BIOEFFICACY OF LION'S EAR (*Leonotis nepetifolia*) AND AFRICAN BASIL (*Ocimum gratissimum*) EXTRACTS AGAINST TWO-SPOTTED SPIDER MITE (*Tetranychus urticae*) ON FRENCH BEANS (*Phaseolus vulgaris L*)

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DECLARATION AND RECOMMENDATION

Declaration

I hereby declare that this Thesis is my original work and to the best of my knowledge has not been presented for an award of a degree in this or any other University.

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Recommendation

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DEDICATION

This Thesis is dedicated to God, my mother and sisters.

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ABSTRACT

French bean is a major export vegetable crop in Kenya that is currently facing a steady decline in production. Two-spotted spider mite, (*Tetranychus urticae* Koch*)* is one of the major pests that has contributed to the fluctuations in French beans production. The conventional management of this pest using miticides has caused serious environmental and safety concerns particulary in the export market. Plant-based products provide safe alternatives that can be included in integrated pest management systems. The objectives of this study were to determine; (i) miticidal, oviposition and repellent effects of *Leonotis nepetifolia* and *Ocimum gratissimum* plant extracts on spider mites (ii) effect of *L. nepetifolia* and *O. gratissimum* plant extracts on quality and yield of French beans. Field and laboratory bioassays were conducted at Egerton University using four concentrations (1.5, 3.0, 6.0 and 12.0 % w/v) of each plant extract in a randomized complete block design and completely randomized design, respectively. Field experiments were replicated three times and laboratory experiments four times. Abamectin (0.6 ml/L) was used as negative while methanol and water were used as positive controls in the laboratory and the field respectively. Laboratory data on mortality, repellence and oviposition were collected 24, 48 and 72 hours after treatment. Field data were collected on spider mite population which was expressed as percent corrected efficacy, percent leaf damage, pod number, pod length, pod diameter and yield. The data was subjected to analysis of variance using SAS. Data on counts were normalized using arcsine data transformation before analysis. Means were separated using Tukey's HSD test ($P < 0.05$). Laboratory bioassay results showed concentration- and exposure time-dependent with increase in efficacy of *L. nepetifolia* and *O. gratissimum* extracts against adult mite. At 12% w/v and 72h, *L. nepetifolia* and *O. gratissimum* extracts produced 93.8 and 96.4% mortality of adult two spotted spider mites, respectively. Both extracts produced 100% reduction in the number of eggs laid. The plant extracts showed 93.8% and 100% repellence respectively at 72 hours exposure time. Abamectin had 43.5 and 45.3 % mortality, and 34.4 and 62.9 % reduction in oviposition, respectively. Results from the field experiments also showed a concentration dependent effect of the plant extracts on mite population, leaf damage, and yield of French beans. Abamectin activity on mite populations was however higher than *L. nepetifolia* and *O. gratissimum* plant extracts at all concentration levels except at 12.0% w/v (82.75 and 69.06 %) mite reduction repectively during the first season. The plant extracts indicated lower leaf reduction and higher pod yield, compared to abamectin at all concentrations. These findings demonstrate the potential use of *L. nepetifolia* and *O. gratissimum* extracts in the management of two-spotted spider mite on French beans.

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LIST OFABBREVIATIONS AND ACRONYMS

AFA	Agriculture and Food Authority
ANOVA	Analysis of variance
CAB	Centre for Agriculture and Bioscience
CIAT	International Center for Tropical Agriculture
CRD	Completely Randomized Design
CV	Coefficient of variation
DW	Distilled water
EPA	Economic partnership agreement
GAP	Good agricultural practices
GDP	Gross domestic product
HAT	Hours after treatment
HCD	Horticultural Crops Directorate
HCDA	Horticultural Crops Development Authority
HSD	Honesty significant difference
IPM	Integrated pest management
KEPHIS	Kenya Plant Health Inspectorate Service
LN	*Leonotis nepetifolia*
Ml/L	Millilitre per litre
MRL	Maximum residue levels
MT	Metric tonnes
OG	*Ocimum gratissimum*
PCPB	Pest Control Products Board
PR	Percent repellence
RCBD	Randomized Complete Block Design
RH	Relative humidity
SAS	Statistical Analysis System
SD	Standard deviation
TSSM	Two-spotted spider mite

CHAPTER ONE

INTRODUCTION

1.1 Background information

Horticulture is an important subsector of agriculture and is among the leading contributors (33%) to Kenya's agricultural gross domestic product (GDP). The sub sector, which primarily comprises of fruits, flowers and fresh vegetables, has grown significantly to become a major employer with over six million Kenyans directly or indirectly. Horticultural exports earn the country considerable amount of income with fresh vegetables exports fetching about 48 % of the foreign exchange. French beans (*Phaseolus vulgaris* L.) is one of the major vegetable exports contributing about 23% of the fresh vegetables exported (HCD, 2017).

French beans production among small-holders who constitute approximately 80% of growers in Kenya has experienced fluctuation in terms of area and volume over the years. The area under production dropped from 7,733 Ha in 2007 to 4,128 Ha in 2012 (HCDA, 2012). There was however a slight increase to 5,983 Ha giving 41,789 tons of French beans worth 1.81 Bilion Kenya shillings in 2016 (HCD, 2017). The major abiotic factors attributed to the fluctuation in acreage and yields of French beans include soil fertility and moisture availability. The major biotic factors undermining French beans production include spider mites, bean fly, white flies and aphids (Monda *et al*., 2003).

The two-spotted spider mite (*Tetranychus urticae* Koch) is one of the most important pest that attack French beans and other crops worldwide (Alzoubi and Cobanoglu, 2008). The pest attacks over 300 host plants ranging from vegetables including French beans (*Phaseolus vulgaris*), egg plant (*Solanum melongena*), peppers (*capsicum frutescens*), tomatoes (*Solanum lycopersicon*) and sweet potatoes (*Ipomea batata*), fruits and ornamental plants (Le Goff *et al*., 2009). The damage from this pest is confounded by the fact that *T. urticae* is highly prolific and has a rapid growth rate (Jovanovic *et al*., 2007). The polyphagous feeding habit coupled with extreme dispersal capacity (Kim *et al*., 2006) enables *T. urticae* to colonise many crops in large areas within a short period. The mites affects the crops through both direct and indirect damage. Direct feeding by the mite results in severe damage such as leaf burning, defoliation and even plant death. Indirect effects caused through feeding by this mites include bronzing (yellow to white discoloration of the leaf) resulting in decreases in photosynthesis, transpiration, loss of quality and yield or death of the attacked plant (Park and Lee, 2002; Badawy *et al*., 2010).

Control of spider mites is mainly achieved by spraying miticides such as lime sulphur, dicofol, propargite and abamectin. Extensive and repeated spraying of these synthetic pesticides over a long period of time could lead to undesirable effects such as environmental contamination, destruction of natural enemies, and development of resistance by the pest and presence of un-acceptable residues in fresh and processed products. The negative effects of the synthetic pesticides therefore creates an impetus to a search for environmentally safe and acceptable alternative control measures against this important pest.

Natural plant extracts are seen as safe alternatives to synthetic pesticides. These plants represent a vast range of potentially useful natural products, which could provide safer products with maximum residue limits that meet GlobalGAP requirements. The botanical pesticides also possess an array of beneficial properties including repellence, antifeedant activity, growth regulatory activity and toxicity to insect and mite pests (Roobakkumar *et al*., 2010). Many laboratory experiments worldwide have screened thousands of species of higher plants both for pharmaceuticals and pest control products (Sarmah *et al*., 2009). This study sought to investigate the pest controlling properties of *Leonotis nepetifolia* and *Ocimum gratissimum* plant extracts against the two spotted spider mites (*Tetranychus urticae* Koch) under laboratory and field conditions.

1.2 Statement of the problem

The two-spotted spider mite (*Tetranychus urticae*) is an important pest that limits the production of French beans among small-holder farmers in Kenya. The pest is also listed as a quarantine pest and hence among the leading causes of rejection of Kenya's fresh produce exports in the European markets. The conventional management of spider mites using broad-spectrum chemicals by small-holder farmers has raised concerns about their threats to the environment and development of pesiticide resistance by the pest. The strict quality and maximum residue limits (MRL) guidelines in the export market has seen many small-holder farmers drop out of French bean production in Kenya. There is an imperative need for an intensive search for safer alternative management strategies for this pest. The development of a plant-based biopesticide would therefore be a valuable alternative tool to synthetic pesticides for use in integrated pest management (IPM) systems to manage the two spotted spider mites on French beans. There is therefore need for scientific rationalization and optimized exploitation of indigenous plant products for the protection of fresh perishable agricultural products against insect pests and diseases of economic value.

1.3 Objectives

1.3.1 Main objective

To contribute to improved yield and quality of French beans in Kenya through use of plant based products in the management of the two spotted spider mites.

1.3.2 Specific objectives

i). To determine the miticidal, repellent and oviposition inhibition effects of *Leonotis nepetifolia* and *Ocimum gratissimum* plant extracts against the two spotted spider mites (*Tetranychus urticae* Koch) on French beans in the laboratory.

ii). To determine the effect of *Leonotis nepetifolia* and *Ocimum gratissimum* plant extracts on the two spotted spider mites *(Tetranychus urticae* Koch) population, quality and yield of French beans in the field.

1.4 Hypotheses

i). *Leonotis nepetifolia* and *Ocimum gratissimum* plant extracts have no miticidal, repellent and oviposition inhibition effects against the two spotted spider mites *(Tetranychus urticae Koch)* on French beans in the laboratory.

ii). *Leonotis nepetifolia* and *Ocimum gratissimum* plant extracts have no effects on the two spotted spider mites *(Tetranychus urticae* Koch) population, quality and yield of French beans in the field.

1.5 Justification of the study

The French beans industry plays a vital role in the Kenyan economy as a very important enterprise and an important cash earner for small-holder farmers who often don't grow traditional cash crops such as tea and coffee. However, French beans varieties grown in Kenya are introductions from developed countries and hence highly susceptible to the local pests and diseases. The two spotted spider mites (*Tetranychus urticae*) ranks high among the pests that attack French beans world wide. Most of the small-holder farmers heavily rely on synthetic miticides to manage the two spotted spider mite pest on French beans. The over-reliance on synthetic miticides to manage this pest has resulted in the development of insecticide resistance. This has forced the farmers to either increase dosage or the frequency of application. The increased dosage and repeated applications of these chemicals have impacted negatively on compliance with GlobalGAP requirements concerning food safety, environmental safety, occupational health and safety and animal welfare (Zhang *et al*., 2003a; Aktar *et al*., 2009; Nderitu *et al*., 2010; Agritrade, 2013). Spider mites have also been listed as quarantine pest thus among the leading causes for Kenya's fresh produce rejection in European markets (Kedera and Kuria, 2003).

Recent research has focused on natural plant products (biopesticides) which are ecologically friendly alternatives for pest control in developing countries such as Kenya. Previous studies on the use of natural plant products to control pests have confirmed their environmental safety compared synthetic pesticides (Hassanali *et al.*, 1990; Ismail *et al.*, 2011). Besides the neem, a number of native plants including seed dust of yellow karavi (*Nerium oleander* L.) and flower juice of akanda (*Calotropis procera* L) were found to provide growth deterrents to spider mites. Plant products have been observed to exhibit high repellence against the stored product pest, red flour beetle (*Tribolium castaneum*) (Pugazhvendan *et al*., 2009). The plant materials were however found to be effective over a short period in both maize and cow peas. Therefore, the residual properties of the plant materials on field crops needs to be tested. Studies have indicated the potential use of plant powders as a novel repellent biopesticide to protect crops against damages caused by pests including the two two-spotted spider mite.The powders and essential oils of Ocimum species have been widely used for control of insect pests and especially storage insect pests. Little information is however available on the use indigenous plant extracts such as *Leonotis nepetifolia* and *Ocimum gratissimum* in the protection of fresh perishable agricultural products against pests of economic value.

CHAPTER TWO
LITERATURE REVIEW

2.1 French beans production in Kenya

The French beans also known as the green bean, string bean, snap bean or fillet bean belongs to the same species as the dry beans *Phaseolus vulgaris* and the family fabaceae. The crop is grown for its immature pods, which are used as a vegetable. The immature pods are grown both for fresh consumption and processing (Hempel and Bohm, 1996). Dry mature seeds are also edible and the French beans leaves are used as pot-herb in both developed and developing countries. French beans have great nutritional importance as human food due to their good protein content (1.83 g/100 g) and digestibility. The crop is also a source of important nutritional components such as vitamin A (12.2 mg/100g), dietary fibres (2.7 g/100g), potassium (211mg/100 g), folate (33 µg/100 g), iron (1.03 mg/100 g), magnesium (21 mg/100 g), thiamine, (0.082 mg/100 g) riboflavin (0.104 mg/100 g) copper (0.06 mg/100 g), calcium (37 mg/100 g), phosphorus (38 mg/100 g) *Omega*-3 fatty acids and niacin (0.734 mg/100 g) (USDA, 2018)

French beans cultivation is wide-spread throughout the world. The most important production areas in the world include Europe and Asia, which account for 76% of the world French beans production (FAO, 2014). Production of French beans in the developing countries is mainly done by small-holder farmers as a high input-output and market oriented crop. In Kenya, French beans immature green fresh or processed pods are mainly grown for export although local consumption is gradualy growing. The crop has a great potential for income generation and poverty alleviation (Ugen *et al.*, 2005). French bean production in Kenya was 41,745 MT in the year 2016 and was valued at KES. 1.81 B (HCD, 2017). European consumers, particularly those in France distinguish among three grades (Fine, extra fine and bobby) French beans. Fine and extra fine are seen as superior to bobby, this makes them to be more income elastic than the bobby (CIAT, 2006a). Bobby beans are typically produced in France, Italy, Spain, Egypt and Morocco, whereas Kenya is the major producer of fine and extra fine beans

2.2 Ecological requirements

French bean is a polymorphic, annual herbaceous plant cultivated in different climatic zones, at varying altitudes and under a variety of management practices. The optimum production of this beans is however attained at elevations ranging between 900-1500m above sea level

(NAFIS, 2017). In Kenya, French beans are mainly grown under irrigation in medium to high altitude areas of the former Central, Rift Valley, Eastern, Western and Coast provinces (Monda *et al*., 2003). The major producing areas include Kirinyaga, Murang'a, Machakos, Nakuru and Meru counties.

The crop is grown under warm temperatures ranging between 12 °C and 34 °C with optimal temperatures of 20 °C. A constant supply of moisture is essential in French beans production because it affects the yields, uniformity and quality of beans. French beans also require a well-distributed rainfall of 600 mm to 1500 mm throughout the growing season. Frost, dry winds, long rain and foggy periods are however harmful ato the crop. Irrigation is therefore vital to maintain continuous production of high qulity pods. The crop grows in a wide range of soils (sandy clay loam, sandy loam), but heavy soils are preferable. The soil type and soil profile should be uniform with a mild acidity (pH of 6.5-7.5) throughout the field but the plants can tolerate up to a pH 4.5 (Nderitu and Anyango, 1993). Harvest time of bean depends on the climatic conditions in which they are grown and variety. The main varieties grown in Kenya are; Monei, Amy, Samantha, Teresa, Julia, Vernando, Bronco, Coby, Espadia, Bakara, Claudia, Tokai, Pekera, Super Monel, Morgan, Paulista, Cupvert, Gloria, Tonivert, Rexas. Picking of French beans begins at 9 weeks after sowing and continues for about 3 weeks when the weather is dry (NAFIS, 2017). The beans are then graded and packed according to the requirements of the export market although market often requires fresh, straight, long, rounded in cross-section beans.

2.3 Production constraints

French beans, which is one of the major foreign exchange earners in Kenya, faces serious production and marketing challenges. Production in small-holder farmers' fields is mainly undermined by major pests like spider mites (*Tetranychus spp*), bean fly (*Ophyiomia spp*), thrips (*Frankliniella occidentalis*), white flies (*Trialeurodes vaporariorum*, *Bemisia tabaci*, *B. argentifolii*) aphids (*Aphis fabae*) and American ballworm (*Helicoverpa armigera*) among others (Nderitu *et al*., 2008; Nderitu *et al*., 2009). The major diseases that affect French bean production include root-knot nematodes (*Meloidogyne ssp*.), anthracnose (*Colletotricum lindemuthianum*), leaf rust ((*Uromyces appendiculatus*), fusarium wilt (*Fusarium oxysporum*) and blights (CIAT, 2006b; Ndegwa *et al*., 2006). The two spotted spider mite (*Tetranychus urticae*) are not only among the major pests causing yield reduction in French beans but has also been listed as quarantine pest in the export market (KEPHIS, 2012).

2.4 Two-spotted spider mites (TSSM)

2.4.1 Taxonomy

The two-spotted spider mite (*Tetranychus urticae* Koch) also known as glass house spider mite is the major mite pest of most ornamental plants and vegetable crops grown all over the world (CABI, 2007). Originally, from Eurasia, the two spotted spider mite has become a cosmopolitan pest with a host range of more than 300 host plants. The pest is described as a serious pest of at least 150 economically important agricultural and ornamental plants including corn, cotton, cucumber, bean, tomato, eggplant, pepper and roses (Mondel and Ara, 2006).

The Two-spotted spider mite belongs to the phylum arthropoda, class arachnida and is a member of the family tetranychidae (Osborne *et al*., 1985). The body of the female mite is oval-shaped and rounded posteriorly. The female adult mite is about 0.5 mm long and has eight short legs. Its colour varies from light yellow or green to dark green, straw colour, brown, black and various shades of orange. The male mite is much smaller and is considerably more active than the female. The body is narrow and distinctly pointed posteriorly. The colour of the male varies from pale to dark green, brownish or orange.

Plate 1: The two-spotted spidermite (*Tetranycus urticae* koch)

2.4.2 Life cycle

Development of the two-spotted spider mite proceeds through egg, larva, protonymph, deutonymph and adult. The larval, protonymphal and deutonymphal stages are further divided into feeding (active) and quiescent (resting) stages. Females mites normally lay clear spherical eggs (about 0.14 mm in diameter) on the underside of leaves (Razmjou *et al.*, 2009), that later turn opaque and glassy as incubation progresses. The larva has three pairs of legs.

The first non-feeding resting stage between the larval and protonymphal stages is called protochrysalis. The protonymph has four pairs of legs and is larger than the larva. At the end of the feeding stage, the protonymph attaches to the substrate, enters the quiescent stage and is later transformed into a deutonymph.

Developmental time of the two spotted spider mites generally vary due to certain factors such as temperature, humidity, host plant, leaf age, among others. Temperature is the most important factor that influences the rate at which spider mite develops. The lower threshold for development is about 12 ^{o}C whereas the maximum upper limit is about 40 ^{o}C with relative humidity ranging from 55 to 98% (El Taj *et al*., 2016). Under these conditions, mites develop from egg to adult on average period of 16.5 days. However, at a temperature of 27 ^{o}C and relative humidity of 90 ± 5%, the two-spotted spider mite can develop from egg to adult within an average period of 7.6 days (Shih *et al*., 1976). An individual female can deposit over 100 eggs in her lifetime. The total number of eggs laid per female and the eggs laid daily per female however depends on age, temperature, host plant species, relative humidity, host plant nutrition and exposure to pesticides (Karban and Carey, 1984; Zhang *et al.*, 2003b). Sex determination in the two spotted spider mite is arrhenotokous i.e. females develop from fertilised eggs and have the normal two sets of chromosomes (diploid) whereas males develop from unfertilised eggs and have only one set of chromosomes (haploid). Unmated females give rise to males only while mated females can produce either females or males (Carey and Bradley, 1982).

2.4.3 Damage caused by TSSM

Spider mites have tiny mouthparts, modified for piercing individual plant cells to remove the contents. This results in tiny yellow or white speckles. When many of these feeding spots occur near each other, the foliage takes on a yellow or bronzed cast. Once the foliage of the host plant becomes bronzed, it often drops prematurely (Badawy *et al*., 2010). Feeding causes the destruction and disappearance of chloroplasts, which then leads to basic physiological changes in the plant like a reduced photosynthetic capacity. The host plant in this case responds by effecting stomatal closure resulting in decreased uptake of carbon dioxide and consequently a marked reduction in transpiration and photosynthesis (Boj *et al*., 2006). Continued feeding may result in the collapse of mesophyll cells. The leaves eventually become bleached and discoloured followed by leaf drop especially under hot, dry conditions (Knapp *et al*., 2003) drastically reducing the crop yield. The two spotted spider mites also release phytotoxic substances into the host plant during feeding (Jeppson *et al*., 1975). The stippling or speckling of the upper leaf surface plus the webbing produced by protonymphs, deutonymphs and adults also leads to aesthetic injury especially in ornamental plants (Lu and Wang, 2005).

2.4.4 Management strategies for TSSM

Several strategies have been used by farmers to manage the two spotted spider mites on French beans and other crops. They include methods such as cultural practices, biological, synthetic pesticides and use of botanical pesticides among others. The different methods have varying degree of control but the best levels have been achieved when most of them are used in combination or in integrated pest management systems (Peairs, 1998; Attia *et al.*, 2013).

a) Cultural management

Cultural management includes practices such as spraying with water, nitrogen fertilization, quarantine and use of pest resistant varieties. Since all Tetranychus species infestations occur under hot, dry conditions, water helps to reduce the mite populations especially during moulting by knocking them off the leaves of infested plants using a forceful jet of water from a hose. Proper irrigation to prevent drought stress is the key cultural practise for avoiding mite outbreaks. However, once mite numbers have established irrigation cannot reduce mite densities (Roseheim and Corbett, 2003). Newly imported plants should be quarantined and confirmed to be free of mites before release.

Crop rotation helps to control weeds, discourages diseases, protect soil from erosion, reduces insect populations and rejuvenates soil organic matter. French beans should not be grown for more than two seasons on the same piece of land without other crops being grown in rotation. Grass/maize-legumes have been recommended because they accumulate a lot of organic matter within a short time (Knapp *et al*., 2003). Some crops have been reported to be resistant to spider mites (Mansour *et al*., 2004). For instance, various cucurbits such as watermelon are resistant to the carmine spider mite. These crops can be used in french bean crop rotations programmes to reduce mite population before susceptible crops are replanted.

b) Biological control

There are many natural enemies such as predators, parasites and pathogens which have been known to control the two spotted spider mite and other phytophahous mites. The natural enemies have shown success under greenhouse but little information or success has been reported under field conditions. The natural enemies include dark coloured lady beetles (*Stethorus sp*), small pirate bugs (*Orius tristicolor*), big-eyed bugs (*Geocoris sp*) and predatory thrips (*Androthrips sp*) have been reported to be good predators for spider mites (Colfer *et al*., 2004). Predatory mites such as *Phytoseiulus persimilis, Amblyseius sp, Neoseiulus californicus, Galandromus occidentalis* and *Metaseiulus occidentalis* are the major species used to control the two spotted spider mites in green-houses. It has however been reported that the presence of alkaloids in many vegetables could be harmful to some predators and this may hamper biological control (Alzoubi and Cobanoglu, 2010).

Some beneficial fungal pathogens have also been reported to be effective in controlling spider mites under different conditions (Maurya, *et al.,* 2013). *Hirsutella thompsonii* and *Beauveria bassiana* are potential entomopathogen for the control of the two spotted spider mites in green-houses (Gardner *et al*., 1982; Seiedy *et al*., 2010). However, their infection and activity on mites is very infrequent in the field thus limiting their usage (Chandler *et al*., 2000).

c) Chemical control

Most phytophagous spider mites can be controlled using soft pesticides such as insecticidal oils and soaps. Horticultural oils applied at higher rates of 3 to 4 % are useful for killing mite eggs and dormant adults. However, this is only effective for a short period of time after which mite resurgence occurs. Chemical pesticides used for the two spotted spider mite suppression are usually weak acaricides and often do not perform well. Some of them have been found to be compatible when used with other products, but have been found to cause reduction in efficacy of partner products (Agostini *et al*., 2014). Some of the active ingredients that have been used by small-holder farmers with little success include abamectin, spiromesifen, dicofol and chlorfenapyr (Elmoghazy *et al*., 2011).

d) Botanical pesticides

Botanical pesticides are naturally occurring chemicals (biopesticides) extracted from plants. Biopesticides are gaining increased attention and interest among those concerned with environmentally friendly, safe, and integrated crop management approaches. Plants generally produce secondary metabolites that are physiologically active on insects, mites and other organisms as a natural defence mechanism (Strauss and Zangerl, 2002). The secondary metabolites have multiple modes of action such as antifeedant and repellent activity, growth and fecundity reduction, disruption of cuticle and activity of the octopamine pathway in the central nervous system (Akhtar and Isman, 2004).

A number of plant substances have been considered for use as insect antifeedants or repellents. Aromatic plants and their volatile constituents are among the most effective botanicals with toxic, repellent, ovicidal and antifeedant properties against many field and storage insect and mite pests of crops. The plant extracts are specific chemicals or mixtures of chemical components derived from a plant. This category of biopesticides is much more diverse in composition, target pest, and mode of action. (Byrappa *et al.,* 2012). The plant extracts and oils are most often used as insecticides, but can also be used as herbicides. The mode of action varies greatly from product to product. Some botanical extracts such as floral essences attract insects to traps. Others such as cayenne can be used as deterrents while those of from lemongrass oil, strip the waxy coating off leaves of weeds to cause dehydration (Kawalekar, 2013) or coat the pest causing suffocation.

The highest plant families known to possess various chemical compounds which act as toxicants, antifeedants, repellents or growth inhibitors to many insect and mite species include Apiaceae, Asteraceae, Combretaceae, Geraniaceae, Gramineae myrtaceae and Lamiaceae among others (Bhattarai and Karki, 2004; Ogendo, 2008). Singh *et al.* (2014) conducted choice assays by exposing *Helicoverpa armigera* larvae on *Ocimum kilimandscharicum* and demonstrated that *O. kilimandscharicum* significantly deters larval feeding. They also reported that the average body weight decreased and mortality of the larvae increased when *Helicoverpa armigera* larvae were fed on *O. kilimandscharicum* leaves. The most commonly found botanical pesticides do not interfere with parasitoid foraging (Charleston, 2004) and can be excellent alternatives for the resource poor farmers in Kenya.

2.4.5 Toxic activity of botanical pesticides against spider mites

Attia *et al.* (2011) reported increased mortality and reduced fecundity of adult *T. urticae* resulting from the use of essential oils from *Deverra scoparia*. Acaricidal activity of plant extracts have been experimented and confirmed in previous studies. Shi *et al.* (2006) reported both contact and systemic acaricidal activity of an annual herbaceous plant *Kochia scoparia* (L) extracts against *T. urticae*. The acaricidal effects of leaf and seed extracts of *Datura stramonium* L. against *T.urticae* are more effective than the seed extracts in acute toxicity tests (Kumral *et al*., 2009).

Mozaffari *et al*. (2013a) reported 100% mortality of *T.urticae* when using *Thuja orientalis* essential oils applied at a concentration of 1000 µl L^{-1}. In another study with *Mentha pulegium* results indicated essential oils exhibiting a high toxicity on adults of *T.urticae* with the LC_{50} value of 2.25 µl L^{-1}. Increasing concern about persistence and environment effects of chemical residues require the development of biodgradable and environmentally friendly alternatives. This motivated the research on the potential of use of fruit extracts of hot pepper (*Capsicum annum*) for controlling the two spotted spider mites (Antonious *et al*., 2006).

2.4.6 Oviposition inhibition activity of botanical pesticides against spider mites

The spread of insect pests is strongly influenced by the female's choice of plant parts for oviposition and other oviposition characteristics. Oviposition deterrence may therefore be of great importance in insect pest management by protecting plants from insects before any feeding damage occurs. Ovicidal inhibition activities of plant extracts or active compounds isolated from extracts have been reported. Mansour *et al*. (2004) reported a significant reduction in the mean number of eggs laid by *Tetranychus cinnabarinus* mites when exposed to *Tamarix aphylla, Cyperus rotundus* and *Punica granatum* plant extracts. Chiasson *et al*. (2004) also reported significant effects on egg hatching of the two spotted spider mite (*Tetranychus urticae*) and European red mite (*Panonychus ulmi*) when exposed to abamectin, insecticidal soap and neem. Sarmah *et al.* (2009) tested four aqueous plant extracts for acaricidal and ovicidal activity under laboratory and field conditions at 2.5, 5.0, and 10.0% (w/v) concentrations against tea red spider mites (*Oligonychus coffea* Nietner). They reported a significant reduction in egg hatching by the spider mites.

Previous studies have also confirmed the ovicidal activities of plant extracts or active compounds isolated from extracts against spider mite management. Neem extracts have been reported as a potent repellent, antifeedant, growth regulator and oviposition deterrent (Ascher, 1993). Kumral *et al*. (2009) reported significant differences between the number of eggs laid by the two spotted spider mites on unsprayed bean leaves compared to those sprayed with thorn apple (*Datura stramonium*) leaf and seed extracts at sub lethal doses of 2500 and 25000 mg/l concentrations respectively. *Mentha pulegium* essential oil also exhibited high toxicity on eggs of the two spotted spider mites at LC_{50} value of 2.57 µl L^{-1} (Mozaffari *et al*., 2013b). Garlic distillate at concentrations of 0.36 and 0.74 mg/l have also been reported to cause a reduction in fecundity by the two spotted spider mites (Attia *et al*., 2011).

2.4.7 Repellent activity of botanical pesticides against TSSM

Studies have indicated the potential use of plant powders as a novel repellent biopesticide to protect crops against damages caused by pests including the two two-spotted spider mite. Dried leaves of *Lippia javanica* and wood of *Spirostachys africana* have varied insecticidal properties and efficacy as grain protectants against stored maize and cowpea insect pests (Chikukura *et al.*, 2011). Crude wild tomato (*Lycopersicon hirsutum*) leaf hexane extracts have the greatest repellence against the two spotted spider mites utilizing the ring bioassay under laboratory conditions. This is due to the presence of trans-caryophyllene in this plant (Antonious and Snyder, 2006). Leaf and seed ethanol extracts of thorn apple have also shown repellent activities against the two spotted spider mite on bean (Kumral *et al*., 2009). *Thuja orientalis* and *Mentha pulegium* plant extracts have also been reported to repel the the two spotted spider mite at different concentrations (Mozaffari *et al*., 2013a; Mozaffari *et al*., 2013b). Methanolic extracts of hot pepper accessions have been documented to have a potential in repelling the mites and thus need to be field-tested on large scale to assess their value in managing exploding populations of spider mites (Antonious *et al*., 2006).

2.4.8 Lion's ear and African basil as botanical pesticides

Lion's ear (*Leonotis nepetifolia*) belongs to the mint family known as lamiaceae. The plant is a robust annual herb that grows to about 1-2 m height. *Leonotis nepetifolia* posses an inflorescence with globose whorls at the upper nodes with orange flowers. The head which is a capitulum has spiny flower heads. The plant is commonly found in waste areas and roadsides. African basil (*Ocimum gratissimum*) is a low growing shrubby species that also belongs to the family lamiaceae. The plant is a slightly hairy annual with much branched angular stems carrying opposite; ovate leaves which is usually less than 1cm long and borne on long petioles. All plant species in the genus ocimum contain strongly scented essential oils (Schippers, 2000)

Leonotis nepetifolia has been reported to have insecticidal, fungal and bacteria activity on animal and plant pests (Hortensia *et al*., 2004; Ayanwuyi *et al*., 2009). Screening of this plants with insecticidal activity in order to isolate, identify and assess the bioactivity of insecticide compounds present against coleopteran pests of stored products has been done. (Asawalam *et al*., 2008) reported. Phytochemical examination of this plant parts and indicated the presence of different diterpenoids and other bioactive compounds. It has been used in the treatment of rheumatism, rickets, headaches and wounds (Burkill, 1995). The anti-bacterial activity of this plant has been experimented using crude extracts from its various parts (Narayan, 2012). Phytochemical studies by de Oliveira *et al*. (2015) also identified and quantified fixed oil components from the leaves of *Leonotis nepetifolia*

Ocimum gratissimum is grown as a pot-herb for local medicines and exists in a diversity of forms and cultivars. It is also been used in the treatment of stomach ache and sores (Ijeh *et al*., 2004).The powders and essential oils of O*cimum* species have been widely used for control of insect pests especially in stored products (Asawalam *et al*., 2008)

Plate 2: Lion's ear plant (*Leonotis nepetifolia*)

Plate 3: African basil plant (*Ocimum gratissimum*)

The genus *Ocimum* contains a reservoir of diverse secondary metabolites, which are known for their defense and medicinal value from several studies. Leaf extract of *Ocimum gratissimum* contain potent bioactive components (essential oils) made up of eugenol and other compounds (Matasyoh *et al*., 2007) with antioxidant and insecticidal properties (Eze *et al*., 2006; Aprioku and Obianime, 2008). Evaluation of insecticidal activities of dried *Ocimum gratissimum* leaves against bruchid (*Callosobruchus maculatus* F.) pests of cow pea resulted in significant increase in the mortality of adult insects compared to the control (Adeniyi *et al*., 2010; Brisibe *et al*., 2011; Koubala *et al.*, 2013). Higher concentrations of *O. gratissimum* equally resulted in increased reduction in the number of surviving bruchids and reduction to seed damage through, lower number of eggs laid and weevil perforation index after 90 days. Water extracts of *O. gratissimum* results in increased egg hatch inhibition of upto 100% after 48 hours and 90% juvenile mortality of *Meloidogyne incognita* with a corresponding higher grain yields (13.1 g) seed weight of cowpea compared to untreated plants (12.3 g) (Claudius-Cole *et al*., 2010). The leaf extracts of *O. gratissimum* also resulted in increased plant growth, vigour and yield by suppressing nematode population on the plant. Onifade (2007) reported the oils of composite parts of *Ocimum sp* to be as effective.

Existence of possible elicitors of defense response in *O. gratissimum* leaf extracts in its modes of action have been reported by Colpas *et al*. (2009). Aqueous extracts of the leaves of *Ocimum gratissimum* tested at 10, 25, 40 and 50% (w/v) concentrations induced the production of phytoalexins in soybean cotyledons and sorghum mesocotyls. The aqueous extracts also induced systemic resistance in cucumber to *Colletotrichum lagenarium*, as was reflected by reduction in disease incidence and an increase in chitinase production. All these studies have demonstrated that if well harnessed and incorporated into existing control and quarantine procedures, the plant products are potential bio-control agents of insect pests and other phytosanitary treatments. The studies further sought confirm the potential of botanical extracts to reduce over reliance on synthetic acaricides in the control of spider mites on crops.

CHAPTER THREE

MITICIDAL, REPELLENCE AND OVIPOSITION INHIBITION OF LIONS EAR (*Leonotis nepetifolia*) AND AFRICAN BASIL (*Ocimum Gratissimum*) EXTRACTS AGAINST TWO-SPOTTED SPIDER MITES (*Tetranychus urticae* Koch)

Abstract

The frequent use of synthetic acaricides to control mite species in crops has raised several concerns related to environment and human health. There is an urgent need to use natural products that possess good efficacy and are environmentally friendly. Among those products, plant based extracts provide a valuable natural resource. Methanol extracts of Lion's ear (*Leonotis nepetifolia* L.) and African basil (*Ocimum gratissimum* L.) were evaluated in the laboratory at Egerton University for toxicity, oviposition inhibition and repellence effects against the adult female two-spotted spider mite (*Tetranychus urticae* Koch). The laboratory bioassays were conducted in a completely randomized design replicated four times using four concentrations (1.5, 3.0, 6.0 and 12.0% w/v) with methanol and Abamectin (0.6 ml/L) being used as negative and positive controls, respectively. Data on mite mortality, oviposition inhibition and repellence indices were recorded 24, 48 and 72 h after treatment and subjected to analysis of variance and means separated using Tukey's HSD test ($P< 0.05$). The median median lethal concentration (LC_{50}) was analyzed using Probit option of SAS. The results showed concentration and exposure time-dependent increase in efficacy of both extracts against the mites. At 12.0% and 72 h, *Leonotis nepetifolia* and *Ocimum gratissimum* produced 93.8% and 96.4% mortality of mites, respectively. Similarly, both extracts produced 100% oviposition inhibition. The extracts also significantly repelled the mites over the exposure periods. Synthetic acaricide, abamectin produced 43.5% and 34.4 % mortality and oviposition inhibition, respectively, compared to the extracts. These findings demonstrate the potential of *L. nepetifolia* and *O. gratissimum* extracts to cause mortality, inhibit oviposition and repel *T. urticae* mites. *L. nepetifolia* and *O. gratissimum* therefore posses' natural products with great potential to provide efficient and safer alternatives in the management of two-spotted spider mite on French beans

3.1 Introduction

The two-spotted spider mites is one of the most important pest that attack French beans in Kenya and other parts of the world. Most of the small-holder farmers who are the major producers of French beans heavily rely on synthetic acaricides to manage this pest on their crops. Controlling mites using chemicals alone is particularly risky because of the ability of mites to become resistant to a wide range of pesticides. This can lead to the evolution of acaricide resistance in *T. urticae* (Dagli and Tunc, 2001; Tirello *et al.,* 2012; Funayama, 2015). The degree of resistance to many established acaricides has resulted in the increased demand for new acaricides as plant derived materials with novel modes of action (George *et al.,* 2014). The resistance is predominantly caused by a less sensitive target site (target site resistance) and enhanced detoxification (metabolic resistance) (Marcic, 2012).

Synthetic acaricides have also caused other serious problems such as environmental contamination, unacceptable pesticide residues in food and lethal effects on non-target organisms (Jovanovic *et al.*, 2007). Adel and Abou (2014) investigated five acaricides (Challenger, Ortus, Vertimec, Delmite and Bioca) for controlling the two spotted spider mites, *Tetranychus urticae* Koch and their side effects on the abundance of the associated natural enemies (insects, mites and true spiders) on cotton seedlings. Their results indicated that the tested compounds induced varying reduction on the population of both *T. urticae* and the predatory mites *Phytoseiidae spp.* The acaricide Challenger was the most effective against *T. urticae* while Delmite recorded the highest reduction in the population of natural enemies. These negative effects have resulted in the increasing interest for natural plant-based pesticides, which might be safer, biodegradable and have shown low pest resistance (Isman and Machial, 2006).

Several studies have pointed to numerous plant species possessing potential pest controlling properties. Studies have also evaluated the potential of natural plant extracts to protect crops from insect and mite pest species such as whiteflies and spider mites (Nomikou *et al.*, 2010). Other studies by Abou-Fakhr *et al.* (2017) reported that 14 extracts out of 42 methanol extracts and 3 extracts out of 13 aqueous extracts of endemic medicinal plants were found to have a potential high bioactivity against *T. urticae* adults. Plant species have been found to contain natural deterrents, which are toxic to various insect and mite pests (Choi *et al.*, 2004) but safe to mammals. The extracts of *Satoreja hortensis* L. (Lamiaceae) was found to be toxic to the two spotted spider mites (Aslan, *et al*., 2004). Similarly the extracts from *Azadirachta* *indica* (Meliaceae), some species of solanaceae, *Capparis aegyptia* (Capparaceae), *Nerium orleander* L. (Apocynaceae) and *Alianthus altissima* L. (Simaroubaceae) have also been found to be effective against the two spotted spider mites (Chermenskaya *et al*., 2010; Bernardi, *et al.*, 2013). Results from a study conducted by Carriço *et al*. (2014) who evaluated the activity of crude aqueous plant extract of *Pouteria sapota* on the the blowfly (*Chrysomya putoria*) larvae treated with different concentrations (5, 10 and 25%) demonstrated the capacity of the extracts to alter post embryonic development of test insect. Phytochemical screening of the crude aqueous extract from *P. sapota* leaves showed that coumarins, reducing sugars, flavonoids and cyanogenic glycosides were the most abundant metabolites that were responsible for the bioavtivity of the extracts against the *H. amigera* larvae.

Medicinal plants are potential sources of microbiocide compounds, which could be used in the management of plant diseases (Amadioha and Obi, 1999). *Ocimum gratissimum* is one such species, and its leaf extracts have been successfully tested for the control of several phytopathogenic fungi in the laboratory. Antifungal, antibacterial and insecticidal antioxidant activities *of L. nepetifoli*a and *O. gratissimum* have also been confirmed in plant health (Hortensia *et al.*, 2004; Ayanwuyi *et al*., 2009; Dhawan *et al*., 2013; Ochola *et al*., 2015). Phytochemical examination of these plants indicated the presence of different diterpenoids and other bioactive compounds. They attributed the insecticidal activity to the presence of secondary metabolites such as saponins, flavonoids, alkaloids, phenolics and terpenes. Similar results with ethanolic extracts of *O. gratissimum* leaves against bean weevil have been documented (Claudius-Cole *et al.*, 2010; Brisibe *et al*., 2011).

This study sought to determine the miticidal, repellence and oviposition inhibition activities of methanol extracts of *Ocimum gratissimum* and *Leonotis nepetifolia* against adult female *T. urticae* under laboratory conditions conditions.

3.2 Materials and Methods

3.2.1 Experimental Site

The laboratory bioassay studies were conducted at Biotechnology laboratory, Egerton University.

3.2.2 Collection and Preparation of test plant materials

The two test plants namely, Lion's ear (*Leonotis nepetifolia* L.) and African basil (*Ocimum gratissimum* L.) were collected in 2013 from fallow fields at Egerton University and its surroundings. The identities of the test plants were confirmed by a taxonomist and a voucher sample kept at Biotechnology Laboratory, Egerton University. Composite fresh leaves and tender stems were were dried in well ventilated room at 18-28 ^oC for two weeks and further oven dried at 35 ^oC for 48 h (Asawalam *et al.,* 2006). The dried leaves were ground into fine powder using an electric laboratory hammer mill. The powder was subjected to methanol (100% AR) extraction at a rate of $200gL^{-1}$ and the extract kept on a magnetic stirring vibrating shaker for 3 h followed by filtration using Whatman No. 1 filter paper. The filtrate was then poured in a round bottom flask and concentrated at 50 ^oC using Büchi Rotavapor (Model R-200, Switzerland). The different extract concentrations were kept in air-tight containers and refrigerated at 4 ^oC for use in the bioassays.

3.2.3 Mass rearing of two-spotted spider mites

The local strain of *T. urticae* was obtained from naturally infested leaves of common beans that had not been sprayed with any pesticide. Rearing was done on 2 – 3 week old beans grown in plastic pots filled with peat moss and soil (2:1) and cultured in the greenhouse at Horticulture research and experimentation farm of Egerton University. Individual mites were then collected and transferred for bioassay studies using a fine hair brush.

3.2.4 Laboratory bioassay tests

a) Contact toxicity test

Leaf dipping method according to Erdogan *et al*., (2012) was used in the contact toxicity experiment which was arranged in completely randomised design (CRD) and replicated four times. Bean leaf discs (3 cm diameter) were cut and dipped into the different extract concentrations (1.5, 3.0, 6.0 and 12.0% w/v) for 30 seconds and allowed to dry under room temperature. Twenty (N_T) adult female mites recognized by their elliptical body and non-tapering caudal end and 12 pairs of dorsal setae (Fasulo and Denmark, 2009) collected from the greenhouse culture were introduced onto each leaf disc placed in petri dishes containing wet cotton wool. Methanol and synthetic acaricide (abamectin EC) were used as negative and positive controls, respectively. Number of dead and live mites were counted by examining the leaves under the stereo-binocular microscope (4× magnification) at 24 h, 48 h and 72 h after introduction. Mites were considered dead when they were not able to move a distance greater than the length of own body after soft contact with a fine haired brush. The mortality was computed after 72 h and corrected percent mortality calculated using Abbott's formula (Abbott, 1925).

$$\text{Corrected \%} = \left(1 - \frac{\text{n in T after treatment}}{\text{n in Co after treatment}}\right) x\ 100$$

Where:

n = Insect population

T = Treated leaves

Co = Control

b) Oviposition inhibition test

The leaf disc according to Zhang *et al*., (2013) method was used to determine the two spotted spider mites oviposition inhibition activity of the test plant extracts. The experiment was laid out in a completely randomised design (CRD) replicated three times. Bean leaves were cut into 3 cm diameter discs and their backs carefully wiped with the different plant extract concentrations using cotton swabs. The leaves were then left to dry under room temperature. Methanol and abamectin were used as negative and positive controls, respectively. Twenty female adult two spotted spider mites were introduced into the different petri dishes containing wet cotton wool. The numbers of eggs laid by the two spotted spider mites in extract treated and the abamectin and untreated control leaves were counted under a microscope at 24 h, 48 h and 72 h after introduction. Oviposition inhibition was expressed as a percentage by considering the number of eggs on the control leaf discs as 100%. Data were corrected by the Abbott (1925)'s equation as indicated above.

c) Repellence test

The repellence test was conducted according to Pontes *et al*. (2011) with modifications. French bean leaf discs (4 cm diameter) were used by immersing half of the disc into the treatments for 30 seconds and dried at room temperature for 30 minutes. The other half of the leaf discs were immersed in methanol or abamectin as positive and negative controls, respectively. Each disc was immersed in such a manner that permitted a free area of 0.5 cm between the two halves where 20 adult two spotted spider mites were initially released. The leaf discs were placed on wet cotton wool placed in petri dishes that were arranged in CRD experimental design with 4 replications. The mites were allowed to move freely. Counting of mites present on each half disc was done at 24 h, 48 h and 72 h after treatment. Percent repellence was computed according to the formula by Echereobia *et al*. (2010):

$$PR = \left(\frac{NC - NT}{NC + NT}\right) x\ 100$$

Where:

PR= Percentage Repellence,

NC= Number of pests on control portion,

NT= Number of pests on treated portion.

3.2.5. Statistical analysis

The data on contact toxicity, oviposition inhibition and repellence against the two spotted spider mites were subjected to analysis of variance (ANOVA) using SAS (SAS Institute, 2011) statistical program. The means were separated using Tukey's HSD test at 0.05% significant level whenever ANOVA showed significant difference. Data on contact toxicity and oviposition inhibition was further subjected to probit analysis to determine median lethal concentration (LC_{50}).

Stastical model

$Y_{ij} = \mu + T_i + Є_{ij}$

Where:

Y_{ij} is the random variable representing the response for treatment i observed in block h

μ is the overall the mean

T_i is the effect of treatment (plant extract concentrations)

$Є_{ij}$ is the random error { $Є_{ij}$ ~N (0σ2)}

3.3 Results

3.3.1 Contact toxicity

The probit analysis showed concentration and exposure time-dependent increase in the efficacy of *Leonotis nepetifolia* and *Ocimum gratissimum* plant extracts against the adult two spotted spider mites in the laboratory (Fig 1 and 2). The 72 h exposure time indicated a higher percent mite mortality (Fig. 1) at 1.5, 3.0 and 6.0 % w/v extract concentrations for *Leonotis nepetifolia*. The shorter exposure time of 24 h and 48 h were not significantly different at 1.5, 3.0 and 6.0 % w/v for *Leonotis nepetifolia* extract concentrations. On the other hand 24 h, 4h and 72 h indicated significant difference in mite mortality at 1.5 %, 3.0 % and 6.0 % w/v for *Ocimum gratissimum* plant extracts concentations (Fig 2). There was however no significant difference in mite mortality after 48 h exposure for both plant extracts at the highest concentrations (12 % w/v).

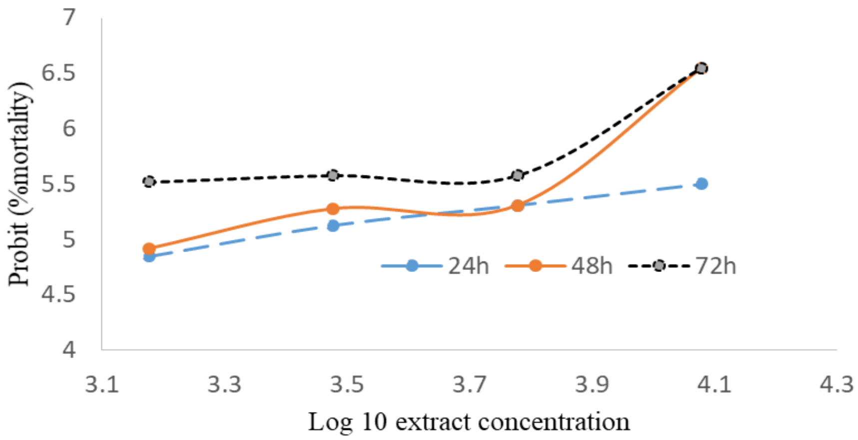

Figure 1: Probit (% mortality) with plant extract concentrations of *Leonotis. nepetifolia* at 24h, 48h and 72h

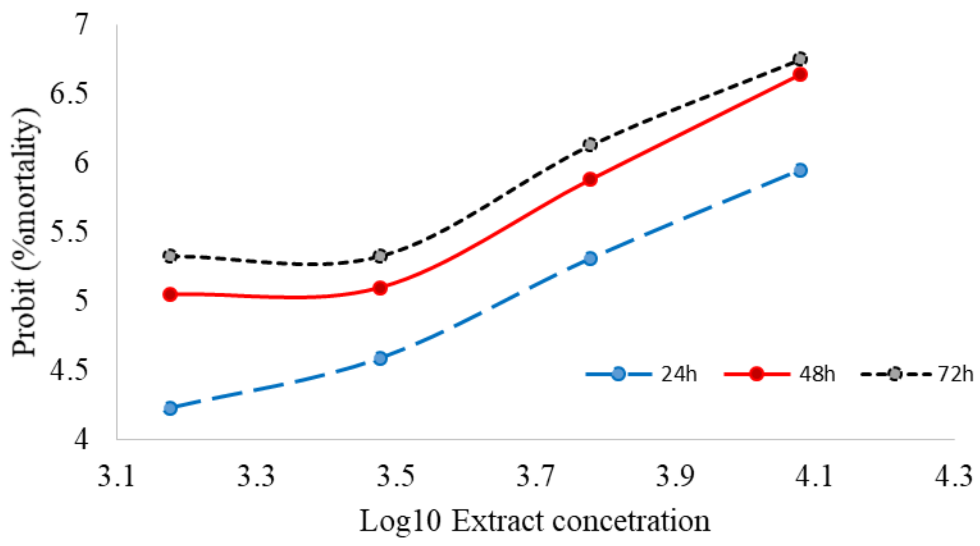

Figure 2: Probit (% mortality) with plant extract concentration of *Ocimmum gratissimum* at 24 h, 48 h and 72 h

The probit regression analysis on the median lethal concentrations (LC_{50}) values for *L nepetifolia* and *O. gratissimum* extracts reduced with exposure time (Table 1). *L nepetifolia* however indicated higher toxicity LC_{50} of 0.48 (480 mg/l) compared to *O. gratissimum* which indicated an LC_{50} of 1.15 (1150 mg/l) at 72h exposure time.

Table 1: LC_{50} values for *Leonotis nepetifolia* and *Ocimum gratissimum* plant extract at 24h, 48h and 72h against TSSM

Plant extract	**LC50**		
	24 h	48 h	72 h
L. nepetifolia	1.77	1.18	0.48
O. gratissimum	4.19	1.80	1.15

Results from the toxicity bioassays of *Leonotis nepetifolia* and *Ocimum gratissimum* varied with plant extracts concentrations and exposure time (Table 2). There was a positive relationship between toxicity and concentration. *Leonotis nepetifolia* plant extracts applied at 12% w/v concentration caused about 94 % adult two spotted spider mites mortality after 48 h exposure time. This was followed by 6% w/v extract concentration, which caused 62% and 71.5% mite mortality at 48 h and 72 h respectively. The two higher plant extracts concentrations indicated more than 60% mortality levels compared to the synthetic acaricide (abamectin) which indicated 43.5% mite mortality. There was however, no significant difference in mite mortality between abamectin and the lower extract concentrations of 1.5% and 3.0% w/v. *Ocimum gratissimum* plant extracts applied at 12% w/v concentration caused 94.3% and 96.4% mortality in the adult two spotted spider mites population at 48 h and 72 h exposure time respectively. This was followed by plant extracts applied at 6% w/v (81.2% and 86.6%) at 48h and 72h, respectively followed by 3% w/v (53.6% and 63.5%) and abamectin (34.4%) which was not significantly different from 1.5% w/v. Abamectin did not indicate further increase in mite mortality after 24h exposure time.

Table 2: Mortality of TSSM adults at different concentration of *Leonotis nepetifolia* and *Ocimum gratissimum* extracts with time

Treatment	Rate (% w/v)	Mortality (%)		
		24 h	48 h	72 h
L. nepetifolia	0.0	$0.0 \pm 0.0^{d*}$	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}
	1.5	43.8 ± 3.9^{bc}	60.8 ± 6.7^{bc}	72.4 ± 6.4^{bc}
	3.0	61.7 ± 4.3^{b}	47.4 ± 3.7^{b}	70.4 ± 5.8^{b}
	6.0	54.5 ± 4.4^{b}	61.7 ± 3.0^{b}	71.5 ± 5.5^{b}
	12.0	68.8 ± 3.6^{a}	93.8 ± 3.4^{a}	93.8 ± 2.7^{a}
Abamectin		43.5 ± 3.6^{c}	43.5 ± 3.6^{c}	43.5 ± 3.6^{c}
Methanol		3.3 ± 1.2^{d}	3.3 ± 1.2^{d}	3.3 ± 1.2^{d}
O. gratissimum	0.0	$0.0 \pm 0.0^{d*}$	0.0 ± 0.0^{d}	0.0 ± 0.0^{d}
	1.5	22.4 ± 1.9^{c}	51.86 ± 2.80^{c}	63.45 ± 2.34^{c}
	3.0	34.0 ± 3.79^{c}	53.64 ± 2.13^{c}	63.45 ± 3.42^{c}
	6.0	61.7 ± 3.0^{b}	81.28 ± 1.32^{b}	86.63 ± 1.53^{b}
	12.0	83.1 ± 2.6^{a}	94.65 ± 7.2^{a}	96.43 ± 9.50^{a}
Abamectin		43.5 ± 3.6^{c}	43.5 ± 3.6^{c}	43.5 ± 3.6^{c}
Methanol		3.3 ± 1.2^{d}	3.3 ± 1.2^{d}	3.3 ± 1.2^{d}

*Means within columns followed by the same letters for each treatment set are not significantly different ($P \leq 0.05$) using Tukey's HSD test.

3.3.2 Oviposition inhibition

The probit analysis showed concentration and exposure time-dependent increase in the oviposition inhibition of the mites (Figure 3 and 4). The 72 hour exposure time indicated a higher egg reduction (Figure 3) at 1.5, 3.0 and 6.0% w/v extract concentrations for *Leonotis nepetifolia*. There was however no significant difference in percent egg reductiom after 48 h exposure time at 12% w/v *Leonotis nepetifolia* plant extract concetration.The shorter exposure time of 24 h and 48 h were not significantly different in percent egg reduction at 1.5, 3.0 and 6.0% w/v *Leonotis nepetifolia* extract concentrations. On the other hand 72 h indicated higher differences in percent egg reduction at 1.5, 3.0 6.0 and 12% w/v for *Ocimum gratissimum* plant extracts concentations (Fig 4). There was however no significant difference in egg reduction between 24 h and 48 h exposure time at 1.5 and 3.0% w/v for *Ocimum gratissimum* plant extracts concentations. Generally, *Ocimum gratissimum* indicated a higher percent egg reduction compared to *Leonotis nepetifolia*.

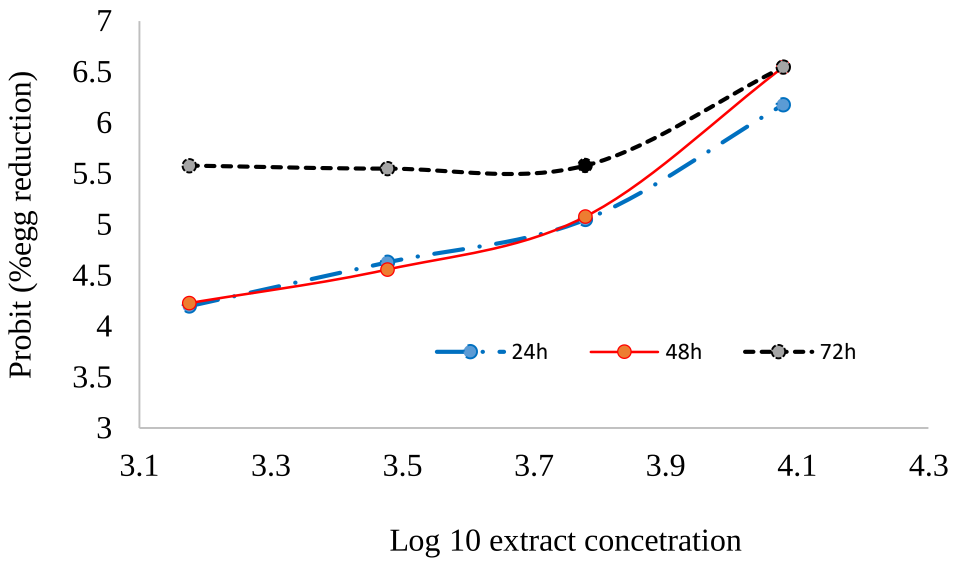

Figure 3: Probit (% egg reduction) with plant extract concentrations of *Leonotis nepetifolia* at 24 h, 48 h and 72 h

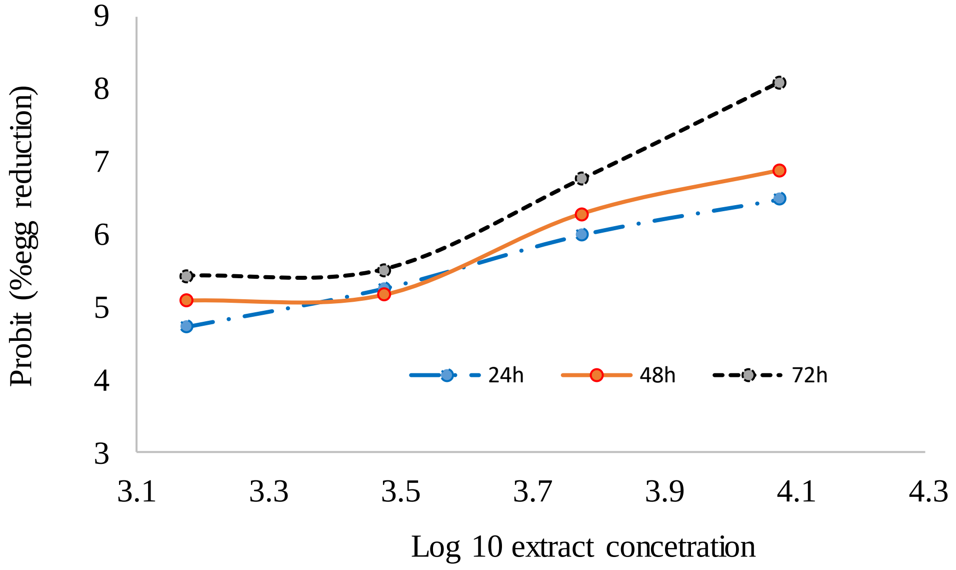

Figure 4: Probit (% egg reduction) with plant extract concentrations of *Ocimmum gratissimum* at 24 h, 48 h and 72h

The probit regression analysis on the median lethal concentrations (LC_{50}) values for *L nepetifolia* and *O. gratissimum* extracts reduced with exposure time (Table 3). *L nepetifolia* however indicated lower ovipsition inhibition LC_{50} of 4.35, 4.11 and 2.50 at 24 h, 48 h and 72 h respectively. *O. gratissimum* on the other hand indicated an LC_{50} 2.11, 1.71 and 1.20 at 24 h, 48 h and 72 h exposure time.

Table 3: LC_{50} values for *Leonotis nepetifolia* and *Ocimum gratissimum* plant extract at 24 h, 48 h and 72 h TSSM oviposition inhibition

Plant extract	**LC50**		
	24 h	**48 h**	**72 h**
L. nepetifolia	4.35	4.11	2.50
O. gratissimum	2.11	1.71	1.20

The laboratory biossays indicated that both plant extracts inhibited egg production in the two spotted spide mites. *Leonotis nepetifolia* plant extracts applied at 12% w/v concentration caused about 95% reduction in the number of eggs laid by the two spotted spider mites after 48 h exposure time (Table 4). This was followed by plant extracts applied at 6% w/v (53.5%) and abamectin (34.4%) which was not significantly different from the plant extracts applied at

3% w/v. On the other hand, *Ocimum gratissimum* plant extracts applied at 12% w/v concentration caused 97.3% and 100% reduction in the number of eggs laid by the two spotted spider mites at 48 h and 72 h exposure time respectively. This was followed by plant extracts applied at 6% w/v (90.3% and 95.8%) after 48 h and 72 h, followed by 3% w/v (56.3% and 68.8) and abamectin (34.4%). Abamectin however did not indicate further egg reduction after 24 h exposure time.

Table 4: Oviposition inhibition of TSSM adults at different concentration of *Leonotis nepetifolia* and *Ocimum gratissimum* extracts with time

Treatment	Rate (% w/v)	Mortality (%)		
		24 h	48 h	72 h
L. nepetifolia	1.5	24.31±1.0^{bc*}	21.53±1.04^{bc}	72.4 ±6.4^{b}
	3.0	31.94±1.8^{b}	33.33±1.90^{b}	71.4 ± 5.8^{b}
	6.0	52.08±3.2^{b}	53.47±3.42^{b}	71.5 ± 5.5^{b}
	12.0	87.5 ± 2.1^{a}	94.4 ± 8.9^{a}	93.8 ± 2.7^{a}
Abamectin		34.4 ± 2.8^{b}	34.4 ± 2.8^{c}	34.4 ± 2.8^{c}
Methanol		3.3 ± 1.2^{c}	3.3 ± 1.2^{c}	3.3 ± 1.2^{d}
O. gratissimum	1.5	38.89±2.29^{d}	53.47±1.57^{c}	65.97±2.32^{c}
	3.0	59.72±2.07^{c}	56.25±2.38^{c}	68.75±2.23^{c}
	6.0	84.03±1.03^{b}	90.28±6.96^{b}	95.83±5.62^{b}
	12.0	93.1 ± 7.0^{a}	97.2 ± 4.1^{a}	100.0±0.0^{a}
Abamectin		34.4 ± 2.8^{d}	34.4 ± 2.8^{d}	34.4 ± 2.8^{d}
Methanol		3.3 ± 1.2^{e}	3.3 ± 1.2^{e}	3.3 ± 1.2^{e}

*Means within columns followed by the same letters for each treatment set are not significantly different (P≤0.05) using Tukey's HSD test

3.3.3 Repellence test

The repellence results showed concentration and time-dependent increase for both *Leonotis nepetifolia* and *Ocimum gratissimum* leaf extracts (Table 5). The results showed spider mites moving towards or away from the treated or untreated side of the leaf disc. Significant observations on the repellence index of 100% and 93.8% was recorded at 72 h exposure time for *Leonotis nepetifolia* and *Ocimum gratissimum* that were applied with 12% w/v plant extracts concentration. The synthetic acaricide Abamectin showed lower significant difference on repellence index at higher exposure time compared to the lower plant extract. concentration. Lethal concentration LC_{50} decreased with exposure time by 67% and 40% for *Leonotis nepetifolia* and *Ocimum gratissimum* extracts, respectively.

Table 5: Repellence of TSSM adults at different concentration of *Leonotis nepetifolia* and *Ocimum gratissimum* extracts over time

Treatment	Rate (% w/v)	Mortality (%)		
		24 h	48 h	72 h
L. nepetifolia	1.5	24.44±3.57^{bc*}	36.67±3.82^{bc}	72.4 ±6.4^{bc}
	3.0	32.78±4.13^{b}	47.78±3.28^{b}	73.4 ± 5.8^{b}
	6.0	38.08±4.31^{b}	50.47±3.86^{b}	71.5 ± 5.5^{b}
	12.0	70.5 ± 2.1^{a}	84.4 ± 5.9^{a}	93.8 ± 2.7^{a}
Abamectin		3.3 ± 1.2^{d}	3.3 ± 1.2^{d}	3.3 ± 1.2^{d}
Methanol		34.4 ± 2.8^{c}	34.4 ± 2.8^{c}	34.4 ± 2.8^{c}
LC_{50}		6.30	3.29	2.06
O. gratissimum	1.5	31.1±3.71^{c*}	38.2±1.3^{c}	42.9±3.8^{c}
	3.0	39.5±2.1^{c}	46.3±3.4^{c}	58.8±3.5^{c}
	6.0	64.0±4.0^{b}	70.3±7.1^{b}	75.8±5.6^{b}
	12.0	83.1±4.4^{a}	97.2 ± 4.1^{a}	100.0±0.0^{a}
Abamectin		3.3 ± 1.2^{d}	3.3 ± 1.2^{d}	3.3 ± 1.2^{d}
Methanol		34.4 ± 2.8^{c}	34.4 ± 2.8^{c}	34.4 ± 2.8^{c}
LC_{50}		3.52	2.66	2.11

*Means within columns followed by the same letters in each treatment set are not significantly different ($P \leq 0.05$) using Tukey's HSD test

3.4 Discussion

The two plant leaf extracts significantly increased the mortality of spider mite compared to abamectin and the untreated controls. Similar results with ethanolic extracts of *O. gratissimum* leaves against bean weevil have been documented (Adeniyi *et al.*, 2010) whereby a high persistence and a mortality of 63.33% was reported after 24 h of exposure. The insecticidal activity in the plant extract was attributed to the presence of secondary metabolites such as saponins, flavonoids, alkaloids, phenolics and terpenes. According to probit analyses, the LC_{50} value of the plant leaf extracts in this study was 480 mg/l and 1150 mg/l for *Leonotis nepetifolia* and *Ocimum gratissimum*, respectively with a 95% confidence interval of 388.3-1200.7 mg/l. Choi *et al*. (2004) observed that *Rosemarinus officinalis* plant extracts had a moderately toxic effect on the adult females of *T. urticae.* Attia *et al.* (2011) also reported increased mortality of adult female *T. urticae* using oil concentration of *Deverra scoparia* with LD_{50} and LD_{90} values of 1.79 and 3.20 mg L^{-1}, respectively. The differential efficacy of *Leonotis nepetifolia* and *Ocimum gratissimum* extracts as indicated by the differences in LC_{50} can be partly attributed to the possible varied quantity and quality of chemical contents responsible for toxicity in insect and mite pests.

The study demonstrated a positive relationship between toxicity and plant extract concentration. The two spotted spider mite mortality was lower at 1.5% w/v plant extract concentrations but increased to more than 95% at the highest extract concentration of 12% w/v indicating a dose dependent response for both plants tested. The increased mortality with increasing plant extract concentration is consintent with the findings of Omodamiro and Jimoh (2015) who investigated the efficiency of ethanol extract of *Ocimum gratissimum* on disease causing pathogens (*Proteus mirabilis*, *Streptococcus pneumonia*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Salmonella spp.)*. They concluded that effectiveness of the plant extracts increases with increasing concentration. Erdogan *et al.* (2012) also reported that the highest mortality effect of five different plant extracts of *Allium sativum* L. *Rhododendron luteum* S., *Helichrysum arenarium* L., *Veratrum album* L. and *Tanacetum parthenium* L. against *T. urticae* larvae and adults occurred at 12% w/v while the smallest effect occurred at 1% w/v.

Similary, the two plant extracts indicated an exposure time response in their toxicity against the two spotted spider mites. The mite mortality was lower at 24 h exposure time but increased to more than 90% at 72 h indicating a time dependent response for both plants tested. The high mortality with exposure time is also consinsitent with studies by Mwandila *et al* (2013) who attributed this to the accumulation of toxic compounds in the plant extracts coupled with longer periods of exposure of the mites to lethal doses as they move and feed on the treated leaf discs.

The two spotted spider mites oviposition was also adversely affected by *Leonotis nepetifolia* and *Ocimum gratissimum* plant extracts. According to probit analysis, the LC_{50} value of the plant extracts for oviposition inhibition was 2500 mg/l and 1200 mg/l for *Leonotis nepetifolia* and *Ocimum gratissimum*, respectively with a 95 % confidence interval of 650.7-1100.2 mg/l at 72h exposure time. Asarone compound extracted from rhizomes of *Acorus calamus* was reported to possess antigonadial activity, causing complete inhibition of ovarian development of different insects. The ovicidal activity of methanolic extracts from several plants were also evaluated by (Yanar *et al*., 2011) and concluded that the leaf extracts had toxic effects on the female mites. Shi *et al.* (2006) also recorded a reduction in fecundity at concentrations of 0.064, 0.08 and 0.26 mg L^{-1} by *Kochia scoparia* extracts against *T. urticae*. In other studies, ovicidal activities of plant extracts or active compounds isolated from extracts have been reported for spider mite management. Kumral *et al*. (2009) reported significant differences between the number of eggs laid by the two spotted spider mites on unsprayed bean leaves compared to the ones that were sprayed with thorn apple (*Datura stramonium* L.) leaf and seed extracts at sub lethal doses of 2500 and 25000 mg/l concentrations respectively.

The study also demonstrated a positive relationship between oviposition inhibition and plant extract concentration. The two spotted spider mite ovioposition was lower at 1.5% w/v plant extract concentrations but increased to 100% at the highest extract concentration of 12% w/v indicating a dose dependent response for both plants tested. Miafo *et al.* (2014) reported higher doses of *O. gratissimum* extracts to be very effective anti-oviposition and ovicidal against *C. maculatus* on cowpea 24 h after treatment. The reduction of spawning activity may have partly been due to the early death of weevils due to the toxicity of the leaf extracts right after their introduction into the treatment (Koubala *et al*., 2013).

The repellence effect indicated by spider movement towards the non-treated side of the bean leaves and away from the leaves treated with higher extract concentrations further indicates the possibility of toxic compounds found in the extracts from the test plants. Studies by Manzoor *et al.* (2011) reported that higher concentrations of the plant extracts from common oleander (*Nerium indicum*) and Chinaberry (*Melia azedarach*) had repellence effect on termites (*Heterotermes indicola* Wasmann) compared to lower concentration. Results from studies by Kheradmand *et al* (2015) also demonstrated that only the two highest concentrations of the oils from *Cuminum cyminum* (Cumin), *Syzygium aromaticum* (Clove) and *Mentha spicata* (Spearmint) indicated repellency effects on *T. urticae*. Koubala *et al.* (2013) also reported higher repellence (78.33%) of *Callosobruchus maculatus* on leaves treated with *O. gratissimum* ethanolic plant extracts under laboratory conditions therefore concluding that increasing the concentration of plant products increased the repellence effects of bioactive plant products.

Results from this study indicate that *Leonotis nepetifolia* and *Ocimum gratissimum* plant extracts have acaricidal properties against the twospotted spider mite and can cause complete mortality in the laboratory at high concentrations and long exposure time. *Leonotis nepetifolia* and *Ocimum gratissimum* may therefore contain certain phytochemicals that may provide potential alternatives in the management of the two spotted spider mites in French beans.

CHAPTER FOUR

EFFECT OF LIONS EAR *(leonotis nepetifolia)* AND AFRICAN BASIL *(Ocimum gratissimum)* PLANT EXTRACTS ON TWO-SPOTTED SPIDER MITES (Tetranychus urticae Koch) POPULATION, YIELD AND QUALITY OF FRENCH BEANS

ABSTRACT

The two spotted spider mite (*Tetranychus urticae Koch*) is an important pest of French beans worldwide. Small-holder farmers who are the major French bean growers often rely on acaricides to manage this pest. Controlling mites by acarides alone pose the risk of mites becoming resistant to a wide range of pesticides. There is need for alternative pest management control measures that include the search for new chemicals of botanical origin. An experiment to evaluate the bio-control potential of *Leonotis nepetifolia* and *Ocimum gratissimum* plant extracts against the two spotted spider mites on French beans was conducted in the field. Five plant extract concentrations (1.5, 3.0, 6.0 and 12.0 % w/v) were tested with water and Abamectin 0.6 ml/L being used as controls. Mite counts were done before and after treatment application and expressed as corrected percent efficacy. The impact of the mites on the French beans was evaluated by recording percent leaf reduction. The quality and quantity was measured by number of pods, pod length, diameter and final yield. There was a dose dependent response in percent mite and leaf reduction, number of pods and yield. Treatments applied at 12% w/v indicated higher mite reduction (82.75%) for *L. nepetifolia* and 69.06% for *O. gratissimum* compared to abamectin (65.76%). The lowest percent leaf reduction of 1.71% for *L. nepetifolia*, 0.39% for *O. gratissimum* compared to abamectin which recorded a leaf reduction of 20.46% was at 12% w/v plant extract concetration. Similarly, the highest number of pods (61.00) for *L. nepetifolia*, 48.67 for *O. gratissimum* and yield (0.88kg) for *L. nepetifolia* and 0.90kg for *O. gratissimum* was recorded at 12% w/v compared to 28.33 pods and 0.36 kg for abamectin. There were no significant differences in pod diameter and pod length between the extracts concentrations and abamectin. The study demonstrated the efficacy of *L. nepetifolia* and *O. gratissimum* in managing two-spotted spider mite and subsequent increase in French bean yield under field conditions

4.1 Introduction

Crop pest management is facing economic and ecological challenges worldwide due to the human and environmental hazards caused by majority of the synthetic pesticides. Identification of novel effective insecticidal compounds is essential to combat increasing resistance rates (Calmasur *et al.*, 2006). Therefore, there is a continuous need to explore new active molecules with different mechanisms of action. Secondary metabolites present in plants apparently function as defense (toxic) substances which inhibits reproduction and other processes. The phytochemical biomolecules could be used for maximizing the effectiveness and specificity in future insecticide design with specific or multiple target sites, while ensuring the economic and ecological sustainability(Strauss and Zangerl, 2002).

The screening of plants with insecticidal activity to isolate, identify and assess the bioactivity of insecticidal compounds present against coleopteran pests of stored products has also been done (Asawalam *et al*., 2008). Plant powders from selected plant species have demonstrated high repellence against the red flour beetle, *Tribolium castaneum* (Pugazhvendan *et al*., 2009). Chikukura *et al.* (2011) reported varied repellent activityof dried leaves of *Lippia javanica* and wood of *Spirostachys africana* against stored maize and cowpea insect pests. However, the study revealed that the plant materials were effective over a short period in both maize and cowpeas. Therefore, the residual properties of the plant materials need to be tested to get the exact time over which the plant materials will be effective and this could help in avoiding unnecessary applications. Thus, plant powders may prove to be a novel biopesticide to protect crops from the damages caused by mite pests such as two-spotted spider mite.

Crude hexane extracts of wild tomato (*Lycopersicon hirsutum*) leaves have been reported to have repellence activities against the two spotted spider mites under laboratory conditions. The repellent activity is mainly attributed to presence of the chemical compound trans-caryophyllene (Antonious and Snyder, 2006). *Thuja orientalis* and *Mentha pulegium* extracts have also been reported to repel the two spotted spider mites at different concentrations (Mozaffari *et al*., 2013a; Mozaffari *et al*., 2013b). Methanolic extracts of hot pepper accessions have been documented to have a potential in repelling the mites and thus should be field-tested on large scale to assess their value in managing exploding populations of spider mites and this could reduce over reliance on synthetic acaricides (Antonious *et al*., 2006).

Marcio *et al.* (2007) reported that hexane extract from seven plant species of *Ocimum selloi* B. *Ruta graveolens* L., *Leonotis nepetaefolia* L., *Datura stramonium* L. *Cordia verbenaceae* L., *Mentha piperita* L. *Mormodica charantia* L. and *Ageratum conyzoides* L. identified three compounds; 5,6,7,8,3_, 4_, 5_-heptamethoxyflavone, 5,6,7,8,3_-pentamethoxy-4_, 5_-methylenedioxyflavone and coumarin. The compound Pentamethoxy-4_, 5_-methylenedioxyflavone did not show any insecticidal activity against the four insect species tested, 5, 6, 7, 8, 3_, 4_, 5_-Heptamethoxyflavone showed low activity against *D. hyalinata* and *R. dominica* and was not toxic to *M. domestica* or *P. americana*. In contrast, coumarin showed insecticidal activity against all four insect pest species tested, with the following order of susceptibility: *R. dominica* < *P. americana* < *D. hyalinata* <*M. domestica* after 24 h exposure.

Other studies have demonstrated the insecticidal, antifungal and antibacteria activities of *Leonotis nepetifolia* and *Ocimum gratissimum* extracts (Hortensia *et al*., 2004; Ayanwuyi *et al*., 2009; Mohamed *et al*., 2010). *L. nepetifolia* exhibits various biological activities such as antifungal, antibacterial and antioxidant activities. Dried *Ocimum gratissimum* leaves have also been reported to have insecticidal activity against bruchid (*Callosobruchus maculatus* F.) of cowpea (Brisibe *et al*., 2011). Phytochemical examination of these plant parts indicates the presence of different diterpenoids and other bioactive compounds (Imran *et al.*, 2012; Veerabadran *et al*., 2013). Leaf extract of *Ocimum gratissimum* contain potent bioactive components (essential oils) made up of eugenol and other compounds (Matasyoh *et al*., 2007), that have antioxidant and insecticidal properties (Eze *et al*., 2006; Aprioku and Obianime, 2008).

The aim of this study was to demonstrate that if well harnessed and incorporated into existing control and quarantine procedures, the plant products of *L. nepetifolia* and *O. gratissimum* are potential bio-control agents of insect pests and phytosanitary treatments. This study thus sought to determine the miticidal activity *L. nepetifolia* and *O. gratissimum* against *T. urticae* population and the resultant effect on the quality and yield of French bean under field conditions.

4.2 Materials and Methods

4.2.1 Experimental site

A field experiment to evaluate the bio-control potential of *L. nepetifolia* and *O. gratissimum* plant extracts was conducted at Horticulture Research and Teaching Farm of Egerton University for two seasons. Egerton farm lies at longitude 36°30'E, a latitude of 0°30'S, and an altitude of 2,238 metres above sea level. The experimental site receives an annual rainfall of 1013 mm and the dominant soil type is mollic andosols (Jaetzold *et al*., 2009).

4.2.2. Crop establishment and maintenance

Certified French bean seeds (variety Teresa) were planted on experimental plots measuring 2 m × 2 m with a spacing of 0.5 m between and 0.2 within the rows. The crop was raised according to recommendations for commercial French bean production. The beans were sown at a seed rate of 55 kg/Ha in single rows spaced at 30 by 15 cm, (1 seed per hole).The first hand weeding was done at 2 weeks after crop emergence. Second weeding was done at the 5th week after emergence. All plants in each plot were covered with a nylon mesh size 0.4 by 0.5 mm and thread thickness of 0.1 mm at the primary leaf stage to avoid natural mite pest infestations.

4.2.3 TSSM Introduction

Twenty adult spider mites from the greenhouse cultures were randomly introduced onto each bean plant at 21 days after planting using a fine hair brush. Six plants from the two middle rows in each plot were randomly selected and tagged for data collection.

4.2.4 Field Bioassays

Methanolic extracts of *Leonotis* and *Ocimum* were evaluated at four different concentrations (1.5 %, 3 %, 6 %, 12 % w/v) in a Randomised Complete Block Design (RCBD) replicated three times. A synthetic acaricide (Abamectin 0.6 ml/L DW) was used as positive control and the untreated where only water was applied as negative control. The plant extracts were applied as spray solutions using a hand held sprayer 14 days after mite infestation. A repeat treatment application was done 14 days after the first treatment giving two spray applications.

a) TSSM population

The population of the mites were assessed three days before treatment application by counting the number of adult mites from the underside of leaves from the six tagged plants in each plot.

A second mite population count was done at 72 h after the second treatment application. The corrected percent efficacy of the plant extracts was then calculated according to Sun-shepard formula (Puntener, 1981)

$$\% \text{ Corrected efficacy} = \frac{\% \text{ change in treated} - \%\text{change in control}}{100 - \text{change in control}}$$

Where:

% change in control = (population in control after treatment – population in control before treatment)/population in control before treatment, in percent

% change in treated = (population in treated plot before treatment – population in treated plot after treatment)/population in treated plot before treatment, in percent.

b) Percent leaf reduction

This was done by counting the number of leaves on the six tagged plants in each plot before treatment and after the second treatment application. The change in number of leaves constituted the damage by the two spotted mites which was calculated as follows,

$$\% \text{ Leaf reduction} = \frac{\text{NL untreated} - \text{NL treated}}{\text{NL in untreated}} \text{x100}$$

Where:

NL= number of leaves

c) Pod length and diameter

This was done by hand plucking all immature green pods from the two middle rows. The length and diameter of the pods were measured using a ruler and a veneer calliper respectively.

d) Number of pods and yield

The harvested pods were counted and weighed when still fresh using an electric weighing balance.

4.2.5 Data analysis

All the data collected was subjected to analysis of variance (ANOVA) ($p \leq 0.05$), and mean separations byTukeys HSD using SAS (2011) statistical program. Data on mite counts was first subjected to log data transformation to correct for heterogeneity of treatment variances before analysis.

Stastical model

$Y_{ij} = \mu + T_i + \beta_j + \epsilon_{ij}$

Where,

Y_{ij} is the random variable representing the response for treatment i observed in block h

μ is the overall the mean

T_i is the effect of treatment (plant extract concentrations) (

β_j is the effect of the block

ϵ_{ij} is the random error { $\epsilon_{ij} \sim N\ (0\sigma 2)$}

4.3 Results

4.3.1 Spider mite population

Results of the field bioassays indicated an increase in mite population expressed as corrected corrected percent efficacy in the untreated plots during the two seasons (Table 6). The plant extract and abamectin treated plots indicated varying percent efficacy levels. The highest efficacy of 82.75% for *Leonotis nepetifolia* and 69.06 % for *Ocimum gratissimum* plant extracts occurred at 12 % w/v concentration in 2014 and 78.0 % for *Leonotis nepetifolia* and 77.56 % for *Ocimum gratissimum* in 2015. The synthetic acaricide (Abamectin 0.6 ml/L) on the other hand showed a corrected percent efficacy of 65.76% and 69.05 % in the year 2014 and 2015 respectively. Abamectin however showed higher corrected percent efficacy when compared with the lower plant extract concentrations of 1.5, 3.0 and 6.0 % w/v during both growing seasons.

Table 6: Mite population reduction (corrected percent efficacy) at different *Leonotis nepetifolia* and *Ocimum gratissimum* plant extracts concentrations in season 1 and 2

Treatment	Rate (% w/v)	**Corrected efficacy (%)**	
		Seaon 1	Season 2
L. nepetifolia	0.0	-14.16±1.35e*	20.00±2.35e
	1.5	30.98±0.00d	31.63±9.12d
	3.0	62.22±7.90b	45.72±7.30c
	6.0	53.82± 5.77c	69.61±6.06b
	12.0	82.75±6.13a	78.63±10.38a
Abamectin		65.76±3.47b	69.05±9.30b
O. gratissimum	0.0	-1.42±2.37e*	-2.34±1.47f
	1.5	40.50±8.92d	44.17±6.13e
	3.0	45.19±2.00c	46.34±1.45d
	6.0	57.57± 5.75b	57.85±7.51c
	12.0	69.06±4.03a	77.56±1.85a
Abamectin		65.76±3.47b	69.05±9.29b

*Means within columns followed by the same letters in each treatment are not significantly different (P≤0.05) using Tukey's HSD test

Results indicated an increase in percent efficacy in the untreated plots during the two seasons (Table 7). The treated plots indicated varying percent efficacy levels. The plant extracts concentrations 12 % w/v, 6 % w/v and 3% w/v showed significantly lower percent leaf reduction 1.71, 4.88% and 5.92% respectively for *eonotis nepetifolia* and 0.39%, 3.5% and 19.86% for *Ocimum gratissimum* compared to abamectin (20.46%) in 2014. The trend was the same in 2015 with *Leonotis nepetifolia* showing 0.58%, 5.09% and 21.72% leaf reduction at concertation levels of 12 % w/v, 6 % w/v and 3% w/v respectively and *Ocimum gratissimum* showed 12.49%, 13.86% and 33.58%. Abamectin on the other hand showed a percent leaf reduction of 20.46 % and 23.94% in 2014 and 2015 respectively. This was however significantly higher than 1.5 % w/v plant extract concentrations of *Leonotis nepetifolia* and *Ocimum gratissimum* in both growing seasons except 2015 when abamectin was not significantly different from 1.5% w/v

Table 7: Percent French bean leaf reduction at different *Leonotis nepetifolia* and *Ocimum gratissimum* plant extracts concentrations in season 1 and season 2

Treatment	Rate (% w/v)	**Leaf reduction (%)**	
		Seaon 1	Season 2
L. nepetifolia	0.0	48.49±8.88a*	53.63±5.47a
	1.5	14.69±1.94c	30.31±6.79b
	3.0	5.92±3.71d	21.72±2.23d
	6.0	4.88± 3.36d	5.09±1.11e
	12.0	1.71±2.16e	0.58±0.19f
Abamectin		20.46±8.09b	23.94±7.56c
O. gratissimum	0.0	48.49±8.88a*	53.63±5.47a
	1.5	37.39±9.49b	22.69±5.27c
	3.0	19.86±5.51d	33.58±2.58b
	6.0	3.50± 4.89e	13.86±7.77d
	12.0	0.39±0.67e	12.49±1.02d
Abamectin		20.46±8.09c	23.94±7.56c

*Means within columns followed by the same letters for each treatment are not significantly different (P≤0.05) using Tukey's HSD test

4.3.3 Pod diameter and pod length

The Plant extracts and abamectin treated plots also recorded wider pod diameter and longer pods compared to the untreated plots (Table 8). Plant extracts applied at 12 % w/v concentration level also indicated the widest pod diameter of 0.80 cm and 0.77 cm for *Leonotis nepetifolia* and *Ocimum gratissimum* respectively. There were however no significant differences in pod diameter between 12 % w/v, 6 % w/v both plant extracts and abamectin acaricide which recorded a pod diameter of 0.70 cm in 2014. The untreated control recorded a pod diameter of 0.5 cm and 0.53 cm in 2014 and 2015 respectively. No significant differences in pod diameter was observed between the plant extracts and abamectin in 2015.

Although *Leonotis nepetifolia* plant extracts caused a significant increase in pod length compared to abamectin and the untreated control, there were no significant differences in pod length across the plant extracts applied either at low or a high concentrations in 2014 and 2015. *Ocimum gratissimum* however recorded significantly longer pods (15.001 cm and 4.77

cm) at plant extract concentration levels of 6 and 12 % w/v respectively in 2014 planting season.

Table 8: Effects of *Leonotis nepetifolia and Ocimum gratissimum* plant extracts on pod diameter and pod length

Treatment (% w/v)		**Pod diameter (cm)**		**Pod length (cm)**	
SEASON 1		*Season 1*	*Season 2*	*Season 1*	*Season 2*
L. nepetifolia	**Control**	*0.50±0.10d	0.53±0.10e	7.83±1.86c	11.33±1.3d
	1.5	0.63±0.11c	0.57±0.06d	10.83±1.0a	13.70±1.5b
	3.0	0.67±0.06bc	0.60±0.10c	11.57±0.0a	13.63±1.7b
	6.0	0.73± 0.06ab	0.60±0.00b	11.57±0.7a	14.97±1.0a
	12.0	0.80±0.10a	0.63±0.06a	11.50±0.2a	14.23±1.6a
Abamectin		0.70±0.17a	0.63±0.06a	9.27±1.44b	12.60±0.2c
O. gratissimum	**Ccontrol**	0.50±0.10d	0.53±0.06d	7.83±1.86c	0.16±0.04d
	1.5	0.66±0.10bc	0.60±0.10c	11.03±0.42a	0.37±0.03c
	3.0	0.67±0.31ab	0.60±0.00b	11.07±1.83a	12.10±1.23b
	6.0	0.67±0.12ab	0.60±0.00b	12.17±0.42a	15.00±0.53a
	12.0	0.77±0.06a	0.67±0.06a	12.60±0.17a	14.77±1.42a
Abamectin		0.70±0.17a	0.63±0.06a	9.27±1.44b	12.60±0.72b

*Means within columns followed by the same letters for each treatment are not significantly different ($P \leq 0.05$) using Tukey's HSD test

4.3.4 Number of pods and pod yield

Higher number of pods and pod yield (kg) was recorded in the plots that were treated with plant extracts compared to those treated with synthetic a.caricide (abamectin) and the untreated plots (Table 9). *Leonotis. nepetifolia* plant extracted recorded 61.00 and 48.33 pods at plant extract concentration levels of 12 % w/v and 6 % w/v respectively. This was followed by *Ocimum gratissimum* plant extract that recorded 48.67 and 35.00 pods respectively. Abamectin and the untreated plots recorded 28.33 and 12.67 pods respectively, during 2014 growing season. The trend was similar in 2015 growing season where *Leonotis nepetifolia* plant extract recorded 41.00 and 27.67 pods at concentrations levels of 12 % w/v and 6 % w/v followed by *Ocimum gratissimum* which recorded 40.67 and 31.00 pods while abamectin and the untreated recorded 18.00 and 11.33 pods.

Results also showed significant differences in pod yields at different plant extract concentrations. The highest pod yield (0.88) kg was recorded at 12 % w/v for *Leonotis nepetifolia* and 0.90 kg for *Ocimum gratissimum* in 2014 and 0.76 kg for *Leonotis nepetifolia* and 0.86 kg for *Ocimum gratissimum* in 2015 respectively. This was followed by 0.65 kg and 0.53 kg for *Leonotis nepetifolia* in 2014 and 0.35 kg and 0.37 for *Ocimum gratissimum* at 1.5 % w/v extract concentrations in 2015. This was significant higher compared to 0.36 kg and 0.25 kg for abamectin and 0.16 kg and 0.13 kg for the untreated plots in 2014 and 2015 respectively.

Table 9: Effects of *Leonotis nepetifolia* and *Ocimum gratissimum* plant extracts on number of pods and Pod yield

Treatment (% w/v)		**Number of pods**		**Pod yield (kg)**	
SEASON 1		*Season 1*	*Season 2*	*Season 1*	*Season 2*
L. nepetifolia	**control**	12.67±1.15f*	11.33±3.79e	0.16±0.04f	0.13±0.11e
	1.5	17.33±4.16e	17.00±1.00d	0.39±0.06d	0.16±0.12e
	3.0	37.00±6.08c	22.33±2.08c	0.52±0.03c	0.35±0.18b
	6.0	48.33± 4.66b	27.67±1.53b	0.65±0.02b	0.35±0.05b
	12.0	61.00±8.19a	41.00±3.11a	0.88±0.02a	0.76±0.06a
Abamectin		28.33±2.58d	18.00±5.20cd	0.36±0.02e	0.25±0.08c
O. gratissimum	**control**	12.67±1.15f	11.33±3.79d	0.16±0.04e	0.13±0.11e
	1.5	19.33±1.53e	12.33±4.73d	0.37±0.03d	0.19±0.02d
	3.0	22.33±1.15d	19.33±4.16c	0.48±0.02c	0.37±0.20b
	6.0	35.00±3.23b	31.00±1.00b	0.53±0.05b	0.37±0.13b
	12.0	48.67±5.51a	40.67±2.52a	0.90±0.03a	0.86±0.20a
Abamectin		28.33±2.58c	18.00±5.20c	0.36±0.02d	0.25±0.08c

*Means within columns followed by the same letters for each treatment are not significantly different ($P \leq 0.05$) using Tukey's HSD test

4.4 Discussion

The study examined the effect of *Leonotis nepetifolia* and *Ocimum gratissimum* extracts on the two spotted spider mite population, damage, yield and quality of French beans. Higher numbers of the two spotted spider mites occurred on the leaves of French beans planted in the untreated (control) plots. The lower numbers of the two spotted mites on the French bean leaves treated with plant extracts is a clear indication of the precense of toxic compounds in the test plants. Plants produce a wide diversity of secondary metabolites which serve them as defense compounds against herbivores (Wink, 2015). The plant extracts from the two test plants therefore demonstrated biological potency against the two spotted spider mites population. Similar data was obtained by Afify *et al.* (2009) who studied the efficiency of three essential oil extracts from Chamomile; *Chamomilla recutita* (L.), Marjoram; *Marjorana hortensis* (L.), Eucalyptus (*Eucalyptus sp*.) against the two spotted spider mites. They reported acaricidal activity of some essential oils against the mites. Calmasur *et al*, (2006) reported various toxic effects on mites by a number of other plant species belonging to the same family of the plant species that were tested in this study.

Ocimum gratissimum has been reported to contain abundant phytochemicals (Phenols) with defensive and medicinal properties (Dinakaran *et al*., 2011). Leaves, stems and roots extract of *Ocimum gratissimum* have been found to contain potent bioactive components (essential oils) made up of eugenol and other compounds such as diterpenes, coumarins, iridoids, saponins, condensed tannins, flavonoids, alkaloids and steroids that have antioxidant and insecticidal properties (Adeniyi *et al.*, 2010; Hernandez-Pinero *et al*., 2016). The current research did not however observe significant differences in the activity of *Leonotis nepetifolia* and *Ocimum gratissimum* on the two spotted spider mite population and subsequent pod yield. The results therefore suggest that there may be similar compounds with similar bioactivities in the plant extracts.

The two plant exracts tested in this study indicated a dose-dependent efficacy against the two spotted spider mites. The highest efficacy of the plant extracts in reducing the two spotted spider mites populations was demonstrated at the highest plant extract concentrations of 12% w/v followed by 6 % w/v. This is consistent with studies by (Ismail *et al.*, 2011; Narayan, 2012) who reported a concentration and exposure time-dependent increase in the efficacy of *L. nepetifolia* and *O. gratissimum* plant essential oils against diferent bacteria isolates in the laboratory.

Higher and significant percent leaf reduction observed in the untreated (control) plots was probably due to the higher population of the two spotted spider mites recorded on the leaves, and their continuous mining of the leaf tissue resulting in leaf death and consequent leaf drop. The phytophagous mite feeds on the leaves causing injuries to the epidermal layer resulting in yellow and brown blotches accompanied by wilting and eventualy, leaf fall. Severe mite-feeding results in the reduction of the quality and quantity of the crop (Gorman *et al.,* 2002; Naher *et al.*, 2006). Conversely, reduced mite populations observed on plots treated with 12% w/v plant extract concentrations corresponded to lower mite damage and lower percent leaf reduction. Reduced leaf loss meant that more leaves were retained on the plant for maximum photosynthesis and hence increased dry matter accumulation resulting in increased pod numbers and yield. A full canopy is required for French bean crop to fully intercept sunlight to produce greater photo assimilates, fill pods and maximize yield (Buntin *et al*., 2004; Leita *et al*., 2006; Abou-Khalifa *et al*., 2008). This is confirmed by the high pod numbers and pod yield observed in plots that were applied with the highest plant extract concentrations of 12% w/v. Similar result were also stated by Rahman *et al.* (2014) who reported that increased vegetation caused increased pod yield, pod weight, pod length and pod diameter in beans. The plant extracts in this study did not however strongly influence the pod diameter and length, which are important French bean quality parameters. All the treated plots recorded a pod diameter and length of about 6-9 mm and 9-12cm respectively, representing extra fine and fine pods categories (grades) of French beans (NAFIS, 2017).

Based on the results of this study, the two plant extracts of *Leonotis nepetifolia* and *Ocimum gratissimum* can be successfully used in managing the two-spotted spider mites (*Tetranichus urticae* Koch) and subsequently, increase the French bean yield under field conditions.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

i). The study demonstrated that the methanol extracts of the *Leonotis nepetifolia* and *Ocimum gratissimum* had miticidal, repellent and oviposition inhibition effects against spider mites (*Tetranychus urticae*) on French beans when applied at the higher concentrations of 12% w/v.

ii). The study also demonstrated that the methanol extracts of the *Leonotis nepetifolia* and *Ocimum gratissimum* applied at higher concentrations of 12% w/v increased the French bean pod yield but not the pod quality parameters (diameter and length). .

5.2 Recommendations

1. Famers should adopt the use of *Leonotis nepetifolia* and *Ocimum gratissimum* plant extracts in managing the two-spotted spider mites on French beans.
2. The two plant extracts from *Leonotis nepetifolia* and *Ocimum gratissimum* can be alternatively used for effective and sustainable management of the the two spotted spider mites on French beans.
3. Further research should be conducted to determine the comparable efficacy of aqueous extracts of the two test plants against the two spotted spider mites to reduce the cost of extraction and allow ease of adoption by the farmers.

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APPENDICES

Appendix 1: Publications from Thesis

A1.1: Paper 1:

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BIOACTIVITY OF *LEONOTIS NEPETIFOLIA* AND *OCIMUM GRATISSIMUM* EXTRACTS IN MANAGEMENT OF *TETRANYCHUS URTICAE* KOCH ON FRENCH BEANS

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ABSTRACT
Methanol extracts of Lion's ear (*Leonotis nepetifolia* L.) (LN) and African basil (*Ocimum gratissimum* L.) (OG) were evaluated in the laboratory at Egerton University for lethal concentrations (LC_{50}), toxic, oviposition inhibition and repellent effects against the adult female two-spotted spider mite, *Tetranychus urticae* Koch. Laboratory bioassays were conducted in a completely randomized design replicated four times using five concentrations (0.0, 1.5, 3.0, 6.0 and 12.0 % w/v) with methanol and Abamectin (0.6 ml/L) as negative and positive controls, respectively. Data on mite mortality, oviposition inhibition and repellence indices were recorded 24, 48 and 72 h after treatment and subjected to analysis of variance and means separated using Tukey's HSD test ($P< 0.05$). The results showed concentration- and exposure time-dependent increase in efficacy of both extracts against the mites. At 12.0% and 72 h, LN and OG produced 93.8 and 96.4% mortality of mites, respectively. Similarly, both extracts produced 100% oviposition inhibition. The extracts also significantly repelled the mites over the exposure periods. Synthetic acaricide, abamectin produced 43.5 and 34.4 % mortality and oviposition inhibition, respectively, compared to the extracts. According to LC_{50} values, LN had the highest percent mortality whereas OG showed highest inhibition and repellence at 24, 48, and 72h after treatment.These findings demonstrate the potential use of LN and OG extracts for the management of two-spotted spider mite on French beans.

KEY WORDS: Toxicity, Oviposition, mortality, French beans, Repellence.

INTRODUCTION

Two-spotted spider mite (TSSM), *Tetranychus urticae* Koch, is one of the major pests that have contributed to the limitation in French beans production[1]. It is not only a major pest causing reduction in French bean yields, but has also been listed as a quarantine pest and hence a leading cause of Kenya's fresh produce rejections in European markets[2]. It attacks over 300 host plant species including vegetables, fruits and ornamental plants[3]. The direct damage includes defoliation, leaf burning and even plant death. Indirect effects of feeding by this spider mite include reduced photosynthesis, transpiration which can lead to yellow to white discoloration of leaves(bronzing) causing loss of yield and quality of host plants[4,5]. Most smallholder farmers rely on synthetic pesticides (acaricides) to manage this pest. However, the available synthetic acaricides used have caused serious problems such as pesticide resistance[5], environmental contamination, unacceptable pesticide residues in food and lethal effects on non-target organisms[6]. Chemical pesticides remain suspended in the atmosphere, on the ground, in waterways and affect humans and beneficial organisms such as predatory mites[7]. These negative effects have resulted in an increasing interest for natural plant-based pesticides which are safer, easily available and easily biodegradable and with no resistance than synthetic chemicals[8]. Studies have also pointed to numerous plant species possessing potential pest controlling properties. These plant species have been found to contain natural deterrents to help them remain healthy. These deterrents are toxic to various insect and mite pests[9]. The extracts of *Satoreja hortensis* L. (Lamiaceae) have been reported to be toxic to TSSM [10]. Similarly the extracts from neem (Meliaceae), some species of solanaceae, *Capparis aegyptia* (Capparaceae), *Nerium orleander* L. (Apocynaceae) and *Aliantkus altissima* L. (Simaroubaceae) have been found to be effective against TSSM[11,15]. The antifungal, antibacterial and antioxidant activities of *Leonotis nepetifolia* L. and *Ocimum gratissimum* L. have also been confirmed in human and animal health[14]. However, limited information exists for usage as crop protectants, hence the objectives of the study was to determine their toxic, oviposition inhibition and repellent effects against adult female *Tetranychus urticae* Koch under laboratory conditions and possibility of using the extracts to manage the pest in the field.

MATERIALS & METHODS

Preparation of test plant materials

The studies were conducted at Biotechnology laboratory, Egerton University. Test plants, Lion's ear (*Leonotis nepetifolia* L.) and African basil (*Ocimum gratissimum* L.) were collected from the wild in Bahati district, Kenya in 2013. Composite of fresh leaves and tender stems were

A1.2: Paper 2:

The Effect of Lion's Ear (*Leonotis nepetifolia*) and African Basil (*Ocimum gratissimum*) Plant Extracts on Two-Spotted Spider Mites (*Tetranychus urticae*) for Improved Yield and Quality of French Beans

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Abstract

An experiment to evaluate the bio-control potential of *Leonotis nepetifolia* and *Ocimum gratissimum* plant extracts against two-spotted spider mites on French beans was conducted in the field. Five plant extract concentrations (1.5%, 3.0%, 6.0% and 12.0% w/v) were applied with water and Abamectin 0.6 ml/L as controls. Mite counts were done before and after treatment application and expressed as corrected percent efficacy. The impact of the mites on the French beans was evaluated by recording percent leaf reduction and quality and quantity by number of pods, pod length, diameter and yield. There was a dose dependent response in percent mite and leaf reduction, number of pods and yield. Treatments applied at 12% w/v indicated higher mite reduction (82.75%) for *L. nepetifolia* and 69.06% for *O. gratissimum* compared to abamectin (65.76%). The lowest percent leaf reduction of 1.71% for *L. nepetifolia* 0.39% for *O. gratissimum* and abamectin (20.46%) was also at 12% w/v. Similarly, the highest number of pod (61.00) for *L. nepetifolia*, 48.67 for *O. gratissimum* compared to 28.33 abamectin and yield (0.88 kg) for *L. nepetifolia* and 0.90 kg for *O. gratissimum* was also recorded at 12% w/v compared to 0.36 kg for abamectin. There were no significant differences in pod diameter and pod length between the extracts concentrations and abamectin. The study demonstrated the efficacy of *L. nepetifolia* and *O. gratissimum* in managing two-spotted spider mite and subsequent increase in French bean yield under field conditions.

Keywords

Leonotis nepetifolia, *Ocimum gratissimum*, *Tetranychus urticae*, French

Appendix 2: Anovas

A2.1 Mortality of TSSM adults at different concentration of *Leonotis nepetifolia* and *Ocimum gratissimum* extracts with time

Source	DF	Type III SS	Mean square	F value	Pr>F
Total	431	834829.4596			
Botan	1	5208.33	5208.33	5.70	0.0175
Trial	2	41883.0310	20941.5155	22.91	<.0001
Time	2	50139.8337	20941.5155	27.43	<.0001
Rep	3	4052.8156	1350.9385	1.48	0.2201
Rate	5	314628.2124	62925.6425	68.84	<.0001
Botan*Time*Rate	27	61502.7400	2277.8793	2.49	<.0001
Error	391	357414.4936	914.1036		
Coefficient of variation =75.75					

A2.2 Oviposition inhibition of TSSM adults at different concentration of *Leonotis nepetifolia* and *Ocimum gratissimum* extracts with time

Source	DF	Type III SS	Mean square	F value	Pr>F
Total	431	739392.3611			
Botan	1	74769.1615	74769.1615	156.97	<.0001
Trial	2	9642.1682	4821.0841	10.12	<.0001
Time	2	23622.6852	11811.3426	24.80	<.0001
Rep	3	5623.0710	1874.3570	3.94	0.0087
Rate	5	413827.1605	82765.4321	173.76	<.0001
Botan*Time*Rate	27	25668.7243	950.6935	2.00	0.0026
Error	391	186239.3904	476.3156		
Coefficient of variation =42.75					

A2.3 Repellence of TSSM adults at different concentration of *Leonotis nepetifolia* and *Ocimum gratissimum* extracts with time

Source	DF	Type III SS	Mean square	F value	Pr>F
Total	431	652981.2500			
Botan	1	752.0833	752.0833	0.98	0.3221
Trial	2	6893.0556	3446.5278	4.50	0.0116
Time	2	11093.0556	5546.5278	7.25	0.0008
Rep	3	311038.1944	62207.6389	81.30	<.0001
Rate	5	993.2870	331.0957	0.43	0.7297
Botan*Time*Rate	27	15381.7130	904.8066	1.18	0.2753
Error	391	306829.8611	765.1617		
Coefficient of variation =72.55					

Appendix 3: Probit Regression Analysis

A3.1: Probit regression Analysis for LC50 on the effect of *Leonotis nepetifolia* on TSSM mortality of TSSM at 72h exposure time

SUMMARY OUTPUT								
Regression Statistics								
Multiple R	0.804581							
R Square	0.647351							
Adjusted R Square	0.471026							
Standard Error	0.360604							
Observations	4							
ANOVA								
	df	*SS*	*MS*	*F*	*Significance F*			
Regression	1	0.477405	0.477405	3.671358	0.195419			
Residual	2	0.26007	0.130035					
Total	3	0.737475						
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*

Intercept	2.083819	1.951732	1.067677	0.397469	-6.31381	10.48144	-6.31381	10.48144
X Variable 1	1.026476	0.535717	1.916079	0.195419	-1.27853	3.33148	-1.27853	3.33148

A3.2 Probit regression Analysis for LC50 on the effect *Ocimum gratissimum* TSSM mortality at 72h exposure time

SUMMARY OUTPUT								
Regression Statistics								
Multiple R	0.948058							
R Square	0.898813							
Adjusted R Square	0.84822							
Standard Error	0.26844							
Observations	4							
ANOVA								
	df	*SS*	*MS*	*F*	*Significance F*			
Regression	1	1.28018	1.28018	17.76547	0.051942			
Residual	2	0.14412	0.07206					
Total	3	1.4243						
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	-0.21268	1.452905	-0.14638	0.897043	-6.46402	6.038668	-6.46402	6.038668
X Variable 1	1.680896	0.398797	4.214911	0.051942	-0.03499	3.396782	-0.03499	3.396782

A3.3 Probit regression Analysis for LC50 on the effect of *Leonotis nepetifolia* on TSSM ovipsition inhibition of TSSM at 72h exposure time

SUMMARY OUTPUT								
Regression Statistics								
Multiple R	0.774274							
R Square	0.599501							
Adjusted R	0.399251							

Square								
Standard Error	0.379947							
Observations	4							
ANOVA								
	df	*SS*	*MS*	*F*	*Significance F*			
Regression	1	0.43218	0.43218	2.993766	0.225726			
Residual	2	0.28872	0.14436					
Total	3	0.7209						
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*
Intercept	2.27208	2.056428	1.104867	0.384351	-6.57602	11.12018	-6.57602	11.12018
X Variable 1	0.976647	0.564454	1.73025	0.225726	-1.452	3.405297	-1.452	3.405297

A3.4 Probit regression Analysis for LC50 on the effect *Ocimum gratissimum* TSSM oviposition inhibition at 72h exposure time

SUMMARY OUTPUT								
Regression Statistics								
Multiple R	0.951825							
R Square	0.90597							
Adjusted R Square	0.858956							
Standard Error	0.473218							
Observations	4							
ANOVA								
	df	*SS*	*MS*	*F*	*Significance F*			
Regression	1	4.315205	4.315205	19.2699	0.048175			
Residual	2	0.44787	0.223935					
Total	3	4.763075						
	Coefficients	*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*	*Lower 95.0%*	*Upper 95.0%*

Intercept	-4.75764	2.561245	-1.85755	0.204349	-15.7778	6.262503	-15.7778	6.262503
X Variable 1	3.086071	0.703018	4.389749	0.048175	0.06123	6.110912	0.06123	6.110912