafminer populations have sometimes been observed to decline naturally after a few years as the result of the action of the natural enemies (Murphy and LaSalle, 1999).

2.6.3.1 *Diglyphus isaea*

Diglyphus isaea is an ectoparasite belonging to the family Eulophidae (Plate 5). It aboriginal home is Europe, North Africa and Japan, but has now been introduced into other regions throughout the world (Koppert, 2003). The parasitoid has been recorded in Senegal, South Africa, Kenya and Zimbabwe._Although this parasitoid is of Palaearctic origin it has been reported to have successfully established in these countries (Musundire *et al.,* 2010). Being an ectoparasite, *D. isaea* lay their eggs on the bodies of the larvae where they feed by puncturing the larval epidermis and abandon the host larva (which then dies), to pupate within the mines. Weeds play an essential role in the biology of some parasites because their flowers provide the nectar necessary for adult fertility and longevity. *Diglyphus isaea* parasitize all four important species of leafminers (*L. huidobrensis*, *L. trifolii*, *L. bryoniae* and *Chromatomyia syngenesiae*) found in Europe (Koppert, 2003).

Plate 5 Adult *Diglyphus isaea*

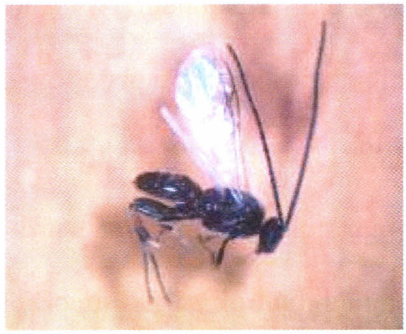

Plate 6 Adult *Phaedrotoma scabriventris*

Diglyphus isaea can parasitize many species of *Liriomyza* leafminer, and has been used commercially since 1984. Above 15°C a population of *D. isaea* grows faster than that of either its hosts or of endoparasitic wasps such as *P. scabriventris*. For this reason, at higher temperatures this ectoparasite gives better control of its hosts (Koppert, 2003). The presence of *D. isaea* in the crop is signaled by short mines in the leaves that contain dead larvae. The parasitic wasp punctures mainly first and second instar larvae in order to feed on their body fluids. Once parasitized by the wasp, the *Liriomyza* leafminer larva ceases feeding. Shortly before the host is finally inactivated, it expels the gut contents; a pierced larva can thus often be recognized by an extra quantity of excreted frass inside the tunnel. A few days later, the larvae turn flaccid and brown. Older larvae of *D. isaea* leave their dead host and crawl back into the mine to pupate. The female is generally rather larger than the male and can be recognized by broad black stripe over the hind leg, whereas males have two small black bands (Plate 5), Koppert, 2003. Despite the difficulties in quantifying the effects of indigenous parasitoids (often due to patterns in pesticides use), they should be treated as a resource and protected as much as possible (Tong-Xian *et al.*, 2009).

Diglyphus isaea, and *Halticoptera arduine* (Hymenoptera: Pteromalidae) have been found to cause 35.0 – 72.9% mortality to *Liriomyza* leafminers in Chile, Peru and Argentina and are of global distribution (Murphy and LaSalle, 1999). In Africa, large-scale mass-production programmes of *D. isaea* have been developed to support biological control of leafminer efforts in Kenya and South Africa through augmentative biological control approaches (Musundire *et al*., 2010).

2.6.3.2 *Phaedrotoma scabriventris*

Phaedrotoma scabriventris is an endoparasitoid belonging to Braconidae family (Plate 6). In this family, only a few species have their biology known. Braconidae are koinobiont Parasitoids of Diptera, especially of mining larvae of Agromyzidae, Anthomyiidae and Drosophilidae among others (Achterberg and Salvo, 1997). *Phaedrotoma scabriventris* originates from the Neotropics and has been reported in several parts of Argentina, Chile, Brasil and Peru (Salvo and Valladares 1995; Mujica and Cisneros 1997) where it is often found as the dominant parasitoid of *L. huidobrensis* representing up to 50 % of the total parasitism (Salvo and Valladares 1995). It has been considered as the most important parasitoid of the leafminer and as a potential agent for its population regulation (Valladares *et al*., 2001). In Argentina, Salvo (1996) recorded *P. scabriventris* as an important parasitoid of leafminer flies in Cordoba, central Argentina (800 m a.s.l.), with an average annual temperature of 16°C (max. 24°C, min. 9°C). In natural habitats of Central Argentina, Salvo and Valladares (2001) examined the temporal community dynamics of Agromyzidae and their parasitoids, and found a frequent dominance of *P. scabriventris* during winter. This could be the result from a close affinity of this polyphagous parasitoid to one of the most common winter host *Haplopeodes lycivorus*, or is just its relatively good performance under cooler winter conditions (CIP, 2008).

Phaedrotoma scabriventris has not yet been fully studied. The total development period (oviposition to adult emergence) of *P. scabriventris* decrease with increasing temperature between 15 and 30 °C. Mean immature developmental time (egg to adult) at 15, 20, 25, 30°C was (for both sexes) 30, 25, 13 and 9 days, respectively. At 10 °C no development was found. The average female longevity observed was 40 (10°C), 27 (15°C), 18 (20°C), 14 (25°C) and 8 (30°C) days. The mean number of progeny produced per *P. scabriventris* was a 33, 120, 100, 80 and 70 individual per female, between 10 and 30°C. No pre-oviposition period between 15 and 30°C was observed. A male: female sex ratio of 2:1 was determined in the offspring of *P. scabriventris*. No significant difference was observed in the mean number of male and female progeny produced between 15 and 30°C. Also host feeding behavior was not observed (CIP, 2008).

2.6.4 Integrated Pest Management

Integrated pest management is defined as the optimization of pest control in an economically and ecologically sound manner. It is a recipe of biological, cultural, genetic, mechanic and chemical tactics used individually or in combination to maintain pest damage below the economic injury level, while providing protection against hazards to humans, animals, plants and the environment (Mwangi, 2004). Some preliminary attempts using the IPM approach were made for *Liriomyza* leafminers on sugar snaps. In those IPM trials Sivapragasam *et al.* (1995) and Myint (1997) proposed a more comprehensive approach for sugar snap which included carrying out sanitation, covering beds with straw, determining adult populations using yellow sticky traps and application of the insecticide abamectin alternating with cyromazine until flowering. Myint (1997) also suggested the economic threshold level to be 13 to 36 (average 26) larvae per 10 leaves or 12 - 22% (average 17) of leaves mined, equivalent to 132 flies per yellow sticky trap per week.

There is a great need to use lower doses of the commercial insecticides in order to reduce the massive deaths of the natural enemies. Probably, the most important strategy to maximize naturally occurring biological control has been the use of selective pesticides, which have minimal impact on LMF parasitoids. This strategy has been used to develop widely used IPM programmes for a variety of crops (Trumble and Rodriguez, 1993).

CHAPTER THREE

3 MATERIALS AND METHODS

3.1 Study site

The field study was conducted in Nyeri County, Central Kenya in three locations; Naromoru (S27°23.2183'W), 2,036 meters above sea level (m. a.s.l); Kabaru (S28°37.2137'W), 2,530 m. a.s.l and Sagana (S35°37.1875'W), 1,208 m a.s.l. (Fig. 3.1). Generally, the area is cool and wet with annual mean temperatures of 23° C (max. 26° C and min. 19° C). It receives annual mean rainfall of 1,700 mm which usually occurs in bimodal patterns of March-July and October-December. These form the main growing seasons in the area. Naromoru has black cotton soil and clay in some areas, Kabaru has loam soil while Sagana has volcanic soil. All laboratory and greenhouse experiments were conducted at the International Centre for Insect Physiology and Ecology (*icipe*) in Nairobi, Kenya (1°, 18'S. 36°49E, 1798m a.s.l).

3.2 Field experiment

The field trials were established on-farm at small scale farmers' fields that produce peas for both export and domestic markets. Farmers were allowed to plant and manage their fields, but all inputs such as seeds, fertilizers, and insecticides were provided. At each location, four farmers; two growing snow peas and two growing sugar snap were selected where out of each category one farmer produced for export market and the other one produced for domestic market.

Each farmer's field was divided into three plots, each plot having different management system as follows:

(a) Farmer's Field system (FF); this is a plot whereby the farmer was allowed to manage his farm as desired. The farmer decided on the method of pest control.

(b) Reduced Pesticides application system (RP); in this plot the researcher did scouting of the pest population and only used the pesticides when the LMF numbers were more than 15 flies per quadrant. Table 3.1 below shows the list of chemicals that were used in this plot as compared to the FF.

(c) Control (C0); the plot was not treated with any chemical.

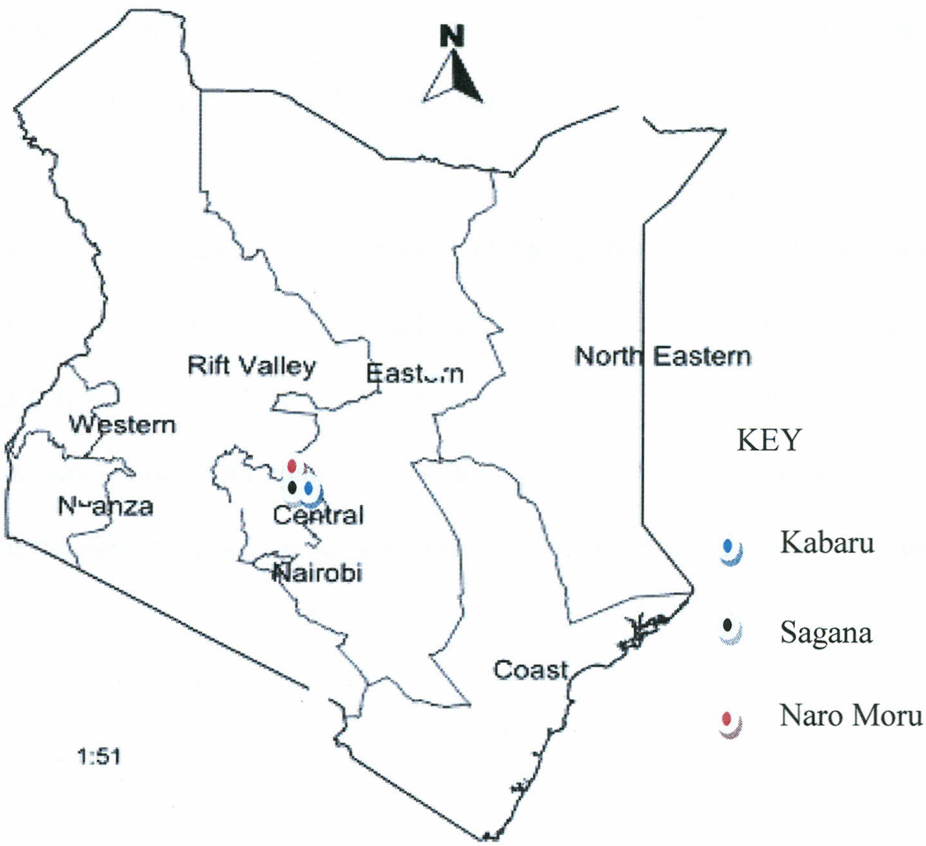

Fig 3.1 A map of Kenya showing study sites

Table 3. 1 Frequency of chemical application in reduced pesticide plot (RP) as compared to farmers plot (FF).

Location	Chemicals used	Frequency of chemical application	
		FF	RP
Sagana	Cyclone, Milraz, Ridomil, Dimethoate, Methane, Folicur, Dithane, Thunder, Bestox, Dynamec	14	10
Kabaru	Antracol, Bulldock, Triger, Thunder, Milraz, Dynamec, Folicur, Dimethoate	13	8
Naromoru	Dimethoate, Milraz, Cyclone, Cumulus, Thunder, Bestox, Folicur, Dynamec	13	6

The field size was approximately 200m^2 in each of the farmer practice plot and about 100m^2 in each of the other two plots. The study was conducted in two cropping periods (under irrigation), from February-April and then from May-August 2009. The distance between farmers was about 200m apart in Sagana and about 500m apart in Kabaru and Naromoru while the shortest distance between the three management systems was about 50m apart.

3.2.1 Determining the effects of Parasitoids, temperature and rainfall on the population dynamics of *Liriomyza huidobrensis*

The population of both the parasitoids and *L. huidobrensis* present in the field was determined from sampled leaves, sticky traps and using the one step-count method.

3.2.1.1 Sampling of leaves

Sampling of infested leaves was done once a week throughout the study period. At every sampling occasion, each plot was subdivided into four equal quadrants and 160 leaflets (40 per quadrant) were plucked at random from FF plot while, 80 leaflets (20 per quadrant) were plucked from the reduced pesticide and control plots. The plucked leaves from each plot were kept in separate plastic bags and kept out of direct sunshine. The plastic bags were marked with the name of the farmer, plot code, date, type of crop (sugar snap or snow pea) and the field number. The samples were taken to the *icipe* laboratory on the same day for further assessment. Sampling was run for 12 weeks until harvest. The population of *Liriomyza* leafminers on the crop leaves was also monitored using a one-step count (0.70 m). In this case, each plot was divided into four quadrants and from each a quadrant, one would make a single step, at random, of approximately 0,70m and then count all the flies occurring within a line transect. This was repeated four times.

In the laboratory, the total number of leaves infested with LMF (those containing a puncture or mine) were counted and recorded. The leaflets per plot were then placed in large plastic container and stored separately for a week. After a week the total LMF pupae and emerging natural enemies were counted and recorded. The LMF pupae were put in a glass vial and stored till the flies emerged. Emerging *Liriomyza* flies and parasitoids were preserved in 95% ethanol for identification. Finally the total LMF adults were counted after about two weeks and a sample of 10 adults were identified per leaf sample.

3.2.1.2 Use of sticky traps

During the first cropping period, two yellow sticky plastic card (40 cm x 40 cm) coated with a layer of polybutene were positioned approximately in the middle of each plot (per farmer) at 40 cm above ground on two wooden sticks making a total of six traps for each farmer. The yellow plastic card was smeared on both sides with adhesive automobile grease. In each plot, one of the traps was placed facing the E-W direction while the other one faced the N-S direction. During the second cropping period, only a single trap (it was not necessary to use many traps as there was no significant difference between them) was placed at the middle of the three plots facing N-S direction. The traps were placed in the fields before crop emergence (5-7 days after planting). The aim of the trapping was to monitor the density of *L. huidobrensis* and its parasitoids. They were removed from plots every 7 days and replaced by new ones. In the laboratory, each trap was divided into 16 cells each of 5 cm x 5 cm. Captured flies were counted and identified at random from 4 cells per trap. This study was run for 12 weeks until harvest.

3.2.1.3 Use of one- step count

The population of *L. huidobrensis* on the crop leaves was also monitored using a one-step count (0.70 m). In this case, each plot was divided into four quadrants and from each a quadrant, one would make a single step, at random, of approximately 0,70m and then count all the flies occurring within a line transect. This was repeated four times.

The daily rainfall and temperature data were collected from the records at the meteorological department at Nyeri district every month.

3.3 Laboratory experiments

The experiments aimed at investigating the effects of insecticides on leafminer flies and their parasitoids so as to evaluate the effectiveness of various doses of chemicals studied. The chemicals tested were the commonly used chemicals in Central Kenya (Nyeri) as reported by Gitonga *et al.* (2010). These pesticides were; Bulldock, Dimethoate, Dynamec, Bestox, Thunder, Cyclone, Folicur and Milraz. They were tested in this study as all were used against leafminer flies except Milraz and Folicur which are fungicides. The concentrations tested in this study, were calculated from the recommended dosages provided by the supplier, where two doses were below the recommended doses and two above the recommended doses. This was in an effort to determine the best dosage for both LMF and the parasitoids. These concentrations were different for the chemicals used since each of them has a different recommended dose from the other. Gitonga *et al.* (2010) also noted that the farmers used a higher rate than the recommended in all the chemicals tested except Folicur and Cyclone (Table 3.2).

Host plants (Snow peas and Sugar snaps) were grown in a greenhouse. The soil used for planting was mixed with the farmyard manure in the ratio of 2:1 and then the seeds were placed about three centimeters down the soil in a 500g pot. About 50 ml of water was added in each pot and the set up was left in the greenhouse for three weeks. The plants were watered daily where each plant received about 50 ml of water until the end of the experiment.

3.3.1 Investigating the effects of pesticide applications on mortality of LMF and their parasitoids in the laboratory.

Peas were planted in 10 pots each week for 8 weeks at the greenhouse. Three weeks after planting, the plants were exposed to about three adult *L. huidobrensis* each (obtained from laboratory cultures at *icipe*) for 24 hours for oviposition to take place. Exposure was done in a holding cage at the greenhouse in *icipe.* The infested plants were transferred to another holding cage until about 6-8 mines had formed in about 10 leaves. The leaves were excised using a pair of scissors and taken to the laboratory.

A total of 100 live larvae were counted on the leaves under a light microscope. The leaves (with a total of 100 larvae) were put together in a glass Petri-dish and treated with the insecticides. Six different Petri-dishes each holding a total of 100 larvae (in the leaves), were used for each insecticide. Each 100 individuals represented different concentrations per each insecticide. Five insecticides, namely; Bulldock, Dimethoate, Dynamec, Thunder and Bestox were used, each with four concentrations one blank (untreated) and one with distilled water.

Table 3. 2 List of pesticides tested against *L.huidobrensis* and its parasitoids in the laboratory experiment at *icipe*

Trade name	Active ingredients	Pesticide Type	Target pests	Recommended rate (ml/l)	Farmers rate (ml/l)
Bulldock	beta-cyfluthrin	I	Cutworm, aphid, lmf	0.75 ml	1.0 ml
Dimethoate	Dimethoate	I	Aphids, cutworm, lmf	1.5 ml	1.6 ml
Milraz	Propineb/cymoxanil	F	Blight, P. mildew	2.0 g	2.95 g
Dynamec	Abamectin	I	*Liriomyza* spp.	0.5 ml	0.75 ml
Folicur	Tebuconazole	F	P. mildew, blight	0.75 ml	0.5 ml
Bestox	Alphacypermethrin	I	Cutworm,thrips,lmf	0.5 ml	0.9 ml
Thunder	Imidacloprid/beta-cyfluthrin	I	Thrips, lmf	0.5 ml	0.75 ml
Cyclone	Chlorpyriphos	I	Cutworm,aphid,lmf	1.5 ml	1.3 ml

Notes: I = insecticide, F = fungicide, lmf = leafminers, P.mildew=Powdery mildew

After preparing the concentrations, the mined leaves in each Petri-dish were immersed for 5 seconds into each pesticide concentration. Each was placed at a time to ensure proper coverage. The treated leaves were then placed in trays and dried in the open for about 15 minutes after which they were put back in the Petri-dish lined with a paper towel and taken to the laboratory (Temperature of 25°C maintained). After about 5 days, the number of pupae that formed were counted from each petri-dish and recorded. They were kept under same temperature conditions until the adult LMF emerged (after approximately a week). These too were counted and recorded. Larval Mortality was determined based on the total number of larvae that failed to pupate. The pupa that failed to develop into adult flies was also recorded. The whole experiment was replicated four times.

3.3.2 Evaluating the optimum dosage for pesticide applications that would control *L. huidobrensis* and protect its natural enemies

The optimum dosage was assessed using different chemical concentrations on *L. huidobrensis* and its parasitoids; *D. isaea* and *P. scabriventris*

3.3.2.1 Evaluating the optimum dosage for adult *Liriomyza huidobrensis*

Adult *Liriomyza huidobrensis* were obtained from the mass rearing stock culture at *icipe*. All the pesticides recorded in the table 1 above (fungicides and insecticides) were used. Each pesticide treatment included four concentrations (calculated from the recommended dose) and one control. For each pesticide treatment, a total of 25 adult *L. huidobrensis* were used.

The procedure involved first preparing the concentrations for each chemical. About 15ml of each of these concentrations were poured separately in a 20ml glass vial, swirled and then emptied. This was done to ensure that the chemical was distributed evenly throughout the vial. The container was then sun dried for 6 hours to prevent the flies from drowning in the chemical. The 25 *L. huidobrensis* adults were placed into the glass vial and the vial covered with a perforated cork to prevent suffocation due to lack of oxygen supply. Cotton swabs saturated with honey/sugar solution were placed at one corner of the cork, to provide food to the flies during testing. The treated vials were taken to the laboratory immediately. Adult mortality was recorded after every 24 hours for three consecutive days. Trials were replicated four times.

3.3.2.2 Evaluating the optimum dosage for adult parasitoid flies

Two parasitoids, *D. isaea* (obtained from Dudutech Kenya Ltd) and *P. scabriventris* (obtained from the laboratory cultures at *icipe*) were used. All the pesticides (insecticides and fungicides) recorded in Table 1 above were used. The same concentrations, methods and replicates used for the adult *Liriomyza huidobrensis* were used for the parasitoids.

3.4 Data Analysis

Data on percentage mortality in the field and laboratory was analysed using ANOVA after angular transformation, a transformation used to normalize percentage data. Graphs were plotted to display parasitoid and leafminer abundance in the field. Probit analysis using procedure probit in SAS software (2008) was used to estimate the LC_{50} and LC_{90}. Means were separated using the Student- Newmans-Keuls (SNK). Correration coefficient was done to test for significance of the abiotic factors (Temperature and rainfall) on the population of LMF.

CHAPTER FOUR

4 RESULTS

4.1 Effects of parasitoids, temperature and rainfall on the population dynamics of *L. huidobrensis*

Biotic (Parasitoids) and abiotic (temperature and rainfall) factors produced varying degrees of mortality on both *L. huidobrensis* and its parasitoids.

4.1.1 Effects of rainfall, parasitoids and temperature on weekly emergence of *L. huidobrensis* from sampled leaves in Kabaru location

In Kabaru during the first cropping season, emergence of *L. huidobrensis* in week 2 was 750 and increased to 800 adult flies in week 3. In the subsequent weeks, the numbers decreased to about 200 to 300 flies although two peaks of 500 and 400 were recorded on week 5 and 12 respectively (Fig. 4.1A). During the second cropping period, the number of leafminer flies that emerged dropped from about 1000 on week 2 to about 50 flies on week 5. On week 6 the numbers increased to about 200 flies and remained so till the end of the cropping period (Fig. 4.1B). More leafminers emerged during the first cropping period as compared to the second.

No rainfall was recorded between weeks 2-7 of the first cropping period (Fig. 4.1A). Thereafter the volume increased gradually to a maximum of about 100cm^3 on week 10 before going down to zero (Fig. 3A). During the second cropping period, a high amount of rainfall (about 800cm^3) was recorded on week 2 before reducing sharply on week 3 to zero (Fig. 4.1B). There was no more rainfall throughout the subsequent weeks of the second cropping period except on week 6 when about 50 cm^3 was recorded (Fig. 4.1B). On week 2 after the rains, the number of LMF drops sharply from about 1000 flies to zero on week 5 (Fig. 4.1B). Very few parasitoids (about 0-5 flies) were recorded between weeks 2-8. As from week 9 (harvest time) however, the numbers increased reaching a peak of 27 parasitoids on week 11 (Fig. 4.1A). During the second cropping period, peaks of 13, 15 and 22 parasitoids were recorded on week 3, 7 and 11 respectively. However, no parasitoids emerged on week 9 (Fig. 4.1B).

Very slight changes in temperatures were observed throughout the study period. These average temperatures appeared to have no relationship with the number of leafminers that emerged from sampled leaves in Kabaru. For example, though there was a slight drop in temperature on week 13, the leafminers still remained low (Fig. 4.2A). In addition, though the leafminers increased from about 150 to over 500 flies (18th-20th weeek) followed by a sharp drop on week 20-22, the average temperatures did not change significantly during that time (Fig. 4.2A). Very little rainfall was recorded throughout the study except on week 13 when about 10ml of rainfall was recorded. As was stated earlier, during and after the rains (13th -18th week) leafminers that emerged dropped sharply (Fig. 4.2A).

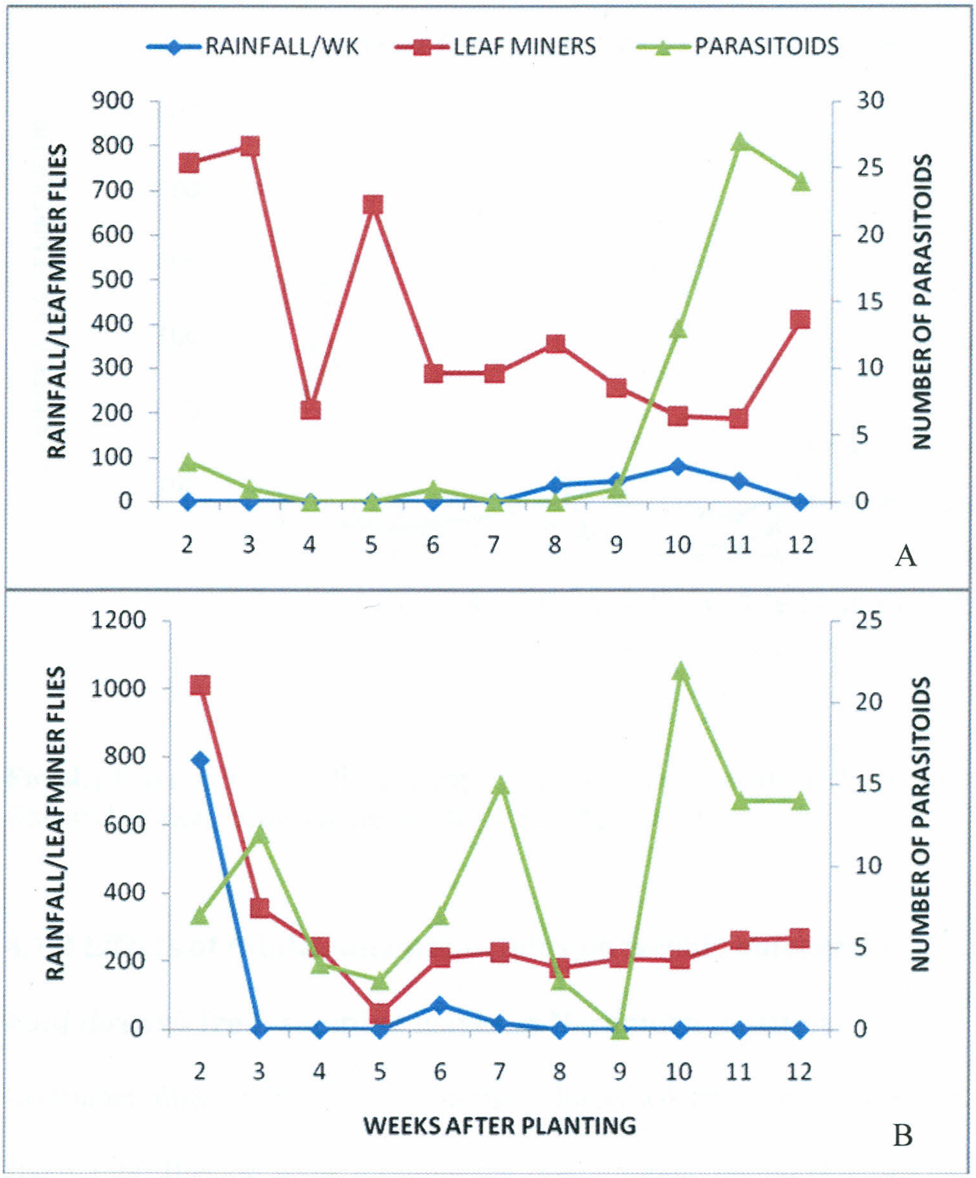

Fig 4.1 Effects of rainfall and parasitoids on emergence of *L. huidobrensis* per week, in Kabaru location. A: First cropping period (Feb-Apr, 2009); B: Second cropping period (May-Aug, 2009).

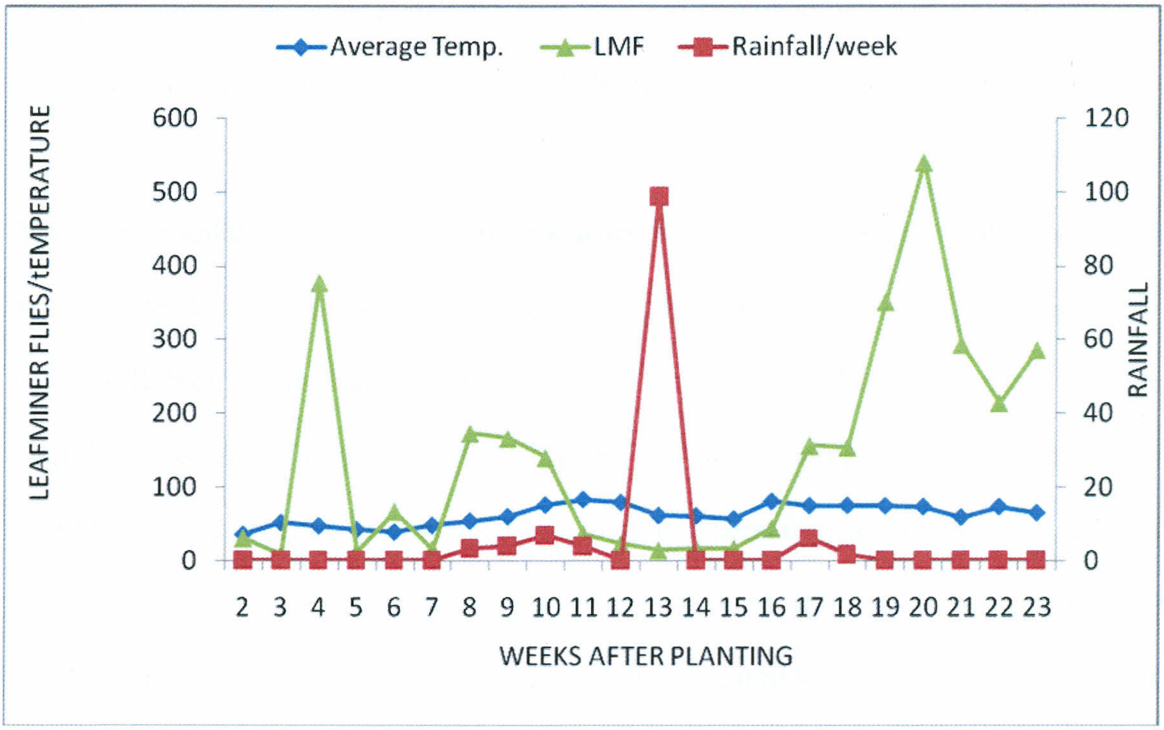

Fig 4.2 Effects of rainfall and temperature on emergence of *L. huidobrensis* per week in Kabaru location, between the month of Feb-Apr, 2009

4.1.2 Effects of rainfall and parasitoids on weekly emergence of *L. huidobrensis* from sampled leaves in Naromoru location

Leafminer flies emergence in Naromoru increased from about 400 flies on week 2 to about 1200 flies on week 3 during the first cropping period (Fig. 4.3A). Thereafter the numbers almost levelled off to about 400 flies except for a sharp drop observed on week 7 from about 400 to 50 flies on week 8 (Fig. 4.3A). A slight increase in LMF emergence was observed on week 9 but the number levelled off threafter till the end of the cropping period (Fig. 4.3A). During the second cropping period, leafminers emergence dropped from about 1300 flies in week 2 to 200 in week 3. On week 4 a sharp rise in LMF emergence was observed (200-1100) before a collapse followed immediately after (Fig.

4.3B). Thereafter the number of LMF that emerged remained between 300 and 400 with exception of week 8 where a peak of about 500 leafminers (Fig. 4.3B).

Very little rainfall was recorded throughout the first cropping period with only a slight rise recorded between week 7-10 having about 100ml on week 10 (Fig. 4.3A). As was the case in Kabaru, immediately after the rains the leafminer flies emergence dropped slightly on week 11 (Fig. 4.3A). During the second cropping period, the amount of rainfall dropped sharply from about 1100 to zero. Thereafter, no rainfall was recorded except on week 6 when about 100ml were registered (Fig. 4.3B). The effects of temperature on the leafminer flies emergence, were negligible as was the case in Kabaru. Though the parasitoids were few, their numbers increased progressively from week 8 reaching a peak of about 27 parasitoids on week 12 of the first cropping period (Fig. 4.3A). The numbers dropped sharply during the second cropping period from about 15 parasitoids on week 2 to zero on week 4 and 5 (Fig. 4.3B). Few parasitoids (about 3) were recorded on week 6 and none at all on week 7. Thereafter, a gradual increase in parasitoid emergence was observed with a maximum of about 30 parasitoids being recorded on week 10. This however, was followed with a sharp drop on week 11 and 12 (Fig. 4.3B).

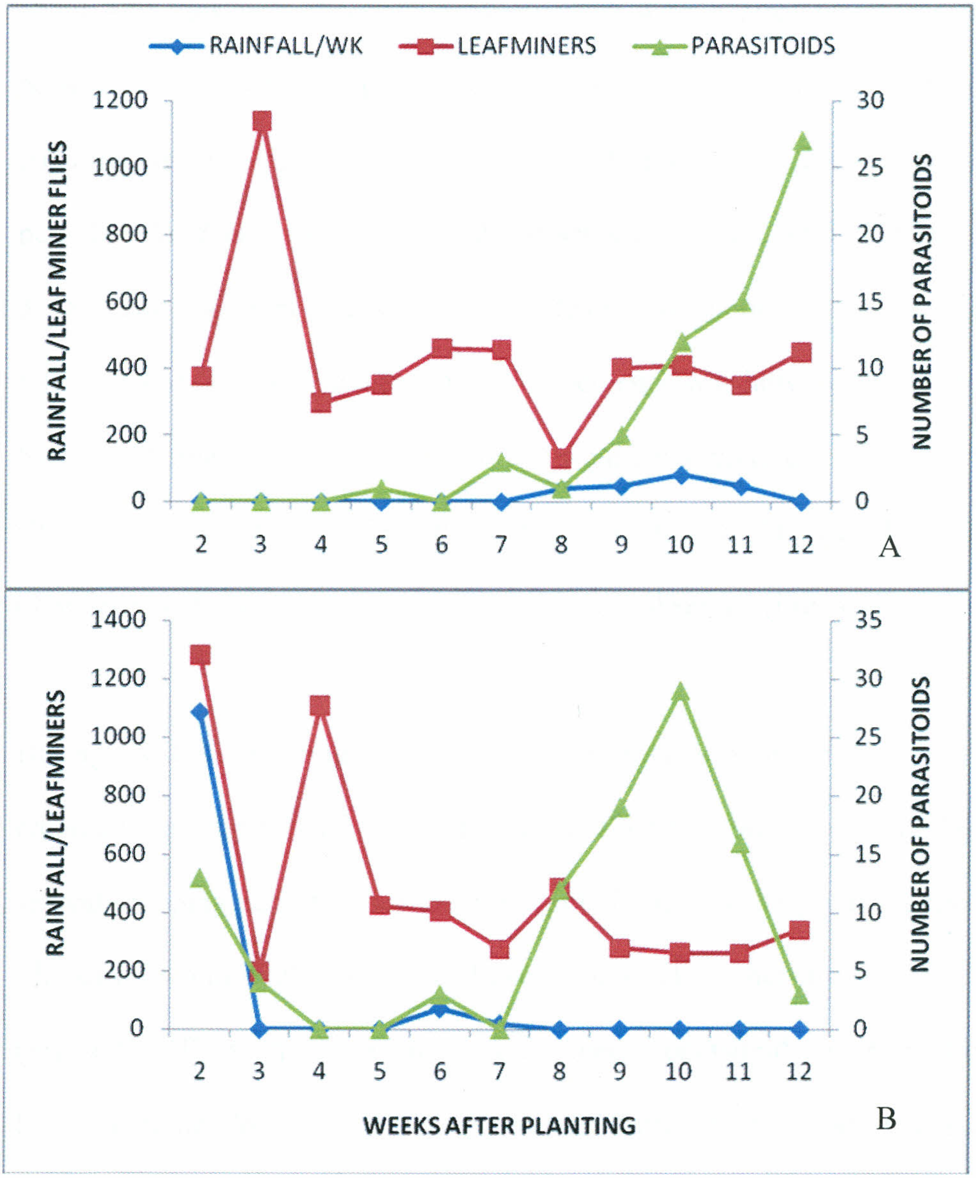

Fig 4.3 Effects of rainfall and parasitoids on emergence of *L. huidobrensis* per week, in Naromoru location. A: First cropping period (Feb-Apr, 2009); B: Second cropping period (May-Aug, 2009).

4.1.3 Effects of rainfall and parasitoids on weekly emergence of *L. huidobrensis* from sampled leaves in Sagana location

The flies emergence fell sharply from about 900 on week 2 to about 100 on week 4 followed by an increase on week 5 (Fig. 4.4A). An increase in numbers from 100 to about 400 flies was observed between week 4 to 5 after which the numbers ranged between 300 to 400 except on week 7 when a slight decrease in the number of flies emerging from sampled leaves was recorded (Fig. 4.4A). During the second cropping period, *L. huidobrensis* emergence decreased sharply from about 1200-200 in week 2 and 3. On week 5 a slight increase was recorded before another drop was registered on week 6 (Fig. 4.4B). A sharp increase in LMF emergence was however observed between week 6 and 7. Thereafter the numbers recorded went down up to the 8th week when an increase (from 400 to about 1000 flies) in was recorded (Fig. 4.4B). A gradual collapse followed on week 9 to 11 before another another rise was observed (Fig. 4.4B).

During the first cropping period, rainfall recorded was minimal with only a slight rise recorded between week 7-10 having about 100ml on week 10 (Fig. 4.4A). During the second cropping period, the amount of rainfall dropped sharply from about 1100 to zero. Thereafter, no rainfall was recorded except on week 6 when about 100ml were registered (Fig. 4.4B). Throughout the study, the number of parasitoids emerging from the sampled leaves remained low. No parasitoids emerged during the first four weeks after planting in the first cropping period (Fig. 4.4A). In week only 2 parasitoids emerged and no more were found till the 8th week. A gradual increase in numbers was registered between weeks 8-10 with the peak of about 14 parasitoids recorded in week 10. A sharp drop followed thereafter (Fig. 4.4A). In the second cropping period, a slight decrease in parasitoid emergence was observed between week 2 and 3. No parasitoids emerged on weeks 4-7 (Fig. 4.4B). A gradual increase in parasitoids numbers was observed between week 9 and 10 where a peak of 25 parasitoids was reached. Thereafter a sharp drop was observed followed by a slight increas on week 12 (Fig. 4.4B).

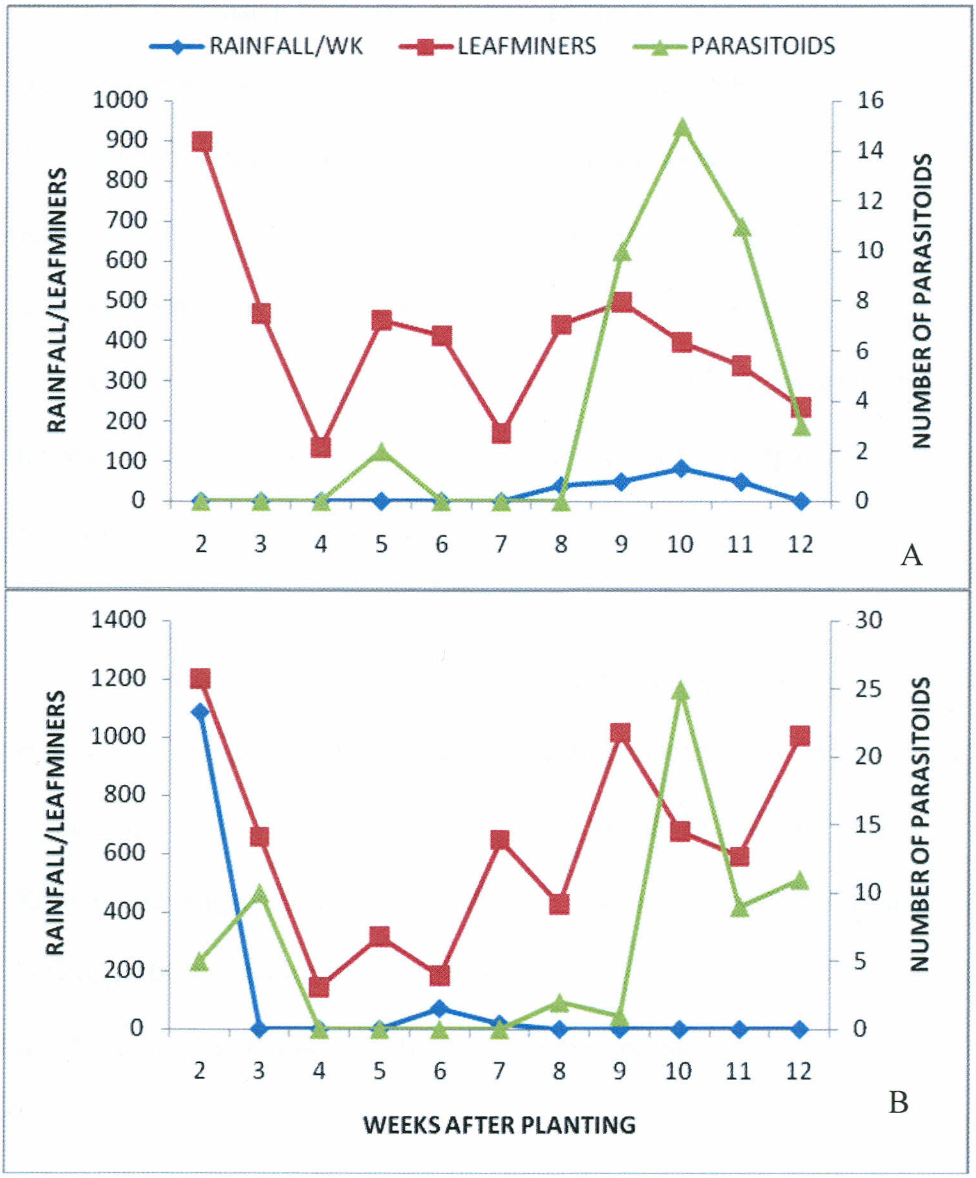

Fig 4.4 Effects of rainfall and parasitoids on emergence of *L. huidobrensis* per week in Sagana location. A: First cropping period (Feb-Apr, 2009); B: Second cropping period (May-Aug, 2009).

NB: In Kabaru, Naromoru and Sagana, 99% of the leafminer flies (LMF) collected from sampled leaves were *Liriomyza huidobrensis*.

4.1.4 Effects of parasitoids on the adult leafminers counted from sticky traps in the field

In first cropping period, the mean adult *L. huidobrensis* adults trapped in the field gradually decreased from the beginning (about 1400 in Sagana; 900 Kabaru; 780 in Sagana) up to the 5th week (about 600 in Sagana; 500 in Kabaru; 300 in Naromoru). Thereafter a significant increase was recorded where in Sagana (600-1000 flies) and Kabaru (500-800 flies) locations between week 5 and 7 (Fig. 4.5A). In Naromoru however, very slight increase was recorded followed by a drop. Thereafter the flies counted dropped gradually between weeks 7-12 in all the locations though in Sagana the numbers remained higher (about 400) in Sagana as compared to 200 and 100 flies in Kabaru and Naromoru respectively (Fig. 4.5A).

During the second cropping period, the mean number of *L. huidobrensis* sampled from sticky traps increased gradually decreased from about 100 in week 2 to about 500 flies in Sagana on week 11 before collapsing on week 12 (Fig. 4.5B). This trend was similar for Kabaru and Sagana but in Naromoru the numbers were low ranging to a maximum of between 100 to about 200 flies (Fig. 4.5B). The emergence during this cropping period was however much less than the first cropping period.

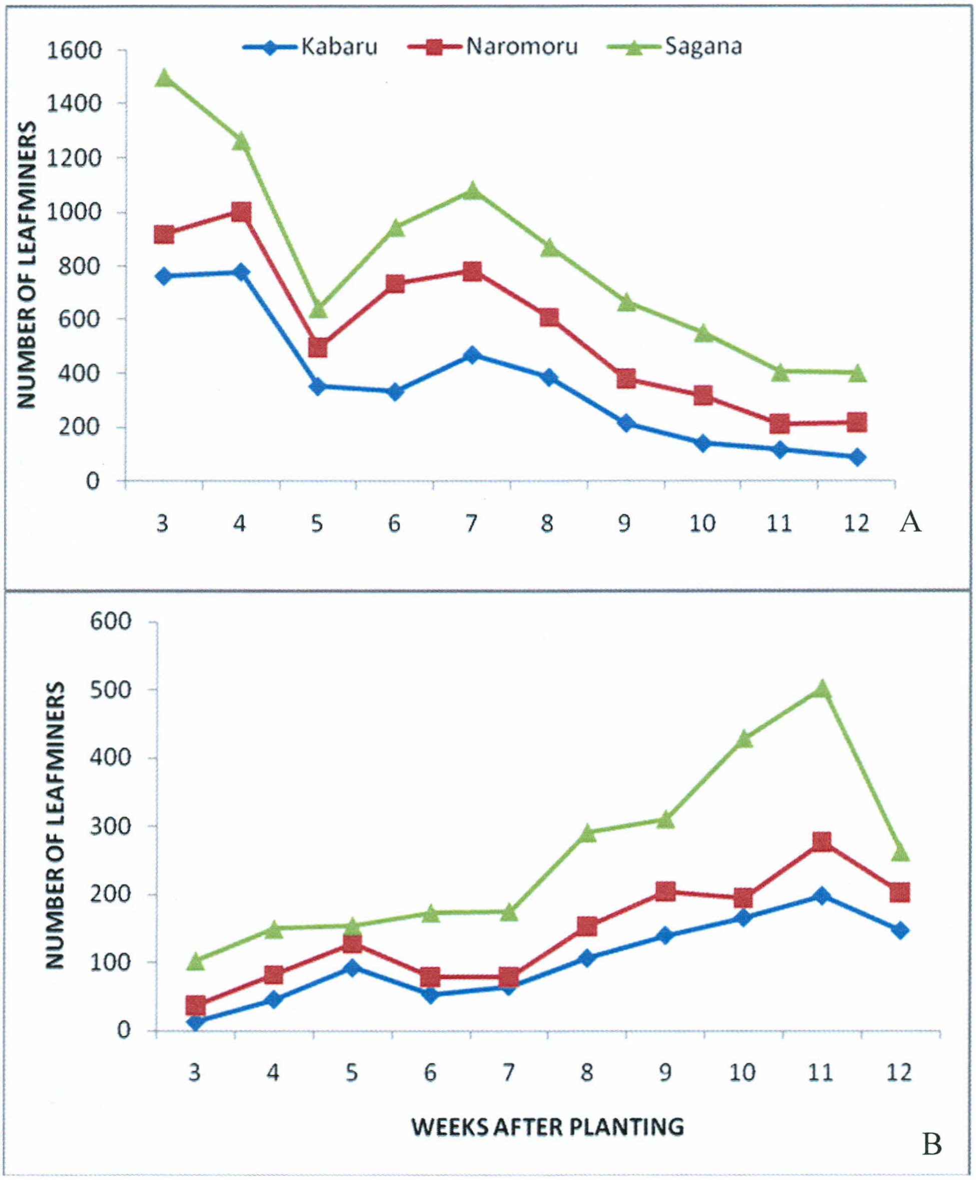

Fig 4.5 Mean numbers of LMF sampled from sticky traps per week. A: First cropping season (February – April, 2009); B: Second cropping season, (May – Aug, 2009)

4.1.5 Effect of parasitoids on the number of leafminers counted on the leaf surfaces using one-step count method in the field

During the first cropping period, the mean adult *L. huidobrensis* counted from the leaf surfaces decreased gradually from the 3rd week (about 2000 in Sagana, 1700 in Kabaru and 1200 in Naromoru) to the 6th week (about 750 in Sagana, 500 in Kabaru and 250 in

Naromoru), Figure 4.6A. However, a slight increase was recorded on week 6-8 in all the locations before a further decrease was observed. Throughout the season, Sagana recorded more adult LMF, followed by Kabaru and Naromoru had the lowest (Fig.4.6 A).

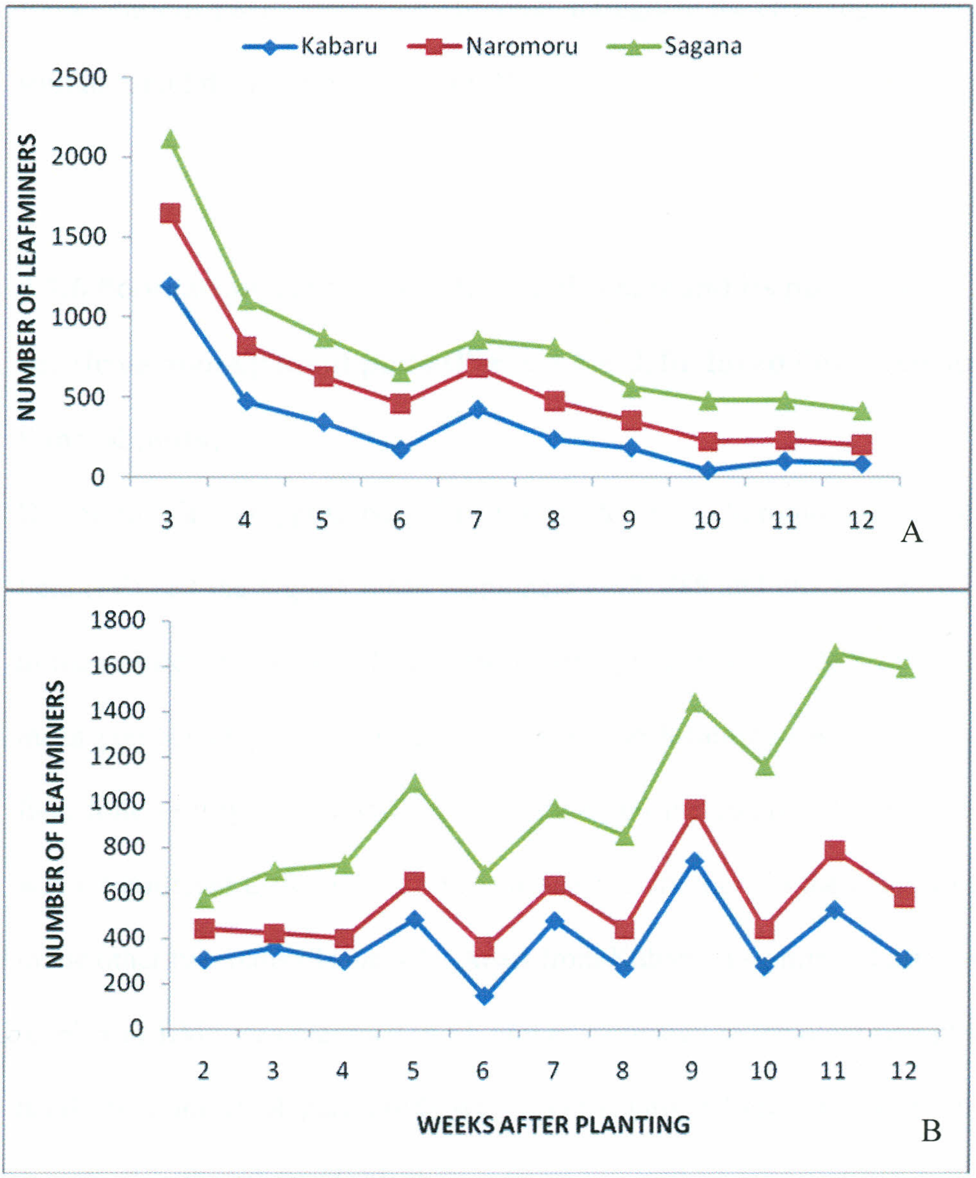

Fig 4.6 Adult LMF counted on the leaf surfaces per week using one-step count. A: First cropping season (February – May, 2009); B: Second cropping season (May – August, 2009).

During the second cropping period, mean adult LMF increased gradually from about 600 flies in Sagana on week 2, to about 1600 flies on week 11 before a slight drop (Fig. 4.6B). In Kabaru and Naromoru, the increase was lower than that of Sagana where the highest number of flies was 800 on week 9 in Kabaru and 700 in Naromoru on the same week. Various LMF peaks were observed throughout the cropping period, which were similar for all the locations (Fig. 4.6B).

4.1.6 Population dynamics of *L. huidobrensis* and its parasitoids in the field in the three management plots (Farmer Field, Reduced Pesticide application and Control plots)

During the first cropping period in Kabaru location, Naromoru and Sagana, the farmer field (FF) had the highest mean leafminers (392, 788 and 667 respectively) as compared to the reduced pesticide (RP) and the control plots (C0) (Table 4.1).On the contrary, the mean number of parasitoids recorded in all the locations was the lowest in the farmer field followed by the reduced pesticide plot and the control plot had the highest. There was a significant difference ($P<0.05$) in mean leafminers in the control plot as compared to the other two plots (Table 4.1). Apart from Kabaru, a significant difference ($P<0.05$) in number of LMF was recorded in FF plot as compared to the other two plots. On the other hand, the number of parasitoids were significantly different ($P<0.05$) in the C0 plot as compared to the others (Table 4.1).

Table 4.1 Leafminer counts and parasitoids numbers from different pea management system at different locations, for the first cropping period (Feb-Apr, 2009).

	Management system	Kabaru	Naromoru	Sagana	Mean
		Location			
Leafminer flies	FF	392±73.7a	788±147.5a	667±124.9a	615 (69.0)a
	RP	314±59.1a	92±17.8b	274±51.8b	216 (25.4)b
	CO	282±53.2b	353±66.5b	120±23.0b	262 (30.6)b
Parasitoids	FF	3.0±1.64a	4.5±3.35a	2.0±1.17a	5.3 (1.63)a
	RP	9.0±4.44a	5.3±2.70a	5.5±2.81a	5.2 (1.72)a
	CO	20.0±9.57b	19.0±9.10b	7.5±3.75b	14.8 (4.5)b

Figures is parenthesis are S.E. for means (SNK). Means with the same letter are not significantly different (approximate 95% Confidence interval). FF is Farmer Practice; RP is Reduced Pesticides and CO is Control – no pesticide use

During the second cropping period, the farmer field also recorded the highest mean leafminers, followed by the control plot and the reduced pesticide plot had the least in both Kabaru and Naromoru. However in Sagana, the control plot had the least mean number of leafminers. There was a significant difference ($P<0.05$) in mean leafminers in the farmer field and the other two plots (RP and C0), (Table 4). The mean number of parasitoids was higher in the control plot in all the locations. Like the first cropping period, Sagana recorded the fewest mean number of parasitoids (3.86 in C0) as compared to Kabaru and Naromoru (13.16 and 10.59). There was a significant difference ($P<0.05$) in mean parasitoids in C0 and other plots (Table 4.2).

Table 1.2 Leafminer counts and parasitoids numbers from different pea management system at different locations for the first cropping period (May-Aug, 2009).

	Management system	Location: Kabaru	Naromoru	Sagana	Mean
Leafminer flies	FF	334±66.9a	716±142.5a	902±180a	650±79.6a
	RP	182±36.8b	149±30.2b	115±23.3b	221±29.8b
	CO	107±21.9b	179±36.1b	406±81.2b	159±18.9b
Parasitoids	FF	7.3±3.17a	5.5±2.47a	3.5±1.67a	7.2±1.83a
	RP	8.5±3.66a	14.0±5.84b	8.8±3.76a	8.7±2.36a
	CO	32.5±13.16b	26.0±10.59b	19.0±9.86b	22.5±5.8b

Figures in parenthesis are S.E. for means (SNK). Means with the same letter are not significantly different (approximate 95% Confidence interval). FF is Farmer Practice; RP is Reduced Pesticides and CO is Control – no pesticide use

4.2 Effects of pesticide application on the mortality of leafminer flies and its parasitoids in the laboratory

4.2.1 *L. huidobrensis* percentage pupation and emergence from treated leaves

In dimethoate, the concentration significantly affected the percentage of pupation (P= 0.0006). There was no difference between the control (0) and the blank which both had higher percentage *L. huidobrensis* pupation (92 and 96.2 respectively) than all other dimethoate treatments. There was no significant difference between the concentrations 1 to 2.5 (Table 4.3).

In Thunder, there was a highly significant effect of the pesticide application and concentration on the pupation (P<0.0001) and in general, increase in the concentration resulted in a corresponding decrease in the number of pupae and adult flies recorded. The control (0), blank and concentration 0.25 were not significantly different in pupation. The pupation following treatment with the concentrations 0.5 and 0.75 were not different but were significantly lower than the control and significantly higher than the concentration 1 (Table 4.3).

In bulldock and bestox the concentrations 0.25, 0.5, 0.75, 1 and 1.25 ml/l were not significantly different in pupation and emergence. The control (0) and blank were significantly different from all the other concentrations. There was no significant difference in pupation and emergence between the control (0) and blank. The ANOVA showed a significant difference (P= 0.0022 and 0.0003) in pupation and emergence (P=0.0194 and P<0.0001) for bulldock and bestox respectively) among the treatments (Table 4.3).

In dynamec, the concentrations significantly affected the pupation and adult emergence (P<0.0001). The two controls were significantly higher than all Dynamec treated samples. All the tested concentrations were significantly different with a continuous decrease of pupation from concentration 0.25 to 1. Dynamec however, recorded a lower percentage pupation and emergence than Dimethoate, Bestox, Bulldock and Thunder (Table 4.3).

Table 4.3 Pupae and adult LMF (%) that pupated/emerged from the treated leaves containing 100 live larvae

Chemical	Concentration	Pupae	Adults
Dimethoate	0	92.0a	89.0a
	1	84.8b	81.0b
	1.5	77.2b	73.0b
	2	68.2b	63.4b
	2.5	63.2b	59.6b
	Blank	96.2a	94.4a
P-Value		0.0006	<0.0001
Thunder	0	97.6a	94.4a
	0.25	92.2a	88.6ab
	0.5	87.8ab	84.8b
	0.75	83b	81.0b
	1	69.6c	68.0c
	Blank	97.9a	95.4a
P-Value		<0.0001	<0.0001
Bulldock	0	96.4a	94.2a
	0.25	81.4b	77.6b
	0.75	76.2b	72.6b
	1	72.0b	69.6b
	1.25	70.2b	76.2b
	Blank	94.8a	92.8a
P-Value		0.0022	0.0194
Bestox	0	93.6a	92.8a
	0.25	81.8b	79.2b
	0.5	76.6b	72.4b
	0.75	73.6b	69.2b
	1	71.6b	68.4b
	Blank	91.0a	88.6a
P-Value		0.0003	<0.0001
Dynamec	0	95.4a	92.2a
	0.5	65.4c	56.0c
	0.75	51.6c	49.6c
	1	40.0d	33.4d
	Blank	91.2a	89.4a
S.E		0.54	0.14
P-Value		<0.0001	<0.0001

Within a column and for each pesticide, means followed by the same lower case letter are not significantly different at $P \leq 0.05$. Test statistic was ANOVA and means separated using SNK.

4.2.2 Effect of pesticides on adult *L. huidobrensis* over time

In Dimethoate, the concentrations significantly affects adult *L. huidobrensis* mortality (P<0.0001). Increase in concentration of the chemicals resulted in a corresponding increase in mortality. At 72hrs, concentration 2 and 2.5 ml/l were not significantly different but the percentage mortality was very high (87.1 and 90) respectively. The C0 had the least mortality (30%) at 72hrs and concentration 1 ml/l had the highest (90%), (Table 4.4).

For Thunder, a significance difference in percentage adult *L. huidobrensis* mortality among the concentrations was recorded at 24, 48 and 72 hrs (P= 0.0002, P< 0.0001 and P< 0.0001 respectively). However, at 72hrs, concentrations 0.25, 0.5 and 0.75 ml/l were not significantly different from each other. The percentage mortality increased with time and also with increase in the concentration of the chemical (Table 4.4).

In Bulldock and Milraz, the control (0) and concentration 0.25ml/l were significantly different in adult *L. huidobrensis* mortality from the other concentrations. Increase in the concentration resulted in an increase in mortality over time (Table 4.4). In Bestox, cyclone and Folicur, the lower concentrations (0, 0.25 and 1 ml/l) were significantly different (P<0.0001) in percentage mortality from the other concentrations (Table 4.4).

In Dynamec, at 24 and 48 hrs, the percentage adult *L. huidobrensis* mortality was significantly different (P= 0.0002 and P< 0.0001 respectively) among the treatments where an increase in the chemical concentration resulted in an increase in mortality. At

72hrs, 0.25, 0.5, 0.75 and 1 ml/l were not significantly different in percentage mortality from each other except the control (0), Table 4.4.

Table 4.4 Percentage mortality (Angular transformed percentage) of Adult *L. huidobrensis* monitored for three consecutive days

Chemical	Concentration	Mortality after 24hrs	Mortality after 48hrs	Mortality after 72hrs
Dimethoate	0	8.22c	21.8d	30.0c
	1	29.8b	56.9c	73.6b
	1.5	39.3b	62.7bc	78.9b
	2	48.7ab	67.5ab	87.1a
	2.5	63.5a	74.9a	90.0a
	F-value	12.2	60.2	118.6
	P-Value	<0.0001	<0.0001	<0.0001
Thunder	0	11.0c	24.2a	36.2c
	0.25	20.1bc	44.4b	61.2b
	0.5	25.2b	56.3ab	65.4b
	0.75	29.1b	54.7ab	66.7b
	1	38.6a	66.5a	90.0a
	F-value	11.05	23.78	14.99
	P-Value	0.0002	<0.0001	<0.0001
Bulldock	0	12.1c	27.6d	36.3b
	0.25	42.7b	58.8c	75.7ab
	0.75	56.2a	78.3b	74.9a
	1	60.0a	80.5a	90.0a
	1.25	71.3a	90.0a	90.0a
	F-value	29.71	126.9	20.13
	P-Value	<0.0001	<0.0001	<0.0001
Milraz	0	9.88c	26.6c	33.7c
	1	36.1b	50.3b	65.3b
	1.5	54.0a	67.3a	74.8ab
	2	57.1a	69.0a	81.4a
	2.5	60.3a	68.4a	85.9a
	F-value	35.3	27.11	28.11
	p-Value	<0.0001	<0.0001	<0.0001

Folicur		0	9.88c	19.9c	33.6c
		0.25	22.4bc	49.7b	69.0b
		0.75	34.7b	55.4b	76.2ab
		1	49.1a	59.6b	80.0ab
		1.25	61.5a	69.9a	90.0a
	F-value		20.38	33.6	29.91
	P-Value		<0.0001	<0.0001	<0.0001
Bestox		0	11.0c	21.3c	34.9b
		0.25	43.0b	50.7b	77.6a
		0.5	59.1a	69.5a	79.2a
		0.75	61.4a	78.6a	83.4a
		1	65.9a	82.1a	90.0a
	F-value		21.65	19.96	18.91
	P-Value		<0.0001	<0.0001	<0.0001
Dynamec		0	9.88d	30.6c	36.3b
		0.25	58.8c	75.8b	84.2a
		0.5	68.1b	83.0ab	87.1a
		0.75	70.0ab	87.1ab	90.0a
		1	76.0a	90.0a	90.0a
	F-value		165.56	57.75	129.73
	P-Value		0.0002	<0.0001	<0.0001
Cyclone		0	7.95c	26.3c	36.7b
		1	39.1b	54.3b	74.4a
		1.5	58.5a	69.8a	82.1a
		2	67.6a	78.9a	90.0a
		2.5	65.1a	77.9a	90.0a
	F-value		44.49	26.28	30.47
	P-Value		<0.0001	<0.0001	<0.0001

Within column and for each pesticide, means followed by the same lower case letter are not significantly different at $P \leq 0.05$. Test statistic was ANOVA and means separated using SNK.

4.2.3 Effect of pesticides on adult Parasitoids, *Diglyphus isaea* and *Phaedrotoma scabriventris* over time

4.2.3.1 *Diglyphus isaea*

In dimethoate, at 24 hrs the recommended dose (1.5ml/l) and other concentration below it, differed significantly (P<0.0001) in mortality of *Diglyphus isaea* compared to the higher doses. However, the mortality at and below the recommended dose was relatively low as compared to higher doses of the chemical (Table 4.5). Increase in the chemical concentration resulted in a corresponding increase in mortality. At 48 and 72 hrs, the mortality was significantly different ($P<0.0001$) from the control in all the concentrations used. All other concentrations were not significantly different from each other (Table 4.5).

For Thunder, all the concentrations used differed significantly from the control ($P=0.0031$, 0.0092 and 0.0169) at 24, 48 and 72hrs respectively. However, Thunder had lower percentage mortality at the highest concentration (1 ml/l) as compared to the other chemicals (Table 4.5). In Bulldock, Milraz, Folicur, Bestox and Dynamec the control was significantly different ($P<0.0001$) from all the other concentrations used. However, there was no significant difference ($P>0.05$) in mortality among the treatments. The percentage adult mortality increased over time in all the chemicals. In dynamec up to 100% mortality was observed at 48 and 72 hrs (Table 4.5).

In Cyclone, at 24 hrs all the concentrations (0, 1, 1.5, 2 and 2.5 ml/l) were highly significantly different ($P<0.0001$) from each other. At 48 and 72hrs however, only the control (0) was significantly different ($P<0.0001$) in percentage *D.isaea* mortality from the other concentrations. Increase in percentage mortality was observed over time even in the control (0), Table 4.5.

Table 4.5 Percentage Mortality (Angular transformed percentage) of *Diglyphus isaea* adults under different chemicals

Chemical	Concentration	Mortality after 24hrs	Mortality after 48hrs	Mortality after 72hrs
Dimethoate	0	10.2c	31.9b	36.2b
	1	66.2b	82.9a	90.0a
	1.5	77.8ab	90.0a	90.0a
	2	90.0a	90.0a	90.0a
	2.5	90.0a	90.0a	90.0a
	F-value	54.92	61.42	1280.73
	P-Value	<0.0001	<0.0001	<0.0001
Thunder	0	12.2b	24.5b	32.2b
	0.25	30.6a	44.4a	63.7a
	0.5	39.3a	47.4a	66.5a
	0.75	36.6a	41.7a	70.8a
	1	45.6a	57.4a	60.0a
	F-value	8.39	6.14	5.09
	P-Value	0.0031	0.0092	0.0169
Bulldock	0	22.9b	29.7b	37.8b
	0.25	67.4a	82.9a	84.9a
	0.75	70.9a	84.9a	90.0a
	1	70.9a	84.9a	90.0a
	1.25	65.6a	79.8a	90.0a
	F-value	28.1	22.01	93.08
	P-Value	<0.0001	<0.0001	<0.0001
Milraz	0	17.3b	31.0b	36.5b
	1	68.9a	83.0a	84.9a
	1.5	70.8a	80.0a	84.9a
	2	72.5a	80.0a	90.0a
	2.5	83.0a	83.0a	90.0a
	F-value	23.53	16.19	48.56
	p-Value	<0.0001	0.0002	<0.0001
Folicur	0	24.8b	31.3b	36.5b
	0.25	72.7a	80.0a	80.0ab
	0.75	66.2a	77.8a	90.0a
	1	75.9a	80.0a	90.0a
	1.25	80.0a	84.9a	90.0a
	F-value	21.49	20.83	96.1
	P-Value	<0.0001	<0.0001	<0.0001

Bestox		0	24.5b	31.3b	36.5b
		0.25	57.9a	79.6a	83.0a
		0.5	67.1a	84.9a	90.0a
		0.75	68.5a	79.6a	90.0a
		1	61.5a	69.9a	90.0a
	F-value		6.35	6.37	52.94
	P-Value		0.0082	0.0082	<0.0001
Dynamec		0	19.2b	29.73b	33.81b
		0.25	84.9a	90.0a	90.0a
		0.5	84.9a	90.0a	90.0a
		0.75	84.9a	90.0a	90.0a
		1	90.0a	90.0a	90.0a
	F-value		54.88	1455.08	2020.68
	P-Value		<0.0001	<0.0001	<0.0001
Cyclone		0	21.0c	29.7b	35.2b
		1	64.3b	84.9a	84.9a
		1.5	70.9ab	81.1a	90.0a
		2	79.8ab	90.0a	90.0a
		2.5	84.9a	90.0a	90.0a
	F-value		29.48	30.76	92.07
	P-Value		<0.0001	<0.0001	<0.0001

Within column and for each pesticide, means followed by the same lower case letter are not significantly different at $P \leq 0.05$. Test statistic was ANOVA and means separated using SNK.

4.2.3.2 Phaedrotoma scabriventris

In *P. scabriventris*, all the chemicals used in this experiment (Bulldock, Milraz, Folicur, Bestox, Dimethoate, Dynamec and Cyclone) except Thunder; showed a highly significant difference (P<0.0001) in percentage adult mortality from all the other concentrations used (Table 4.6). In these chemicals up to 100% adult mortality was recorded in all the concentrations except the control (0) at 24, 48 and 72hrs. Increase in the chemical concentration resulted into an increase in the mortality of the parasitoid with time (Table 4.6). In Thunder, no adult *P. scabriventris* mortality was recorded in the control (0) at 24hrs. A significant difference (P<0.0001) in percentage mortality was recorded among the treatments at 24 hrs. At 48 and 72 hrs, the adult mortality in all the concentrations used for Thunder differed significantly (P<0.0001) from the control (Table 4.6). Any concentration below the recommended dose (0.5ml/l) showed a lower percentage (44.5 and 72.3) adult *P. scabriventris* mortality at 24 and 48 hrs respectively, as compared to other concentrations above the recommended (Table 4.6).

Table 4.6 Percentage mortality (Angular transformed percentage) of *Phaedrotoma scabriventris* adults under different chemical concentrations

Chemical	Concentration	Mortality after 24hrs	Mortality after 48hrs	Mortality after 72hrs
Dimethoate	0	10.2b	30.8b	41.2b
	1	67.6a	90.0a	90.0a
	1.5	67.6a	90.0a	90.0a
	2	90.0a	90.0a	90.0a
	2.5	90.0a	90.0a	90.0a
	F-value	5.17	583.92	2278.9
	P-Value	0.016	<0.0001	<0.0001
Thunder	0	0.00d	10.2b	26.12b
	0.25	44.5c	72.3a	90.0a
	0.5	70.7b	90.0a	90.0a
	0.75	90.0a	90.0a	90.0a
	1	90.0a	90.0a	90.0a
	F-value	58.7	57.79	535.59
	P-Value	<0.0001	<0.0001	<0.0001
Bulldock	0	10.2b	26.1b	40.3b
	0.25	90.0a	90.0a	90.0a
	0.75	90.0a	90.0a	90.0a
	1	90.0a	90.0a	90.0a
	1.25	90.0a	90.0a	90.0a
	F-value	243.4	535.59	1829.04
	P-Value	<0.0001	<0.0001	<0.0001

Milraz		0	5.11b	21.1b	35.0b
		1	81.1a	90.0a	90.0a
		1.5	90.0a	90.0a	90.0a
		2	90.0a	90.0a	90.0a
		2.5	90.0a	90.0a	90.0a
	F-value		66.05	452.7	494.43
	p-Value		<0.0001	<0.0001	<0.0001
Folicur		0	5.11b	24.3b	32.1b
		0.25	90.0a	90.0a	90.0a
		0.75	90.0a	90.0a	90.0a
		1	90.0a	90.0a	90.0a
		1.25	90.0a	90.0a	90.0a
	F-value		275.59	427.67	1557.02
	P-Value		<0.0001	<0.0001	<0.0001
Bestox		0	0.00b	19.2b	20.3b
		0.25	79.6a	90.0a	90.0a
		0.5	90.0a	90.0a	90.0a
		0.75	90.0a	90.0a	90.0a
		1	90.0a	90.0a	90.0a
	F-value		71.05	1445.7	1982.64
	P-Value		<0.0001	<0.0001	<0.0001
Dynamec		0	0.00b	22.9b	34.5b
		0.25	90.0a	90.0a	90.0a
		0.5	90.0a	90.0a	90.0a
		0.75	90.0a	90.0a	90.0a
		1	90.0a	90.0a	90.0a
	F-value		Infinity	1367.5	1484.2
	P-Value		<0.0001	<0.0001	<0.0001
Cyclone		0	5.11b	19.3b	38.6b
		1	90.0a	90.0a	90.0a
		1.5	90.0a	90.0a	90.0a
		2	90.0a	90.0a	90.0a
		2.5	90.0a	90.0a	90.0a
	F-value		275.59	52.66	229.1
	P-Value		<0.0001	<0.0001	<0.0001

Within column and for each pesticide, means followed by the same lower case letter are not significantly different at $P \leq 0.05$ Test statistic was ANOVA and means separated using SNK.

4.3 The evaluation of the best dosage for chemical application.

The larva and adults of *L. huidobrensis* together with their parasitoids were exposed to different chemical concentrations to evaluate the best dosage to use on these.

4.3.1 Effects of chemical concentrations on larvae of *L. huidobrensis*

Dynamec caused the highest percentage larval mortality (about 60%) reaching its peak at a concentration of 1.5 ml/l followed by Bestox, Bulldock, Dimethoate and Thunder caused the lowest larval mortality (about 20%) at the highest concentration (Fig. 4.7). Increase in concentration resulted into a corresponding increase in larval mortality for all the chemicals tested. Larval mortality was low (< 10%) in the control (0), which was not treated with the chemical (Fig 4.7). The ANOVA showed a significant difference (P<0.0001) in larval mortality among the concentrations and also among the chemicals used.

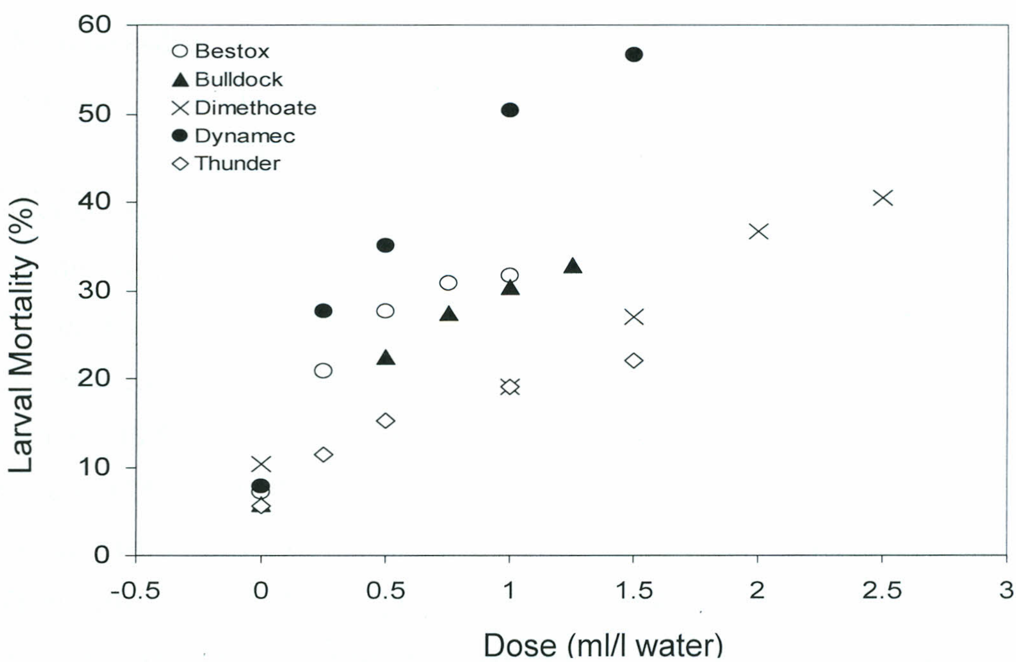

Fig. 4.7 Percentage *L. huidobrensis* larval mortality after exposure to various chemical concentrations.

4.3.2 Effects of chemical concentrations on adult *Liriomyza huidobrensis*

In the lethality tests, all the chemicals tested caused at least 50% adult *L.huidobrensis* mortality at a concentration below the recommended dose (shown in brackets after each chemical), except Folicur and Thunder (Table 4.7). Dynamec caused 50% adult mortality at a concentration (0.24 ml/l) slightly below the recommended dose (0.5 ml/l). However, for all the chemicals used a concentration above the recommended dose (shown in brackets) was required to kill at least 90% of the flies. In Thunder, the concentration that killed at least 50% of the flies (1.63 ml/l) was more than three times the recommended dose (0.5 ml/l). Dynamec, recorded the lowest concentration (0.65ml/l) that caused at least 90% mortality followed by Bestox (0.92 ml/l), Table 4.7.

Table 4.7 LC_{50} and LC_{90} values for the chemicals sprayed on *L. huidobrensis* flies

Chemical (Recommended dose, ml/l	LC_{50}		LC_{90}	
	Mean	CI	Mean	CI
Bestox(0.5)	0.32	[0.26, 0.38]	0.92	[0.83, 1.06]
Bulldock(0.75)	0.50	[0.43, 0.57]	1.20	[1.09, 1.35]
Cyclone(1.5)	1.23	[1.10, 1.35]	2.41	[2.22, 2.67]
Dimethoate(1.5)	8.03	-*	14.78	-*
Dynamec(0.5)	0.24	[0.19, 0.28]	0.65	[0.58, 0.73]
Folicur(0.75)	0.87	[0.80, 0.95]	1.63	[1.48, 1.86]
Milraz(2.0)	1.29	[1.14, 1.42]	2.72	[2.48, 3.06]
Thunder(0.5)	1.63	[1.27, 2.59]	3.14	[2.31, 5.49]

*Dashes indicate areas where unrealistic values were obtained such as a negative value for confidence interval

4.3.3 Effects of chemical concentrations on *Diglyphus isaea*

For all the chemicals tested, a dose well below the recommended dose (shown in brackets after the name of the chemical) was able to cause at least 50% adult *D. isaea* mortality, except Thunder. For bestox, Bulldock and Dynamec the concentration that killed 50% of the flies was less than 10 times the recommended dose (in brackets). Cyclone, Dimethoate, Folicur and Milraz respectively were able to kill atleast 50% of the parasitoid at a dose less than 3 times the recommended (Table 4.8).

Dynamec also caused at least 90% *D. isaea* mortality at the lowest concentration (0.39 ml/l) below the recommended dose (0.5 ml/l), followed by Dimethoate, Cyclone and Milraz. For Bestox, Bulldock, Folicur and Thunder; a concentration above the recommended dose (in brackets) was required to kill at least 90% of the adult parasitoid. Thunder required more than 4 times the recommended dose in order to cause 90% mortality (Table 4.8).

Fig. 4.8 LC_{50} and LC_{90} values for the chemicals sprayed on *Diglyphus isaea* adults

Chemical(Recommended dose, ml/l)	LC_{50}		LC_{90}	
	Mean	CI	Mean	CI
Bestox(0.5)	0.02	[-0.37, 0.20]	1.15	[0.89, 1.82]
Bulldock(0.75)	0.01	[-0.38, 0.22]	1.27	[1.00, 1.85]
Cyclone(1.5)	0.32	[0.05, 0.53]	1.38	[1.14, 1.72]
Dimethoate(1.5)	0.28	[0.10, 0.44]	0.95	[0.75, 1.25]
Dynamec(0.5)	0.02	[-0.09, 0.09]	0.39	[0.30, 0.53]
Folicur(0.75)	0.11	[-0.10, 0.24]	0.85	[0.70, 1.08]
Milraz(2.0)	0.43	[0.12, 0.65]	1.71	[1.45, 2.12]
Thunder(0.5)	0.80	[0.63, 1.11]	2.06	[1.56, 3.42]

4.3.4 Effects of chemical concentrations on *Phaedrotoma scabriventris*

Dynamec recorded the lowest concentration below the recommended dose (shown in brackets after the chemical), that caused at least 50% *P. scabriventris* mortality followed by Bulldock and Folicur, Dimethoate, Milraz, Cyclone and Bestox respectively. Thunder required a dose above the recommended (0.5 ml/l) to be able to kill 50% of the flies. Apart from Thunder, all the other chemicals were lethal to at least 90% of the parasitoid at a dose below the recommended (Table 4.9).

Fig. 4.9 LC50 and LC_{90} values for the chemicals sprayed on *Phaedrotoma scabriventris* adults

Chemical (Recommended dose, ml/l)	LC_{50}		LC_{90}	
	Mean	CI	Mean	CI
Bestox(0.5)	0.27	[0.19, 0.34]	0.71	[0.61, 0.86]
Bulldock(0.75)	0.29	[0.19, 0.38]	0.81	[0.69, 1.00]
Cyclone(1.5)	0.61	[0.42, 0.77]	1.33	[1.15, 1.59]
Dimethoate(1.5)	0.50	[0.33, 0.66]	1.15	[0.97, 1.40]
Dynamec(0.5)	0.14	[0.09, 0.19]	0.37	[0.31, 0.47]
Folicur(0.75)	0.29	[0.20, 0.37]	0.63	[0.54, 0.75]
Milraz(2.0)	0.63	[0.44, 0.79]	1.45	[1.25, 1.72]
Thunder(0.5)	0.77	[0.62, 1.02]	1.91	[1.48, 2.98]

CHAPTER FIVE

5 DISCUSSION

5.1 Effects of biotic and abiotic factors on the population dynamics of leafminers and its parasitoids

The number of leafminer flies that emerged from sampled leaves in Kabaru, Naromoru and Sagana were found to be high at the beginning of the first cropping period but gradually decreased towards the end of the season. This same trend was observed during the second cropping period in Kabaru and Naromoru. However, in Sagana a gradual increase in the number of these flies was observed throughout the study. Similar results were obtained from experiments on the sticky traps and one-step count method during the second cropping period. This gradual decrease in number of leafminers towards the end of the first cropping period could have been caused by the differences in the weather patterns especially rainfall which was experienced at the end of the first cropping period. Alternatively, leafminer flies may have sought alternative hosts nearby until pea crop was available, confirming therefore the polyphagous nature of this pest as pointed out earlier by Parella, 1987; Kang, 1996 and Koppert, 2003.

A sharp drop in numbers of leafminers emerging from sampled leaves was observed in all locations on the fourth week of the first cropping period. This may have been caused by the effects of the pesticides which were applied on the third week in efforts to control the then increasing numbers of leafminers. This conforms with earlier reports by Salvo *et al*

(2005) who observed that the population of leafminers was affected by pesticides use among other factors.

The parasitoids found in the field were few, especially during the first cropping period where in most locations no parasitoid was recorded during the first 9 weeks. As from week 9 to the end of both the first and second cropping periods, a reasonable number of parasitoids were recorded. The absence of parasitoids was probably as a result of effects of the pesticides applied during the growing period in an effort by the farmers to control other pests. Week 9 marked the beginning of harvesting period and farmers ceased the use of pesticides which explains why some parasitoids were recorded at that time. Djoko et al (2004) observed that the effects of chemicals on beneficials were more severe than on target pests, resulting in pest outbreaks. Therefore, the pesticides and other chemicals used on crops need to be considered in relation to parasitoids if they are to have a role in IPM (Bjorksten *et al.,* 2005).

The absence of parasitoids during the growing period indicates that natural control was not effective and augmentative release of natural enemies would be important to increase the efficiency of the existing natural enemies. Some local natural parasitoids such as *Diglyphus isaea* have been identified already and can be responsible for very high levels of parasitism of leafminers. It is widely used locally and overseas and is even cultured for release (Tong-Xian *et al.,* 2009). Other parasitoids can also be imported for use such as the *P. scabriventris* (not present in the field during this study), because laboratory experiments have shown that it is effective against the leafminers. In Italy, parasitoids reduced *L. huidobrensis* populations on lettuce (Burgio *et al*., 2005). Paul and Kevin (2002) reported that though repeated release of natural enemies through augmentative or inundative biological control offered a solution for pest control, it was often prohibitively expensive relative to chemical control programs in terms of production and implementation costs. However, the effectiveness of these parasitoids would depend on the development of an effective integrated pest management (IPM) that would enhance the natural populations of parasitoids (Salvo *et al.,* 2005). Preservation of natural enemies would not only reduce the population of leafminers, but would also increase the yield from pea hence better living standards.

Very little amount of rainfall was recorded during the the entire study except on week 2 of the second cropping period when a high amount of rainfall was recorded. The leafminers were also found to decrease at this period which probably indicates that some of them may have drowned in the rain especially the adults flies. However, temperature did not vary significantly during the study which probably shows that it had little to do with the population of both the leafminers and its parasitoids. This probably means that the major factor affecting the build-up of Leaf miners was the pesticides use and lack of biological control methods using the parasitoids. However, more detailed studies concerning the abiotic factors are required in order to explore the potential use of Agromyzid parasitoids for biological control programs. Valladares and Salvo (2001), observed that the parasitoids were fewer during the less favourable trial (winter), which combines low temperatures, almost no precipitation, and great scarcity of tender green leaves for leaf miners to exploit.

5.2 Effects of pesticide applications on mortality of leafminer and its parasitoids

The farmer field had the highest number of leafminers as compared to the other plots even after chemical application. This probably means that the leafminers had developed resistance to the pesticides resulting in outbreaks. In addition, the farmer field had the lowest number of parasitoids in all the locations. Lack of parasitoids in the field may be attributed to continuous use of chemicals by the farmers. Earlier reports showed that applications of pesticides have deleterious effects on the parasitoids, reducing parasitism (Hidrayani *et al.*, 2005; Salvo *et al.*, 2005). Though the control plot had more parasitoids than all the other plots, the reduced pesticide plot (RP) showed the least number of leafminers as compared to the other plots. This probably indicates that the parasitoids being few were not effective on their own for a complete elimination of the pest under the current production system.

The current study revealed than even under laboratory conditions, none of the pesticides used successfully controlled the larval stage of the pest when the recommended doses were used. Effective control in the laboratory was only found on adult flies but in field condition, the adults are mobile and could easily move to wild vegetation. This probably may be the reason why the control of the pest was difficult in the field. Ferguson (2004) indicated that the effectiveness of the insecticides had been reduced by their indiscriminate use, which had adversely impacted on natural enemies resulting in the development of resistance to several groups of insecticides (Ferguson, 2004).

The study has shown that leafminers are serious pests whose control is of utmost importance. The most critical time for its control, is immediately after seed germination because at this time the pest density is very high probably due to the softness of the tissues. If this is not done, the entire plant could be lost (Bjorksten *et al*., 2005). Due to its polyphagous nature, *L. huidobrensis* always finds an alternative host which helps to maintain its numbers in the field even in the absence of pea. Therefore, complete elimination of the pest may be difficult (Kang, 1996). Thorough investigation of the crop is also needed to determine the extent of damage because the pest, usually feeds on the lower surface of the leaf where it forms tunnels hence difficult to see. These tunnels not only act as entries for other opportunistic pests but also cause cosmetic damage to the crop (Bjorksten *et al*., 2005).

Field studies showed that the pest density is influenced by one or more factors including level of insecticide use, parasitism, crop type and cropping system (Salvo *et al*., 2005). High level of LMF in pesticide used areas in Central Kenya could be as a result of the development of resistance by the pest to the pesticides being used and/or the elimination of natural enemies of the pest. In order to achieve an effective control of leafminers, farmers must all work at preserving the natural enemies by using chemicals which do not destroy them such as Thunder.

As new spray practices are being adopted, it is necessary that the aspect of conserving the natural enemies is considered. The retention and the management of wild plants within field margins can be crucial tools to enhance the populations of biological control agents of Agromyzids and to conserve rare parasitic wasp species (Giovanni *et al.*, 2007).

5.3 Evaluation of the best dosage for control with minimal effects to parasitoids

In the laboratory, Dynamec, Cyclone and Bulldock were found effective against *L. huidobrensis* since they eliminated 50% of the pest at a concentration below the recommended. These results coincide with research in other countries indicating that some of those chemicals are effective in controlling *L. huidobrensis* (Prijono *et al.*, 2004). Laboratory tests on Indonesian leafminers indicate that Abamectin (Dynamec) and Cryomazine can be effective in controlling leafminers if administered correctly (Hidrayani *et al.*, 2005). However, these pesticides and the fungicides used in the experiment, adversely affected the parasitoids resulting in over 90% mortality at the recommended dosage except Thunder. The potential effects of fungicides on the parasitoids of leafminers have been largely overlooked. Cyclone and Milraz were found to be lethal to the parasitoids of leafminers in this experiment. Therefore care should be taken when using them in field conditions. Tong-Xian *et al.*, (2009) observed that effects of herbicides and pesticides on leafminer parasitoids were overlooked and only Mancozeb had been tested in literature. Thunder on the other hand cannot be effectively used as a control since it could not control the pest. This calls for an integrated approach of control which encompasses the parasitoids and the pesticides favourable to thes parasitoids.

The parasitoids *D. isaea* and *P. scabriventris* were the most affected where up to 100% mortality was recorded even at concentrations below the recommended dose. The effects of chemicals on beneficials are more severe than on target pests, resulting in pest outbreaks (Djoko *et al.*, 2004). Thunder was the most favourable insecticide to both leafminers and its parasitoids. However, sub lethal effects remain to be investigated particularly because there is evidence from other studies that such effects can occur in parasitoids that survive a pesticide application (Umoru and Powell, 2002). Dynamec (Abamectin), was harmful to all the parasitoids which is in agreement with data from other studies (Weintraub, 2001). In laboratory studies, it was shown to kill Indonesian parasitoids but only at rates above those applied in the fields (Hidrayani *et al*, 2005). These results showed that the mortality of Leafminers increased with the increase in the concentration of the pesticide. A survey carried out in Nyeri showed that farmers usually used a dose above the recommended one in their efforts to reduce pest damage (Gitonga *et al.,* 2010). This is a reflection of the indiscriminate use of pesticides by the farmers in Central Kenya. In Thunder a dose well above the recommended, is needed for elimination of the leafminers. This suggests that the chemical could only be effective if used with another control such as a biological control using parasitoids, since it was found to be less lethal to the parasitoids when tested in the lethality tests.

For biological control of *Liriomyza spp.* to be cost-effective, developing IPM programmes that incorporates pesticides with minimal effects on leafminer parasitoids is a viable strategy (Tong-Xian *et al.,* 2009). *Phaedrotoma scabriventris* researched in this study would be a viable option for *L.huidobrensis* since like the pest, it is also exotic.

Phaedrotoma scabriventris possesses many qualities that make it a very potential candidate in classical biocontrol programs for *L. huidobrensis;* this includes its wide range of geographical and ecological distribution, its occurrence in natural and urban habitats and its significant importance as parasitoid of Agromyzidae flies that occur as pests in agricultural crops (Salvo 1996, Salvo *et al.* 2005). In Mexico, an IPM programme for spring plantings of tomatoes produced a reduction in leafminer puparia collection trays from 185.1/tray/day in a conventional pesticide programme to 4.1/tray/day (Trumble and Rodriguez, 1993). Developing an IPM programme will not only reduce the leafminer populations and potential environmental problems, but also will go a long way in increasing net profits from pea.

CHAPTER SIX

6 CONCLUSIONS, RECOMMENDATIONS AND SUGGESTIONS

6.1 CONCLUSIONS

1) The level of parasitoids was low in the field hence not effective for control of leafminers. The parasitoids may have been killed by overuse of pesticides which was rampant in the field. Therefore, pesticides and other chemicals used on crops need to be reconsidered in relation to parasitoids if they are to have a role in IPM.
2) Apart from Thunder all the other pesticides were harmful to the parasitoids at the recommended dose. Therefore for effective control of the pest, other less harmful pesticides such as botanicals could be a viable option.
3) Temperature range in the experimental sites was narrow hence had little to do with the population dynamics of leafminers and its parasitoids in Central Kenya.
4) The mean numbers of leafminer flies were significantly higher in the farmers' field as compared to the reduced pesticides and the control plots. This demonstrates decreased effectiveness of biological control and also resistance developed by the leafminers to pesticides.
5) None of the pesticides was successful in controlling larva of the leafminers and their LC50 was above the recommended dose.
6) The best dosage for Leafminer control is one slightly lower than the recommended dose for most chemicals in order to protect the parasitoids.

6.2 RECOMMENDATIONS

1. Augmentative and classical biological control should be carried out where local parasitoids will be boosted and efficient parasitoids from the origin zone of the pest introduced against the leafminer flies in Kenya.

2. Farmers should be discouraged from using doses above the recommended dose since it is harmful to the parasitoids. They should also be trained on ways of conserving biocontrol agents.

3. Abiotic factors such as temperature, rainfall and relative humidity is necessary to confirm their role in the abundance of leafminers in the field.

6.3 SUGGESTIONS FOR FURTHER WORK

1. Both local and exotic parasitoids of leafminers need to investigated in order to know the most effective in the control of *L.huidobrensis* and possible synergetic effects.

2. An integrated approach of leafminer control comprising of lower doses of pesticides, parasitoids and cultural control methods which destroy the pest should be investigated.

3. Further studies are requires to identify pesticides that effectively kill the larva and adult leafminer and at the same time are less harmful to natural enemies

4. Farmers should be advised on how biological control programs works so that they can be in a position to apply it in Integrated Pest Management programs.

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