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Effects of pesticidal plants on legume pollinators' attraction in the field and bruchids control in storage

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**EFFECTS OF PESTICIDAL PLANTS ON LEGUME POLLINATORS'
ATTRACTION IN THE FIELD AND BRUCHIDS CONTROL IN
STORAGE**

Juliana Godifrey

**A Dissertation Submitted in Partial Fulfilment of the Requirements for the Degree of
Master's in Life Sciences of the Nelson Mandela African Institution of Science and
Technology**

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ABSTRACT

A randomized complete block design was used to set up field and storage experiments to evaluate the effects of pesticidal plants on common bean pollinators' attraction in the field and *Callosobruchus maculatus* control in cowpea storage. Pesticidal plants *Hyptis suaveolens*, *Osimum suave*, *Dysphania ambrosioides* and *Sphaeranthus suaveolens* were planted as field margin plants (FMPs) in a plot size of 5 m x 5 m in a study area of 75 m x 75 m. Pesticidal leaf powder of *H. suaveolens*, *O. suave* and *D. ambrosioides* were used at the rates of 0, 30, 60 and 90 g 1.5 kg⁻¹ of cowpea seeds in storage. The results showed that, all FMPs attracted a good number of pollinators, but *O. suave* attracted more pollinators than the rest of FMPs. More pods per plant were produced in open pollinated bean plants than in self-pollinated (netted plants) and the control plots (no margins). Higher dosages of plant leaf powders at 60 and 90 g for *H. suaveolens* and *D. ambrosioides* and 90 g for *O. suave* significantly ($P \leq 0.001$) affected insects' mortality, survival and reduced seed damage. Comparatively, *H. suaveolens* and *D. ambrosioides* at 90 g was more effective in inhibiting egg deposition by *C. maculatus* just as successful as the positive control. Therefore, the findings of this study indicated *O. suave* to have high influence in attracting pollinators, while *H. suaveolens* and *D. ambrosioides* at high dosage were effective in protecting the stored cowpea seeds against *C. maculatus*.

DECLARATION

I, Juliana Godifrey, do hereby declare to the Senate of the Nelson Mandela African Institution of Science and Technology that this dissertation is my own original work and that it has neither been submitted nor being concurrently submitted elsewhere for degree award in any other institution.

.....
Juliana Godifrey
Name and signature of candidate

Date:

The above declaration is confirmed

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Prof. Patrick. A. Ndakidemi
Name and signature of supervisor

Date:

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Dr. Ernest R. Mbega
Name and signature of supervisor

Date:

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CERTIFICATION

The undersigned have read and certify that, this is the original work of Juliana Godifrey and that, it is fully adequate in scope and quality, as a dissertation to be accepted in partial fulfillment of the requirements for the Degree of Master's in Life Sciences of the Nelson Mandela African Institution of Science and Technology, Arusha, Tanzania.

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DEDICATION

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LIST OF ABBREVIATIONS

PPs	Pesticidal plants
FMPs	Field margin plants
PFMPs	Pesticidal field margin plants
TaCRI	Tanzania Coffee Research Institute
TARI	Tanzania Agricultural Research Institute
DAP	Diammonium Phosphate
RCBD	Randomized Complete Block Design
NM-AIST	Nelson Mandela African Institution of Science and Technology
RH	Relative humidity
ANOVA	Analysis of Variance
LSD	Least Significance Difference
PFMPs	Pesticidal field margin plants

CHAPTER ONE

INTRODUCTION

1.1 Background information

Plant extracts have been used by man since immemorial to control insect pests and diseases (Arannilewa *et al.*, 2006; Offor *et al.*, 2014). Some common examples of plant-based compounds used for such purposes include pyrethrin, nicotine and rotenone from pyrethrum, tobacco and tephrosia, respectively (Henn and Weinzierl, 1989). Studies by several authors including Obeng-Ofori *et al.* (2000), Adebayo and Eyo (2014), Ojianwuna and Umoru (2010), Tapondjou *et al.* (2001), Koono and Dorn (2005) and Roy *et al.* (2005) have demonstrated efficacies against pests from a number of plants such as *Hyptis suaveolens*, *Ocimum suave*, *Dysphania ambrosioides*, *Nicotiana tabacum*, *Azadirachta indica*, *Tephrosia vogelii*, *Annona squamosa*, *Capsicum frutescens* and *Allium sativa* on field crops especially beans and cowpeas. Beans and cowpeas are among the major important legume's food crop grown as the source of protein to the average people (Jaetzold and Schmidt, 1983; Brisibe *et al.*, 2011; Ileke *et al.*, 2013). Popularity of botanical pesticides for pest management has been due to their little or no threat to the environments as well as human health (Obeng-Ofori *et al.*, 2000; Isman, 2006; Arannilewa *et al.*, 2006; Denloye *et al.*, 2010; Mkenda *et al.*, 2015; Hassan *et al.*, 2018).

Recently, the roles of pesticidal plants (PPs) have been extended from insect pest control in the field and storage to pollinators attraction in agricultural fields (Carvell *et al.*, 2006; Smith and Liburd, 2012; Karani *et al.*, 2017). Pesticidal plants have been intercropped with crop plants and found to attract a number of beneficial insects including pollinators (Smith and Liburd, 2012). For instance, Karani *et al.* (2017) revealed that *Hyptis suaveolens*, *Ocimum suave*, *Bidens pilosa*, *Tagetes minuta*, and *Ageratum conyzoides* influenced the population of pollinators while reducing the number of pests in common bean intercrop. The volatiles compounds produced by these non-crop plants have been reported to play a dual function of repelling insect pests and promoting population of beneficial insects (Nderitu *et al.*, 2009). According to Carvell *et al.* (2006), pesticidal plants such as *Trifolium hybridum*, *Cirsium vulgare*, *Onobrychis viciifolia*, *Lotus corniculatus*, *Leucanthemum vulgare* and *Achillea millefolium* have shown influence in attracting pollinators when planted as field margin plants. Some studies have shown that common bean regardless of being self-pollinated crop, pollinators and some natural enemies such as hoverflies, have been observed to improve its

pollination which in turn increase yields (Ibba-Perez, 1999; Kelly, 2010). Klein *et al.* (2007) quantified that about 5 % of beans yield is contributed by insect pollination. Therefore, this study assumed that inclusion of pesticidal plants (PPs) around the field crop would increase the population of insect pollinators and later on, the same PPs can be harvested and their leaves ground into powder to be used as grain protectants against storage insect pests. Common beans were selected for field experiment due to their flower's nature that allow insect tripping whereas cowpea for storage experiment because they are more susceptible to insect pest attack particularly in storage.

1.2 Problem statement and justification

Factors such as extensive use of synthetic pesticides, monoculture cropping and clearing of uncultivated land around cropped fields amongst others have been reported to associate with pollinators decline (Kevan, 1999; Carvell *et al.*, 2006; Valladares *et al.*, 2006; Henry *et al.*, 2012; Whitehorn *et al.*, 2012). The later lead to decrease in the floral diversity as well as the foraging and nesting sites for wild species of insects leading to reduction in diversity of pollinating insects (Steffan-Dewenter *et al.*, 2005). Management strategies which focus on restoring and conserving diversity of beneficial plants such as the use of pesticidal plants in the cropped fields or around the fields are important. Different pesticidal plants have been used and found to have promising results in attracting a good number of beneficial insects within agro-ecosystem (Carvell *et al.*, 2006). This seems to be an interesting area for increasing the number of pollinators in agricultural fields. Thus, proper selection of these PPs may offer foraging for pollinators while assisting in biological pest control in the field and later on harvested to be used in storage. This would somewhat minimize the use of synthetic pesticides in agriculture production which eventually would minimize the negative impacts associated with their use. In this regard, inclusion of annual PPs as field margin plants would help to rectify the shortage of pollinators and afterward the plant's leaves can be harvested and ground into powder after the cropping seasons, to be used as biopesticide in controlling bruchids (*Callosobruchus maculatus*) in cowpea and other related leguminous crops. Therefore, this study aimed at evaluating the effects of selected pesticidal plants, *Hyptis suaveolens*, *Ocimum suave*, *Dysphania ambrosioides* and *Sphaeranthus suaveolens* on legume pollinators' attraction in the field and bruchids control in storage.

1.2 Research objectives

1.3.1 General objective

To evaluate the effects of pesticidal plants (*Hyptis suaveolens*, *Ocimum suave*, *Dysphania ambrosioides* and *Sphaeranthus suaveolens*) on legume pollinators' attraction in the field and bruchids control in storage.

1.3.2 Specific objectives

- i. To assess the influence of selected field margin pesticidal plants (*H. suaveolens*, *O. suave*, *D. ambrosioides* and *S. suaveolens*) in enhancing the number of pollinators and their contribution in common bean pods formation.
- ii. To assess the effects of *H. suaveolens*, *O. suave* and *D. ambrosioides* on reducing the infestation of cowpea weevils (*Callosobruchus maculatus*) in storage.

1.3.3 Research questions

- i. What is the influence of selected field margin pesticidal plants (*H. suaveolens*, *O. suave*, *D. ambrosioides* and *S. suaveolens*) in enhancing the number of pollinators and their contribution in common bean pods formation?
- ii. What is the effect of *H. suaveolens*, *O. suave* and *D. ambrosioides* on reducing the infestation of cowpea weevils (*Callosobruchus maculatus*) in storage?

1.3.4 Significance of the study

The findings from this study contributes into;

- i. Increasing ecosystem services for production process that will contribute into increased yield and quality of the produce.
- ii. Come up with good agricultural practices that will enhance pollinator attraction in agricultural fields.
- iii. Provision of knowledge to the small holder farmers and the society in broad range about the cheap, effective and environmentally friendly control technology of the storage insect pests affecting the stored cowpea.

CHAPTER TWO

LITERATURE REVIEW¹

2.1 Introduction

Some non-crop plants have a significant role to crop pollinators and other flower visitors and can be useful in making margins for flower-rich crops to encourage populations of beneficial insects (Gurr *et al.*, 2005). Marshal *et al.* (2003) indicated that some weeds are potential for the survival of beneficial insects in agricultural systems. In this case, introducing some specific weeds in agricultural fields has been indicated to boost floral resources to beneficial insects as well as providing nests and nesting materials for refuges (Marshal *et al.*, 2003). In addition, retaining hedge-rows and insectary flowering plants in agro-ecosystems and leaving uncultivated lands around the crop fields help in provision of shelters, micro-climates and resources for pollinators and eventually increase the diversity of beneficial insects relative to monocropping (Dufour, 2000). It is well known that nectar or pollen feeding is vital for the reproductive success of many insect predators and parasitoids (Wäckers and van Rijn, 2005). However, shortage of pollinators and the services they offer to the environments have increased for a long period due to habitat loss and degradation, as well as the increased use of synthetic pesticides (Allen-Wardell *et al.*, 1998; Nicholls and Altieri, 2013).

Monoculture cropping practices are reported to be associated with decline in the population of pollinators in different parts of the world (Öckinger and Smith, 2007). Nevertheless, the removal of weeds around the cropped fields decreases the floral diversity as well as the foraging and nesting sites for wild species of insects. This results into reduction in diversity of pollinating insects, which in turn leads to decline in populations of pollinators (Steffan-Dewenter *et al.*, 2005). Therefore, management strategies which focus on restoring and conserving diversity of beneficial plants such as the use of pesticidal plants in the cropped fields or around the fields are important. However, in facilitating diversity of plants in cropped field margins, appropriate manipulation strategies should be employed to avoid resource competition with the crop plants. Rahat *et al.* (2005) indicated that specific plants attracted different groups of insects and therefore, in habitats manipulation, it is critical to select flowering plants while targeting a specific insect (Table 1). Insects pollinators are attracted to

¹ Part of this chapter is published in the American Journal of Plant Sciences. 9(13), 2659-2675, December 2018.

flowers by various characteristics including floral morphology, scenting odor, petal colour, taste of nectar, and texture of pollen (Colley and Luna, 2000; Fenster *et al.*, 2004). Considering these characteristics, pesticidal plants which are commonly grown within agro-ecosystems can potentially be utilized as important floral resources. Therefore, the inclusion of flowering pesticidal plants as part of cropped field margins deemed useful habitats to pollinators while providing additional benefits as biological pest control. It is under these explanations that this study, sympathized the importance of including pesticidal plants along field margins to enhance the number of pollinators in common bean fields and their later utilization as grain protectants in cowpea storage.

2.2 Importance of including pesticidal plants in margins of cropped fields

Flowering plants favor existence of beneficial insect species in the fields resulting into optimized and sustainable crop productivity. Different non-crop plants have been reported to attract beneficial insects in crop ecosystems due to ecological relationships between the plant resources and insect biology (Van Emden, 1965).

Table 1: Common plant species attracting pollinators

Plant species	Visiting Pollinators	Reference
<i>Trifolium pratense</i> , <i>Ballota nigra</i> , <i>Lamium album</i> , <i>Teucrium scorodonia</i> , <i>Centaurea nigra</i>	Bumble bee (<i>Bombus spp</i>)	(Carvell, 2006)
<i>Trifolium hybridum</i> , <i>Cirsium vulgare</i> , <i>Onobrychis viciifolia</i> , <i>Lotus corniculatus</i> , <i>Leucanthemum vulgare</i> and <i>Achillea millefolium</i>	Most hymenopteran	(Kassina <i>et al.</i> , 2006)
<i>Fagopyrum sagittatum</i> , <i>Lobularia maritima</i> and <i>Agastache foeniculum</i>	Cresson (<i>Microplitis croceipes</i>)	(Nafziger and Fadamiro, 2011)
<i>Sium suave</i> (Apiaceae) and <i>Solidago Canadensis</i> (Asteraceae)	Most hymenopteran including Wasps species <i>Myzinum quinquecinctum</i> (tiphiid) and <i>Scolia bicincta</i> (scoliid)	(Patt <i>et al.</i> , 1997; Tooker and Hanks, 2014)
Coriander, phacelia, alyssum, fennel, buckwheat, mustard	Hoverflies	(Colley and Luna, 2000)
<i>Allium cepa</i> , <i>Daucus carota</i> , <i>Coriandrum sativum</i> , <i>Cirsium arvense</i> , <i>Launaea procumbens</i> , <i>Ranunculus muricatus</i> and <i>Prosopis juliflora</i>	Hoverflies	(Sajjad and Saeed, 2010)
<i>Glebionis segetum</i> Corn marigold, <i>Coriandrum sativum</i> Coriander, <i>Foeniculum vulgare</i> Fennel, <i>Phacelia tanacetifolia</i> (Phacelia)	Wasps and Hoverflies	(Sievwright <i>et al.</i> , 2006)
Fennel, cosmos hypericum, yarrow, lavender, bishop's weed, petunia, chamomile	Hoverflies	(Martini <i>et al.</i> , 2014)
<i>Tagetes erecta</i> , <i>Foeniculum vulgare</i> , <i>Ocimum</i> , <i>Ziziphora interrupta</i>	Syrphidae, Anthocoridae and Coccinellidae	(Saidovand Douglas, 2008)
<i>Aster pilosus</i> (Asteraceae) and <i>Heracleum maximum</i> , <i>Pastinaca sativa</i> , <i>Cicuta maculata</i> (Apiaceae)	Syrphidae and tachnid flies	(Tooker <i>et al.</i> , 2014)
<i>Hyptis suaveolens</i> , <i>Tagets minuta</i> , <i>Ageratum cinyzoides</i> , <i>Ocimum suave</i> , <i>Bidens Pilosa</i>	Stingless bee and butter flies	(Karani <i>et al.</i> , 2017)

Understanding of the biology and ecology of different crop and non-crop plants is relevant in designing valuable vegetative barriers in cropped fields (Molthan and Ruppert, 1988). Diversity of field margin plants across the cropping seasons can have a major influence on insect dynamics (Altieri and Letourneau, 1982). Kasina *et al.* (2006) confirmed the diversity of beneficial arthropods to be enhanced by the field margin plants.

Different species of flowering plants with pesticidal properties have been reported to show promising results in attracting pollinators when planted as field margin plants. These plants include *Trifolium pratense*, *Ballota nigra*, *Centaurea nigra*, *Teucrium scorodonia*, *Lamium album*, *Trifolium hybridum*, *Cirsium vulgare*, *Onobrychis viciifolia*, *Lotus corniculatus*, *Leucanthemum vulgare*, and *Achillea millefolium* (Carvell *et al.*, 2006). Karani *et al.* (2017) found that *Hyptis suaveolens*, *Osimum suave*, *Bidens pilosa*, *Tagetes minuta*, and *Ageratum conyzoides* influenced the population of pollinators while reducing the number of pests in cultivated fields.

Pollinators such as parasitic wasps perform their full role of biological control and pollination when provided with essential sugar resources for their survival (Wäckers, 2001). Wasps are attracted by volatiles that are produced by plant tissues of pesticidal plants (Brodmann *et al.*, 2008). The contribution of pesticidal plants that produce secondary metabolites in form of volatile organic compounds to attract pollinating insects is widely documented (Carvell *et al.*, 2006; Karani *et al.*, 2017; Wäckers, 2001; Brodmann *et al.*, 2008; Saidov and Douglas, 2008). Therefore, if pesticidal plants are well utilized as field margin plants, they are expected to attract diverse species of pollinators due to their aroma characteristics.

Nafziger and Fadamiro (2011) investigated the suitability of buck-wheat (*Fagopyrum sagittatum*), sweet alyssum (*Lobularia maritima*) and licorice mint (*Agastache foeniculum*) as nectar sources for Cresson wasp (*Microplitis croceipes*) a potential parasitoid of some caterpillar pests and a pollinator. Their study found that the longevity of adult *Microplitis croceipes* was enhanced by buckwheat and licorice mint but females outperformed the males. They attributed these observations with the amount of energy needed for the host location and oviposition by females.

The use of pesticidal plants as artificial pesticide replacers has also been reported (Mkindi *et al.*, 2015). The pesticidal plants also provide ecosystem services like pollination and biological pest control in agricultural fields (Ndakidemi *et al.*, 2016). Tooker and Hanks (2014) identified several species of hymenopteran which visited the pesticidal flowering plant hosts. The host

plants visited was *Sium suave* (Apiaceae), *Solidago canadensis* (Asteraceae) and the wasp's species were *Myzinum quinquecinctum* (tiphiid) and *Scolia bicincta* (scoliid). Wasps have also been indicated to visit Apiaceae plants due to exposed anthers and nectar since mouthparts of these insects are not adopted specifically for extracting floral resources (Patt *et al.*, 1997).

The importance of flowering plants as both attractant to natural enemies and pollinators is widely investigated (Colley and Luna, 2000; Sajjad and Saeed, 2010; Sievwright *et al.*, 2006; Martini *et al.*, 2014; Tooker *et al.*, 2014). Some plant species were potential floral resource to hoverflies (Colley and Luna, 2000) an effective pollinator and a natural enemy of aphids (Barbir *et al.*, 2015). Martini *et al.* (2014) reported the importance of plant species such as fennel, cosmos hypericum, yarrow, lavender, bishop's weed, petunia and chamomile in attracting hoverflies species. Sajjad and Saeed (2010) reported *Allium cepa*, *Daucus carota*, *Coriandrum sativum*, *Cirsium arvense*, *Launaea procumbens*, *Ranunculus muricatus*, and *Prosopis juliflora* to be the potential attractants of syrphid species under natural conditions.

Siewwright *et al.* (2006) investigated the attractiveness of *Coriandrum sativum* Coriander, *Glebionis segetum* Corn marigold, *Foeniculum vulgare* Fennel and *Phacelia tanacetifolia* Phacelia on lacewings, parasitic wasps, ladybirds and hoverflies, as key natural enemies of pests and pollinators in agricultural fields. Saidov and Douglas (2008) studied the key natural enemies and pollinators including Syrphidae, Anthocoridae and Coccinellidae using pesticidal plants such as *Tagetes erecta*, *Foeniculum vulgare*, *Ocimum basilicum* and *Ziziphora interrupta* which showed promising performance. Tooker *et al.* (2014) studied the plant species preferred by syrphid and tachinid flies and found that most syrphid and tachinid flies visited *Aster pilosus* (Asteraceae), *Heracleum maximum*, *Pastinaca sativa* and *Cicutam aculata* (Apiaceae). Therefore, inclusion of strips of pesticidal plants as a field margin could offer a multiple purpose in reducing number of pests whilst favoring beneficial insects most of them being pollinators. Table 1 shows various studies reported on usage of pesticidal plants in attracting pollinators.

2.3 Role of pollinators in crop productivity

Pollination services are referred to as the transfer of pollen grains from the floral anthers to the floral stigma of a different plant (cross-pollination) or the same plant (self-pollination) (Willmer, 2011). Kron *et al.* (2001) reported that pollinators take pollen from anthers and deliver them to the stigma through foraging. Pollination depends on the plant-animal association, whereby both plants and animals benefit from the service.

There is an interaction between floral signals and the senses of the pollinators (Kevan and Menzel, 2012). Floral signals are delivered by the synthesized volatile organic compounds and some of them are derivatives of fatty acids, some nitrogenous compounds, terpenoids and benzenoids (Knudsen *et al.*, 1993). Floral volatiles emitted by the plants have potential in attracting specific groups of pollinators, some being common to most plants while others differ from plant to plant (Pichersky and Gershenzon, 2002). Due to this chemical prompt the pollinators such as honey bees can fly long distances in attraction of such floral resources (Theis, 2006). In addition, flowers provide amino acids and carbohydrates as sources of energy for reproduction, oviposition, development and survival of beneficial insects including the pollinators (Stubbs and Falk, 1983; Landis, 2000). Since, pestiferous plants produce these volatile compounds as secondary metabolites, if well maintained within the agricultural landscape they would be a good floral resource for pollinators.

Ecosystem services such as biological control of pests, pollination, soil formation and nutrient cycling are provided by pollinators and natural enemies in many agricultural fields (Ndakidemi *et al.*, 2016). Beneficial insects-mediated services such as pollination are essential for livelihoods improvement as they provide assurance of food security. Subsistence agriculture is the backbone of smallholder farmers in most African countries and thus, pollination is the key and essential service for boosting the economies through cultivation of different crops and products (Munyuli, 2011; Munyuli, 2013). Studies have revealed that 75 % of agricultural crops are insect pollinated, in which up to 87.5 % of flowering plants in the tropics and temperature zones benefit from insect pollinators which are naturally found in the environment (Wardhaugh, 2015). Bees are key pollinators of many crops and hence it is important to provide comfortable environment and resources such as nectar, pollen, places for overwintering for the insects for their sustainable ecosystem services (Greenleaf and Kremen, 2006; Kosior *et al.*, 2007; Winfree *et al.*, 2007). Thus, pollinators require specific recognition in agro-ecological system because of their importance in pollination process in agriculture and natural ecosystems.

Generally, quality and yield of different crops are reported to increase when there is pollinators' involvement (Potts *et al.*, 2010). For instance, in self-pollinated crop like beans yield has been reported to increase by 5 % in presence of insect pollinators (Klein *et al.*, 2007). Aouar-sadli *et al.* (2008) investigated the pollination potential of wild bees (*Eucera pulveracea*), honey bees (*Apis mellifera*) and carpenter bees (*Xylocopa violacea*) in relation to seed production on the broad bean (Fabaceae). Their findings revealed that, the wild bees made frequent visits to broad bean but the honey bees and the carpenter bee made several visits to forage. Another

study by Barbir *et al.* (2015) observed that, the presence of bees increased yield in cross-pollinated coriander than in self-pollinated. Also, Stein *et al.* (2017) found that, cross-pollination by honey bees and wild bees successfully improved the quality of cotton and sesame products.

Bischoff *et al.* (2013) investigated the visits of Syrphid flies (*Allograpta* spp) and solitary bees (*Hylaeus matamoko*) on two New Zealand alpine herbs; *Ourisiagla ndulosa* and *Wahlenbergia albomarginata* and found that, both pollinators had equal frequencies of visit to *Ourisia glandulosa*, while the solitary bee had more frequencies of visits to *Wahlenbergia albomarginata*. In this regard, insect pollinators have a lot to do with the reproduction potential of flowering plants regardless of the mode of reproduction of a particular crop plant. Thus, there is a continuous need of considering and investigating the relative attractiveness of the field margin plants to pollinators for sustainable crop production in agricultural systems.

In addition to optimized crop productivity, pollination enhances food security as well as genetic variation among crops, which lessens inbreeding depression and accelerates resistance to environmental changes (Naylor and Ehrlich, 1997; Aizen *et al.*, 2009; Garibaldi *et al.*, 2011). Therefore, the knowledge on management techniques that attract different pollinators in the agricultural fields is an important way forward to the enhanced agro-ecosystems for increased crop production.

2.4 Roles of selected pesticidal plants in controlling pests and attracting pollinators

This study provides detailed explanations to four pesticidal plants namely *Hyptis suaveolens*, *Ocimum suave*, *Dysphania ambrosioides* and *Sphaeranthus suaveolens* as the representatives of the diverse flower producing pesticidal plants that could be used as field margin plants. These pesticidal plants are mostly used by farmers as plant protectants against insect pests and their occurrence is abundant in local settings (Ngamo *et al.*, 2007; Mkenda *et al.*, 2015).

Considering the use of these plants in biological pest control and the association of pollinators with the volatile organic compounds produced by different plants, it deemed useful to include them as field margin plants to enhance the population of insect pollinators in cultivated fields. The odor characteristic of most pesticidal plants provides them with added advantage to be attracted by the senses of pollinators. In addition, among the selected plants *H. suaveolens* and *O. suave* have been reported to have influence on attracting many stingless bees and butterflies in common bean intercrops (Karani *et al.*, 2017). However, based on farmers' field experience, *O. suave* fresh leaves are used by bee keepers in cleaning the beehives because of its ability to

attract many honey bees. Despite the potential influence of these plants to pollinators, little is known on their potential role in attracting pollinators in agricultural fields.

2.4.1 *Hyptis suaveolens* as a beneficial pesticidal plant

Hyptis suaveolens belongs to the family Lamiaceae and has been traditionally used as a botanical pesticide in many developing countries due to its insecticidal and repellent properties against several field and storage insect pests (Aizen *et al.*, 2009). More than 400 species of the genus *Hyptis* are characterized by high aromatic and grow in tropical regions, mostly in Africa and America and it is not commonly found at an altitude above 500 m. The plant is normally restricted to places where soils have been intensely disturbed, and may be considered as a ruderal species (Wulff, 1973). *Hyptis suaveolens* is found around villages, along road-sides, on farmsteads and on bushes. Its oil constituents have been used in controlling stem borer in maize intercrop (Adda *et al.*, 2011). Chemical screening for the chemical constituent of its aqueous extracts revealed that the plant is rich in flavonoids and alkaloids (Fig. 1). Other secondary compounds include tannins and phenols (Edeoga *et al.*, 2006). When tested against *Fusarium oxysporum* in Gladiolus corms, it significantly reduced the pathogen population during storage (Sharma and Tripathi, 2008). In addition, an extract from the fresh leaves were reported to have larvicidal and repellence properties against the Asian tiger mosquito, *Aedes albopictus* Skuse (Diptera: Culicidae) (Ndakidemi and Dakora, 2003).

Ofuya (2010) evaluated the efficacy of the *H. suaveolens* extracts on storage pests, namely *Sitophilus oryzae*, *Sitophilus zeamais* and *Callosobruchus maculatus*. The results of this study revealed that methanolic extract of the plant at 100 % concentration was able to cause mortality of all exposed insect pests after 5 seconds. Chi and Apiiah (2012) tested the toxicity and feeding deterrent using *H. suaveolens* ethanol, distilled water, chloroform, petroleum, ether and methanol extracts on cowpea weevils, *C. maculatus*. Their findings indicated that chloroform extracts at the concentrations of 250 μgml^{-1} and 500 μgml^{-1} showed 100 % deterrent effect to the weevils whereas, the chloroform extract at the concentration of 125 μgml^{-1} showed the least deterrent effect. When compared, chloroform extracts caused the highest mortality at an average of 41 % whereas ethanol extract had the lowest average mortality of 29 %. Contrarily, the flowers of *H. suaveolens* have been reported to provide pollen and nectar to bees and butterfly for its pollination process by hovering around the flowers and touching the carinal-corolla with their proboscis (Aluri, 1992). However, the potential role of this plant in attracting populations of pollinators in agriculture production is underestimated in most parts of the world

where similar studies have been conducted (Aluri, 1992; Rani and Raju, 2016). Thus, further research needs to be done to investigate the importance of these plants in attracting pollinators to increase crop productivity.

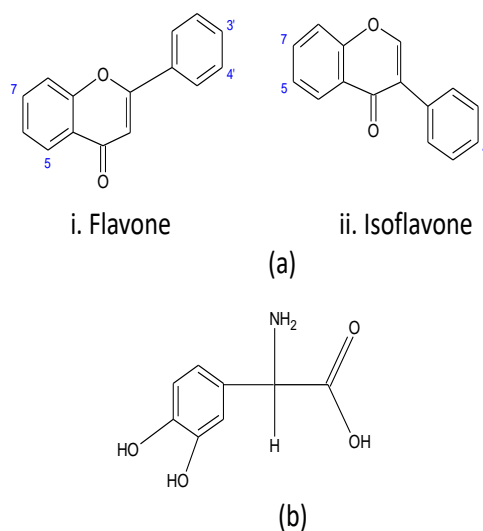


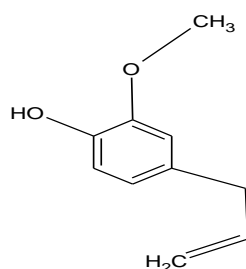
Figure 1: Chemical structures of (a) Flavonoid compounds; (b) Alkaloid compound (Ndakidemi and Dakora, 2003)

2.4.2 *Ocimum suave* as a beneficial pesticidal plant

Ocimum suave is also known as Wild basil and it belongs to the family Lamiaceae or Labiatae. Lamiaceae family has been used since early times because of its medicinal properties and many of these species are distributed in Mediterranean and tropical countries across the world (Pandey *et al.*, 2014). The three main centres of *Ocimum* diversity has been reported as tropical and subtropical parts of Africa and America and tropical Asia (Chowdhury *et al.*, 2017). The phytochemical analysis (Fig. 2) has identified eugenol as the major component of *O. suave* essential oil (Chogo and Crank, 1981; Obeng-Ofori *et al.*, 2000).

Several studies have been conducted on the toxicity of the leaf oil on important agricultural pests. Ojuanwuna *et al.* (2013) tested the toxicity of the plant oil extracts on the bruchid (*Callosobruchus maculatus*), which is a cowpea weevil, and a major problem in storage of cowpea seeds in the tropics. Their study revealed that, the crude oil extracts had a potential insecticidal activity on the weevil and the mortality increased with extract concentration from 0.02 to 0.08 mg 20 ml⁻¹ of water. However, the period of exposure from 24 to 96 h was an important factor for the mortality of the insects. Obeng-Ofori and Reichmuth (1997) investigated the toxicity of eugenol against four coleopteran species of stored-products, which

are *Sitophilus granarius*, *Sitophilus zeamais*, *Tribolium castaneum* and *Prostephanus truncates*. Their study found that mortality effect on the beetles increased with extract dosage and exposure time. High mortality occurred on *S. granarius*, *S. zeamais* and *T. castaneum* at higher dose. The eugenol also significantly inhibited the development of eggs, larvae, and pupae and was highly repellent to the Coleopterans. Similar findings were obtained by Obeng-Ofori *et al.* (2000) when investigating the effectiveness of essential oil of the *Ocimum* plant species namely *O. kenyense*, *O. suave*, and *O. kilimandscharicum* against storage pests of *S. zeamais* and *P. truncates*. The essential oils from all species extracts indicated a dose-dependent mortality effect against the pests. The oils also resulted into inhibition of developments of the eggs, larva and pupa, oviposition by the adults, deterrence and the repellence. However, there is limited understanding of the role of *O. suave* plant in supporting beneficial insects (pollinators). Thus, future research should focus on *O. suave* to determine its potential role for promoting diversity of populations of pollinators.



Eugenol

Figure 2: Chemical structure of Eugenol (Gülçin, 2011)

2.4.3 *Dysphania ambrosioides* (*Chenopodium ambrosioides*) as a beneficial pesticidal plant

Dysphania ambrosioides belongs to Chenopodiaceae, a family of varieties of herbaceous weedy plants (Smith and Liburd, 2012). The genus *Chenopodium* comprises about 250 species (Ruas *et al.*, 1999) in which most species are annuals, distributed in the Americas, Asia, and Europe. *Dysphania ambrosioides* has been used for medicinal purposes mainly for treating intestinal parasites (Salimena *et al.*, 2015). However, its use ranges from pharmaceutical purposes to pest control in agricultural fields (Wohlenberg and Lopes-da-Silva, 2009). Reported bioactive compounds of *D. ambrosioides* essential oil includes, ascaridole, isoascaridole, α -terpinene, Isoascaridolnene, 2-carinene and p-cymene (Mwanauta *et al.*, 2014) of which ascaridole is the major compound constituting 40 % - 70 % of the total active compound present (Barbosa *et al.*, 2011) (Fig. 3).

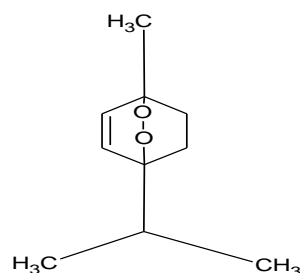


Figure 3: Chemical structure of Ascaridol (Dembitsky *et al.*, 2008)

The activities of the plant extracts and its essential oil against different agricultural pests have been studied. Vázquez-Covarrubias *et al.* (2015) tested the effects of essential oils and the aqueous extracts of Chenopodiaceae plants including *D. ambrosioides* on the development and reproductive potential of Lepidopteran *Copitarsia decolora*. This is a serious pest of several plants including Brassicaceae species (Suarez-Vargas *et al.*, 2006). The results indicated that the essential oils of *D. ambrosioides* at 0.5 % significantly reduced larval weight to 33 % compared with the control ($F = 2.1$, $df = 5, 328$, $P > 0.05$). The essential oil also increased duration of the larval period at 0.1 % concentration compared with the control by 20 % ($H = 60.9$, $df = 6, 400$, $P \leq 0.001$), and this was the largest while all the essential oils at the concentration of 0.5 % increased the duration of the larval period in relation to the control ($F = 74.917$, $df = 6, 172$, $P < 0.001$). It was further observed that the essential oils at a concentration of 0.5 % significantly reduced fecundity by 88 % ($F = 38.5$, $df = 6, 74$, $P < 0.001$) whereas 0.5 % of aqueous extracts reduced the fecundity by 70 % ($F = 14.4$, $df = 5, 97$, $P < 0.001$). Furthermore, *D. ambrosioides* essential oils significantly decreased survival time for *Copitarsia decolora*. At 0.5 % concentration, the oils significantly reduced the number of fertile eggs by 93 % ($F = 36.6$, $df = 6, 74$, $P < 0.001$) while at 75 % caused significant largest reduction in fertility ($F = 13.4$, $df = 5, 97$, $P < 0.001$).

Insecticidal properties of a *Chenopodium*-based botanical effects on different pests including green peach aphid (*Myzus persicae*), greenhouse whitefly (*Trialeurodes vaporariorum*) and flower thrips (*Frankliniellaocci dentalis*) are also reported. A mixture of UDA-245 which was based on an essential oil extracts from *D. ambrosioides* had potential in controlling aphids, thrips and whiteflies compared with neem oil, insecticidal soap and endosulfan. Insecticidal soap exhibited high mortality of the parasitoid *Encarsia formosa* (Aphelinidae) than emulsifiable concentrate but UDA-245 was safer to the parasitoid (Chiasson *et al.*, 2004).

Denloye *et al.* (2010) investigated toxicity of *Chenopodium ambrosioides* powder extracts and essential oil against storage insect pests namely *C. maculatus* (Bruchidae), *S. zeamais* (Curculionidae) and *T. castaneum* (Tenebrionidae). Their study found that *D. ambrosioides* powder induced toxicity to *S. zeamais* compared with other test organisms. Ethanol extract and essential oils were more effective against *C. maculatus* compared with other test organisms. Based on these explanations, there is limited scientific data on the use of this herb in attracting beneficial insects to promote crop pollination. Hence, it is crucial to undertake studies so as to generate data on the role of *D. ambrosioides* in enhancing populations of pollinators.

2.4.4 *Sphaeranthus suaveolens* as a beneficial pesticidal plant

This is a herb that belongs to the family Asteraceae (Compositae) (Ahmed and Mahmoud, 1997). The phytochemical analysis of *S. suaveolens* essential oils showed high variability in the secondary metabolites, which are biologically active. They include isopinocampone, α -pinene, thymohydroquinone dimethlether, 1, 8-Cineole, γ -Terpinene (De Pooter *et al.*, 1991) (Fig. 4). It has been reported by Hashim *et al.* (2006) that ethanol, ethyl acetate, methanol and aqueous extracts of the plant parts showed antibacterial activity. The association of *S. suaveolens* and pollinators in bean fields is considerably unstudied Therefore, there is a need to generate information on the importance of this plant in attracting pollinators.



Figure 4: Chemical structures of Isopinocampone and α -pinene (Wang *et al.*, 2014)

2.5 Summary

This chapter has demonstrated that ecosystem services such as pollination are interfered by habitat manipulation and landscape disturbance, which ultimately leads to disruption of the communities of plant pollinators. Agricultural intensification has led to reduction in floral resources, nesting places for pollinators and thus decreases pollinator abundance and diversity. This has created a need for appropriate habitat management practices such as the use of field margin plants as a mitigating strategy in reducing pollinator decline for crop production. For development of sustainable conservation practices and increasing productivity, it is important

to understand and identify plants that play role in the maintenance of the pollinators populations to improve the ecosystem services while boosting the biological pest control. In this case, various pesticidal plant species can be fully utilized to provide dual function within agro-ecosystem. To date, few studies have been done on the potentials of some native pesticidal plants in promoting the diversity of the agents of pollination. Therefore, further research is needed in identifying specific pesticidal plants species that potentially influence pollinators population and the volatiles that enhance their visits. Again, studies on proper design of these plants are of high importance to avoid competition with crop plants. Among pesticidal plant species used, *Hyptis suaveolens*, *Osimum suave*, *Dysphania ambrosioides* and *Sphaeranthus suaveolens* have been fully utilized in the control of crop storage pests due to their secondary compounds that are responsible for insecticidal activities which are also likely to have influence in attraction of beneficial insects including pollinators. These plant species may therefore be important as resources in promoting the diversity of pollinators for increasing crop productivity and in the control of legume storage pests.

CHAPTER THREE

MATERIALS AND METHODS

3.1 To assess the influence of selected field margin pesticidal plants (*H. suaveolens*, *O. suave*, *D. ambrosiodes* and *S. suaveolens*) in enhancing the number of pollinators and their contribution in common bean pods formation

3.1.1 Study area

The field work was conducted at Tanzania Coffee Research Institute (TaCRI) in a single cropping season from May to July 2018, to assess the influence of selected field margin pesticidal plants in enhancing the number of pollinators and their contribution on common bean pods formation. The study area TaCRI (Fig. 5) was located at the base of Mount Kilimanjaro at the elevation of 1330 m above the sea level in Kilimanjaro region, Tanzania having latitude (3°13'58.99'S) and longitude (37°14'53.03'E). The field experiment was conducted in an area with mean annual rainfall of 1200 mm.

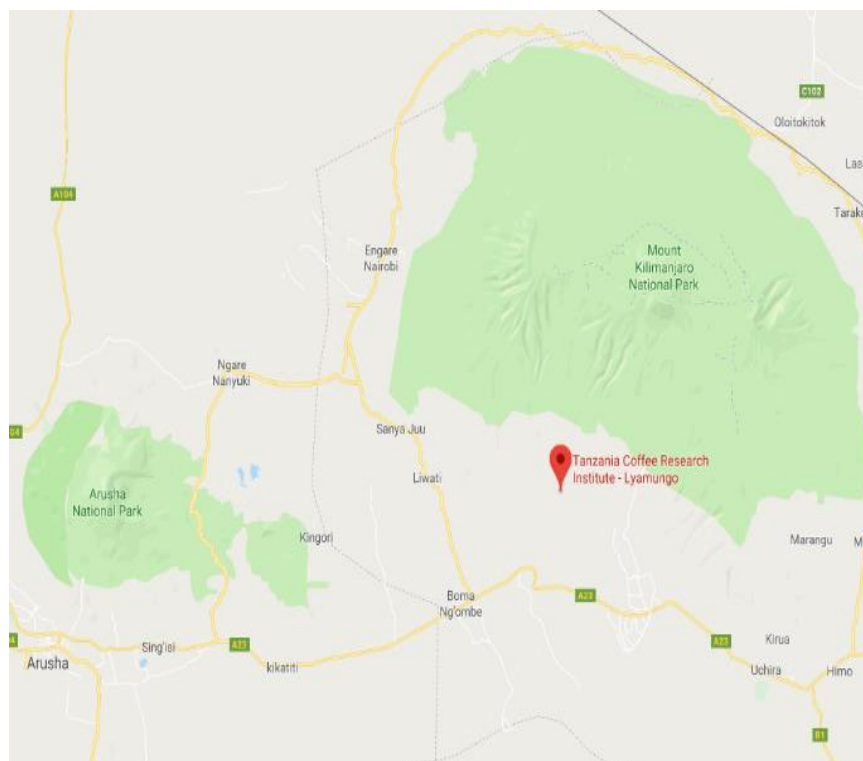


Figure 5: A map showing the study area (Field survey, 2018).

3.1.2 Study materials

The crop plant used in this experiment was common bean variety Lyamungo 90 purchased at Tanzania Agricultural Research Institute (TARI) - Selian. Pesticidal plant seedlings of *Ocimum suave*, *Hyptis suaveolens*, *Dysphania ambrosioides* and *Sphaeranthus suaveolens* were obtained from Kibosho village in Moshi Tanzania. The fertilizers used in this experiment were Diammonium Phosphate (DAP) during planting. The screen house net was obtained from Balton – Tanzania and was used for bagging experiment.

3.1.3 Experimental design and treatments

The field experiment was designed in a randomized complete block design (RCBD), in a plot size of 5 m x 5 m with 5 treatments replicated five times in a study area of 75 m x 75 m. All plots were planted with pesticidal field margin plants (PFMPs) except control plots, at a spacing of 50 cm wide from the bean field and 40 cm from each other. Pesticidal field margin plants were planted 3 weeks before the sowing of common bean seeds to ensure them flower at the same time with the common bean plants. The bean seeds were planted in each of the 25 established plots at a spacing of 50 cm between rows and 20 cm between plants. The experimental plots were located at a distance of 10 m apart spatial separation, to minimize the synergetic effect of one pesticidal plant by another. Two bean seeds were sown per holes. The design and treatment randomization were as in Table 2.

Table 2: Experimental layout

R1	R2	R3	R4	R5
1	2	5	1	4
2	5	1	3	3
3	4	3	5	2
4	1	4	2	1
5	3	2	4	5

R1 = Replication 1; R2 = Replication 2; R3 = Replication 3; R4 = Replication 4; R5 = Replication 5. For PFMPs, 1 = *Ocimum suave*; 2 = Control; 3 = *Hyptis suaveolens*, 4 = *Dysphania ambrosioides* and 5 = *Sphaeranthus suaveolens*.

3.1.4 Sample selection

At a very early stage of plant growth, nine bean plants were randomly selected in each plot. The sampled plants were used for three pollination methods (Open pollination, Hand pollination and Self-pollination) in which three plants were used for each method. In Open pollinated bean plants, all the flowers of each plant were accessible to autonomous self and insect-pollination whereas, for the netted treatments, all plants were bagged with a screen net. Thus, in the netted plants all flowers were exposed to only self-pollination, and the other three were hand pollinated. The difference between these plants represents the contribution from insect pollination. Bag manipulations were done carefully and in most cases before anthesis to avoid increased levels of self-pollination (Bartomeous *et al.*, 2014). The nets and bags were removed immediately after fruit set when petals began to wither and fall off. Bean pods were left in the field up to maturity and harvest.

3.1.5 Data collection

i. Insect sampling

In each of the experimental plots, the major groups of insects visiting flowers, including bees (Hymenoptera: Apoidea: Apiformes), hoverflies (Diptera: Syrphidae), wasp (Hymenoptera), butterflies, solitary bees, carpenter bees and moth (Lepidoptera) were assessed both in the FMPs and inside bean field. Physical observation was used in which a researcher walked around the study plot for 10 minutes identifying visiting insects at species level by tallying them and catching unidentified species within and along the margin 50 cm wide. This was done between 09.00 AM to 12.00 noon and repeated from 02.00 to 05.00 PM only on sunny day, with no precipitation, dry vegetation, and low wind speed (Westphal *et al.*, 2008). The assessment was done at two days interval during the main flowering period for the maximum number of 10 days. The collection and recording of pollinators were done after 35, 38, 41 and 44 days from the sowing date of common bean seeds and were named as early, mid, maximum and late flowering phases respectively.

ii. Yield data collection

The number of pods per plant at physiological maturity were counted in all sampled bean plants from all treatments and recorded.

3.2 To assess the effects of *H. suaveolens*, *O. suave* and *D. ambrosioides* on reducing the infestation of cowpea weevils (*C. maculatus*) in storage

3.2.1 Experimental site

The experiment was carried out in a special storage room designed at Nelson Mandela African Institution of Science and Technology, Arusha-Tanzania. The room was well ventilated with enough air circulation inside.

3.2.2 Insects

The Adult cowpea weevil (*C. maculatus*) used to establish a colony was obtained from highly infested cowpea seeds bought from the local market in Moshi Tanzania. Insects were identified with a University Entomologist in the laboratory of Life Science at the Nelson Mandela African Institution of Science and Technology (NM-AIST), Arusha, Tanzania. Rearing of cowpea weevil was done in ventilated 10 L plastic containers half filled with 5 kg of uninfested cowpea seeds, covered on top with 10 mm mesh sieve to allow free air movement while restricting weevils from escaping. Rearing was carried out at room temperature 25 ± 3 °C and relative humidity (RH) 75 ± 5 %. Adult weevils were kept for 20 days to allow their multiplication, after which they were harvested and used in the experiment.

3.2.3 Insecticidal plant materials

Plant materials used in this study were *H. suaveolens*, *O. suave*, and *D. ambrosioides*. *Hyptis suaveolens* and *Ocimum suave* were handpicked from farms at Kibosho village where as *Dysphania ambrosioides* was collected at Lyamungo village both in Moshi Tanzania. These plant species were selected for this trial because they are traditionally used by farmers in the local areas as medicines and their readily available in the northern Tanzania (where this research was conducted). Matured plants leaves were dried in shed to reduce photolysis of bioactive compounds and then ground into a fine powder by using an electric mill, then packed into a plastic container with airtight lid to maintain the aroma and stored in the dark at ambient conditions of 25 ± 3 °C and 75 ± 5 % RH.

3.2.4 Cowpea seeds

Cowpea seeds used in this experiment were newly harvested by farmers from Tunduru District, in Ruvuma–Tanzania. The seeds were free from insecticides. They were cleaned thoroughly by winnowing and mechanical sorting to remove infested and damaged grains. The clean seeds

were sterilized by placing them into a freezer at 7 °C for 24 hours, and then heated in an oven at 60 °C for 24 hours to kill any larvae and adult cowpea weevils that might remain in the process of cleaning.

3.2.5 Testing the effect of leaf powder to *C. maculatus*

The dosages were set at different rates (0 g-negative control, 30, 60 and 90 g 1.5 kg⁻¹ of cowpea seeds) for each of the insecticidal plant powder used, so as to obtain the effective dose. To test the effect of leaf powder, 1.5 kg of healthy, fresh, clean, and unbroken cowpea grains were loaded into 2 kg cotton storage bags (Fig. 6). Each bag with cowpea seeds was placed inside another bag of the same volume and the leaf powder of *H. suaveolens*, *O. suave* and *D. ambrosioides* at different rates (30, 60 and 90 g) were then spread on the outer surface of the inner bags (Double bagging experiment).

Three treatments (*H. suaveolens*, *O. suave* and *D. ambrosioides*) leaf powder at rates 30, 60 and 90 g 1.5 kg⁻¹ cowpea seeds plus the positive and negative control were arranged in Randomized Complete Block Design (RCBD) in 3 replicates. Experimental bags in each treatment (including the controls) were arranged to surround a single plastic container containing heavily infested cowpea seeds left opened to allow movement of weevils to the surroundings.

3.2.6 Data collection

A subsample of 1000 cowpea seeds was drawn out from each bag for insect pest's assessment. Counting of *C. maculatus* (live and dead) seeds with eggs on the surface, damaged seeds (seeds with holes and/or larval inside) was done after every 21 days. After assessment everything were taken back into the respective bag and the bags were sealed. The experiment ran for three consecutive months.

3.3 Data analysis

Data collected were subjected to STATISTICA (data analysis software system Version 8.0) to test for treatment effects over the study period. One-way analysis of variance (ANOVA) was used to analyze the collected data and Fisher's Least Significant Difference (LSD) test was used to compare significant treatment means at 5 % confidence interval ($P = 0.05$)

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Results

4.1.1 The influence of selected field margin pesticidal plants (*H. suaveolens*, *O. suave*, *D. ambroseiodes* and *S. suaveolens*) in enhancing the abundance of pollinators and their contribution in common bean pods formation

Generally, pesticidal field margin plants (PFMPs) attracted different kinds of pollinators at flowering period of common bean. The recorded pollinators during this study period were honey bees, minute bees, solitary bees, carpenter bees, hoverflies, wasps, butterflies and moth. However, carpenter bees, solitary bees, moth and wasps were not significant and hence were excluded in the analysis. Therefore, mean number of four species of pollinators over two weeks in two days interval is described below:

i. Mean number of honey bees recorded in the field over two weeks

Results indicated significant difference on the mean number of honey bees between treatments in different flowering phases from early to late flowering phases (Appendex 1). The highest mean number of honey bees was observed in plots surrounded by *O. suave* while, the lowest mean number was seen in control plots (no margins) throughout the study. At early and mid-flowering phases *O. Suave* and *S. suaveolens* perform better by attracting a good number of honey bees followed by *H. suaveolens*. During maximum and late flowering phase only *O. suave* performs better than the rest of the treatments (Fig. 6). Among the FMPs *D. ambrosioides* attracted fewer numbers of honey bees and this was almost similar to the

control.

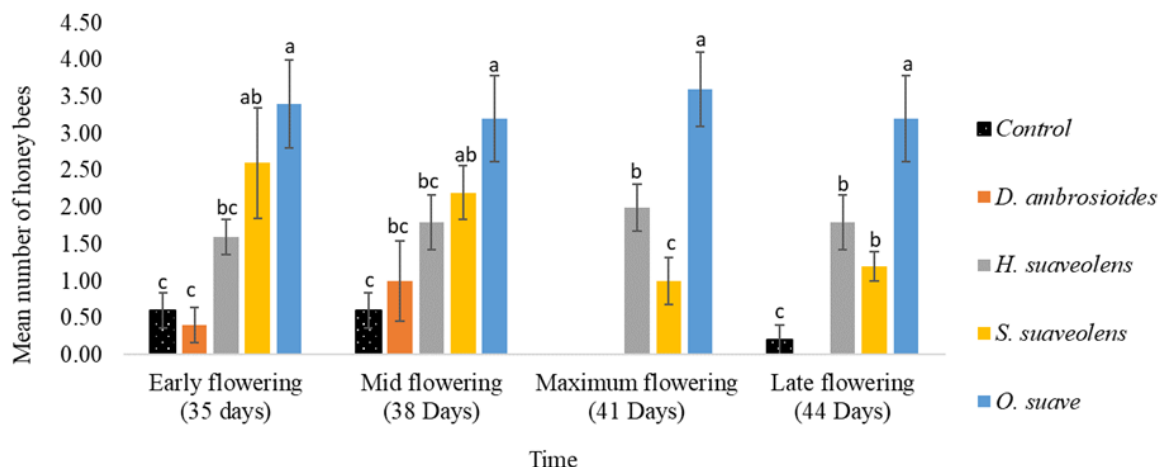


Figure 6: Mean number of honey bees over two weeks

*Mean values indicated by different letter (s) are significant at $P \leq 0.05$ among treatments in the same day of collection and the days were named from the sowing date of common bean seeds to the first flowering day.

ii. Mean number of small bees recorded in the field over two weeks

There was significant difference in the mean number of small bees between different treatments throughout the study period (Appendix 2). Among the PPs *O. suave* attracted large number of small bees in comparison with the rest of the FMPs. However, *O. suave* and *H. suaveolens* attracted almost similar number of small bees during early, mid and late flowering phases followed by *S. suaveolens* and *D. ambrosioides* while the control plots (no margins) were the least. At maximum flowering phase only *O. suave* attracted the highest number of small bees than the rest of FMPs that attracted similar number of small bees as in control plots (Fig. 7).

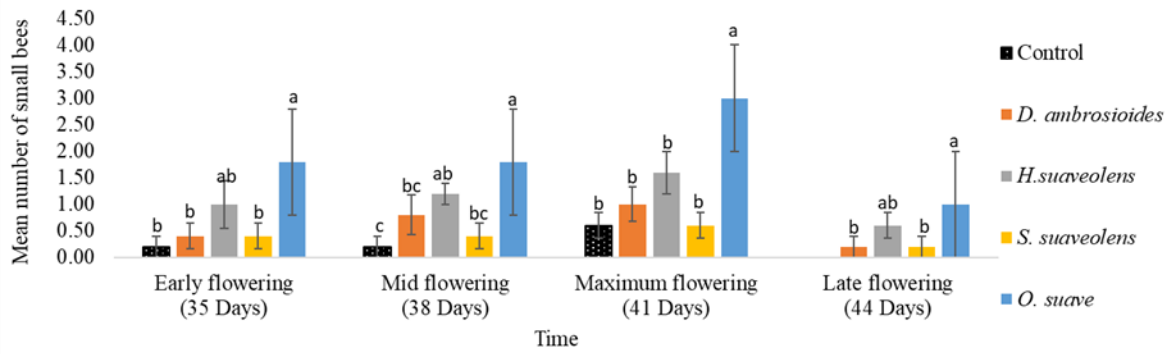


Figure 7: Mean number of small bees over two weeks

*Mean values indicated by different letter (s) are significant at $P \leq 0.05$ among treatments in the same day of collection and the days were named from the sowing date of common bean seeds to the first flowering day.

iii. Mean number of hoverflies recorded in the field over two weeks

Results showed significant difference on mean number of hoverflies between treatments throughout the study period (Appendex 3). During the early and mid-flowering phases *O. suave*, *H. suaveolens* and *S. suaveolens* attracted similar number of hoverflies followed by *D. ambrosioides* and the control plots. During the maximum flowering phase *O. suave* attracted large number of hoverflies followed by *H. suaveolens*. Although, in the late flowering all PFMPs except *O. suave* attracted the same number of hoverflies as in control plots (Fig. 8). There were increased numbers of hoverflies visiting the flowers of the common bean in all treatments at maximum and late flowering phases.

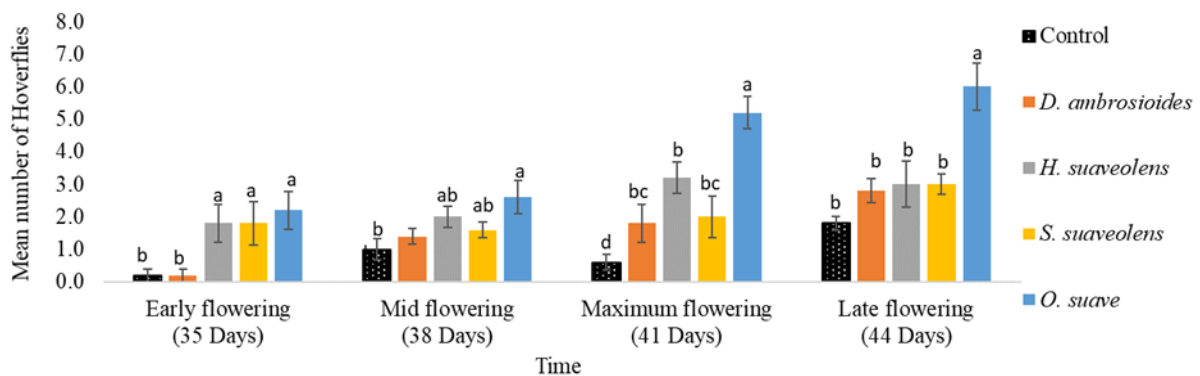


Figure 8: Mean number of hoverflies over two weeks

*Mean values indicated by different letter (s) are significant at $P \leq 0.05$ among treatments in the same day of collection and the days were named from the sowing date of common bean seeds to the first flowering day.

iv. Mean number butterfly recorded in the field over two weeks

The result was significant on the mean number of butterflies among different treatment throughout the study (Appendix 4). Statistically *O. suave* and *H. suaveolens* attracted similar number of butterflies during the early flowering period. However, in the proceeding phases, *H. suaveolens*, *O. suave* and *S. suaveolens* attracted butterfly nearly at similar level and the lowest mean number were counted in plots planted with *D. ambrosioides* and the control plots throughout the study period (Fig. 9).

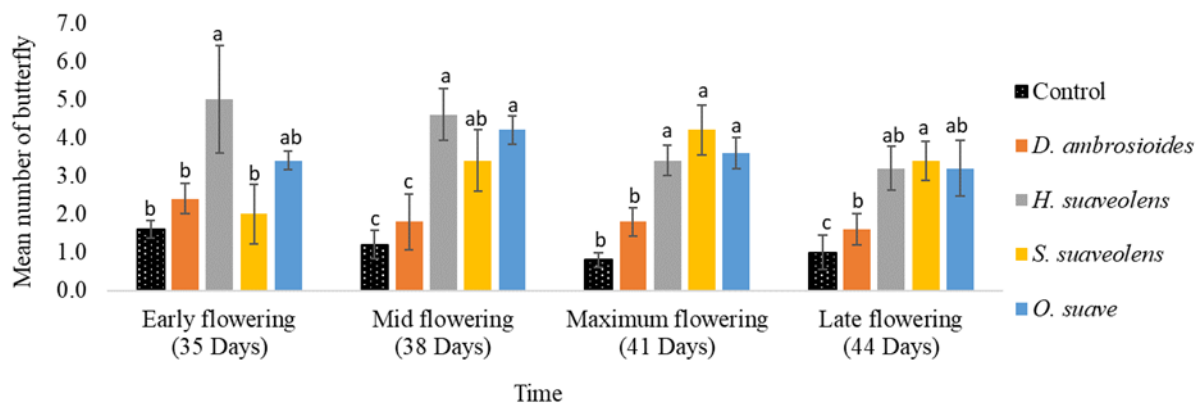


Figure 9: Mean number of butterflies over two weeks

*Mean values indicated by different letter (s) are significant at $P \leq 0.05$ among treatments in the same day of collection and the days were named from the sowing date of common bean seeds to the first flowering day.

v. The contribution of insect pollinators in common bean pods formation

Number of pods per plant formed varied significantly ($P \leq 0.001$) in relation to pollination method in which the highest pods number per plant was observed in open pollinated bean plants followed by hand pollinated bean plants and the lowest pods number per plant were counted in self-pollinated bean plants (Appendix 5). However, differences in mean number of pods per plant can be well explained based on the type of pesticidal field margin used. Comparatively, greatest number of pods per plant were counted in plots surrounded by *O. suave* (24) followed by *H. suaveolens* and *S. suaveolens* (19 and 16) respectively for open pollinated bean plants while the lowest count was in control plots (10) with no margins (Fig. 10). Self-pollinated bean plants depicted almost similar number of pods per plant as in control plots.

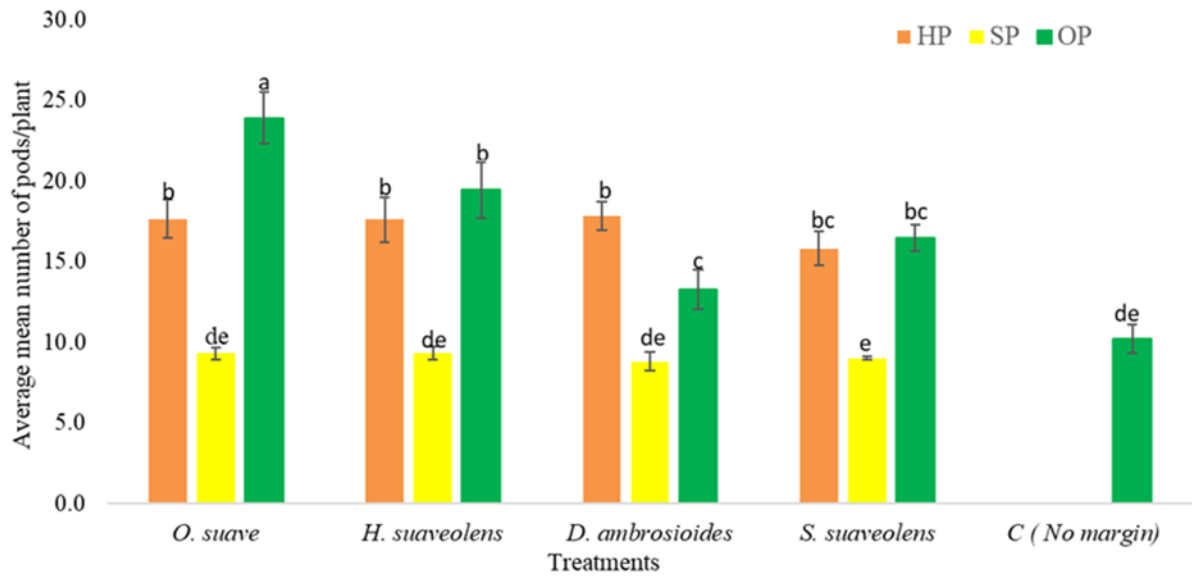


Figure 10: Mean number of pods per plant formed between treatments in different pollination methods

HP = Hand pollination; SP = Self-pollination; OP = Open pollination; C= Control; *mean values indicated by different letter (s) indicate significant difference at $P \leq 0.05$ among treatments in different pollination methods.

4.1.2 Effects of *H. suaveolens*, *O. suave* and *D. ambrosioides* on reducing the infestation of cowpea weevils (*C. maculatus*) in storage

i. Effect of pesticidal leaf powders on live adult *C. maculatus*

The application of plant leaf powders at rates of 30, 60 and 90 g 1.5 kg⁻¹ of cowpea seeds, showed varied pesticidal effects on the survival of adult *C. maculatus*. Their differences were statistically significant ($P \leq 0.001$) (Table 3). The highest mean numbers of live *C. maculatus* were recorded in the bags with negative control (8.00±1.53, 10.67±1.20, 12.67±0.88 and 85.67±5.21) all over 12 weeks of insect assessment. For the first three weeks all tested plants leaf powder at the rates of 30, 60 and 90 g 1.5 kg⁻¹ of cowpea seeds effectively inhibited adult *C. maculatus* emergence similar to the seeds treated with Actellic® dust (positive control). However, from 6 to 12 weeks, the effects were dose dependent whereby increased rate of leaf powder consequently increased pesticidal effect against *C. maculatus* on stored cowpea. *O. suave* at 90 g and *H. suaveolens* and *D. ambrosioides*, at 60 and 90 g significantly ($P \leq 0.001$) inhibited the emergence of adult *C. maculatus* when compared with their respective lower rates.

Table 3: Mean number of live *C. maculatus* in stored cowpea treated with pesticidal leaf powder

Treatments	Rates (g)				
	1.5 kg ⁻¹	3weeks	6weeks	9weeks	12weeks
Control (-ve)	0	8.00±1.53a	10.67±1.20a	12.67±0.88a	85.67±5.21a
<i>Ocimum suave</i>	30	0.33±0.33b	5.00±1.53b	6.00±0.58bc	26.33±4.91b
<i>Ocimum suave</i>	60	0.33±0.33b	5.00±0.58b	5.00±0.58c	17.67±1.86b
<i>Ocimum suave</i>	90	0.33±0.33b	1.00±0.58c	0.67±0.67d	3.00±0.58c
<i>Hyptis suaveolens</i>	30	1.67±0.33b	6.33±0.88b	7.33±0.88b	20.33±1.45b
<i>Hyptis suaveolens</i>	60	0.00±0.00b	0.67±0.33c	0.67±0.33d	1.00±0.58c
<i>Hyptis suaveolens</i>	90	0.67±0.67b	0.33±0.33c	0.33±0.33d	0.67±0.33c
<i>Dysphania ambrosioides</i>	30	1.00±0.58b	6.33±1.20b	7.67±0.88b	22.00±3.21b
<i>Dysphania ambrosioides</i>	60	0.00±0.00b	0.33±0.33c	0.67±0.67d	1.00±0.58c
<i>Dysphania ambrosioides</i>	90	0.67±0.00b	0.67±0.33c	0.33±0.33d	0.33±0.33c
Actellic dust (+ve)	2	0.00±0.00b	0.00±0.00c	0.00±0.00d	0.00±0.00c
One Way ANOVA					
(F-statistics)		12.71***	30.15***	36.05***	54.65***

Control (-ve) = negative control, +ve = positive control; *** significant at $P \leq 0.001$ and means within the same column followed by the same letter (s) are not significantly different at ($P = 0.05$) from each other using Fisher's Least Significant Difference (LSD) test.

ii. The effect of pesticidal leaf powder on the mortality of adult *C. maculatus*

Different effects were observed by supplying 30, 60 and 90 g of plant leaf powder on the mortality of adult *C. maculatus*. The results showed significant difference ($P \leq 0.001$) in the mean number of dead adult *C. maculatus* across treatments all 12 weeks (Table 4). The highest mortality was observed outside the bags treated with Actellic® dust (2 g) (19.00 ± 1.15 , 21.67 ± 2.73 , 26.33 ± 2.91 and 29.67 ± 4.98) throughout the study period. The result of the first week observation for *H. suaveolens* and *D. ambrosioides* at 90 g was statistically similar to positive control followed by 90 g for *O. suave* and 60 g for *H. suaveolens* and *D. ambrosioides*. There were increased numbers of dead insects in treatments involving *H. suaveolens* and *D. ambrosioides* plant leaf powder at the rates of 60 and 90 g and *O. suave* at 90 g 1.5 kg^{-1} of cowpea seeds after the first 3 weeks up to 9 weeks of treatment indicating high effectiveness of these plants materials in increasing the number of dead adult *C. maculatus*. However, mortality started to decrease after 12 weeks of treatment contrary to Actellic® dust 2 g that maintained its effectiveness. This showed that, mortality of adult *C. maculatus* treated with plant leaf powder was affected by increased rate of plant leaf powder and its exposure time to cowpea seeds. There were no dead insects found in untreated bags (negative control) throughout 12 weeks of assessment. In this case, the lowest mortality was observed in a negative control while the highest number was observed in the bags treated with Actellic® dust where dead insects were observed outside the bags. Additionally, 30 g of all plant leaf powder had a low pesticidal effect on the mortality of *C. maculatus*.

Table 4: Mean number of dead *C. maculatus* in stored cowpea treated with pesticidal leaf powder

Treatments	Rates (g) 1.5 kg ⁻¹	Week 3	Week 6	Week 9	Week 12
Control (-ve)	0	0.00±0.00e	0.00±0.00d	0.00±0.00f	0.00±0.00f
<i>Ocimum suave</i>	30	6.67±1.20d	8.67±1.20c	12.00±1.15e	11.67±2.33e
<i>Ocimum suave</i>	60	9.00±0.58cd	13.00±2.08bc	17.67±0.88cde	12.33±1.76de
<i>Ocimum suave</i>	90	15.67±0.88b	17.00±1.53ab	24.33±2.03ab	17.33±0.88bcd
<i>Hyptis suaveolens</i>	30	9.33±0.88c	12.00±2.52bc	12.00±1.73e	13.00±2.08cde
<i>Hyptis suaveolens</i>	60	16.00±1.00b	20.00±1.53a	21.67±2.19abcd	17.33±1.20bcd
<i>Hyptis suaveolens</i>	90	17.67±0.88ab	21.00±1.73a	23.33±5.36abc	19.33±0.33b
<i>Dysphania ambrosioides</i>	30	7.67±0.88cd	10.67±1.45 c	16.00±1.53de	13.33±1.85cde
<i>Dysphania ambrosioides</i>	60	15.67±1.45b	16.67±3.38ab	19.33±2.03bcd	18.33±1.45bc
<i>Dysphania ambrosioides</i>	90	17.00±1.15ab	19.67±2.33a	21.33±1.20abcd	18.33±0.88bc
Actellic dust (+ve)	2	19.00±1.15a	21.67±2.73a	26.33±2.91a	29.67±4.98a
One-Way ANOVA (F-statistics)		61.54***	18.01***	19.96***	21.54***

Control (-ve) = negative control, +ve = positive control; *** significant at $P \leq 0.001$ and means within the same column followed by the same letter (s) are not significantly different at ($P = 0.05$) from each other using Fisher's Least Significant Difference (LSD) test.

iii. The effect of pesticidal leaf powder on the oviposition of *C. maculatus*

Plants leaf powder supplied at rates of 30, 60 and 90 g displayed different effects on oviposition of *C. maculatus*. Although, the effectiveness depends on the dosage of plant leaf powder and its exposure time to *C. maculatus* yet, different rates of pesticidal leaf powder were statistically significant ($P \leq 0.001$) when compared with negative control which had highest mean number of cowpeas with eggs on the surface throughout the study (Table 5). After the first 6 weeks of treatment, all pesticidal leaf powder at 60 and 90 g effectively inhibited eggs laying capacity by *C. maculatus* almost in a similar manner to the positive control. Generally, *H. suaveolens* and *D. ambrosioides* at the highest rate of 90 g tested in this study maintained its effectiveness up to 12 weeks of treatment. The trend was different from *O. suave* which showed less pesticidal activity after 6 weeks of treatment. *Ocimum suave* at 30 g displayed the least pesticidal effect in affecting egg laying capacity by *C. maculatus*. Actellic® dust 2 g showed the greatest capacity to inhibit oviposition by *C. maculatus* than the rest of the treatments.

Table 5: Mean number on oviposition by *C. maculatus* in stored cowpea treated with pesticidal leaf powder

Treatments	Rates (g)				
	1.5 kg ⁻¹	Week 3	Week 6	Week 9	Week 12
Control (-ve)	0	50.33±4.91a	98.67±7.22a	234.33±3.53a	536.33±9.39a
<i>Ocimum suave</i>	30	20.00±1.53b	47.00±4.16b	139.00±23.12b	246.67±41.91b
<i>Ocimum suave</i>	60	6.67±1.45cd	12.67±3.18d	70.67±6.44c	124.00±22.54de
<i>Ocimum suave</i>	90	2.33±0.33d	3.00±1.00de	64.67±23.47c	60.67±24.34f
<i>Hyptis suaveolens</i>	30	18.33±6.36bc	31.67±1.86c	88.33±5.78c	210.00±25.32bc
<i>Hyptis suaveolens</i>	60	6.67±3.18cd	3.33±1.20de	6.00±3.46d	56.33±4.33fg
<i>Hyptis suaveolens</i>	90	1.33±1.33d	0.67±0.67de	5.33±1.76d	3.33±1.45h
<i>Dysphania ambrosioides</i>	30	20.33±6.06b	31.33±3.48c	59.33±20.30c	172.00±20.26cd
<i>Dysphania ambrosioides</i>	60	6.67±2.40cd	1.67±0.88de	5.67±1.20d	106.00±5.13ef
<i>Dysphania ambrosioides</i>	90	3.3±2.40d	0.67±0.67de	4.67±1.20d	4.67±0.88gh
Actellic dust (+ve)	2	0.00±0.00d	0.00±0.00e	0.33±0.33d	2.00±0.58h
One-Way ANOVA (F-statistics)		19.38***	87.87***	49.82***	129.58***

Control (-ve) = negative control, +ve = positive control; *** significant at $P \leq 0.001$ and means within the same column followed by the same letter (s) are not significantly different at ($P = 0.05$) from each other using Fisher's Least Significant Difference (LSD) test.

iv. The effect of pesticidal leaf powder on cowpea damage by *C. maculatus*

Treating cowpea seeds with plant leaf powder at rates 30, 60 and 90 g, displayed varied pesticidal effects on protection of cowpea grains against *C. maculatus* damage. Actellic® dust at a rate of 2 g showed high effectiveness in controlling cowpea damage by *C. maculatus* (Table 6). The highest seed damage was observed in a negative control (27.67±4.98, 33.00±3.06, 107.67±13.13 and 132.67±16.60) throughout the study period. All plants leaf powder at rates 30, 60 and 90 g 1.5 kg⁻¹ of cowpea seeds displayed similar effect in reducing cowpea seeds damage during the first 3 weeks of treatment. However, from 6 to 12 weeks, their differences were statistically significant ($P \leq 0.001$) and were dose dependent. Treatments with *O. suave* at 90 g, *H. suaveolens* and *D. ambrosioides* at rates 60 and 90 g showed promising results in protecting the cowpea seeds against *C. maculatus* damage similar to synthetic chemical.

Table 6: Mean number of damaged seeds by *C. maculatus* in stored cowpea treated with pesticidal leaf powder

Treatments	Rates (g) 1.5 kg ⁻¹	Week 3	Week 6	Week 9	Week 12
Control (-ve)	0	27.67±4.98a	33.00±3.06a	107.67±13.13a	132.67±16.60a
<i>Ocimum suave</i>	30	12.00±1.73b	18.33±1.45b	39.33±1.86b	77.00±6.25b
<i>Ocimum suave</i>	60	10.67±0.88b	12.00±1.53bc	15.00±2.00c	68.67±6.57b
<i>Ocimum suave</i>	90	7.00±1.15bc	8.67±0.88bcd	10.00±1.53c	9.67±0.67c
<i>Hyptis suaveolens</i>	30	10.33±1.45b	11.00±1.15bcd	36.00±6.03b	70.00±7.77b
<i>Hyptis suaveolens</i>	60	9.33±0.88b	7.33±1.20bcd	7.00±2.65c	7.67±0.88c
<i>Hyptis suaveolens</i>	90	8.67±2.03b	6.67±1.20cd	7.00±2.08c	5.00±1.15c
<i>Dysphania ambrosioides</i>	30	11.00±1.00b	12.67±2.73bc	33.33±8.37b	60.00±4.36b
<i>Dysphania ambrosioides</i>	60	8.67±1.20b	8.00±1.53bcd	10.67±0.88c	8.33±0.67c
<i>Dysphania ambrosioides</i>	90	6.33±2.85bc	7.33±2.03bcd	5.00±0.58c	5.00±0.58c
Actellic dust (+ve)	2	0.00±0.00c	0.33±0.33d	1.00±0.58c	1.33±0.33c
One-Way ANOVA (F-statistics)		15.77***	9.66***	49.01***	47.84***

Control (-ve) = negative control, +ve = positive control; *** significant at $P \leq 0.001$ and means within the same column followed by the same letter (s) are not significantly different at ($P = 0.05$) from each other using Fisher's Least Significant Difference (LSD) test.

4.2 Discussion

4.2.1 The influence of selected field margin pesticidal plants (*H. suaveolens*, *O. suave*, *D. ambrosioides* and *S. suaveolens*) in enhancing the number of pollinators and their contribution in common bean pods formation

The present study on effects of selected pesticidal plants on legume pollinators attraction in the field and bruchids control in storage generally indicated that FMPs attracted a number of pollinators which consequently increased pods formation in common bean. Increased number of pollinators might be attributed by the presence of pesticidal field margin plants (PFMPs) that are assumed to offer forage, a good micro climate, nest and nesting materials for survival of pollinators in agricultural settings. This is similar with the study by Dufour (2000) which describe the various manipulation strategies in restoring the population of beneficial insects within agro-ecosystem. Also, study by Carvell *et al.* (2006) and Tooker and Hanks (2014)

reported on various PPs which showed promising results in attracting pollinators when used as field margin plants.

Among pollinators, honey bees, small bees, hoverflies and butterflies were mostly attracted by pesticidal field margins used and their mean number significantly differed among treatments (Appendices 1- 4). Different PPs attracted different number of pollinators in different flowering phases and *O. suave* was seen to attract almost all kinds of pollinators than the rest of the PPs used. This implies that the aroma or floral volatiles released by PPs have high potential in attracting diverse number of pollinators. *Ocimum suave* might have volatile organic compounds that triggers the senses of pollinators and make them attracted to their flowers compared with other PPs used. This is in line with the study by Pichersky and Gershenzon (2002) and Theis (2006) which revealed that chemical compounds produced by different plants can activate the senses of pollinators and make them fly a long distance in attraction to such floral resources. Also, another study by Steffan-Dewenter *et al.* (2005) indicated that specific plants attracted different groups of insects and thus, in habitats manipulation, it is crucial to select flowering plants while targeting a specific insect. In this case PPs, display floral diversity for insect pollinators and therefore proper design of these plants within agro-ecosystem will enhance their visitation rate as pollinators are attracted to different plants by a range of characteristics including the floral signals.

Generally, the most abundant insect pollinator was the honey bee throughout the study period. This can be explained by the fact that bees are the key pollinators for most agricultural crops once provided with comfortable environment and necessary resources (Winfree, 2007). Furthermore, the number of hoverflies increased during maximum and late flowering in all treatments and even in control plots. This might be contributed by food availability in bean field due to insect pest infestation. Hoverfly being a natural enemy and pollinator feed on insect pests like aphids which were plenty in the bean field at this particular time. In this case, hoverfly play a dual role of biological pest control as well as pollination of crop plants (Colley and Luna, 2000).

The highest number of small bees was observed during the maximum flowering phase which might be contributed by presence of nectar and pollen resources because there were plenty of flowers at this period. *Dysphania ambrosioides* attracted almost similar number of pollinators as the control plots perhaps due the nature of flowers as well as the strong odor released by this plant which make it less attractive to pollinators.

Likewise, there was significant difference ($P \leq 0.001$) in number of pods per plant formed among different treatments in relation to pollination methods used. The highest mean number of pods per plant were observed in plots surrounded by *O. suave* (24) followed by *H. suaveolens* and *S. suaveolens* (19 and 16) respectively for Open-pollinated bean plants (Fig. 10). This implies that, insect pollinators contributed in the pollination process of common bean. Different treatments depicted different number of pods per plant and therefore, the more visited PPs the more it influenced insect visits to common bean flowers and eventually contributed to more pods' formation. Less number of pods per plant was observed in self-pollinated and in control plots (no margins) implying that the greater number of pods per plant observed in open pollinated bean plants might be contributed by the insect pollinators that visited the flowers of the common beans. This result is in consistent with the study by Bartomeus *et al.* (2014) that reported on the increased yield of bean field on the presence of insect pollinators. Hand pollination was done to determine pollen deficit in self-pollinated crops like common bean when insects are not involved in the pollination process.

4.2.2 Effects of *H. suaveolens*, *O. suave* and *D. ambrosioides* on reducing the infestation of cowpea weevils (*C. maculatus*) in storage

In this study, the pesticidal activities of *H. suaveolens*, *O. suave* and *D. ambrosioides* were evaluated on *C. maculatus* adult mortality, eggs deposition, seed damage (seeds with holes or larval) and number of adult emergences under a special storage room with ambient temperature and relative humidity. The plant leaf powders used in this study displayed different pesticidal effect on *C. maculatus* which depended on dose and exposure time in stored cowpea. The extracts and/or leaf powders derived from various plants including the three pesticidal plants used in this experiment have been found to be effective in managing a number of stored product insect pests (Obeng-Oforri and Reichmuth, 1997; Obeng-Ofori *et al.*, 2000; Asawalam *et al.*, 2007; Sharma and Tripath, 2008; Chiasson *et al.*, 2004; Adebayo and Eyo, 2014).

i. Performance of *H. suaveolens* on *C. maculatus*

Hyptis suaveolens is known to contain sabinene (41.0 %), terpinen-4-ol (12.31 %), β -pinene (10.0 %) and β -caryophyllene (8.0 %) as four major compounds with biological activities against various stored product insect pests (Tripathi and Upadhyay, 2009). In this study, leaf powder of *H. suaveolens* showed high effectiveness on reducing oviposition, adult emergence, seed damage and increasing mortality of *C. maculatus* at the higher dosages over the period of 12 weeks. Only few adult's emergence of *C. maculatus* were recorded (Table 3) indicating that

H. suaveolens induced high mortality and reduced oviposition by *C. maculatus*. The repellency property of *H. suaveolens* contributed to the reduced oviposition which consequently resulted into inhibition of larval development that could grow into pupa and hence low number of adult cowpea emergence. Hassan *et al.* (2018) reported on 100 % of eggs' mortality when leaf powder of *H. suaveolens* was tested on *C. maculatus* (F.) (Coleoptera: Bruchidae) eggs to test the ovicidal activity. Also, many other scholars reported on similar effect of the *H. suaveolens* plant materials on controlling cowpea weevils (Tripathi and Upadhyay, 2009; Adebayo and Eyo, 2014). However, the plant materials have also been observed to be effective as grain protectants not only to cowpea weevils but also to maize weevils (Asawalam *et al.*, 2007). In this regards, *H. suaveolens* seems to have protectants ability against storage insect pests for a longer period than *O. suave* and hence can be used as an alternative to synthetic pesticide.

ii. Performance of *O. suave* on *C. maculatus*

Ocimum suave contains phytochemical eugenol as a major bioactive compound that excite insecticidal activities such as repellency, fumigant and contact toxicity against storage insect pest, *Sitophilus zeamais* and *Prostephanus truncatus* (Obeng-Ofori *et al.*, 2000). In this present study, *O. suave* was identified as having pesticidal activities against *C. maculatus* at high dosage (60 and 90 g) of plant leaf powder that effectively reduces oviposition and the number of adult *C. maculatus* emergence up to 6 weeks after treatment (Table 3 and 5). This indicates that the plant materials can be used as a grain protectant in a short-term storage. In order for *O. suave* to have effectiveness in a long-term storage, need to be mixed with other pesticidal materials. Ojianwuna (2010) applied a mixed powder of *Cymbopogon citratus* and *Ocimum suave* against *C. maculatus* and found highly effective in reducing oviposition and number of adult emerged. At higher dose of 90 g plant leaf powder of *O. suave*, showed effectiveness in increasing adult mortality of *C. maculatus*. This result is similar to the work of Bekele *et al.* (1999) in which *O. suave* ground leaves worked best at the higher dosage and evoked 100 % mortality of three stored insect pests *Sitophilus zeamais*, *Rhyzopertha dominica* and *Sitotroga cerealella*. *Ocimum* species are known to have repellent and toxicant effect against various stored insect pests (Bekele *et al.*, 1999; Hassanali *et al.*, 1990) which might be the reason for high mortality and reduced oviposition caused by this plant material to *C. maculatus*. The protection of grains against insect damage by the leaves of *O. suave* form the basis for their use by small scale farmers as traditional grain protectant in short -term storage.

iii. Performance of *Dysphania ambrosioides* on *C. maculatus*

The chemical screening of *D. ambrosioides* identified the plant to have several chemical compounds including ascaridole (54 %), isoascaridole, (28 %) and p-cymene (8 %) (Dembitsky *et al.*, 2008; Mkenda *et al.*, 2015). At the rates of 60 and 90 g of *D. ambrosioides* exhibited high effectiveness on increasing adult mortality, reduced seed damage and inhibition of adult emergences of *C. maculatus*. Similar to other plant materials used in this study, *D. ambrosioides* also was very effectively at higher dosage of plant leaf powder. Mkenda *et al.* (2015) reported on complete seed protection, mortality and lower number of holes per cowpea seeds using powder from five pesticidal plants against *C. maculatus*. The effectiveness of *D. ambrosioides* could be as a result of biological activity of ascaridole compound that contains insecticidal activity against insects' pests. Chu *et al.* (2011) showed ascaridole to be the active component against the storage weevil *Sitophilus oryzae*. Effectiveness of *D. ambrosioides* plant materials against several insect pests have been widely reported (Tapondjou *et al.*, 2002; Chiasson *et al.*, 2004; Denloye *et al.*, 2010; Mkenda *et al.*, 2015).

Generally, the selected three pesticidal leaf powder evaluated, provide protection to cowpea seeds from *C. maculatus* at the high rates of plant leaf powder which may be as a result of ovicidal and larvicidal properties possessed by the tested plant materials that killed few eggs laid while preventing the few once that hatched to grow into larva. Furthermore, high mortality caused by plant leaf powder might also be due to contact toxicity of the tested plant powders which affect insect's survival thus kills them. The toxins released by plant materials affects the respiratory tract of the insects leading to death (Adedire *et al.*, 2011).

However, the aromatic nature of pesticidal plants used in this study might also be the cause for insect pest's suffocation which consequently results to death (Adedire *et al.*, 2011; Ileke, 2011; Ileke and Olotuah, 2012).

The efficacy of botanical pesticides decreases with increased exposure time of the leaf powder to the insect pest which suggests that there should be periodic reapplication of plant powders in order to offer continual protection of the grain against cowpea weevils (Muzemu *et al.*, 2013). This is due to the nature of the active ingredients presents in these plants and their fast-degradable ability (Reuben *et al.*, 2006; Belmain *et al.*, 2012).

The reduced oviposition at high dosage of plant leaf powder could be as a result of high mortality of weevils as observed in the synthetic pesticide treatment in which the cowpea weevils were observed to die while outside on the surface of bags which might be contributed by high toxicity of the Actellic® dust that prevent insect pests from entering the bags. Among the pesticidal leaf powder used, *H. suaveolens* and *D. ambrosioides* at 90 g effectively reduced the oviposition in stored cowpea similar to Actellic® dust. The bio-active ingredients present in pesticidal plants have been reported to caused mortality, oviposition deterrence and or ovicidal action resulting in reduced progeny production of stored insect pests (Aswalam, 2007; Kamanula *et al.*, 2010; Grzywacz *et al.*, 2014; Oni, 2014). Therefore, this might contribute to such high effectiveness of these plant powders and hence, small holder farmers can utilize these plant materials as grain protectants in stored cowpea.

CHAPTER FIVE

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

In this study, all the PFMPs used with the exception of *D. ambrosioides* attracted a reasonable number of pollinators. Their identified role as attractant plants to pollinators, signify also their importance within agro-ecosystem for increased agro-biodiversity. These pesticidal plants in the past were mainly used for biological pest control in the fields and storage. However, the finding from this research indicated that, these plants can also be used in attracting pollinators in agricultural bean fields and hence contribute in increased pods formation. *Ocimum suave* was the best plant that attracted a good number of different kinds of pollinators throughout the assessment period. Plots surrounded by *O. suave* also produced a greater number of pods per plant for open-pollinated beans compared with the rest of the plots. Moreover, the general trend showed large number of pods produced in open-pollinated beans where insect's pollinators were allowed to visit flowers of common beans than in self-pollinated and the control beans plots. This revealed that, insects contribute to the pollination process of self-pollinated crop such as common bean and thus habitat manipulation to restore or increase pollinator's population is of crucial importance.

The three selected pesticidal plants (*H. suaveolens*, *O. suave* and *D. ambrosioides*) when tested against *C. maculatus* using double bagging in stored cowpea seeds, effectively reduced adult weevils' emergence, oviposition and seed damage while increasing their mortality. Pesticidal leaf powder of *H. suaveolens* and *D. ambrosioides* at the rate of 90 g 1.5 kg⁻¹ of cowpea seeds showed promising results in inhibiting egg laying capacity by *C. maculatus*. This implies that these plant materials are good storage grain protectants in cowpea seeds, as adult cowpea weevils do not feed on grains but rather deposit their eggs. Thus, having good means to prevent or inhibit eggs deposition on seeds will completely or to some extent reduce development of larvae that will grow into pupae and therefore, reduced grain damage. Furthermore, these plant materials at the same rate of application were able to cause high mortality to weevils similar to positive control. This showed that, remote farmers can use these plants for storing their grains as the plants are, readily available, easily biodegradable and poses no danger to humans and other mammals. Therefore, this study revealed important technique of using botanical

pesticides through double bagging for effective control of cowpea weevils. reduces contamination level of grains with insecticides.

5.2 Recommendations

- i. Among the FMPs used *H. suaveolens*, *O. suave* and *S. suaveolens* attracted a good number of pollinators in common bean fields that potentially boosted the pollination and eventually increased pods number in common bean production.
- ii. Timely planting of PPs is required to ensure flowering at the same time with the focal bean crop for better results.
- iii. Further studies are recommended using the same PPs to assess their impact on grain yield.
- iv. For effective protection of cowpea grains using *H. suaveolens* and *D. ambrosioides* pesticidal leaf powder, continuously reapplication to stored grains at high dosage (90 g 1.5 kg⁻¹ of grains) is recommended for long term storage.
- v. *Ocimum suave* leaf powder can be used for short-term grains storage otherwise; periodic reapplication of *O. suave* powder should be adopted if the grains are to be stored for more than six weeks.
- vi. Double bagging is recommended for effective storage results using pesticidal leaf powder.
- vii. Also, further research to test the effect of the higher dosages (90 g) of these plant materials to human health using different models is recommended.

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APPENDICES

Appendix 1: Mean number of honey bees over 2 weeks of insect counting

Treatments	Early flowering (35 Days)	Mid flowering (38 Days)	Maximum flowering (41 Days)	Late flowering (44 Days)
Control	0.60±0.24c	0.60±0.24c	0.00±0.00d	0.20±0.20c
<i>Dysphania ambrosioides</i>	0.40±0.24c	1.00±0.55bc	0.00±0.00d	0.00±0.00c
<i>Hyptis suaveleons</i>	1.60±0.24bc	1.80±0.37bc	2.00±0.32b	1.80±0.37b
<i>Sphaeranthus suaveolens</i>	2.60±0.75ab	2.20±0.37ab	1.00±0.32c	1.20±0.20b
<i>Ocimum suave</i>	3.40±0.60a	3.20±0.58a	3.60±0.51a	3.20±0.58a
One - WAY ANOVA (F - statistics)	7.51***	7.55**	25.13***	15.11***
P value	0.000729	0.00706	0.000000	0.000008

35 Days = early flowering; 38 Days, 41 Days = Maximum flowering and 44 Days = late flowering named from the sowing date of common bean seeds to the first flowering day. Values presented are means ± Standard Error; Means with different letter (s) in the same column are significantly different at $P = 0.05$ according to Fisher's Least Significance Difference (LSD).

Appendix 2: Mean number of small bees over 2 weeks of insect counting

Treatments	Early flowering (35 Days)	Mid flowering (38 Days)	Maximum flowering (41 Days)	Late flowering (44 Days)
Control	0.20±0.20b	0.20±0.20c	0.60±0.24b	0.00±0.00b
<i>Dysphania ambrosioides</i>	0.40±0.24b	0.80±0.37bc	1.00±0.32b	0.20±0.20b
<i>Hyptis suaveleons</i>	1.00±0.45ab	1.20±0.20ab	1.60±0.40b	0.60±0.24ab
<i>Sphaeranthus suaveolens</i>	0.40±0.24b	0.40±0.24bc	0.60±0.24b	0.20±0.20b
<i>Ocimum suave</i>	1.80±0.37a	1.80±0.37a	3.00±0.71a	1.00±0.32a
One - WAY ANOVA (F - statistics)	4.28**	4.90**	5.73**	3.33*
P value	0.01157	0.006396	0.003068	0.030233

35 Days = early flowering; 38 Days, 41 Days = Maximum flowering and 44 Days = late flowering named from the sowing date of common bean seeds to the first flowering day. Values presented are means ± Standard Error; Means with different letter (s) in the same column are significantly different at $P = 0.05$ according to Fisher's Least Significance Difference (LSD).

Appendix 3: Mean number of hoverflies over 2 weeks of insect counting

Treatments	Early flowering	Mid flowering	Maximum flowering	Late flowering
	(35 Days)	(38 Days)	(41 Days)	(44 Days)
Control	0.20±0.20b	1.00±0.32b	0.60±0.24a	1.80±0.20b
<i>Dysphania ambrosioides</i>	0.20±0.20b	1.40±0.24b	1.80±0.58bc	2.80±0.37b
<i>Hyptis suaveleons</i>	1.80±0.58a	2.00±0.32ab	3.20±0.49b	3.00±0.71b
<i>Sphaeranthus suaveolens</i>	1.80±0.66a	1.60±0.24ab	2.00±0.63bc	3.00±0.32b
<i>Ocimum suave</i>	2.20±0.58a	2.60±0.51a	5.20±0.49a	6.00±0.71a
One - WAY ANOVA (F - statistics)	3.87*	3.21*	11.83***	9.73***
P value	0.017431	0.034576	0.000043	0.000154

35 Days = early flowering; 38 Days, 41 Days = Maximum flowering and 44 Days = late flowering named from the sowing date of common bean seeds to the first flowering day. Values presented are means ± Standard Error; Means with different letter (s) in the same column are significantly different at $P = 0.05$ according to Fisher's Least Significance Difference (LSD).

Appendix 4: Mean number of butterflies over 2 weeks of insect counting

Treatments	Early flowering (35 Days)	Mid flowering (38 Days)	Maximum flowering (41 Days)	Late flowering (44 Days)
Control	1.60±0.24b	1.20±0.37c	0.80±0.20b	1.00±0.45c
<i>Dysphania ambrosioides</i>	2.40±0.40b	1.80±0.73bc	1.80±0.37b	1.60±0.40b
<i>Hyptis suaveleons</i>	5.00±1.41a	4.60±0.68a	3.40±0.40a	3.20±0.58ab
<i>Sphaeranthus suaveolens</i>	2.00±0.77b	3.40±0.81ab	4.20±0.66a	3.40±0.51a
<i>Ocimum suave</i>	3.40±0.24ab	4.20±0.37a	3.60±0.40a	3.20±0.73ab
One - WAY ANOVA (F - statistics)	3.22*	5.69**	10.57***	4.04**
P value	0.034268	0.003166	0.000091	0.014653

35 Days = early flowering; 38 Days, 41 Days = Maximum flowering and 44 Days = late flowering named from the sowing date of common bean seeds to the first flowering day. Values presented are means ± Standard Error; Means with different letter (s) in the same column are significantly different at $P = 0.05$ according to Fisher's Least Significance Difference (LSD).

Appendix 5: Mean number of common beans pods counted in different treatments

Treatments	HP	SP	OP
<i>O. suave</i>	17.62±1.19a	9.32±0.38a	23.88±1.60a
<i>H. suaveolens</i>	17.60±1.39a	9.32±0.39a	19.42±1.73b
<i>D. ambrosioides</i>	17.80±0.88a	8.8±0.58a	13.26±1.24bc
<i>S. suaveolens</i>	15.80±1.05a	9.00±0.09a	16.46±0.84c
Control			10.20±0.90
One Way ANOVA F- Statistics			4.80***
P value			0.000357

*** Significant at $P \leq 0.001$; Values presented are means \pm Standard Error; Means with different letter in the same column are significantly different at $P = 0.05$ according to Fisher's Least Significance Difference (LSD).

RESEARCH OUTPUTS

Output one: A review paper presentation entitled “Potentials of pesticidal plants in enhancing diversity of pollinators in cropped fields” published in *American Journal of Plant Sciences*. <https://www.scirp.org/journal/ajps>.

Godifrey, J., P. A Ndakidemi. and E. Mbega. (2018). Potentials of pesticidal plants in enhancing diversity of pollinators in cropped fields. *American Journal of Plant Sciences*. **9**(13), 2659-2675.

Output two: A research paper presentation entitled “Effect of selected pesticidal plants on *Callosobrunchus maculatus* in stored cowpea (*Vigna unguiculata* L.) Walp” accepted in the *International Journal of Biosciences*.

Output three: Poster presentation.