INTEGRATED STRATEGIES FOR MANAGEMENT OF *LIRIOMYZA SPP* (DIPTERA: AGROMYZIDAE) ON BASIL (O*CIMUM BASILICUM*)

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Dedication

This milestone is dedicated to my beloved wife Lydia Kerubo for the constant love, support and moral encouragement in every step of the way.

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Abbreviations and acronyms

BCPC	British Crop Protection Council.
CABI	Centre for Agriculture and Biosciences International
DAFF	Department of Agriculture, Forestry and Fisheries Republic of South Africa
DAP	Di- ammonium Phosphate
FPEAK	Fresh Produce Exporters Association of Kenya
IFOAM	International Federation of Organic Agriculture Movements
KEPHIS	Kenya Plant Health Inspectorate Service
NPK	Nitrogen, Phosphorous and Potassium
PCPB	Pest Control Products Board
OMRI	Organic Materials Review Institute
RCBD	Randomized Complete Block Design

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ABSTRACT

Kenya exports basil and other fresh produce to the European Union commanding about 35% of the total market share. Export of basil has constantly been under threat due to interceptions resulting from various live stages of *Liriomyza spp* and exceedances in pesticide residue levels. The study aimed at reducing infestation of by basil by *Liriomyza spp* through sustainable and food safe integrated management options. This was done by determining the effect of soil amendments and foliar feed sprays on damage by *Liriomyza spp* in addition to evaluating the efficacy of selected bio-pesticides together with polythene mulch and sticky traps in controlling infestation. Two separate field experiments were conducted in line with specific objectives and repeated once. Both experiments were laid out in a randomized complete block design (RCBD). Three treatments replicated three times were applied in the first experiment while nine treatments replicated three times were applied in the second. Soil amendments and foliar feed sprays significantly ($P \leq 0.05$) influenced the level of damage by *Liriomyza spp* on basil. The combined application of Di-ammonium phosphate with Chicken manure and foliar fertilizer recorded the highest number of leaflets (3.34 and 2.52) damaged during the first season and second season, respectively. Spraying neem alternated with spinosad combined with polythene mulch and yellow sticky traps significantly ($P \leq 0.05$) recorded the lowest number of damaged leaflets (1.47 and 1.46) in season 1 and 2, and the least number of *Liriomyza spp* larvae (1.07 and 1.15) damaging basil leaflets in season 1 and 2, respectively. The integrated application of neem, spinosad, polythene mulch and yellow sticky traps reduces infestation and damage of *Liriomyza spp* in basil and is recommended for addressing food safety, pesticide resistance and environmental concerns arising from the persistent use of chemical pesticides. Farmers while combining different nutrition regimes to realize high yields in basil should take necessary considerations not to increase infestation and damage by *Liriomyza spp.*

CHAPTER ONE

INTRODUCTION

1.1 Background information

Basil (*Ocimum basilicum* L.) is an important herb that belongs to the Lamiaceae family. It is also known by the synonym *Ocimum americanum* (CABI, 2017). Basil is native to central Asia and northwest India (CABI, 2017). It is widely grown in Asia, Africa and Central and Southern America. The leading producers of basil in the world include Israel, Morocco, France, United States of America, Greece and Indonesia (DAFF, 2012). Many varieties are grown in the world with diversity in growth habits, leaf size, flower colour, physical appearance and aroma (Miele *et al*., 2001). It is an annual or short lived perennial herb which thrives in warm growing conditions. The herb has been naturalized in the tropics, subtropics and temperate areas. In Kenya due to good climatic conditions, it is grown throughout the year either in the open fields or in green houses mainly by large scale farmers who are interested in the export market. The leading export companies are based in Kiambu, Nyeri, Meru and Kajiado counties (KEPHIS, 2015). The bulk of produce is for export though a section of small scale farmers produce the herb to sell locally.

Leaves of basil are utilized while fresh or dried for spicing and flavoring foods in addition to production of essential oils. Besides its use as a condiment, historically basil has been used as a treatment for ailments such as insomnia, skin infections, depression and snake bites (Bora *et al*., 2011). Basil has essential oils that are good in smell, therefore utilized in perfumes and cosmetics as part of fragrance composition (Khatri *et al*., 1995). Low yield of basil is a result of many constraints which include imbalanced use of fertilizers with the most significant being insect pests and disease attack. Pests and diseases and the strict requirements on food safety due to application of pesticides are major limitations to basil production and export in Kenya. The common diseases of importance in basil include Botrytis (*Botrytis cinerea*), Fusarium (*Fusarium oxysporum*), *Rhizoctonia solani*, *Pythium spp*. and *Phytophthora spp* (CABI, 2017). Various insect pests attack basil. The common ones include beetles, thrips, whitefly, leafhoppers and leafminers. Leafminers (*Liriomyza spp*) are a major constraint because of the quarantine importance in Europe where most of the Kenyan basil is exported.

1.2. Problem statement

Kenya exports basil and other fresh produce to the European Union commanding about 35% of the total market share (KEPHIS, 2014). Fruits, herbs and spices, vegetables and cut flowers are the key components of Kenya's export bringing in about 100 billion shillings annually into the economy (FPEAK, 2016). The leading importers of Kenya's fresh produce in Europe include: The United Kingdom, Germany, France, Switzerland, Belgium, The Netherlands and Italy (KEPHIS, 2014).

Kenya's fresh produce export dominance in the European market is constantly under threat due to low yields, pest and diseases, presence of pesticide residues and interceptions resulting from presence of harmful (quarantine) organisms. Various species of *Liriomyza* are quarantine to the European Union in line with EU directive 2000/29/EC. The commonly intercepted species on consignments from Kenya include *Liriomyza huidobrensis, Liriomyza trifolii* and *Liriomyza sativae* (KEPHIS, 2016). The three species are listed as quarantine pests therefore, their introduction and spread within all European member states is not acceptable by law.

Table 1.1: Interception notifications due to *Liriomyza spp* on basil (*Ocimum basilicum*)

Year	No. of interceptions in the European Union
2012	14
2013	8
2014	3
2015	0
2016	1
2017	4

https://ec.europa.eu/food/plant/plant_health_biosecurity/europhyt/interceptions_en.

In 2014, a total of 108 interception notifications due to harmful organisms were received on Kenyan consignments in Europe with more than half of the interceptions occurring due to *Liriomyza spp* (EUROPHYT, 2018). The bulk of the interceptions occurred on cut flower (Gypsophilla and Eryngium) and basil (*Ocimum basilicum*). In the same year, Kenya was also placed on high alert list as a risky source of fresh produce due to the frequent interceptions (KEPHIS, 2015). As a mitigation measure, Kenya through the National Plant Protection Organization (KEPHIS) instituted stringent phytosanitary measures. Key among the measures was immediate suspension from export market for growers whose produce was inspected and found non-compliant in regard to pests of concern and exceedances of pesticide residues. Basil growers and exporters were hit hard by that requirement on compliance. Consequently many exporters opted out of business while others lost millions of shillings due to destructions of consignments locally and abroad. The volume of basil and other herbs exported to the EU from Kenya is approximated to have declined from 2% of total export volumes in 2012 to less than 1% in 2015 (KEPHIS, 2015).

1.3. Justification

From the interception trends, there exists a knowledge gap on the part of the Kenyan growers in the management of *Liriomyza spp* and/or the current farmer practices are no longer effective. Growers could be over-relying on the use of chemicals as the sole approach of management. The situation in Kenya is complicated further because there is no diversity of the registered and approved chemical products for control of *Liriomyza spp* on basil (PCPB, 2016). Growers are compelled to use active ingredients that are not approved for use in basil. Besides, their efficacy and safety is not ascertained in regard to basil.

Farmers in Kenya have limited their approach for the management of *Liriomyza spp* to routine insecticide application. Gitonga *et al.* (2010) observed that imidacloprid, alphacypermethrin and abamectin are commonly used insecticides against *Liriomyza spp* in production of vegetables in Kenya. Past studies show that alphacypermethrin and abamectin are effective against *Liriomyza* (Kaspi and Parella, 2006). However due to persistent use of chemicals, the pest is quickly developing resistance. Studies have shown that none of the listed insecticides (imidacloprid, alphacypermethrin and abamectin) kills the larvae of *L. huidobrensis* (Wentraub, 2001). *L. huidobrensis* is the most intercepted *Liriomyza spp* on Kenyan produce in the European Union market (KEPHIS, 2014).

1.4. Objectives

1.4.1 Broad objective

The overall objective of the study was to contribute to reduced infestation of basil leaves by *Liriomyza spp* through sustainable and food safe integrated management options.

1.4.2 Specific objectives

Specific objectives of the study were:

i. To determine the effect of soil amendments and foliar feed sprays on the level of damage and the onset of infestation by *Liriomyza spp* on basil

ii. To evaluate the efficacy of selected bio-pesticides, polythene mulch and sticky traps in controlling *Liriomyza* infestation on basil

1.4.3 Hypothesis

i. Soil amendments and foliar feed sprays increases the level of damage by *Liriomyza spp* on basil

ii. Neem applied alternately with spinosad integrated with polythene mulch and yellow sticky traps is efficacious in controlling *Liriomyza spp* on Basil.

CHAPTER TWO

LITERATURE REVIEW

2.1. Origin, distribution and ecological requirements of basil

Basil is thought to have originated from Africa though it is also thought to have been first cultivated in India (Putievsky and Galambosi, 1999).The name basil is borrowed from the Greek word “basileus”. The name means “king” because of its sweet smell (Jacqueline, 2011). It is commonly called sweet basil and belongs to the lamiaceae family. Basil is wide spread in Asia, Africa and Central and South America. Over 50 species of basil exist differing in growth habits, chemical and aromatic compositions and physiological appearances (Svecova and Neugebauerova, 2010).

Basil is grown in Asia and Mediterranean countries with extensive production occuring in Indonesia, Greece, Hungary, France, Morocco, Egypt, United States of America and Israel (Putievsky and Galambosi, 1999). In Kenya basil is mainly cultivated in Kiambu, Meru, Laikipia and Kajiado counties (KEPHIS, 2015). In South Africa, basil is recorded to yield 10-20kg oil per Hectare and between 2.5 to 7.5 t/ha of fresh biomass (DAFF, 2012).

Basil is an erect herbaceous annual plant growing between 30 to 130 cm tall and bushy in shape. The stems are herbaceous when young and become woody as the plant matures. The leaves are light green to purple in colour depending on species, variety and soil fertility. They are broad to oval shaped 5-8cm long. The leaf texture is silky to shiny, dull to crinkly. Flowers are white to purple in colour and are arranged in whorls on the end of branches. The flowers produce oblong seeds which vary in colour from brown to black. The essential parts of basil are the leaves, stems and flowering tops (CABI, 2017).

Basil requires warm conditions to do well. It germinates best at optimum temperatures of 20^0C and requires a temperature range of 7-30^0C for better growth (CABI, 2017). Cold temperatures affect basil. It thrives in sunny conditions but cannot tolerate stress due to drought. A minimum of 700mm annual rain is suitable for dry land cultivation. Drip irrigation is best in supplementing rainfall for constant growth of basil compared to overhead irrigation. Drip irrigation reduces the development of foliar diseases due to reduced contact between the water and foliage. Black polythene mulch enhances yields and produces leaves that are clean and of high quality (DAFF, 2012). Basil prefers fertile, well drained soils and high nitrogen and water (DAFF, 2012). Basil also does well in well compost soils with a pH range of 5.5-6.5 (CABI, 2017).

2.2. Cultivation practices of basil

Basil is propagated through seed or cuttings. Seeds are sown in seed trays before they are transplanted or directly seeded. With direct seeding, 80 to 90 % germination can be achieved though it takes a shorter time to get a crop by pre-germinating seeds. In warm conditions, seeds take 4-6 days to germinate while at lower temperatures they take 8-14 days before germination occurs (CABI, 2017). Transplanting is done after three to six weeks when the seedlings are about 15 cm in height. Cultivation of basil can be done in the open field or in the greenhouse (DAFF, 2012). A spacing of 30-45 cm is used between rows and 10-20 cm between plants (CABI, 2017). A total plant populations of 60 000 to 90 000 per hectare is mostly achieved (DAFF, 2012).

At about 15cm from the ground, seedlings are pruned to encourage lateral branching and growth. Fertilizer application is determined by the type of soil and the recommendations arising from soil analysis. Basil responds well to moderate fertility depending on the purpose of cultivation. Fragrance of its oils varies with the quantity of fertilizer applied. Large amount of fertilizers cause decline in the fragrance of oils (CABI, 2017). Basil can be grown through over-head irrigation, drip irrigation or can be rain fed. Weed management is critical to reduce competition because weeds decrease the quality of basil leaves (CABI, 2017). Weeds can be controlled mechanically or through manual weeding and mulches can be used to supplement weed control strategies (DAFF, 2012).

2.3. Basil production constraints in Kenya

A number of constraints limit basil production in Kenya. The major constraints include pest and diseases and the strict requirements on food safety due to application of pesticides. Important diseases of basil include Botrytis (*Botrytis cinerea*), Fusarium (*Fusarium oxysporum*), *Rhizoctonia solani*, *Pythium spp*. and *Phytophthora spp* (CABI, 2017). Basil is also attacked by various insect pests that include beetles, slugs, thrips, whitefly, leafhoppers and leafminers. Leafminers (*Liriomyza spp*) are a major biotic constraint due to their quarantine importance in Europe where most of the Kenyan basil is exported (EPPO, 1992).

2.4. Description, classification and distribution of *Liriomyza*

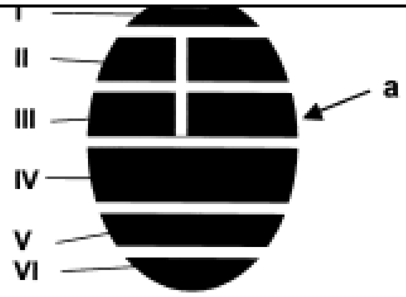
Leafminers belong to the genus *Liriomyza* (Diptera: Agromyzidae). The genus contains over 300 species which are distributed worldwide (Parella, 1987). The genus contains 23 species of economic importance that cause damage to a wide range of agricultural, horticultural and ornamental plants. Adult leafminers are small 1-3mm in length. When observed from above, they are largely black with a bright yellow scutellum. Three highly polyphagous and economically important species occur in Kenya (CABI, 2017). They include the American serpentine leaf miner (*L. trifolii*), the vegetable leaf miner (*L. sativae*) and the serpentine leafminer (*L. huidobrensis*).

L. trifolii © CSL, York (GB) British Crown | *L. huidobrensis* © CSL, York (GB) - British Crown | *L. sativae* © Lyle J. Buss, University of Florida.

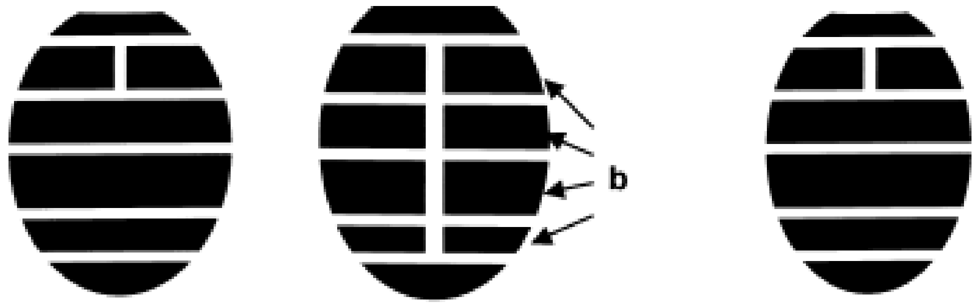
Figure 2.1: Morphological distinctions between the adult *Liriomyza spp*. *L.huidobrensis* and *L. sativae* have similar tergite patterns while for *L. trifolii* the second to fifth tergites are divided by the yellow medial furrow. Source (Shiao, 2004).

It is not easy to distinguish among *Liriomyza spp*. *Liriomyza trifolii* is smaller and more yellow than *Liromyza huidobrensis* and *Liriomyza sativae.* Mines made by *Liriomyza trifolii* are commonly based on the upper surface of the leaf while those made by *Liriomyza huidobrensis* occur on the lower side of the leaf. Mines caused by *Liriomyza trifolii* are narrow and occurs near the leaf margin whereas those caused by *L. huidobrensis* are frequently found near the base of the leaf mostly along the middle veins (EPPO, 2005).

Abdominal colour patterns are commonly used in morphological identification. The overall appearance of *Liriomyza huidobrensis* adult is that of a small dark fly while *Liriomyza sativae* is smaller and yellow in colour. For *Liriomyza trifolii*, the second to fifth tergites are divided by the yellow medial furrow which is distinct from the other two that have the second visible tergite divided by a yellow medial furrow (Shiao, 2004).



I to VI above indicate the first to sixth visible abdominal tergites



L. huidobrensis *L. trifolii* *L. sativae*

Figure 2.2: Tergite patterns as a mark of morphological distinctions between the species.

2.5. Biology and ecology of *Liriomyza spp*

The typical life cycle for *Liriomyza* comprises of the egg, larvae, pupa and adult stages. Egg stage lasts 3 to 4 days. The larval stage has up to third instars. The first-instar lasts 3 to 4 days; the second-instar lasts 2 to 3 days while the third instar lasts 3 to 4 days. The pupal stage lasts 12 to 18 days after which adults emerge (CABI, 2017). Female adults can live for 3 to 28 days while male adults live 2 to 6 days (CABI, 2017). Development occurs well in optimal temperatures of 20-30^{o}C (CABI, 2017). With suitable temperatures, many generations occur throughout the year. The larval stage is noted as the most destructive.

Females puncture leaf tissue by their ovipositors to lay eggs. The eggs are 0.25 mm in length, white in colour and oval in shape. The larvae feed as they tunnel through the leaf tissue. Emerging larvae are about 0.5 mm long and get to 3.0 mm when fully grown. The larvae that are fully grown drop to the ground to pupate. They do so by cutting a slit in the leaf surface where they exit. In some cases, the larvae may pupate within the mines. Pupae are oval in shape about 2.0 mm in length varying in colour from yellow to orange to dark brown (CABI, 2017).

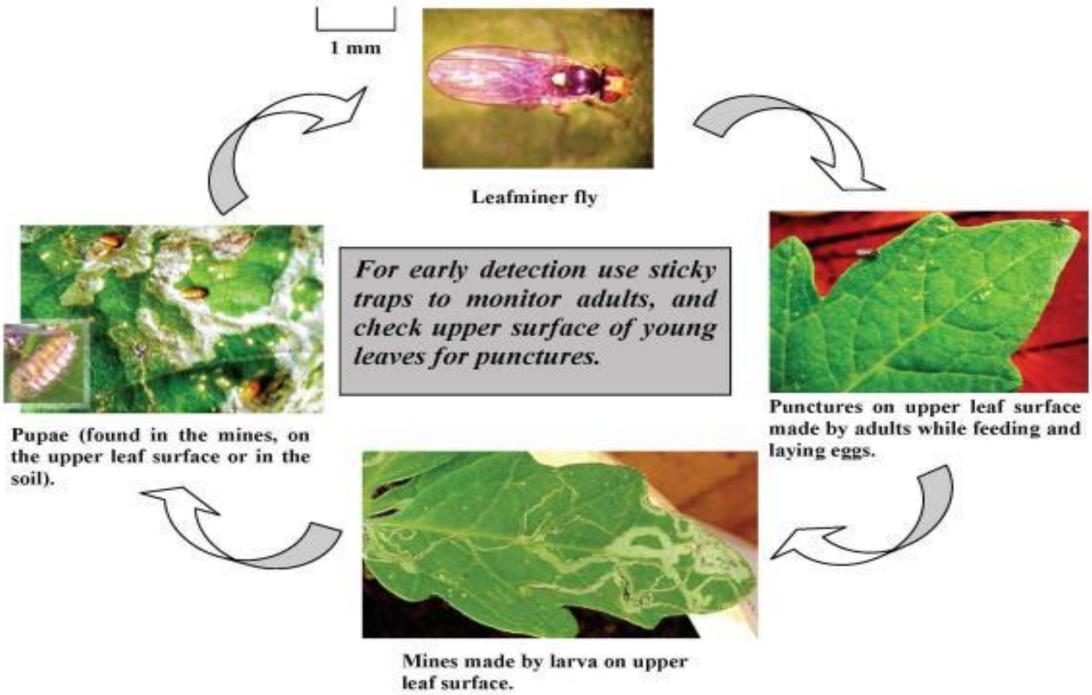


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Figure 2.3: Life cycle of *Liriomyza spp***.**

2.6. Damage symptoms and economic importance of *Liriomyza spp*

The symptoms of *Liriomyza* attack include feeding punctures and leaf mines. Feeding punctures are 0.2 mm in diameter and round in shape (Spencer, 1989). The punctures are white speckles occurring on the upper surface of the leaf and are similar cutting across species (Spencer, 1989). Leaf mines occur when the larvae feed through the palisade tissue in the upper part of the leaf while mining through. The mines are white in colour and inside them may occur trails of broken frass along their length. *Liriomyza trifolii* form tightly coiled almost blotch-like mines. *Liriomyza sativae* form a more loosely, irregular serpentine mine while *Liriomyza huidobrensis* form irregular serpentine mine which is restricted by veins within segments of the leaf with a wave like appearance between upper and lower leaf surface (OEPP/EPPO, 1992). Mines configuration form a guide in identification of agromyzid species. However, the mines are affected by the physical and physiological condition of each leaf, the host type and the number of larvae mining the leaf (EPPO, 2005).

Liriomyza spp attack host plants during the seedling, vegetative, flowering and fruiting stages (CABI, 2017). Leaves and pods are the plant parts mainly attacked. Larva is the most damaging stage of the pest. Adults and larvae cause direct damage to plants. Female adults puncture plant tissues with their ovipositors where they insert eggs or create open wounds where they feed on the oozing plant sap (EPPO, 2005). In case of laid eggs, it is the hatched larvae that tunnel through the leaf for feeding (EPPO, 2005). Tunneling affects plants by reducing their photosynthetic capacity. Attack also causes leaf abscission (Parrella, 1987) and death of young seedlings (EPPO, 2005). Aesthetic value of the plants is also reduced (Parrella, 1987) and adults can transmit viral diseases (Zitter and Tsai, 1977) in addition to causing decline in crop yields (Weintraub and Horowitz, 1997). According to Civelek and Onder (1997) *Liriomyza huidobrensis* adults transmit viruses such as Tomato Mosaic virus in plants of solanaceae and chenopodiacea families. In trade, eggs and larvae are carried through leaves, stems, shoots, trunks and branches of the host plant (CABI, 2017).

Liriomyza spp are polyphagous. They feed on plants cutting across various families: Solanaceae, Liliaceae, Compositae, Umbelliferae, Chenopodiaceae and Curcurbitaceae (CABI, 2017). In Colombia, infestation by *Liriomyza huidobrensis* (0.7 adults/leaf) decreased production quantities by 38-47% in Gypsophila as well as delayed the start of harvesting (Torres *et al*., 1995). Losses of 100% in potato yield were recorded in Indonesia (Shepard *et al*., 1998) while losses of 30% were also recorded on potato yields in Israel (Weintraub, 2001). Wolfenbarger (1954) reported an increase in yield of 25% with reductions in leafminer infestation in potato.

It is reported that in 1976, *Liriomyza trifolii* introduced from Florida through contaminated cuttings caused closure of a chrysanthemum nursery in Masongaleni Kenya (CABI, 2017). IPPC (2005) reported losses of crop and markets oversee due to infestation by *Liriomyza trifolii* due to quarantine requirements. In the USA *Liriomyza trifolii* is recorded to have caused huge losses on celery estimated at US$ 9 million in 1980 (Spencer, 1982). *Liriomyza trifolii* is also reported as being a vector of plant viruses (Zitter *et al*., 1980). *Liriomyza sativae* is the most serious of the leaf miners. It is recorded to have caused severe damage and loss of yield in many of the southern states of America and South America. Losses of 80% were reported on celery in Florida (CABI, 2017). *Medicago sativa* in Argentina was seriously damaged with estimates of up to 80% losses (Spencer, 1973).

2.7. Control methods of *Liriomyza spp*

Various approaches have been applied in the control and management of *Liriomyza spp*. The approaches include cultural, chemical, biological and phytosanitary measures.

2.7.1 Cultural control

Burning is effective on imported plant consignments that are checked and found with signs and symptoms of *Liriomyza* (Velez *et al*., 1980). Weeds are controlled because they act as secondary hosts to *Liriomyza*. Mined leaves including infested plant materials after harvest are hand-picked and destroyed. Ploughing to expose pupae to desiccation and predators is applied in reducing emergence population of adults (University of California, 2012). Where nursery stock is used as seedlings, infested plants are checked and destroyed. The pupa in the soil is killed through solarisation. Planting of susceptible hosts of *Liriomyza* continuously leads to pest build up. Rotation with non-host plants is applied to reduce pest levels and the risk of infestation. Cold storage at 0°C for 1-2 weeks is effective at killing *Liriomyza* larvae in chrysanthemum cuttings (Webb and Smith, 1970).

Maintaining hygiene contributes to the control of leafminers. Clearing all weeds and treating the soil destroys the pupae. For effective control, infested weeds and waste plant material are burned and not composted (Velez *et al*., 1980).

2.7.1.1. Polythene mulch as cultural control for *Liriomyza spp*

Reflective polythene mulches have been applied to protect crops from insect pests or diseases (Summers and Stapleton, 2002) and has been observed that the control of silver leaf whitefly population by reflective plastic mulch equals that provided by imidacloprid treatment. Visual cues are used by insects to find their host plants. Csizinszky *et al.* (1995) observed that reflective polythene mulches interfere with such cues thus causing increased attraction or repulsion to crops. According to Matteson *et al*. (1992), western flower thrips (*Frankliniella occidentalis*) are attracted to low UV reflective blue, yellow and white colours. Matteson *et al*. (1992) also observed a lower count of thrips on black, green, red and substrates that were highly UV reflective. Bare soil and colours like green and yellow have been observed to attract aphids while silver colours repulse them (Webb *et al*., 1994). Various insect-vectored diseases have been delayed or prevented on their onset by the application of coloured plastic mulches. Jones (1991) observed that when reflective mulch was applied, the number of aphid vectors reduced which also reduced the spread of cucumber mosaic virus and bean yellow mosaic viruses. Reflective mulches have also been evaluated for reducing landing rates of flying insects such as aphids (Kumar and Poehling, 2006). Murphy *et al.* (2009) reported that reflective mulches reduce the incidences of vector borne viral diseases in crops by repelling vectoring insects.

2.7.2. Physical control

2.7.2.1. Mass trapping by use of sticky traps

Yellow sticky traps have been used in monitoring population of various insect pests. Yellow traps are attractive to many insect species (Moreau and Isman, 2011). In various researches, they have been utilized in monitoring leafminers, aphids and whiteflies (Gu *et al*., 2008). Use of yellow sticky traps is an important element in IPM strategy on *Liriomyza spp* control. They are applied in assessing the presence of adult *Liriomyza* and to gauge the best time to apply control measures (CABI, 2017). They are also applied in direct suppression of adults by mass trapping. Yellow sticky traps effectively control leafminers at low densities (Valenzuela, 2010).

Arida *et al*. (2013) observed a significant decline in the average number of mines per leaf after 50 days when yellow sticky traps were applied in the management of *Liriomyza trifolii*. In another study, damage by *Liriomyza sativae* was reduced when yellow stick traps were placed above the growing host plants (Liu and Wang, 1992). The reduction was attributed to the fact that male *Liriomyza sativae* fly low between plants and are more mobile than females. Trapping, therefore, reduced the number of active males and the mating probability thereby reducing damages. Gu *et al*. (2008) recommended the use of yellow sticky traps and parasitoids to control *Bemisia tabaci* in green house production. According to Abdel-Megeed *et al*. (1989) yellow sticky traps are effective in controlling *Bemisia tabaci* in field production too. Yellow sticky traps are applied while targeting leaf-miners, aphids, whiteflies, thrips and sciarids (https://www.koppert.com/products).

2.7.3. Chemical control

Translaminar insecticides such as abamectin, growth regulator cyromazine, neem and spinosad are presently effective against *Liriomyza* (Weintraub and Mujica, 2006). This is due to their ability to penetrate the leaf cuticle into the plant tissues. *Liriomyza spp* have developed resistance against chemical pesticides such as carbamates, organophosphates and pyrethroids that were effective before 1990s (Nuessly and Webb, 2013). Gamma irradiation of first larval stage or eggs is reported to be effective control on *Liriomyza* (Yathom *et al*., 1991). Chemical products registered in Kenya and recommended for the control of *Liriomyza spp* are listed in Table 1 below. However, none is registered specifically for use on basil.

Table 2.1: Registered pesticide products against *Liriomyza spp* in Kenya

Product Name (Active Ingredient)	**Purpose for registration**
Abamectin 18% w/w (18g/L)	Control of *Liriomyza spp* on Roses, French beans, snow peas, tomatoes and ornamentals/flowers
Acetamiprid 25g/L	Control of Liriomyza spp on French beans.
Thiocyclam- hydrogen oxalate 50% w/w	control of *Liriomyza spp* on horticultural crops and flowers
Deltamethrin 100g/L	Control of *Liriomyza spp* on tomatoes
Lambda-cyhalothrin 50g/L	Control of *Liriomyza spp* on Broccoli

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2.7.3.1 Challenges of using pesticides in controlling *Liriomyza* in basil

Besides development of resistance by *Liriomyza*, food safety concerns have led to increased restrictions on the use of pesticides in pest management on basil and other fresh produce. There are currently 473 active ingredients screened for MRLs in basil by the European Food Safety Authority (EU Pesticides database - European Commission, 2016). The tolerable maximum level for spinosad (Sum of spinosad A and spinosad D) is fixed at 15mg/kg of basil while that of Azadirachtin is fixed at 1mg/kg of basil (EFSA, 2016). Compared to the other 471 active ingredients, spinosad and neem have the highest acceptable limits because of their relative low toxicity. Codex Alimentarius Commission of the World Health Organization fixed the maximum acceptable level of Thiocyclam-hydrogen oxalate (Active Ingredient for Evisect®) as 0.3mg/kg in cabbages, 0.5 mg/kg in brinjals and 0.5 mg/kg in tomatoes (CODEX, 2010).

2.7.4. Use of bio-chemical pesticides

Bio-chemical pesticides are based on substances that occur naturally and control pests by non-toxic mechanisms unlike chemical pesticides that kill pests through active ingredients that affect certain physiological functions (Sporleder and Lacey, 2013). Biochemical pesticides are categorized into various classes that function biologically: Plant extracts (botanicals), natural insect growth regulators, pheromones and other semio-chemicals. Neem and spinosad fall into this category (Sporleder and Lacey, 2013).

Use of conventional pesticides is less advantageous compared to bio-pesticides (Kaya and Vega, 2012). Bio-pesticides are safe to the environment and humans. They do not have residues that are harmful in food. Besides, they are more specific to target pests. Due to safety to the environment and consumers plus their sustainability in use, bio-pesticides like neem and spinosad application are now becoming an important component in Integrated Pest Management (IPM) strategies (Sporleder and Lacey, 2013).

2.7.4.1 Use of neem

Neem is one of the desirable bio-pesticides (botanical insecticide) for control of agricultural pests. Because of its low toxicity, neem based insecticides are recommended and approved for organic agriculture (OMRI, 2007). Neem is obtained from *Azadirachta indica* (A) Juss (Meliaceae). Several limonoids are extracted from the neem tree which can be used for pest control (Khan *et al.*, 1974). They include: Azadirachtin, salannin, nimbin, ammonia formaldehyde, phenols, fatty acids and tannins. Azadirachtin is reported as the most effective amongst the limonoids (Kraus, 1995). Neem is effective in control of over 400 species of insect pests that belong to the Lepidoptera, Hemiptera and Diptera orders (Schmutterer and Singh, 1995). Other studies have shown that neem is effective against nematodes, fungi, viruses and protozoa (Mordue and Blackwell, 1993). Neem has various attributes which make it attractive for use in control of agricultural pests compared to synthetic products like Lambda- cyhalothrin. It's effective against many kinds of pests, degrades fast, and has minimal human and mammal toxicity and very low incidence of resistant biotypes (Niemann and Hilbig, 2000).

Azadirachtin the active ingredient in neem controls pests in several ways. It has anti-feedant, repellant and ovipositional deterrent properties. The insect of concern stops feeding on the host plant to which neem has been applied and starves to death. Neem also affects growth and reproduction of insects. It has a direct effect on cell division thereby affecting growth of insects (Mordue and Blackwell, 1993). Neem is reported to hinder development of larval stages of whiteflies into adults by inhibiting the activities of the insect molting hormone (Mordue and Blackwell, 1993). Azadirachtin has also been shown to inhibit vitellogenesis (yolk deposition*)* in adult females of *Labidura riparia* leading to low fecundity (Sayah *et al.,* 1996).

For centuries, Neem has been used for the control of insect pests in Agriculture where azadirachtin and neem oil have been used to kill aphids (Immaraju, 1998). When azadirachtin was systemically applied to rape plants, the population of cabbage aphids (*Brevicoryne brassicae* L.) was reduced, more nymphs were killed and the adult fecundity was reduced (Pavela *et al*, 2004). A formulation containing neem oil has been proved effective in the control of fruit flies (*Ceratitus capitata*) by causing adult mortality (Ad´an *et al*., 1998). Neem seed oil is reported to be highly effective against the brown plant-hopper nymphal stages (Saxena and Khan, 1985). According to Thoeming *et al*. (2006), neem is also efficacious in the control of thrips.

2.7.4.2. Use of spinosad

Spinosad is a product of bacterial fermentation. It is a metabolite obtained from a spinosyn-producing soil actinomycete bacteria *Saccharopolyspora spinosa*. Spinosad commercially comprises of spinosyn A and spinosyn D in the ratio of about 85:15. Structurally Spinosyn A and D are similar except for the additional methyl group on spinosyn D (BCPC, 1997).

Spinosad kills through contact and stomach poisoning (Salgado, 1998). The central nervous system of target insect is activated through interaction with the nicotinic acetylcholine receptors. Contact with spinosad causes tremors, trembling, paralysis and eventual death of target pest. Spinosad is very effective against most caterpillar pests, thrips, fruitflies, sawflies, some beetles e.g. Colorado beetles and leaf miners in vegetables and ornamental plants (Miles, 2003).

Spinosad is classified as a "reduced-risk" compound by U.S. Environmental Protection Agency (www.epa.gov/opprd001/factsheets/spinosad.pdf) because it has low impact and it is obtained naturally. Spinosad product labels bear the word "Caution", the lowest human hazard label assigned by the U.S. EPA. In Sweden, spinosad was approved for organic agriculture in 2001(IFOAM, 2000).

2.7.5. Biological control

Parasitoids, fungi, nematodes and bacteria have been used biologically to control *Liriomyza spp* (Abd El-Salam *et al*., 2013). Use of parasitoids on different *Liriomyza spp* both in the open fields and greenhouses are recorded (Liu *et al*., 2011). Over 150 species of parasitoids for *Liriomyza spp* are reported globally (Liu *et al*., 2011). Use of parastoid wasps such as *Diglyphus isaea* and *Dacnusa sibirica* are recommended for green house crops (Leuprecht, 1992). *Diglyphus isaea* is commercially present in Kenya and approved for control of *Liriomyza spp* on flowers and vegetables (www.pcpb.or.ke). *Diglyphus isaea* parasitizes

Liriomyza larvae by laying its eggs alongside them. On hatching, *Diglyphus isaea* larvae feed on the *Liriomyza* larvae from the outside consequently killing them. *Dacnusa sibirica* lays its eggs in the *Liriomyza* larvae. The eggs hatch and develop emerging as adults having killed *Liriomyza* pupae.

Successful control of leafminers by use of nematodes has also been achieved by foliar application of *Steinernema feltiae* (Williams and Macdonald, 1995). Williams and Walters (2000) observed that all the three larval instars of *Liriomyza huidobrensis* are killed by *Steinernema feltiae*. *Steinernema feltiae* swims into the *Liriomyza* mines, penetrates the larvae and releases a bacterium that kills them. Bordat *et al*. (1988) studied the effect of entomogenous fungal strains of *Beauveria bassiana*, *Metarhizium anisopliae*, *Paecilomyces farinosus* and *P. fumosoroseus* on *Liriomyza sativae* pupae and found great reductions of up to 80% in the emergence of adults. Releasing sterile *Liriomyza trifolii* males is reported to reduce the population fitness and fecundity of subsequent offspring (Kaspi and Parrella, 2006).

2.7.6 Integrated Pest Management (IPM)

Prokopy (2003) defined IPM as a tactic applied in the control of pests based on coordinated use of various tactics that are ecologically and economically sound. IPM for control of leafminers involves application of a range of techniques. Chemical pesticides are used selectively to reduce costs and related safety concerns (Nath and Singh, 2006). IPM-compatible techniques include cultural methods, host plant resistance, biological control and physical control strategies.

IPM approaches on *Liriomyza* involves use of botanical extracts, minerals, soaps, growth regulators and entomopathogenic insecticides (Yildirim and Baspinar, 2012). Spinosad is recommended for integration in IPM program because it displays high level of selectivity towards most beneficial insects (Mayer *et al.*, 2001). Spinosad is safe for use and does not kill honey bees (Mayer *et al.*, 2001). Ester (1993) recommended physical barriers as effective against *Liriomyza spp* and a critical element in IPM. Ogbuewu *et al.* (2011) recommended incorporation of neem in IPM programs because of its economic advantages, safety to humans and non-target organisms.

2.7.7. Phytosanitary measures

Phytosanitary measures have been applied in the control of *Liriomyza*. Storage of infested produce at 0°C for 1-2 weeks has been proved to kill all stages of *Liriomyza* (CABI, 2017). Field inspections are mandatory for propagation material entering the EU originating from countries where *Liriomyza spp* occur. It is a requirement that host plants are inspected at least every month to check freedom from *Liriomyza spp* (EPPO, 2014). A phytosanitary certificate is issued to accompany such produce as a proof of compliance.

2.8. Nutrient supply and insect pest incidences

Nitrogen, phosphorus and potassium are fundamental macro nutrients for plant growth and development. They are critical in metabolism and energy production which enhances crop yield. Various researchers have demonstrated that high nitrogen levels in plants tissue decreases plant resistance to diseases and increases susceptibility to pest attack. Uhart and Andrade (1995) observed that leaf area index (LAI) and crop photosynthetic rate reduced under nitrogen stress while excess nitrogen led to increased pest problems. Soil nutrients enhance plant growth while increasing vegetative growth make plants more attractive to phytophagous insects. Ramzan *et al*. (1992) stated that high infestation of pest is associated with the high use of nitrogenous fertilizers. Setamou *et al*. (1995) observed that damage to crops by insect pests is amplified by the application of fertilizers. Tanzubil *et al*. (2006)

observed that stem borer populations increased in a millet crop with high nitrogen application. Saha and Saharia (1970) reported increased incidences of stem borers on rice with increased supply of nitrogen fertilizer rates. According to Cisneros and Godfrey (2001), nitrogen decreased plant resistance against aphids in cotton. In other studies, it was observed that addition of nitrogen fertilizers in cotton, tomato, lettuce, chrysanthemum and poinsettia crops favoured attack by *B. tabaci*, *B. argentifolii* and *T. vaporariorum* (Bi *et al*., 2001). Yildrim and Unay (2011) in a study observed that the combination of foliar fertilizers of fulvic acid and calcium nitrate applied on tomato resulted in reduction of *Liriomyza trifolii* population. Application of potassium silicate foliar on *Gerbera jamesonii* reduces *Liriomyza trifolii* populations (Mortezaiefard *et al*., 2012).

CHAPTER THREE

EFFECT OF SOIL AMENDMENTS AND FOLIAR FEED SPRAYS ON THE LEVEL OF DAMAGE AND ONSET OF INFESTATION BY *LIRIOMYZA SPP* ON BASIL

Abstract

Basil is one of the economically important herbs grown in Kenya. Besides its condiment and medicinal importance, it is locally sold and exported as a source of income. *Liriomyza spp* is one of the biotic factors constraining basil production in Kenya. Female *Liriomyza* puncture leaf tissues by their ovipositors to lay eggs while larvae feed by tunneling through the leaves and is the most destructive stage. Attack by *Liriomyza spp* reduces the net volume available for sale especially in Europe where the pest is regulated as quarantine. The study was conducted to determine the effect of soil amendments and foliar feed sprays on the level of damage and onset of infestation by *Liriomyza spp*. An experiment was conducted in the field and arranged in a randomized complete block design (RCBD) with three replicates and three treatments. The treatments involved varying the crop nutrition components as undertaken by farmers. Treatments applied were: (i) Control (DAP fertilizer only) (ii) DAP fertilizer + chicken manure (iii) DAP fertilizer + chicken manure + foliar feed spays (applied weekly). Data on the number of leaflets damaged by *Liriomyza spp* was collected weekly from three plants randomly selected per treatment. Analysis of Variance using SAS version 9.4 was carried out and the treatments were compared using least significant difference ($LSD_{0.05}$) pair-wise comparisons at 5% significance. The results showed that *Liriomyza spp* attacked basil in the second week after transplanting and the level of damage was significantly ($P \leq 0.05$) higher on the plots treated with DAP fertilizer, chicken manure and foliar fertilizer. These findings will help growers in aligning monitoring and management strategies of leafminer to comply with phytosanitary standards.

3.1 Introduction

Basil is one of the major herbs grown in Kenya. The herb is utilized locally for spicing but it is largely grown for the export market mainly the European Union. In 2015, fresh produce including basil and other herbs was recorded to have fetched about 100 billion into Kenya's economy (FPEAK, 2016). *Liriomyza spp* is one of the pests that limit basil production and hindrance to market access. Female *Liriomyza* puncture leaf tissues by their ovipositors for laying eggs while the larvae mine through the leaves. Severe attack causes leaves to fall and young plants to die. *Liriomyza* is also reported to transmit viral diseases besides creating wounds that form entry points for disease causing pathogens (Parella, 1987). *Liriomyza huidobrensis, Liriomyza trifolii* and *Liriomyza sativae* are quarantine to the European Union (EU, 2000).

Consignments of basil from Kenya have frequently been intercepted in Europe. Strict requirements have resulted to the loss of millions of shillings which is a threat to business and Kenya's foreign exchange. The volume of basil and other herbs exported to the EU from Kenya is reported to have declined approximately from 2% of the total export volumes in 2012 to less than 1% in 2015 (KEPHIS, 2015). Following the economic importance of basil, it is critical that growers intervene with management measures against *Liriomyza spp* early enough in order to increase yield and to eliminate phytosanitary incompliance. Therefore, the objective of the study was to determine the effect of soil amendments and foliar feed sprays on the level of damage and the onset of infestation by *Liriomyza spp.*

3.2. Materials and methods

3.2.1. Location and description of the experimental site

The experiment was undertaken at the University of Nairobi College of Agriculture and Veterinary Sciences field station in Kabete for two seasons. The first season commenced in late February until June 2016 and the second season commenced July until November 2016. Kabete and the larger Kiambu County are agriculturally rich and some of the basil which is exported from Kenya is grown in these areas. Kabete is located at an altitude of 1900 m above the sea level (m.a.s.l) with annual rainfall average of 1,200 mm. The area is largely covered by reddish friable volcanic clay soils that are well drained with moderate fertility (Jaetzold *et al*., 2006). The farming system in this area is a continuous cropping of various crops in separate rotation and commercial fields. Kabete is generally a warm area with a mean temperature of 22°C. July and August are the coldest months while January to March is the hottest period. During cold months temperatures can fall to 7°C while during hot months they can rise up to 34°C.The average relative humidity of Kabete ranges from 54% in the dry months to about 100% during wet months of March up to August (Jaetzold *et al*., 2006).

3.2.2 Establishment of crop, experimental layout and application of treatments

A case study was conducted outside this experiment in five large scale farms to establish the agronomical practices which are applied on basil. The farms selected were based on the volumes grown and the history of regular export of basil to the European Union. The study established that basil is pre-germinated on trays and raised in a nursery before seedlings are transplanted to the field. In the field, growers amended the soil through the application of organic manure (decomposed cattle or chicken manure) and applied inorganic fertilizer at planting. To enhance vegetative growth and correct any nutritional deficiencies, some growers boosted their crops weekly or bi-weekly with foliar sprays rich in Nitrogen, Phosphorous, Potassium and micro nutrients.

Basil seeds of Bonanza® variety were purchased from a local agro dealer and pre-germinated on trays containing coco peat media. Coco peat is widely used in Kenya for pre-germination and pre-rooting in green house productions. Germination occurred after seven (7) days and the germinated seedlings were maintained under shade net conditions of room temperature (about 25°C) and relative humidity (approximately 60%). The seedlings were fertigated once a day with NPK (19:19:19) with incorporated micro nutrients.

In the field, the experimental site was demarcated and the field was ploughed by a tractor and plots were manually prepared for planting. Each plot measured 2m by 2m. The experimental plots were arranged in a randomized complete block design with three replicates. The treatments consisted of (i) Control (DAP only) (ii) DAP + Chicken manure (iii) DAP + Chicken manure + foliar feed sprays (applied weekly). Chicken manure was in-cooperated in the soil at a rate of 5t/ha. Di-Ammonium Phosphate (DAP {18:46:0}) fertilizer was broadcasted at a rate of 50kg/acre during transplanting. Seedlings were transplanted after two weeks with each plot consisting of 6 rows at spacing of 30 cm between the rows and 20 cm between plants. A maximum of 60 plants were raised per plot. A foliar spray consisting of NPK (24:24:18), magnesium and chelated trace elements was applied weekly where applicable.

Data on the number of leaflets damaged by *Liriomyza spp* was taken from the middle four rows in each treatment weekly for a period of seven weeks. Three plants were randomly sampled and tagged in each treatment for weekly data collection. Tagging was done to accord each plant an equal chance of being sampled for data collection. The three plants were randomly selected using random numbers generated through MS Excel 2010 between 1 and 40. From the tagged plants, all leaflets bearing larvae, tunnels and punctures due to *Liriomyza* infestation on each plant were counted and their totals recorded per week.

3.2.3 Statistical Analysis

Data on the number of leaflets damaged by *Liriomyza spp* was subjected to the analysis of variance (ANOVA) using SAS version 9.4 to assess the effects of treatments on the quantity of damage. The data was transformed (using the formula: square root + 1) to normalize the distribution for analysis. Least significant difference ($LSD_{0.05}$)-Pair-wise comparisons was used to differentiate the means among the treatments. The transformed data was used in presenting the dynamics in regard to the time when *Liriomyza spp* attacked the basil crop in the field.

3.3 Results

3.3.1 Time of attack by *Liriomyza spp*

During the first season, *Liriomyza spp* attacked the basil crop during the second week after transplanting. Peak *Liriomyza* damage occurred at the fourth week. The treatment comprising of Di-ammonium phosphate (DAP) with chicken manure and foliar feed sprays recorded the highest leaf damage while the control (Di-ammonium phosphate only) had the least leaf damage throughout the research period. In general, leaf damage increased with increase in time in all the treatments (Figure 3.1).

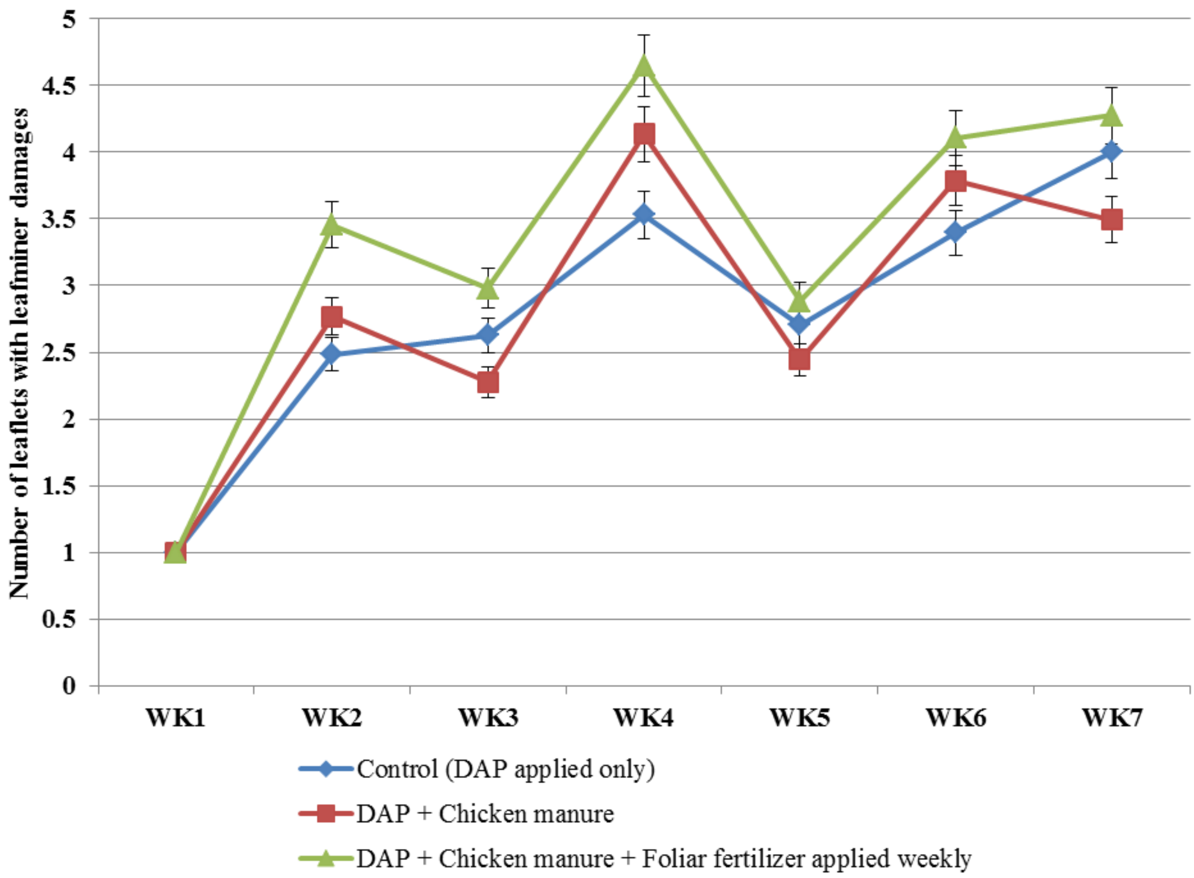

Figure 3.1: Leaf damage on treatments during the Feb to June 2016 season (Data transformed as square root (x + 1))

In the second season, a similar trend was observed like in the first season. *Liriomyza spp* attacked basil during the second week after transplanting. Di-ammonium phosphate with chicken manure and foliar feed spray treatment recorded the highest number of leaflets with *Liriomyza* damage while the control (Di-ammonium phosphate only) had the least number of leaflets bearing *Liriomyza* damage throughout the season. Peak leaflet damage occurred in the seventh week for all the treatments and the damage increased generally with increase in time throughout the season (Figure 3.2).

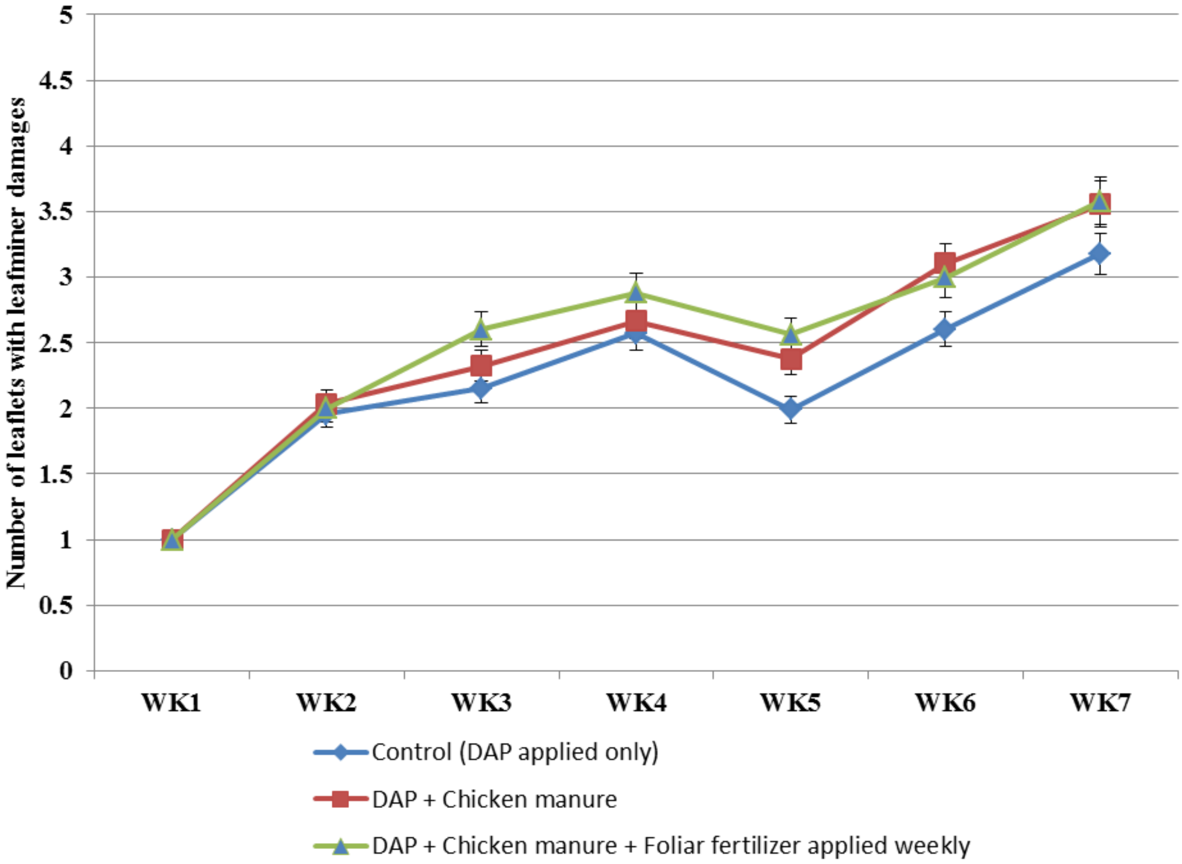

Figure 3.2: Leaf damage on treatments during the July to Nov. 2016 season (Data transformed as square root (x + 1))

3.3.2 Treatment effect on the number of leaflets damaged by *Liriomyza spp*

In the first season, the number of leaflets damaged in Di-ammonium phosphate with chicken manure and foliar feed spray treatment was significantly ($P \leq 0.05$) higher than the treatments that comprised of Di-ammonium phosphate and chicken manure, and the control (Di-ammonium phosphate only). There were no significant differences in the number of leaflets damaged in the control (DAP only) compared to DAP and chicken manure treatments (Table 3.1).

In the second season, the number of leaflets damaged in Di-ammonium phosphate with chicken manure and foliar fertilizer treatment was significantly ($P \leq 0.05$) different from the control (Di-ammonium phosphate only). There was no significant difference in the number of leaflets damaged in the control (Di-ammonium phosphate only) compared to Di-ammonium phosphate and chicken manure. In addition, there was no significant difference between the number of leaflets damaged in Di-ammonium phosphate and chicken manure compared to Di-ammonium phosphate with chicken manure and foliar feed spray. In both seasons, Di-ammonium phosphate with chicken manure and foliar feed spray treatment recorded the highest mean of the number of leaflets damaged by *Liriomyza spp* (Table 3.1).

Table 3.1: Effect of soil amendments and foliar feed spray on the mean number of leaflets damaged by *Liriomyza*

Treatment	**Season 1**	**Season 2**
Control (DAP only)	2.82^{b}	2.21^{b}
DAP + Chicken manure	2.84^{b}	2.44^{ab}
DAP + Chicken manure + Foliar fertilizer	3.34^{a}	2.52^{a}
P Value	0.0008	0.028
CV%	15.12	15.72
LSD	0.28	0.23

Means followed by the same letter in each column are not significantly different.at $P \leq 0.05$ Season 1 period from Feb-June 2016, Season 2 period from July-Nov. 2016

3.4. Discussion

The results obtained from this study show that soil amendments and foliar feed sprays increase the level of damage by *Liriomyza spp* and that infestation of basil occurs soon after transplanting. This could have happened because at the second week after transplanting and in suitable conditions, the crop is established, adjusted to the field conditions and at least two leaves are developed (CABI, 2017). Adults are attracted to the seedlings to feed while female adults besides feeding are in search of suitable sites for oviposition. Feeding leads to leaf mines due to the feeding effect of the hatched larvae while oviposition leads to punctures. According to CABI (2017), *Liriomyza* attacks host plants during the seedling, vegetative, flowering or fruiting stages.

From this study, it was also observed that the application of soil amendments and foliar fertilizer enhanced the magnitude of damage by *Liriomyza spp*. In this case the damage was higher in the crop where Di-ammonium phosphate, Chicken manure and foliar feed sprays were applied. This could be attributed to the increased supply of nitrogen and other nutrient elements as provided by Di-ammonium phosphate, chicken manure and the foliar fertilizer. Cisneros and Godfrey (2001) argued that fertilizers reach in the supply of free amino acids, sugars and nitrates provide nutrients to plants making them more attractive to insect pests. Adeniyan and Ojeniyi (2003) observed that soil fertility and crop yields are improved with the application of poultry, sheep and pig manure. Chicken manure increases soil pH, Nitrogen, Organic Carbon, Potasium, Calcium, Phosphorus, Magnesium, Sodium and CEC (Ewulo, 2005).

The results obtained in this research are in agreement with the findings of many other authors: Jahn (2004) reported that application of soil amendments stimulate plants to produce more broad, succulent and fresh leaves which form suitable oviposition sites for various insect pests. Ukwungwu (1985) and Setamou *et al*. (1995) demonstrated that damage to crops by insect pests increased with the application of nitrogen fertilizers. Monchiah *et al*. (2011) observed an increase in the population of insect pests on a cabbage crop upon the addition of organic or synthetic fertilizers. Stone *et al*. (2000) reported that increased supply of nitrogen from organic manure decreases resistance of plants to insect pest. Asawalam *et al*. (2007) observed that soil amendments with high nitrogen content increased infestation of insect pests on Okra. Mattson (1980) established that nitrogen content in plants is a limiting factor that affected host selection by phytophagous insects. Sseruwagi *et al*. (2003) observed that *Bemisia tabaci* populations increased significantly with Nitrogen, Phosphorous and Potassium fertilizer application on cassava. According to Habibibullah *et al*. (2007) high doses of nitrogen on cotton results to an increased population of *Bemisia tabaci* compared to lower doses. Ortega (2002) observed that increased application of nitrogen fertilizers increased severity of damage by whiteflies in ornamental plants.

From the findings of this study, it is important that growers of basil start monitoring *Liriomyza spp* as early as possible in addition to instituting control strategies immediately the presence of *Liriomyza* is detected. Farmers should also be weary of applying too much nitrogen fertilizers which in this case is attracting more infestation by *Liriomyza*.

CHAPTER FOUR

EFFICACY OF SELECTED BIO-PESTICIDES, POLYTHENE MULCH AND STICKY TRAPS IN CONTROLLING *LIRIOMYZA* (DIPTERA: AGROMIZIDAE) INFESTATION ON BASIL

Abstract

The leaves of basil (*Ocimum basilicum*) are used to add aroma and flavor to food. The herb is also medicinal applied in the treatment of various ailments. *Liriomyza spp* attack basil reducing its market value. The study aimed at evaluating the efficacy of neem and spinosad integrated with yellow sticky traps and polythene mulch in the management of *Liriomyza spp* on basil. Eight management options compared with the untreated control were tested to evaluate their efficacy in reducing the number of leaflets damaged by *Liriomyza spp* and the number of larvae that hatched on the oviposited leaflets. The two sets of data were obtained from three tagged plants randomly selected per treatment on a weekly basis. Analysis of variance using SAS version 9.4 was done and least significant difference ($LSD_{0.05}$) pair-wise comparisons at 5% significance used to compare treatments. The treatment comprising of neem alternated with spinosad sprayed weekly with polythene mulch and yellow sticky traps significantly ($P \leq 0.05$) reduced the number of leaflets damaged and the number of *Liriomyza* larvae hatched on the infected leaves. It was observed that the integrated application of neem, spinosad, polythene mulch and yellow sticky traps is efficacious in the management of *Liriomyza spp.* This IPM tool can be adopted as an option in addressing phytosanitary incompliance, food safety, pesticide resistance and environmental concerns that occur with the use of conventional chemical pesticides in basil production systems in Kenya.

4.1. Introduction

Basil production is constrained by a variety of insect pests including *Liriomyza spp. Liriomyza* affect the quality of leaves by direct feeding and by creating wounds that form entry points for disease causing pathogens. Besides feeding, adult females cause damage during oviposition (Parrella *et al*., 1985). Larva is the most destructive stage causing mining of leaves. The presence of eggs, larvae, pupa or even adults in consignments during international trade is a major phytosanitary concern (EU, 2000).

Various *Liriomyza spp* management practices have been recommended by a range of authors. Gitonga *et al.* (2010) observed that farmers in Kenya mostly limited their approach for the control of *Liriomyza* to the application of insecticides which have lately become subjects of pesticide residues exceedance and pest resistance. Bio-pesticides are generally acceptable in organic farming due to their low toxicity (Sporleder and Lacey, 2013). Neem and spinosad incorporated in this research are bio-pesticides categorized as bio-chemical pesticides. Neem is documented to control over 400 species of insect pests including *Liriomyza spp* (Schmutterer and Singh, 1995). Besides, neem is recorded to exhibit extremely low mammalian toxicity (Champagne *et al*., 1989). Spinosad is a product of bacterial fermentation which kills through stomach poisoning and contact activity (Salgado, 1998).

When applied in mass trapping, yellow sticky traps effectively control *Liriomyza spp* at low densities. Yellow sticky traps are also applied in pest monitoring. In monitoring, they give an indication of the adult *Liriomyza* population trends to determine the timing of enhanced control measures. Arida *et al*. (2013) depicted that when yellow sticky traps were applied in the management of *L. trifolii*, the average number of mines per leaf were significantly lower in the fields with yellow sticky traps after 50 days.

Reflective polythene mulches have been shown to reduce the population of certain insect pests and diseases on crops (Ngouajio and Ernest, 2008). Fully grown *Liriomyza* larvae mostly exit host plant leaves and drop to the ground to pupate. Polythene mulch may not provide conducive environment for the pupae to molt. The pupae are exposed to desiccation and adverse temperatures resulting to death, hence reducing the overall number of emerging adults.

There is scarce information in regard to the management of *Liriomyza spp* using bio-pesticides integrated with sticky traps and polythene mulch on basil. This study was undertaken to determine efficacy of the above approaches for *Liriomyza* management while addressing food safety concerns resulting from traditional pesticide use.

4.2. Materials and methods

4.2.1. Location and description of the experimental site

This study was undertaken in the field station of the University of Nairobi College of Agriculture and Veterinary Sciences Kabete. It comprised of two seasons of three months each from late February to November 2016. Kabete lies at an altitude of 1900 m above the sea level (m.a.s.l) with average annual rainfall of 1,200 mm. The soils are volcanic well drained with moderate fertility suitable for tea, pyrethrum, coffee and horticultural production (Jaetzold *et al.*, 2006). Farmers in this locality mainly practice continuous cropping of various crops undertaken in separate rotation and commercial fields. Temperatures are generally warm with a mean of 22°C. The hot period range from January to March while July to August is the coldest period. Temperatures fall to 7°C during cold months and can rise up to 34°C during hot months. The average relative humidity ranges from 54% in dry months to about 100% during wet months (Jaetzold *et al.*, 2006).

4.2.2 Establishment of the crop and experimental layout

Before undertaking the study, a case study survey was conducted outside this experiment in five large scale growers' fields to establish the management practices which were applied on *Liriomyza*. The growers selected were based on volumes grown and the frequency of export to foreign market. Across the farms, it was established that the companies commonly applied evisect (thiocyclam- hydrogen oxalate) for *Liriomyza* management while yellow sticky traps were largely applied in pest monitoring.

Seedlings of Bonanza® variety were rooted on trays using coco peat media in a nursery. At the field, the experimental site was demarcated and the field was ploughed by a tractor and plots manually prepared by use of hoes. Each plot measured 2m by 2m. As part of the farmer practice, chicken manure was in-cooperated in the soil at a rate of 5t/ha. Di-Ammonium Phosphate (DAP {18:46:0}) fertilizer was broadcasted at a rate of 50kg/acre before transplanting. Polythene mulch was locally purchased. It was cut into pieces enough to stretch over the selected 2M by 2M plots and secured by soil on the edges. Each plot consisted of 6 rows with a spacing of 30 cm between the rows and 20 cm between plants. A maximum population of 60 plants was raised per plot. On the plots bearing polythene mulch, hills were made on the polythene based on the required spacing to allow for planting and irrigation.

The experiment was arranged in a randomized complete block design with three replicates. Nine treatments comprising of: (i) Untreated control (ii) Yellow sticky traps only (iii) Polythene mulch only (iv) Spinosad sprayed weekly with yellow sticky traps (v) Neem sprayed weekly with yellow sticky traps (vi) Neem alternated with spinosad sprayed weekly with yellow sticky traps (vii) Evisect® (Thiocyclam 50% w/w of thiocyclam- hydrogen oxalate) only sprayed weekly (viii) Polythene mulch with yellow sticky traps (ix) Neem alternated with spinosad sprayed weekly with polythene mulch and sticky traps were allocated randomly to the plots using random numbers that were generated using MS Excel 2010 between 1 and 9 per block.

A commercial product Tracer® 480SC containing spinosad was applied. It is a suspension concentrate containing 480 g/litre (44.03% w/w) of spinosad applied at a rate of 0.2-0.5L/ha at intervals of 7-10 days with a pre-harvest interval of 10 days in most crops. Neem based product Nimbecidine was locally purchased. It is a liquid product containing Azadirachtin 0.03% applied at a rate of 50ml/20L or 2.5-3L/Ha at a pre-harvest interval of 7 days. Evisect® (Thiocyclam 50% w/w of thiocyclam-hydrogen oxalate) was also locally purchased. It was applied at the rate of 500-800g/ha or 7-10g/20L weekly with a pre-harvest interval of 7 days. Yellow sticky traps of sizes 25 by 10 cm were purchased locally. They were used as per the manufacturer's recommendation of 1 trap per 2 m^2. Spraying of pesticide treatments was done in the evening when winds were low. It was done using a hand sprayer while keeping in mind that good coverage of the crop by the applied products increased the efficacy of the active ingredients. Wind direction was also considered in avoiding contamination of neighbouring plots. Yellow sticky traps were monitored and changed weekly and were adjusted accordingly as the crop increased in height.

Two parameters were evaluated: the number of leaflets with *Liriomyza* damages and number of larvae on damaged leaves. The data was collected from the middle four rows of each plot weekly for a period of seven weeks. Three plants were randomly sampled and tagged weekly in each plot using random numbers generated through MS Excel 2010 between 1 and 40.

4.2.3 Sampling for Pesticides residue Analysis

At the fifth week during the first and second season, leaf samples were collected for pesticide residue analysis using the Codex Alimentarius guidelines on sampling for food safety. Nine samples of 1kg of fresh leaves were randomly collected representing each treatment. The samples were analyzed at the Analytical Chemistry Laboratory of the Kenya Plant Health Inspectorate Services.

4.2.4 Statistical Analysis

The data collected on the number of leaflets damaged by *Liriomyza spp* and the number of larvae on damaged leaves were transformed (using the formula: square root + 1) and later subjected to the analysis of variance (ANOVA) using SAS version 9.4 to assess the effects of treatments on the quantity of damage and the number of larvae hatched after oviposition. Least significant difference ($LSD_{0.05}$)-Pair-wise comparisons test was used to compare significant differences among treatment means ($P \leq 0.05$).

4.3 Results

4.3.1 *Liriomyza* damage on leaves

In season one, *Liriomyza* infested the crop naturally and the various treatments tested, significantly ($P \leq 0.05$) reduced the number of leaflets damaged by *Liriomyza* compared to the untreated control. The untreated control registered the highest mean number of leaflets damaged and was significantly ($P \leq 0.05$) different from all the other treatments applied. The treatments comprising of yellow sticky traps only, polythene mulch only and polythene mulch with yellow sticky traps were not different from each other. Evisect® only (farmer practice), neem applied weekly with yellow sticky traps and spinosad applied weekly with yellow sticky traps registered a lower mean number of leaflets damaged and were not different from each other. The treatment comprising of neem with spinosad (alternated weekly) and yellow sticky traps was significantly different ($P \leq 0.05$) from Neem with Spinosad (alternated weekly) together with Polythene mulch and yellow sticky traps. The treatment comprising of neem with spinosad (alternated weekly) together with polythene mulch and yellow sticky traps was significantly ($P \leq 0.05$) different from all the other treatments and it is the treatment that recorded the lowest mean number of damaged leaflets in the season (Table 4.1).

In season two, the infestation by *Liriomyza* also occurred naturally and the various treatments tested significantly reduced ($P \leq 0.05$) the number of leaflets damaged by *Liriomyza* compared to the untreated control. The untreated control was significantly ($P \leq 0.05$) different from all the other treatments applied. Yellow sticky traps only, polythene mulch only and polythene mulch with yellow sticky traps treatments were not different from each other. Evisect® only (farmer practice), neem weekly and yellow sticky traps, and spinosad weekly and yellow sticky traps were not significantly different from each other. Neem with spinosad (alternated weekly) and yellow sticky traps was significantly ($P \leq 0.05$) different from neem and spinosad (alternated weekly) together with polythene mulch and yellow sticky traps and the later recorded the lowest mean number of the damaged leaflets in the season (Table 4.1).

Table 4.1: Effect of treatments on *Liriomyza* damage on leaves

Treatment	**Season 1**	**Season 2**
Untreated control	3.21^{a}	3.25^{a}
Yellow sticky traps only	2.83^{b}	2.95^{b}
Polythene mulch only	2.80^{b}	2.84^{b}
Polythine mulch + Yellow sticky traps	2.63^{b}	2.90^{b}
Evisect® only sprayed weekly (farmer practice)	2.01^{c}	1.98^{c}
Neem Weekly + Yellow sticky traps	1.89^{cd}	1.99^{c}
Spinosad weekly + yellow sticky traps	1.87^{cd}	1.91^{c}
Neem + spinosad (Alternated weekly) + yellow sticky traps	1.79^{d}	1.62^{d}
Neem + Spinosad (alternated weekly)+ Polythene mulch + yellow sticky traps	1.47^{e}	1.46^{d}
P Value	<0.0001	<0.0001
LSD	0.2025	0.2035
CV%	14.56	14.35

Within column, means followed by the same letter are not significantly different at $P \leq 0.05$. Season 1 period from Feb-June 2016, Season 2 period from July-Nov. 2016

4.3.2 Effect of treatments on larvae damaging leaves

In the first season, the mean number of larvae observed on the damaged leaves was highest in the untreated control and was significantly ($P \leq 0.05$) different from all the other treatments. The mean number of larvae observed on yellow sticky traps only, was not significantly different from that observed on polythene mulch only. Polythene mulch with yellow sticky traps significantly ($P \leq 0.05$) differed from all other treatments and was fourth from the untreated control in terms of the mean number of larvae on damaged leaves. Evisect® only (farmer practice), neem weekly with yellow sticky traps, spinosad weekly with yellow sticky traps and neem and spinosad (alternated weekly) with sticky traps were not significantly different from each other. Neem and spinosad (alternated weekly) together with polythene mulch and yellow sticky traps had the least mean number of larvae on damaged leaves but was not different from neem weekly with yellow sticky traps, spinosad weekly with yellow sticky traps, and neem and spinosad (Alternated weekly) with sticky traps (Table 4.2).

In the second season, the treatments tested also significantly ($P \leq 0.05$) reduced the number of larvae observed on damaged leaves. The mean number of larvae in the untreated control was significantly ($P \leq 0.05$) different from all the other treatments. The treatments comprising of yellow sticky traps only, polythene mulch only and polythene mulch with yellow sticky traps did not differ significantly from each other. Evisect® only (farmer practice) and neem weekly with yellow sticky traps were not also different. Neem weekly with yellow sticky traps was not different from neem and spinosad (alternated weekly) with sticky traps. Spinosad weekly with yellow sticky traps and neem and spinosad (alternated weekly) together with polythene mulch and yellow sticky traps were not different though the latter recorded the lowest mean on the number of larvae on damaged leaves (Table 4.2).

Table 4.2: Effect of treatments on the larvae damaging leaves

Treatment	Season 1	Season 2
Control	1.96[a]	2.56[a]
Yellow sticky traps only	1.68[b]	2.28[b]
Polythene mulch only	1.72[b]	2.31[b]
Polythene mulch + Yellow sticky traps	1.48[c]	2.34[b]
Evisect only sprayed weekly (farmer practice)	1.30[d]	1.59[c]
Neem Weekly + Yellow sticky traps	1.22[de]	1.50[cd]
Spinosad weekly + yellow sticky traps	1.13[de]	1.22[ef]
Neem + spinosad (Alternated weekly) + yellow sticky traps	1.19[de]	1.34[ed]
Neem + Spinosad (alternated weekly)+ Polythene mulch + yellow sticky traps	1.07[e]	1.15[f]
P Value	<.0.0001	<.0.0001
LSD	0.17	0.17
CV%	19.48	14.92

Means followed by the same letter in each column are not significantly different at P≤0.05. Season 1 period from Feb-June 2016, Season 2 period from July-Nov. 2016.

4.3.3 Maximum residue levels

Fresh leaf samples were analyzed for pesticide residue levels. In the season Feb - June 2016 (Table 4.3), contamination attributable to drift of spinosad (Spinosyn A & D) was detected in the following treatments: control, yellow sticky traps, polythene mulch, neem applied weekly with yellow sticky traps and on the farmer's practice (evisect® applied only). Contamination due to drift of Azadiractin was also detected on the control and on the treatment where polythene mulch and sticky traps was applied. Treatments were tested statistically and in the two seasons, the quantity of spinosad (spinosyn A & D) and Azadiractin (A & B) residues detected in the three treatments; neem and spinosad (alternated weekly) together with polythene mulch and yellow sticky traps, spinosad sprayed weekly with yellow sticky traps and neem alternated with spinosad sprayed weekly and yellow sticky traps were significantly (P≤0.05) differently from each other. Thiocyclam- hydrogenoxalate was detected in the treatment where the farmers practice (Evisect® only) was applied and was statistically different (P≤0.05) from all other treatments (Table 4.3 & 4.4).

In both seasons, no sample exceeded the maximum acceptable limit of 1mg/kg for Azadirachtin (Sum of Azadiractin A & B) analytes. In addition, no sample exceeded the acceptable limit of 15mg/kg for spinosad (Sum of spinosyn A and spinosyn D) analytes. The samples obtained from Evisect® (farmer practice) treatment tested positive for Thiocyclam hydrogen oxalate at 0.401mg/kg in the season of Feb-June 2016 and 0.3652mg/kg in season July-Nov 2016. The two samples exceeded the World Trade Organization limit of 0.3mg/kg in cabbage (CODEX, 2010).

Table 4.3: Pesticide residue levels (micro-gram/kg) for the basil crop in Feb-June 2016 season

Treatment	Spinosyn A	Spinosyn D	Azadiractin A	Azadiractin B	Thiocyclam - hydrogenoxalate
Untreated control	0.10[f]	0.78[g]	0[b]	0.4[d]	0[b]
Yellow Sticky Traps only	0.10[f]	0.21[h]	0[b]	0[e]	0[b]
Neem alternated with Spinosad sprayed weekly + Polythene mulch + Sticky traps	387[a]	471[a]	0[b]	19.1[c]	0[b]
Neem alternated with spinosad sprayed weekly + Yellow sticky traps	309[c]	216[c]	0[b]	0[e]	0[b]
Polythene mulch only	1.20[ef]	0[i]	0[b]	0[e]	0[b]
Spinosad sprayed weekly + yellow sticky traps	358[b]	333[b]	0[b]	0[e]	0[b]
Neem sprayed weekly + Yellow sticky traps	3[d]	94[d]	4[a]	46.3[a]	0[b]
Polythene mulch + yellow sticky traps	1.2[ef]	24[f]	0[b]	35.2[b]	0[b]
Evisect® (Thiocyclam- hydrogenoxalate) only sprayed weekly	1.6[e]	72[e]	0b	0e	401[a]
P Value	<.001	<.001	<.001	<.001	<.001
LSD	1.17	0.014	0.577	0.194	1.731
CV%	0.6	1	25	1	2.2

The figures are the mean residues in micrograms detected in the samples tested for the various active ingredients. Means followed by the same letter in each column are not significantly different at $P \leq 0.05$.

Table 4.4: Pesticide residue levels (micro-gram/kg) for the basil crop in July-November 2016 season

Treatment	Spinosyn A	Spinosyn D	Azadiractin A	Azadiractin B	Thiocyclam - hydrogenoxalate
Untreated control	0^{d}	0^{d}	0^{b}	0^{d}	0^{b}
Yellow Sticky Traps only	0^{d}	0.01^{d}	0^{b}	0^{d}	0^{b}
Neem alternated with Spinosad sprayed weekly + Polythene mulch + Sticky traps	165.2^{c}	321.3^{a}	0^{b}	18.71^{c}	0^{b}
Neem alternated with spinosad sprayed weekly + Yellow sticky traps	230.1^{b}	184.2^{c}	0^{b}	52.41^{b}	0^{b}
Polythene mulch only	0^{d}	0^{d}	0^{b}	0^{d}	0^{b}
Spinosad sprayed weekly + yellow sticky traps	327.4^{a}	295.3^{b}	0^{b}	0^{d}	0^{b}
Neem sprayed weekly + Yellow sticky traps	0^{d}	0^{d}	24.8^{a}	68.20^{a}	0^{b}
Polythene mulch + yellow sticky traps	0^{d}	0^{d}	0^{b}	0^{d}	0^{b}
Evisect® (Thiocyclam- hydrogenoxalate) only spayed weekly	0^{d}	0^{d}	0^{b}	0^{d}	365.2^{a}
P Value	<.001	<.001	<.001	<.001	<.001
LSD	1.168	0.577	0.058	0.1169	0.577
CV%	0.8	0.4	1.2	0.4	0.8

The figures are the mean residues in micrograms detected in the samples tested for the various active ingredients. Means followed by the same letter in each column are not significantly different at P≤0.05.

4.3.4. Discussion

The results showed that neem alternated with spinosad integrated with polythene mulch and yellow sticky traps are effective for the control of *Liriomyza* on basil and this is in line with the hypothesis of the study. The strategy reduced the adult damage on leaves in addition to the reduction of the number of larvae which hatched after oviposition. The reduction on damage and the drop in the number of larvae could be attributed to the individual complimentary or synergistic effects of each component of the management incorporated.

Neem contributed to the results due to its anti-feedant, repellant and anti-molting properties (Vietmeyer, 1992) on the adults and the other growth stages of *Liriomyza*. In repelling the adults, punctures due to feeding and oviposition are greatly reduced. It is reported that neem works systemically and penetrates the leaf surface to act as an insect growth in-hibitor (Yildirim and Civelek, 2010). By inhibiting growth, damages due to larvae mining on the leaf surfaces are reduced. In a study by Webb *et al*. (1983) water solutions of neem were found effective in killing both eggs and larvae of *Liriomyza sativae* on lima beans. Therefore, it is suggested that some eggs and larvae might have been killed during the application of treatments. Neem injected into conifers and birch trees was also observed to significantly reduce *Liriomyza* infestation (Helson *et al*., 2001). In regard to other insect pests, Servicio de Sanidad Vegetal- Murcia (2008) in his study recommended the use of neem (Azadirachtin) as a preventive spray for the reduction of the infestation rates of *T. absoluta* larvae.

Spinosad has been applied in the control of caterpillar on fruits and vegetables. It has been found effective against thrips in tomatoes, peppers and ornamental plants. In addition, spinosad is effective on leafminers on vegetables and ornamental plants. The efficacy of spinosad on the control of dipterous leafminers is recorded as equivalent to synthetic pyrethroids, most organophophates and carbamates (Bret, 1997). Through its contact activity, spinosad acts fast in killing the leaf miners. The larvae and adults upon contact cease from feeding, become paralyzed and die. As a result, the level of damage to the crop due to the feeding of *Liriomyza* adults and the larvae is considerably reduced. Its activity on nicotinic acetylcholine receptors has been identified as the cause of death (Salgado, 1998). Due to the mode of action, spinosad is also recommended as a suitable tool for resistance management (Salgado, 1998).

Polythene mulches have been tested for insect control giving varying results. Reflective polythene mulch has been observed to reduce the population of thrips, aphids, spidermites and whiteflies on plants (Gilreath *et al.*, 2005). The reflected light is thought to confuse the pests and is recorded as more effective than insecticides (Summers and Stapleton, 2002). During this study, it is suggestive that reflections from the polythene mulch might have contributed to the disruption of the *Liriomyza* from feeding and oviposition. The polythene mulch also changes the background from the brown colour of soil and this might have interfered with the migration and landing patterns of *Liriomyza* as it was observed on aphids by Prokopy and Owens (1983). *Liriomyza* pupae mostly fall from the host plant leaves into the soil to pupate. With the polythene mulch underneath the plants, pupae are exposed to dessication and predators thus few adults that create damages emerge.

Sticky traps are hailed as an important component in an Integrated Pest Management (IPM) program. Yellow and blue sticky traps are applied in early detection of flying adult insect pests and management especially in green houses. They are more effective in the early detection of infestations than intensive plant sampling. It has been scientifically proved that sticky traps reflect certain wavelengths which attract insects. Thrips for example detect and respond to UV light and colours using receptors (Norris *et al.*, 2002). The yellow colour attracts a number of insect pests and the sticky substance applied on the material traps the insects. Traps that reflect the wavelengths of yellow are used in monitoring and management programs that include aphids, whiteflies and *Liriomyza spp***.** By trapping, the yellow sticky traps control target pest or at least slow their multiplication rates. Insects that do not fly like mites, mealybugs, scales, wingless aphids and immature stages of thrips and whiteflies will not be caught by the use of yellow sticky traps. Traps can be applied in insect pest management by mass trapping adults as a way of suppressing target insect populations. According to Sampson and Kirk (2012) mass trapping of thrips (*F. occidentalis*) by use of blue sticky traps reduce the adult thrips per flower in semi-protected strawberry crops by 61%.

Integrated Pest Management (IPM) is a recommended technology to manage pests, reduce pesticide use and managing pesticide resistance. Application of neem, spinosad, polythene mulch and yellow sticky traps provides an IPM arrangement that is effective in pest management due to their different modes of action in killing *Liriomyza,* hence the compliance to food safety limits. Neem and spinosad have been recommended in organic production (OMRI Products List, 2007; IFOAM guidelines, 2000) while polythene mulch and yellow sticky traps have zero risks on pesticide residue levels unlike Evisect® (Thiocyclam-hydrogenoxalate) that is commonly used by basil farmers.

CHAPTER FIVE

GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

This study confirmed the presence of the three species of *Liriomyza* (*L. sativae, L. huidobrensis* and *L. trifolii*) that are of phytosanitary importance in Kenya. The three species infested basil at the second week after transplanting and the intensity of damage was enhanced by the application of soil amendments and foliar fertilizer application. The damage was more in the crop where Di-ammonium phosphate, chicken manure and foliar fertilizer sprays were applied. This is attributed to the possibility of increased supply of nutrients which could have enhanced vegetative growth of basil leading to increased succulent surface area for *Liriomyza* attack (Jahn, 2004). Di-ammonia phosphate, chicken manure and the applied foliar fertilizer may have contributed to ready supply of free amino acids, sugars and nitrates, which may have made the crop's sap more attractive to *Liriomyza* as was observed by Natarajan (1986) while studying whitefly population density on cotton upon fertilization with Nitrogen, Phosphorous and Potassium (NPK).

In the second experiment, the integrated management approach of utilizing neem, spinosad, polythene mulch and yellow sticky traps emerged superior above the other options including farmer practice. The approach is sustainable in terms of pest management, pesticides use and the management of pest resistance due to the use of pesticides. In the long term this approach is also cheaper due to the reduced risks of contamination by *Liriomyza spp* that are the major cause of frequent interceptions in international markets for basil from Kenya. As observed from the pesticides residue analysis, farmer practice when applied poses higher chances of interceptions due to exceedances in pesticide residues. Neem and spinosad due to quick degradation and non-mammalian toxicity are safer when incorporated in basil production

(Niemann and Hilbig, 2000) compared to evisect (thiocyclam hydrogen oxalate). They have a wide range of acceptable limits which reduce chances of detection. Repeated application of thiocyclam-hydrogen oxalate (Evisect®) creates auxiliary challenges of pest resistance making its usage environmentally unsafe.

Besides pest control, polythene mulch has a number of other benefits to the basil crop. Water deficit is among environmental stresses that affect agricultural productivity resulting to reduction in growth and yields. Use of polythene mulch is popular in vegetable production in conserving soil moisture (Anikwe *et al*., 2007). Polythene mulch increases soil temperature, control weeds, increases crop yields and leads to an efficient use of soil nutrients (Kumar and Lal, 2012). In this study, crops generally grown on polythene mulch were observed to be more vigorous, healthy, vegetative and taller; traits attributable to benefits of using polythene mulch.

5.2 Conclusion

This study revealed that *Liriomyza* infested basil two weeks after transplanting and the magnitude of leaf damage was enhanced by the application of soil amendments and foliar fertilizer. The incorporation of Di-ammonia phosphate and chicken manure in the soil combined with weekly application of foliar fertilizer to stimulate growth and enhance yield results to increased infestation. Therefore in a crop of basil, early monitoring against *Liriomyza* is necessary and there should be a balance on the quantity of soil amendments to be applied to improve on yield while reducing incidences of *Liriomyza* attack.

The results of this study also revealed that the integrated application of Neem, spinosad, polythene mulch and yellow sticky traps is efficacious in reducing leaf damage and the number *Liriomyza* larvae on infested basil. The approach takes advantage of the different ways *Liriomyza* survives i.e. flight, feeding, oviposition and pupation in the ground. By using neem, spinosad, polythene mulch and yellow sticky traps, *Liriomyza* survival and multiplication is counteracted. Therefore, the approach forms a good IPM technology that is suitable for management of *Liriomyza* in compliance to phytosanitary standards, food safety concerns, pest resistance and environmental concerns.

5.3 Recommendations

- Farmers while combining the application of Di-ammonia phosphate, chicken manure and foliar fertilizer on basil should take necessary caution or apply sparingly because the application leads to increased infestation and damage by *Liriomyza spp.*
- The integrated application of neem, spinosad, polythene mulch and yellow sticky traps is recommended to growers as a suitable IPM approach for the management of *Liriomyza spp* in basil.
- The demonstration that the level of damage by *Liriomyza spp* may be partly accelerated by nutrient sources provided to basil crop provides a future research area to evaluate how various soil nutrient amendments influence other pest attack in basil.

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APPENDICES

Appendix I: Analysis of variance on the number of leaflets with *Liriomyza* damages for the season Feb –June 2016 (Objective 1)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Block	2	0.70911530	0.35455765	1.72	0.1915
Treatments	2	3.55400572	1.77700286	8.64	0.0008
TIME	6	62.18267584	10.36377931	50.37	<.0001
Treatments*TIME	12	2.56565729	0.21380477	1.04	0.4341

Appendix II: Analysis of variance on the number of leaflets with *Liriomyza* damages for the season July –Nov 2016 (Objective 1)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Block	2	0.73974299	0.36987150	2.63	0.0848
Treatments	2	1.09790224	0.54895112	3.90	0.0284
TIME	6	31.97183219	5.32863870	37.83	<.0001
Treatments*TIME	12	0.61328184	0.05110682	0.36	0.9692

Appendix III: Analysis of variance on the number of leaflets with *Liriomyza* damages for the season Feb –June 2016 (Objective 2)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Block	2	0.13202009	0.06601005	0.60	0.5500
Treatments	8	59.83864917	7.47983115	68.06	<.0001
TIME	6	57.07986836	9.51331139	86.57	<.0001
Treatments*TIME	48	13.27786532	0.27662219	2.52	<.0001

Appendix IV: Analysis of variance on the number of leaflets with *Liriomyza* damages for the season July –Nov. 2016 (Objective 2)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Block	2	0.03551618	0.01775809	0.16	0.8523
Treatments	8	72.94587655	9.11823457	82.18	<.0001
TIME	6	69.89853270	11.64975545	104.99	<.0001
Treatments*TIME	48	21.62768132	0.45057669	4.06	<.0001

Appendix V: Analysis of variance on the number of *Liriomyza* larvae on damaged leaves for the Feb –June 2016 (Objective 2)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Block	2	0.01706887	0.00853443	0.11	0.8943
Treatments	8	15.93956177	1.99244522	26.11	<.0001
TIME	6	15.14344644	2.52390774	33.07	<.0001
Treatments*TIME	48	13.33005516	0.27770948	3.64	<.0001

Appendix VI: Analysis of variance on the number of *Liriomyza* larvae on damaged leaves for the July –Nov. 2016 (Objective 2)

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Block	2	0.07172984	0.03586492	0.49	0.6130
Treatments	8	51.83157888	6.47894736	88.75	<.0001
TIME	6	31.81225344	5.30204224	72.63	<.0001
Treatments*TIME	48	17.30276782	0.36047433	4.94	<.0001

Appendix VII: Pest diagnosis report - Feb-June 2016

KENYA PLANT HEALTH INSPECTORATE SERVICE (KEPHIS)
PLANT QUARANTINE AND BIO-SECURITY STATION - MUGUGA

P.O. Box 49421 – 00100, Nairobi, Kenya ♦ Tel: 020 3597204/5/0722 209505/0734330017 ♦ Fax: 020 3536176 ♦ E-mail: pqs@kephis.org

PEST DIAGNOSIS REPORT

Our Ref: KEPHIS/PQS/98/240 **Date:** 10th September 2016
Your Ref:

Alfayo Ombuya
College of Agriculture and Veterinary Science
University of Nairobi
P.O. Box 29053, Loresho Ridge,
Nairobi.

RE: LEAFMINER SAMPLES

Reference is made to two leaf miner samples received in our Entomology laboratory for pest diagnosis. The specimens were collected on basil at the University of Nairobi; kabete

Method
Using a dissecting microscope, the specimens were morphologically identified with the guidance of a diptera identification key.

Results

County	Location	Host plant	Farm	Plot number	Date collected	Collector	Identification
Kiambu	Kabete	Basil	Uon – Kabete	C1 -27	5/11/16	A.Ombuya	Non target dipteral
Kiambu	Kabete	Basil	Uon – Kabete	C1- 27	5/11/16	A.Ombuya	*Liriomyza sativae*- 19
							Liriomyza huidobrensis- 11
							Liriomyzae trifolii-7

Analyzed by: Hellen Heya and Alfayo Ombuya **Confirmed by:** George Ngundo
Sign: **Sign:**
Analyst **Lab. Manager**

Appendix VIII: Pest Diagnosis report - July- Nov 2016

KENYA PLANT HEALTH INSPECTORATE SERVICE (KEPHIS)
PLANT QUARANTINE AND BIO-SECURITY STATION - MUGUGA

P.O. Box 49421 – 00100, Nairobi, Kenya ♦ Tel: 020 3597204/5/0722 209505/0734330017 ♦ Fax: 020 3536176 ♦ E-mail: pqs@kephis.org

PEST DIAGNOSIS REPORT

Our Ref: KEPHIS/PQS/98/240 **Date:** 18th May 2016

Your Ref:

Alfayo Ombuya
College of Agriculture and Veterinary Science
University of Nairobi
P.O. Box 29053, Loresho Ridge,
Nairobi.

RE: LEAF MINER SAMPLES

Reference is made to two leaf miner samples received in our Entomology laboratory for pest diagnosis. The specimens were collected on basil at the University of Nairobi; kabete

Method

Using a dissecting microscope, the specimens were morphologically identified with the guidance of a diptera identification key.

Results

County	Location	Host plant	Farm	Plot number	Date collected	Collector	Identification
Kiambu	Kabete	Basil	Uon – Kabete	C1- 27	11/07/16	A.Ombuya	*Liriomyza sativae*- 10
							Liriomyza huidobrensis- 12
							Liriomyzae trifolii-5

Analyzed by: Hellen Heya & Alfayo Ombuya **Confirmed by:** George Ngundo

Sign: **Sign:**

Analyst **Lab. Manager**

Appendix IX: Maximum residue Analysis report - Feb-June 2016

KEPHIS ANALYTICAL CHEMISTRY LABORATORY

Oloolua Ridge, Karen • P.O. Box 49592 Nairobi, Kenya •Tel: 020 6618000• 0709 891 000
E-mail:director@kephis.org • Website: www.kephis.org

Date: 1st April 2016

Client Information
Client: Alfayo Ombuya

Sample Details
Sample Type: Basil
Sample size: 1 kg
Sample Condition: Fresh
Date start of Analysis: 28/03/2016

REPORT OF ANALYSIS

The following are the results for the Basil sample you submitted to KEPHIS Analytical Chemistry Laboratory on 23rd March 2016 for pesticides residue analysis.

NOTE:

- The results mentioned in this report only relate to the sample received by the laboratory.
- This report should not be reproduced/copied/scanned except with written approval of the Head of Laboratory.
- LOQ – Limit of Quantitation
- U: the expanded measurement uncertainty U (obtained by multiplying the measurement uncertainty with factor 2 that produces a 95% reliability interval) is expressed as % of the analysis result x. Result to be read as x ± U. This value is available in the laboratory on your request.
- Correspondences regarding these results shall be channeled through KEPHIS Managing Director’s office using the address provided above.

Summary of Results

Plot No.	Treatment type	Spinosyn A (mg/kg)	Spinosyn D (mg/kg)	Azadiractin A (mg/kg)	Azadiractin B (mg/kg)	Thiocyclam (mg/kg)
18	Untreated control	0.00013	0.00078	0.0000	0.0004	0.0000
17	Yellow Sticky Traps only	0.0010	0.00021	0.0000	0.0000	0.0000
1	Neem alternated with Spinosad sprayed weekly + Polythene mulch + Sticky traps	0.387	0.471	0.0000	0.0191	0.0000
7	Neem alternated with spinosad sprayed weekly + Yellow sticky traps	0.309	0.216	0.0000	0.0000	0.0000
24	Polythene mulch only	0.0012	0.0000	0.0000	0.0000	0.0000
3	Spinosad sprayed weekly + yellow sticky traps	0.357	0.333	0.0000	0.0000	0.0000
23	Neem sprayed weekly + Yellow sticky traps	0.0030	0.00094	0.0040	0.04630	0.0000
21	Polythene mulch + yellow sticky traps	0.0012	0.00024	0.0000	0.0352	0.0000
19	Evisect (Thiocyclam hydrogen oxalate) only spayed weekly	0.0016	0.00072	0.0000	0.0000	0.4010

Analyst	**Technical Signatory**	**Authorised Signatory**
Samuel Kikuvi	Dominic Indasio	Onesmus Mwaniki
Lab Technologist	**Senior Lab Technologist**	**Laboratory Manager**

Appendix X: Maximum residue Analysis report - July- Nov. 2016

KEPHIS ANALYTICAL CHEMISTRY LABORATORY

Oloolua Ridge, Karen • P.O. Box 49592 Nairobi, Kenya •Tel: 020 6618000• 0709 891 000
E-mail:director@kephis.org • Website: www.kephis.org

Date: 31st July 2016

Client Information
Client: Alfayo Ombuya

Sample Details
Sample Type: Basil
Sample size: 1 kg
Sample Condition: Fresh
Date start of Analysis: 22/07/2016

REPORT OF ANALYSIS

The following are the results for the Basil sample you submitted to KEPHIS Analytical Chemistry Laboratory on 23rd March 2016for pesticides residue analysis.

NOTE:

- The results mentioned in this report only relate to the sample received by the laboratory.
- This report should not be reproduced/copied/scanned except with written approval of the Head of Laboratory.
- LOQ – Limit of Quantitation
- U: the expanded measurement uncertainty U (obtained by multiplying the measurement uncertainty with factor 2 that produces a 95% reliability interval) is expressed as % of the analysis result x. Result to be read as x ± U. This value is available in the laboratory on your request.
- Correspondences regarding these results shall be channeled through KEPHIS Managing Director's office using the address provided above.

Summary of Results

Plot No.	Treatment type	Spinosyn A (mg/kg)	Spinosyn D (mg/kg)	Azadiractin A (mg/kg)	Azadiractin B (mg/kg)	Thiocyclam (mg/kg)
13	Untreated control	0.0000	0.0000	0.0000	0.0000	0.0000
11	Yellow Sticky Traps only	0.0000	0.00001	0.0000	0.0000	0.0000
6	Neem alternated with Spinosad sprayed weekly + Polythene mulch + Sticky traps	0.1652	0.3213	0.0000	0.01871	0.0000
21	Neem alternated with spinosad sprayed weekly + Yellow sticky traps	0.2301	0.1842	0.0000	0.05241	0.0000
16	Polythene mulch only	0.0000	0.0000	0.0000	0.0000	0.0000
7	Spinosad sprayed weekly + yellow sticky traps	0.3274	0.2953	0.0000	0.0000	0.0000
27	Neem sprayed weekly + Yellow sticky traps	0.0000	0.00000	0.0248	0.06820	0.0000

8	Polythene mulch + yellow sticky traps	0.0000	0.00000	0.0000	0.0000	0.0000
1	Evisect (Thiocyclam hydrogen oxalate) only spayed weekly	0.0000	0.00000	0.0000	0.0000	0.3652

Analyst	**Technical Signatory**	**Authorised Signatory**
Samuel Kikuvi	Dominic Indasio	Onesmus Mwaniki
Lab Technologist	**Senior Lab Technologist**	**Laboratory Manager**