EFFICACY OF Tagetes *minuta* L AND Tephrosia *vogelii* Hook CRUDE LEAF EXTRACTS ON Tetranychus *urticae* Koch AND Aphis *fabae* Scopoli

BY

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DECLARATION

Declaration by the candidate

This thesis is my original work and has not been presented for a degree in any other university. No part of this thesis may be reproduced without the prior permission of the author and/ or University of Eldoret.

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DEDICATION

I dedicate this work to my loving husband, Ani Sabwa, daughter, Shmneh Aima and son Israel.

ABSTRACT

Red spider mites and aphids are examples of agricultural pests which are very problematic to many farmers in Kenya and further Sub- Saharan Africa. Efforts research wise are focused on the bioactivity, application methods, cost-effectiveness and sustainable use of botanical pesticides against insect pests. The insecticidal and repellent properties of *Tagetes minuta* L and T*ephrosia vogelii* Hook collected from KARI, Kitale botanical garden were evaluated for their efficacy and repellence against red spider mite *Tetranychus urticae* Koch and aphids *Aphis fabae* Scopoli of broad beans (*Vicia faba*) in the laboratory and in the field. Mortality potential of the two plants was determined by spraying the crude extracts on the insects. *Tagetes minuta* crude extracts at concentrations of 50 g/L, 100 g/L, 150 g/L soaked for 24 hours and also 150 g/L soaked 48 hours; *Tephrosia vogelii* crude leaf extract of 10 g/L, 30 g/L and 60 g/l soaked 12 hours and also 60 g/l soaked 48 hours in distilled water, synthetic insecticides (Alpha cymba 10 EC for aphids and Disect for mites) were used to investigate treatment efficacy on mortality of the adult insects. *Tetranychus urticae* and *Aphis fabae* were artificially infested in the screen house on potted bean plants placed on a bench in a screen house at KARI Kitale. Treatment using *T.minuta* at concentration 150 g/L soaked for 48 hours caused a percent mortality of 90% in aphids and 69% in mites, while same concentration at 24 hour soaking caused percent mortality of 84% in aphids and 35% in mites. *T. vogelii* at concentration 60 g/l soaked 48 hours in water caused percent mortality of 98% in aphids and 92% in mites at the end of 24 hour exposure to the herb. The same concentration at 12 hour soaking caused percent mortality of 94% in aphids and 60% in mites. Repellency tests were performed using filter- paper circles on mites and aphids. The extracts of *T.minuta* and *T. vogelii* were applied around the periphery of the filter papers. Percentage repellency (PR) was determined for each extract. *T.minuta* at concentration 150 g/L soaked 48 hours had the strongest PR on mites at 55% followed by that soaked 24 hours PR at 12%. *T. vogelii* at 60 g/L soaked 48 hours had a moderate repellent effect on mites at PR 17% while same concentration after 12 hours of soaking had PR of 8% on mites. However aphids were not repelled by any of the extracts used. The two botanicals are promising insecticides which can be used by subsistence farmers as alternatives to synthetic pesticides.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ACRONYMS AND ABBREVIATIONS

KARI………………………………Kenya Agricultural Research Institute

DAP………………………………..Di-ammonium Phosphate

BBLRV…………………………….Broad bean leaf roll virus

BYMV…………………………..….Bean yellow mosaic virus

USD………………………………...United States of America Dollar

EC……………………………..….Emulsifiable concentrate

PR…………………………..…….Percentage Repellency

g/l.…………………………………gram per liter

^{O}N……………………………..….Degrees North

^{O}E……………………………..….Degrees East

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CHAPTER ONE

INTRODUCTION

Red spider mites and aphids are some of the agricultural pests which are very problematic to many farmers in Kenya and further Sub- Saharan Africa. In recent years, research has focused on the bioactivity, application methods, cost-effectiveness and sustainable use of botanical pesticides against insect pests (Ogendo *et al.,* 2003; Yallapa *et al.,* 2012). The prohibitive costs of commercial synthetics, the increasing development of insect resistance to pesticides, toxicity concerns, the frequent erratic supply of pesticides (Ogendo *et al.,* 2003), the destruction of beneficial insects, pesticide residue magnification in humans and wildlife and disruption of ecosystem (Georghiou, 1986; Clergy and Mackean, 1994, Ruchika and Kumar, 2012), have increased the need to search for alternative insect control methods.

Today there is an ever-growing awareness of the importance for safer and more rapidly biodegradable pesticides, such as plant derived natural pesticides. These higher plants are a rich source of novel natural substances that can be used to develop environmental safe methods of insect control (Arnason *et al.,* 1989). These plants produce chemicals that function as defense mechanisms to reduce feeding injury by phytophagous organisms. Insecticidal activity of many plants against several insect pests has been demonstrated (Jilani and Su, 1983; Carlini and Grossi-de-sa, 2002). Botanical insecticides have been reported to have a wide range of biological activities against insects. These include repellence and anti-feedant activities (Viglianco *et al.,* 2005), oviposition deterrence, toxicity, sterility, growth regulatory and fecundity reduction, molting and respiration inhibition, and cuticle disruption (Tinzaara *et al.,* 2006). This combination of multiple modes of action is advantageous because it delays the development of resistance among arthropod pest populations. Two other advantages are their low level of environmental pollution and low toxicity to humans.

Today, researchers are seeking new classes of naturally occurring insecticides that might be compatible with newer pest control approaches (Rajashekar *et al.,* 2012). Among the plant species with potential to be used as natural pesticides are Mexican marigold (*Tagetes minuta* L) and Fish poison bean (*Tephrosia vogelii* Hook) that have been introduced into many different parts of the world.

With increasing public concern over the use of synthetic pesticides and their negative effects such as fears relating to build-up of chemical residues and also effects on non-target species and the environment, devising new methods of pest control is therefore a prerequisite to prudent pest control. This involves moving away from the reliance on broad spectrum synthetic pesticides while encouraging the use of botanical plants to help control pest species populations. This awareness has created a world-wide interest in the development of alternative strategies, including the discovery of newer insecticides (Rajashekar *et al.,* 2012; Jan, and Juraj, 2013). This will reduce the build-up of pesticide resistance within pest species and also reduce the amount of chemical pesticides required for their control, therefore helping to form a more environmentally sustainable ecosystem.

The present study therefore investigated the efficacy of the two plant extracts and involved the crude leaf extracts on red spider mites (*Tetranychus urticae* Koch) and Black bean aphids (*Aphis fabae* Scopoli) that affect many agricultural crops in the entire world.

1.1 Problem statement

Presently, there are major concerns about the use of synthetic pesticides and their increasing resistant on pests and their cost which is very expensive especially to the resource poor traditional farmers. There is therefore the need to find newer pesticides to be used in order to increase the yield of crops and hence improve on the food security in the country. Botanical plant pesticides therefore can be used as one of the alternatives to synthetic pesticides to achieve better yields of crops. The use of botanicals to control pests remains rather under-utilized in Kenya by agricultural communities. This may be related to the fact that there is limited research on the correct concentration of botanical plants extracts that can be used against pests that affect many agricultural crops. The use of botanical plants extracts will reduce the build-up of pesticide resistance within pest species and therefore this will help to form a friendlier environment since the pollution from synthetic pesticides will be reduced.

1.2 Significance and justification for the study

Red spider mites and black bean aphids are commonly controlled using synthetic pesticides, which are expensive, not easily accessible and unaffordable to the resource-limited farmers. Mites attack crops such as tomatoes, African night shade, amaranth, eggplant, tomatoes, beans, coffee, water melon, papaya, peas, and passion fruit. Black bean aphids attack crops such as; beans, spinach, potatoes, sunflower, carrots, tomatoes and cowpeas. Commercial growers of crops use synthetic organic pesticides to control agricultural pests but small scale growers and subsistence farmers, faced with limited access to financial resources cannot afford them. Due to the continuous use of synthetic pesticides, the pests have developed resistance, pesticides and their degradation products dissipate into other environmental compartments including ground water, surface water, and the atmosphere. The use of pesticides also kills both the target and non-target organisms, hence driving the need for the farmers to devise new methods of pest control.

Because of these problems, use of botanicals as one of the alternatives being sought for in insect pest control. This involves moving away from the reliance on synthetic pesticides and encouraging the use of botanical plants to help control pest species populations. This will reduce the build-up of pesticide resistance within pest species and also reduce the amount of chemical pesticides required for their control, therefore helping to form a more environmentally sustainable ecosystem. Unfortunately not much has been documented on these practices. This necessitated the documentation and validation of the potential pesticides properties of these botanicals. This will assist to reserve the local knowledge in pest control for future generations and other communities in the region.

1.3 Objectives of the study

1.3.1 Overall objective

The overall objective of the study was to determine the effect of different concentrations of Mexican marigold (*Tagetes minuta* L) and Fish poison bean (*Tephrosia Vogelii* Hook) on the red spider mites (*Tetranychus urticae* Koch) and black bean aphids (*Aphis fabae* Scopoli)

1.3.2 Specific objectives

The specific objectives were:

- To determine the efficacy of different concentration of Mexican marigold and fish poison bean crude leaf extracts on red spider mites and black bean aphid in broad beans.
- To investigate the repellence properties of Mexican marigold and fish poison bean on red spider mites and black bean aphids.

1.4 Research hypothesis

Ho: Different concentrations of crude extracts of Mexican marigold have no significant effect on red spider mite and black bean aphid populations

Ho: Different concentrations of the crude extracts of Fish poison bean have no significant effect on red spider mite and black bean aphid numbers.

CHAPTER TWO

LITERATURE REVIEW

Pesticide use in Kenya is one of the highest in sub-Saharan Africa with a market share of approximately USD 40.4 million by 1992 (Odipo *et al.,* 2003). After farm application, pesticides and their degradation products dissipate into other environmental compartments including ground water, surface water, and the atmosphere. These chemicals are also environmentally unfriendly and thus the environment has been continually polluted (Reddy *et al.,* 2011).

The annual amount of synthetic pesticides applied worldwide is reported at 5,000,000 metric tons. The yearly consumption of pesticides in developing countries was estimated at 600,000 metric tonnes in 1988 with a drastic increase of 184 % during 1980. However, besides the cost factor, synthetic organic chemicals have proved not to be the absolute solution to pest problems (Berger, 1994, Schwab *et al.,* 1995). They agreeably have a high knock down effect on pest organisms but concern about the long term consequences of using synthetic pesticides has arisen for several ecological reasons. These include; destruction of predators that control pests or pollinators of fruit trees, resistance development and pesticide residue bio magnifications in humans and wildlife, (Georghiou, 1986, Clegg and Mackean, 1994).

Epidemiological data shows that workers who handle synthetic organic pesticides more than twenty days a year have an increased risk of developing certain types of cancer, (Katsvanga and Chigwaza, 2004). Significance inhibition of plasma and red blood cells cholinesterase activity has also been recorded in workers exposed to an average of 0.7

mg/m^3 of chemical pesticides (Menz *et al.,* 1974). Hence the increasing discovery and use of plant based pesticides by farmers which are friendlier to the environment and humans.

There are different types of pests that attack plants, causing a lot of damage and hence heavy losses to farmers. An example of such pests is red spider mites, *T. urticae* and black bean aphid, *A. fabae* that attack many agricultural plants. The red spider mites and black bean aphids are some of the serious agricultural pests in the tropics. Although these pests can be controlled using synthetic organic pesticides, the majority of farmers are resource- poor, have neither the means nor the skills to obtain and handle pesticides appropriately.

Pesticidal control of mites is not only expensive, but also when used indiscriminately and frequently the use causes several hazards (Taleb and Sardar, 2007). Furthermore, frequent use of synthetic pesticides from the same group has resulted in the development of strains of two spotted mites that are highly resistant to almost all classes of pesticides (Yong-Heon, 2001). Thus the use of plant extracts to control these pests may prove to be effective and therefore cheaper for farmers in the long run.

2.1 Mexican marigold (*Tagetes minuta* L)

In recent years, many species and chemo types of *Tagetes* have been re-evaluated as sources of different classes of biologically active secondary metabolites that can be used in industry and medicine. *Tagetes* is a genus of 56 species (Soule, 1996) of annual and perennial mostly herbaceous plants in the sunflower family (*Asteraceae or compositae*). *T.minuta* is a weed which has been reported to contain the chemicals 5-(3-buten-1-ynyl)

2, 2-bithienyl and alpha therthienyl which have pesticidal properties (Morallo-Rejesus and Decena, 1982). Alpha terthienyl, for example in its synthetic form has been reported to be the active component in marigold (Kanagy and Kaya; 1996, Olabayi and Oyedunmade, 2007).

Marigolds have several compounds in their tissue which have biological activity against a range of organisms (Vasudevan *et al*., 1997). One such compound, thiophene, demonstrates antiviral, antibacterial, antifungal, nematicidal, and insecticidal properties (Marles *et al*., 1992, Margl *et al*., 2001, Riga *et al*., 2005). The thiophene concentration in the marigold plants varies according to the marigold species, the stage of the plant development, and the vegetative organ (Margl *et al*., 2001; Gil *et al*., 2002). For example, Jacob *et al*., (1994) reported higher thiophene concentrations in *Tagetes patula* than in *Tagetes erecta*. In addition, marigold roots contain the highest diversity and contents of thiophene (Tosi *et al.,* 1991) and their concentration levels increase as the plant gets older, reaching a maximum during the reproductive stages (Gil *et al*., 2002). Marigolds have the potential to control a range of plant pests including insects and plant parasitic nematodes. Marigolds can be used safely by home gardeners and growers wishing to avoid the use of synthetic pesticides, which can be especially important if the growers live on the farm or farm close to communities.

2.1.1 Description of *T.minuta* L

T. minuta L is an annual herb that belongs to the Asteraceae family. It consists of 30 species (Loockerman *et al.,* 2003). It is commonly known as Khaki weed/ Mexican marigold. It has a very strong and sharp smell. The plant is an erect herb, 1-2m tall with yellow-orange ray florets and 10 to 15 yellow-orange disk florets per capitula. Leaves are slightly glossy green and pinnately dissected into 4 to 6 pairs of pinnae. Leaf margins are finely serrate (Plate 2.1). The undersurface of the leaves bear a number of small, punctuate, multicellular glands, orange in colour, which exudates a licorice-like aroma when ruptured (Rao *et al*., 1999). *T. minuta* possesses insecticidal activities (Tomova *et al*., 2005) and is at times referred to as the African marigold.

Plate 2.1: Mexican marigold (*Tagetes minuta* L) plant. (Source: Author, 2011)

2.1.2 Origin and geographic distribution

T. minuta L, African marigold, is native to the temperate grasslands and mountain regions of South America (McVaugh, 1943; Soule, 1993), and most probably naturalized in the rest of Central America and the western Andes of South America. It became naturalized also elsewhere in the tropics and is widely cultivated all over the world (including Africa and the Indian Ocean islands) as a popular garden ornamental (Chiu and Chi-Hsiung, 2005). It is cultivated commercially for its dye mainly in Latin America. In Kenya it is widely distributed in Uasin Gishu, Thika, and Taita Taveta (Odipo *et al.,* 2003).

2.2 Fish poison bean (Tephrosia Vogelii Hook)

The plant genus Tephrosia is a legume with about 300 species found in the tropical and sub-tropical regions of the world, some of which have been used for many beneficial purposes (Barnes and Freyre 1967, Gaskins *et al.*, 1972). *Tephrosia* is well known as a rich source of flavenoids, comprising the isoflavonoid rotenone among their secondary metabolites (Beal and Anderson, 1993; Andel, 2000). *T. Vogelii* is a tall, robust legume found in many areas of tropical Africa up to an elevation of approximately 2,000 meters. The plant may attain a height of 2 to 3 meters in a growing season of 5 to 7 months but seedling development is slow. Flower color may be purple, red, or white. The compound leaves contain 7 to 12 pairs of leaflets.

It is often grown in villages and sometimes in forest areas as a fish poison. The white flowered form predominates in East Africa, but the purple-flowered form is more abundant in West Africa. The plant is used as a green-manure crop, and its use for windbreaks and as an ornamental has been reported (Ogendo *et al.*, 2003). Extracts have been used for wound dressings and other medicinal purposes (Dzenda *et al*., 2007), for arrow poisons, and for insect and fish poisons (Ekanem *et al*., 2004). It has a pungent odor which is due to a volatile oil known as Tephrosal (Dalziel, 1937) and contains an active substance known as Tephrosin that is a crystline substance only slightly soluble in water (Ekanem *et al*., 2004).

The plant also contains a number of different compounds commonly known as rotenoids, which are present in its leaves, and it is the chemical responsible for the pesticidal property of *T. vogelii* (Andel, 2000). The chemical has an acute oral toxicity of 132-1500 mg/kg, which is capable of killing fish and vegetable pests. *T. vogelii* can also be used for the protection of stored grain against pests since rotenone breaks down within 3-5 days after application thereby leaving no residues unlike synthetic pesticides (Blommaert, 1950, Barnes and Freyre, 1967, Gaskins *et al.,* 1972). Other than rotenone, other compounds found in *T. vogelii* include: deguelin, and tephrosin. Rotenone is selective non-systemic insecticide, containing some acaricidal properties. Although rotenone is highly toxic to numerous insects, it is of low toxic to most mammals (Jones, 1931, Fukami *et al.,* 1970). In addition to insecticidal compounds, the leaves of *T.vogelii* also contain 5-methoxyisolonchocarpin, a highly effective antifeedant (Shimmond *et al.,* 1990).

2.2.1 Description of *Tephrosia vogelii* Hook plant

Tephrosia vogelii Hook plant is a leguminous plant. It is a much branched shrub reaching up to 4 m high. It is always under cultivation, and ubiquitous in tropical Africa and India (Burkill, 1995). The plant is easy to propagate by seed, seeding at 6-7 months, but taking about 3 years to reach maturity (Chadha, 1976, Burkill, 1995). The stems are more or less erect and the leaflets are five or more pairs.

According to Gaskins *et al.,* (1972), the flower of *T. vogelii* is typically papilionaceous, about 2.5 cm across and purple with white markings or white. The flowers are 2 cm or more and are densely crowded, borne on compact racemes that bloom over a 3 to 6 week period. There may be 20 to 30 flowers per raceme with up to 200 flowers per plant (Plate 2.2). Fruits of Tephrosia vogelii are large and 2-12 cm long, very densely villous or tomentose (Hutchinson and Dalziel, 1958). Pods usually contain 8 to 16 seeds. Seeds are dark brown to black with a distinctive indurate white funiculum that is open along the lotoid groove in the hilum. The flowers have a faint but definite pleasant aroma, and bees visit them freely for both nectar and pollen. Flowering occurs on decreasing day lengths

Plate 2.2: Fish poison bean (*Tephrosia vogelii* Hook*)* plant. (Source: Author, 2011)

2.2.2 Origin and Geographical distribution

Fish poison bean **(***Tephrosia vogelii* Hook*)* plant is a native to West Africa, including Nigeria, and other regions of Tropical Africa in general, but is now found in India, Asia and other tropical regions (Dalziel, 1937; Lambert *et al.,* 1993). According to (Burkill, 1995), the exact origin of the plant is uncertain. It is cultivated throughout tropical Africa, particularly in West Africa and chiefly in the forest regions, but also in the Savannah Zones (Lambert *et al*., 1993). Tephrosia is cultivated around many villages either casually or by riverine people in fields for use in stupefying fish (Dalziel, 1937; Lambert *et al*., 1993; and Bajaj, 1998).

According to Martin and Cabanillas (1970), the plant requires a tropical home for seed production.

2.3 The Red spider mite (*Tetranychus urticae* Koch)

The red spider mite (Plate 2.3), is an important agricultural pest with a global distribution. Its phytophagous nature, high reproductive potential and short life cycle facilitate rapid resistance development to many acaricides often after a few applications (Devine *et al.,* 2001; Stumpf and Nauen, 2001). They have evolved resistance to almost eighty acaricides to date, for example, Carbaryl, some Organophosphates and some pyrethroids and resistance has been reported from about sixty countries (Saber and Murray, 2006). The mites inflict physical damage as they feed on the cell sap, punching leaf surfaces and leaving tissue blemishes, first as yellow spotting, later bronzing, desiccation and finally defoliation (Anyango and Nakhumicha, 2003).

Plate 2.3: Red spider mite (*Tetranychus urticae* Koch). (Source: Author, 2012)

2.3.1 Biology of the red spider mites

Tetranychus urticae (Family: Tetranychidae*)* is one of many species of plant-feeding mites found in dry environments, and generally considered as a pest. It is extremely polyphagous, it can feed on hundreds of plants, including most vegetables, fruit crops and ornamentals for instance roses. *T. urticae* is mostly found in glass house, on house plants and can also be found on outdoor plants. When active, the red spider mites are pale green-brown in color and can be recognized by the two dark dots on their backs. They often live underneath leaves, and are only active when it is warm. During cold season, they hibernate either as eggs or inactive newborns which can be spotted as tiny red or orange shiny dots. *T. urticae* is extremely small, barely visible with the naked eye as reddish or greenish spots on leaves and stems, the adults' measure about 0.5 mm. They are always seen spinning a fine web on and under leaves. The two spotted spider mites lays its eggs on the leaves, and it poses a threat to host plants by sucking cell contents from the leaves cell by cell, leaving very tiny, pale spots or scars where the green epidermal cells have been destroyed. Although the individual lesions are very small, commensurate with the small size of the mites, the frequently observed attack of hundreds or thousands of spider mites can cause thousands of lesions and thus can significantly reduce the photosynthetic capability of plants, greatly reducing their production of nutrients, sometimes even killing the plants. Adult females of red spider mites are about 0.4 mm long, body oval, strongly convex and dark red with long setae arising from light-colored pinacula (Afford, 1984). They have eight legs and are tiny resembling tiny moving dots to the naked eye. The male is similar to the female only smaller but have a more pointed abdomen. The female mite deposits round orange or red eggs of about 0.14 mm long. The eggs are tiny, spherical, pale-white, and are laid on the underside of leaves often under the webbings. They can only be seen with a magnifying lens. Eggs hatch in 4 or 5 days into six legged larvae with an average length of 0.17 mm (Blair and Groves, 1952). On hatching, juvenile mites move to the underside of the leaves and begin feeding. They reach maturity in approximately three weeks after undergoing three molts. The larvae are light green or pinkish, slightly larger than the eggs. Subsequently, the larva develops into a protonymph, followed by a deutonymph and an adult stage. The nymphs look similar to the adults but are smaller. They are green or red in color and have eight legs. The nymph feeds continually, and this can take 8 days to 2 months depending on temperature**.**

The development stages are separated by a quiescent stage, during which the mite settles immobile on the leaf with its legs drawn in. The adults mate soon after emerging from the last resting stage, and in warm weather, the females soon lay eggs. Each female may lay over 100 eggs in her life and up to 19 eggs per day, and the mites live for about a month. Development is most rapid during hot, dry weather. A single generation may require as many as 20 or as few as 5 days to reach adult hood before it begins producing offspring.

In a population of red spider mites, there are about 3 times more females than males. Generally male spider mites can be found in close association with quiescent female deutonymph, waiting for the latter to complete their development. Unfertilized females only give birth to males. The female lays her eggs during 10 days (at 35°C) to 40 days (at 15°C). At 20°C she lays about 40 eggs in total, but under optimal circumstances this can mount up to 100. Especially at dry and warm weather red spider mites can reproduce very rapidly (Hail and Ja'far, 2007).

When temperature and photoperiod drop, fertilized females enter diapause. Such females turn orange-red. They hide in all kinds of cracks in the greenhouse, to appear again early in the following season when circumstances improve (Odipo *et al.*, 2003).

Mites can overwinter without feeding, and emerge in the spring and summer to infest crops once again and with artificial feeding, they may only hibernate for very short period of time (between crops), or they may not even hibernate at all. Humid environments that are higher than 60 % can diminish the egg production of a mite (Hail and Ja'far, 2007).

2.3.2 Damage caused by red spider mites

The damage is caused by direct feeding of the mites on plant leaf tissue. They prefer undersides of leaves, but in severe infestation will occur on both leaf surfaces as well as on the stems and fruits. They suck the sap of plant tissues. Infestations are most serious in hot and dry conditions. Because they multiply very fast they are able to destroy plants within a short period of time. Spider mites spin red silk threads that anchor them and their eggs to the plant. The fine web produced by spider mites protects them from some of their enemies and even from pesticide application.

The red spider mites feed on plant sap and unless they are crowded they will mostly be found alongside the veins on the underside of the leaves (Blair and Groves, 1952). They puncture plant cells with their stylets and the contents are exuded due to turgor pressure. The depth reached by the stylets is approximately seventy to 120 micrometer, (Avery and Briggs, 1968). Depth at which injury occurs is related to length of stylets, the feeding time and population density.

Continuous feeding leads to regular spots being formed (Plate 2.4) and when these coalesce characteristics chlorotic areas develop often referred to us the ‘bronzing’ effect. (Jeppson *et al.*, 1975).At high density mites reduce the photosynthetic activities of the leaves (Mobley and Marini, 1990).

The damage caused by mites can lead to current-year or delayed second-year effects. Current-year damage, depending on the timing, duration, and severity can reduce levels of foliar nitrogen, cause premature leaf fall (Baker, 1984), reduce shoot growth and trunk diameter (Chapman *et al*., 1952) and most importantly lower fruit yields (Croft *et al*., 1983, Baker, 1984, Hardman *et al*., 1985). It can also adversely affect fruit quality size (Hoyt *et al.*, 1979), skin color, titrate able acids and firmness (Ames *et al*., 1984). Delayed second year effect of mite damage are reduced bloom with consequent reductions in numbers of fruits and yield (Baker 1984).

Plate 2.4: Red spider mite injury to eggplant. (Source: Reddy *et al.*, 2012)

The two spotted spider mite (*T. urticae*) causes yield loss on tomatoes only in exceptional cases such as; very hot and dry conditions, destruction of natural enemies, the presence of other highly infested crops in the near vicinity and insufficient water supply to the crop. Damage of spider mites on beans is most severe when mite feeding occurs early in the vegetative period. Plant cells turn yellow, which can be seen on the upper surface of the leaf as small yellow spots. The feeding of red spider mites causes injury to chlorophyll containing mesophyll cells within the leaf tissue, which results in a decrease of photosynthetic capacities of infested leaves. Early infestations cause reductions in photosynthesis and transpiration at a much lower population that causes the same level of injury later in the season (Elena *et al.*, 2006). Moreover, the webs made by spider mites reduce the aesthetic value of ornamentals.

2.4 The Black bean aphids (*Aphis fabae* Scopoli)

Black bean aphid (Plate 2.5), is a true bug in the order Hemiptera. It has specialized piercing and sucking mouthparts, which are used to suck plant juices. It is a tiny insect about two millimeters long with a small head and bulbous abdomen. The body is blackish or dark green in colour and the membranous wings, when present, are held angled over the body. The legs and antenna are light yellow. The siphunculi are twice as long as the finger-like tail and both are brownish-black. There are two tube-like protrusions at the rear of the abdomen known as cornicles which are the openings of wax glands.

Plate 2.5: Black bean aphid (*Aphis fabae* Scopoli) (Source: Ruchika and Kumar, 2012)

2.4.1 Biology of Black bean aphids

Black bean aphid, *A. fabae* Scopoli, occurs nearly world-wide, feeding on about 200 host plants in different botanical families. This aphid species is among the most harmful pests of broad bean, *Vicia faba*, in many countries. *A. fabae* can achieve very dense populations, particularly when infestation of the crop occurs before flowering. As a result, heavy yield loss may occur, where some plants can be killed by direct feeding (Hail and Ja'far, 2007).

The eggs overwinter on host plants. The aphids that hatch from these eggs in the spring are wingless females known as stem mothers. These are able to reproduce asexually giving birth to live offspring through a process known as parthenogenesis. The life span of the parthogenetic female is about 50 days and during this period, each can produce as many as 30 young ones. The offspring are also females and able to reproduce without mating but the next generation to be produced are typically winged forms. These migrate to new host plants such as beans, and sugar beet. Further parthenogenesis on these hosts allows large populations of aphids to build up quickly. Winged and wingless forms are produced throughout the summer, the winged forms dispersing to new host plants. As autumn approaches, the winged forms migrate back to the primary hosts. Both males and sexual females are produced here, mating takes place and the females lay eggs which over winter ready to repeat the life cycle the following year (Reddy *et al.*, 2012).

2.4.2 Damage caused by black bean Aphid

Aphids are considered to be a serious pest to almost all agricultural crops and alternative host plants because of their polyphagous feeding habits (Ruchika and Kumar, 2012).They cause direct injury to the host plant by the removal of the plant sap, which contains essential food materials that promote aphid and plant growth. Affected leaves wrap or curl inwards around the clusters of aphids. Because phloem sap is richer in sugars than amino acids, which aphids need for growth, much of the sap is excreted as honey dew. When aphid populations are extremely large, this sugar-rich honey dew can cover the leaf surface, forming an ideal substrate for the growth of sooty mould fungi, which affect the quality of produced pods. In addition the fungi, along with honey dew, reduce the efficiency of respiration and photosynthesis of the plant and thereby the final yields. Besides the direct feeding activity, black bean aphid is capable of transmitting over 42 plant viruses, non-persistent for example; Broad bean leaf roll virus (BBLRV) and Bean yellow mosaic virus (BYMV) .The honey dew produced attracts a large number of pests like ants and fruit flies that in turn spread young aphids (Roderick,1992). Growth of beans is affected and flowers abort due to the action of toxic saliva (Viglianco *et al*., 2008).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study area

The experiment was conducted at Kenya Agricultural Research institute- KARI, Kitale center, between the months of June to December 2011. Kitale is situated in North West Kenya in Trans-Nzoia County whose latitude is 1^{o}N, longitude, 35 ^{o} E, at an altitude of 6150m above sea level. The average annual rainfall is 1143 mm per annum which is fairly evenly distributed but with peaks in May and August and relatively dry season from December to March. The average maximum air temperature is 27.8^{o}C and the minimum is 10^{o}C. The soil at KARI, Kitale is classified as red loam (ferralsols). It is well drained, responding mainly to phosphate and nitrogen fertilizers, and tending to be deficient in calcium (DAO, 1999-2005).

3.2. Rearing of the black bean aphid (*Aphis fabae* Scopoli)

Rearing took place in the screen house which had been prepared earlier. Fifty pots were filled with black loam soil up to three quarter level then water added to soak the soil before planting the broad beans (*Vicia faba*). The pots were placed in a screen house bench in KARI Kitale. The bean seeds were soaked in warm water for one hour before planting in the pots. Two bean seeds were planted per pot and one teaspoon of D.A.P fertilizer added during planting. Watering was done daily using a bucket.

After two weeks of germination, the shoots were infested with 50 black bean aphids, which had been collected from KARI Kitale bean plants garden. The reared aphids were as shown in the Plate 3.1. The black bean aphids were reared in the screen house in order to get a bigger population to be used in the laboratory experiments.

Plate 3.1: Colonies of *Aphis fabae* on potted bean plants inside the screen house (Source: Author, 2011)

3.3 Rearing of the red spider mites (*Tetranychus urticae* Koch)

Rearing took place in the screen house which had been prepared earlier. Fifty pots were filled with black loam soil up to three quarter level then water added to soak the soil before planting the broad beans (*Vicia faba*). The pots were placed on a screen house bench in KARI Kitale. The bean seeds were soaked in warm water for one hour before planting in the pots. Two bean seeds were planted per pot and a teaspoonful of D.A.P fertilizer added during planting. Watering was done daily using a bucket. After two weeks of germination; the shoots were infested with 200 red spider mites' colonies (Plate 3.2) which had been earlier collected from Kenya Seed Company green house Kitale on egg plant. The red spider mites were reared in the screen house in order to get a bigger population to be used in the laboratory experiments.

Plate 3.2: Red spider mites' colonies on potted bean plants inside the screen house (Source: Author, 2011)

3.4 Preparation of crude leaf botanical extracts

3.4.1 Preparing Mexican marigold (*T.minuta* L) crude leaf extracts

The leaves of Mexican marigold, *T. minuta*, at flowering time were collected from KARI, Kitale center botanical garden and weighed to get 50 g, 100 g, and 150 g respectively. After being weighed, each set of the weighed leaves were put in a separate thick plastic bag then pounded until they were soft.

The pounded leaves were then put into separate plastic containers and one litre of distilled water added to make extracts of 50 g/L, 100 g/L and 150 g/L (50 g/L represents an extract made with 50 g of Mexican marigold leaves in 1 liter of water). The containers were covered tightly because the botanicals lose activity if left open and degrade rapidly in sunlight, air and moisture (Buss and Park-Brown, 2002),then soaked for 24 hours under ambient conditions. After 24 hours, the extracts were filtered using a sieve to remove the large pieces of leaf material. The resulting filtrate was then mixed with 3ml of Teepol detergent per liter (potash based soap) to assist in dispersion of the spray on the plant and pest surface since it acts as a sticking agent (Viglianco *et al*., 2008). Another extract of 150 g/L was also soaked for 48 hours to be used on the two pests. The filtrate was then put into a 1 liter plastic hand sprayer and used immediately on the pests.

3.4.2 Preparing Fish poison bean (*T. vogelii* Hook) crude leaf extract

Leaves of *T. vogelii* were collected from KARI, Kitale centre botanical garden. The entire compound leaf was cut and weighed to get 10 g, 30 g and 60 g respectively. After being weighed, the weighed leaves were put in a thick plastic bag separately then pounded until they were soft. The crushed leaves were then put into separate plastic containers and one litre of distilled water added to make 10 g/L, 30 g/L and 60 g/L of crude extract (10 g/L represents an extract made with 10g of *T. vogelii* leaves in 1 liter of water). The containers were then covered tightly because the botanicals lose activity if left open and degrade rapidly in sunlight, air and moisture (Buss and Park-Brown, 2002), and soaked for 12 hours under ambient condition. After 12 hours, the extract was then filtered using a sieve to remove the large pieces of leaf material. Another extract of 60 g/L of *T. vogelii* crude leaf extract was also soaked for 48 hours and used on the two pests. The resulting filtrate was then mixed with 3ml of Teepol detergent per liter (Potash based soap) to assist in dispersion of the spray on the plant surface and on the pests, since the detergent acts as a sticking agent (Katsvanga and Chigwaza, 2004; Viglianco *et al*.,2008)

The filtrate was then put in a 1 liter plastic hand sprayer and used immediately on the pests.

3.5 Laboratory experiments

This part of the study was done in order to determine how crude leaf extracts reduced mite and bean aphid populations in the laboratory.

3.5.1 Application of botanical extracts on Black bean aphids

The Black bean aphids that had been reared earlier in the screen house were collected within 30 minutes of the beginning of each experiment. They were tested in plastic containers. The aphids on the bean leaves were sprayed with different concentrations of *T. minuta* and *T. vogeli* before being placed into the containers. The containers were then sealed using a well-ventilated lid. Each treatment test with different concentrations of the botanical was replicated four times and in each replicate, a total of 50 aphids were used. There was also a container for positive control, where Alpha cymber insecticide 10 EC was used as standard treatment and distilled water used as negative control.

Observations were made at 1,2,4,6,8,10, and 24 hours after spraying the aphids and numbers of dead aphids were recorded at each observation. The bean aphids were considered alive if they exhibited normal behavior when breathed upon or physically stimulated with a wooden stick. For each time point, if bean aphid were incapable of moving, maintaining a normal posture, leg coordination, or any signs of life they were considered dead (Panella *et al*., 2005).

3.5.2 Application of botanical extracts on the Red spider mites

The red spider mites that had been reared in the screen house were collected within 30 minutes of the beginning of each experiment. They were tested in plastic containers. The bean leaves which had been heavily colonized with red spider mites were sprayed with different concentrations of *T. minuta* and *T. vogelii* which had been prepared earlier then air dried for five minutes before being placed into the containers. The containers were then sealed using a well-ventilated lid. Each treatment test with different concentrations of the botanical was replicated four times and in each replicate, a total of 100 red spider mites were used. There was also a container for positive control where Disect 10 EC Bifenthrin insecticide was used as a standard treatment while the other container distilled water was used as negative control.

Observations were made at 1,2,4,6,8,10, and 24 hours after spraying the red spider mites and numbers of dead mites were recorded at each observation. The mites were considered alive if they exhibited normal behavior when breathed upon or physically stimulated with a wooden stick. For each time point, if the mite were incapable of moving, maintaining a normal posture, leg coordination, or any signs of life they were considered dead (Panella *et al.*, 2005).

3.5.3 In vitro repellency bioassay

The repellency method described by Thorsel *et al*., 2005 was used in this bioassay. The adults of *T. urticae* and *Aphis fabae* were used to test the repellency properties of *T. minuta* and *T. vogelii* crude leaf extracts. Two filter papers (Whatman No. 1) were placed inside a petri dish with an inner diameter of 9.5 cm. The treatments of 10 g\L, 30 g\L, 60 g\L *T. vogelii* crude leaf extracts soaked 12 hours and 60 g\L extract soaked 48 hours were used against *T. urticae* and *Aphis fabae. T.minuta* crude leaf extracts of 50 g\L, 100 g\L, 150 g\L soaked 24 hours and 150 g\L soaked 48 hours were also used against *T. urticae* and *Aphis fabae.* The extracts were applied along the periphery of the filter papers. The filter papers were then air dried for 2 minutes. Each treatment test was replicated three times.

Ten adults of *T. urticae* and *A. fabae* were placed respectively at the centre of each of the treated filter papers and their movement with regard to avoiding the treated area was physically observed. The pest that continued its motion beyond the periphery of the treated area within 5 minutes was indicated as non-repelled, and the pest that reversed its direction before reaching the periphery of the treated area was considered as repelled.

The repellency was expressed as number of red spider mites or black bean aphids avoiding the treated area to the total number of red spider mites or black bean aphids at every observation. Thus 10 species avoiding out of a total of 10 was recorded as 100% repellency.

Mean percentage values were classified according to the following Table 3.1 (Adriana *et al.*, 2008)

The data was then subjected to one-way ANOVA for the significance test and further, Fisher's LSD test for mean comparison test for separation was applied.

Table 3.1: Six repellency classes

Class	**Repellency rate (%)**
0	>0.01 to < 0.1
1	0.1 to 20
2	20.1 to 40
3	40.1 to 60
4	60.1 to 80
5	80.1 to 100

3.6 Field preparation

The size of experimental plot that was prepared was 32m x 32m which was divided into two equal halves measuring 32m x 16m respectively. Each plot was first ploughed, harrowed and ridged during the month of August before planting the beans. Each plot was divided into three replicates and each replicate had eight treatment plots including control replicated thrice. Each treatment plot measured 3m x 3m.The inter-spacing between replicates was 1 meter and spacing between each treatment was 0.75m (Plate 3.3).The bean seeds bought from the market were planted on 13th September 2011 at 40cm x 20cm spacing between rows and plants respectively. One tablespoon DAP fertilizer per hole was used during planting of bean seeds. On 27th September 2011, the bean seedlings in the field were sprayed using Folicur 250 EW (a broad spectrum systemic fungicide for the control of fungal diseases) and Bull dock (a systemic insecticide) and weeding was done one week later. On 5th October 2011, one half of the plot (32m x 16m) that was planted with beans, (had been divided into 24 treatment plots) was infested with 500 black bean aphids from the screen house by physically introducing them in the field on sampled plants while the other half with 24 treatment plots was infested with 500 red spider mites.

The two pests black bean aphids and red spider mites were left to multiply in the field.

Plate 3.3: Preparation of experimental plots in the field at KARI Kitale. (Source: Author, 2011)

3.6.1 Field experiment

Bean plants which had been infested with black bean aphids and red spider mites were sprayed with *T. vogelii* of 10 g/L and 60 g/L soaked 12 hours and 60 g/L soaked 48 hours. An extract of Mexican marigold of 50 g/L and 150 g/L soaked 24 hours; 150 g/L Mexican marigold soaked 48 hours was also sprayed.

Disect 10 EC Bifenthrin insecticide was used as positive control for red spider mites while Alpha cymber insecticide 10 EC was used as positive control on black bean aphids. Three plots for red spider mites and three for black bean aphids were not treated, hence used as negative controls. Observations were made at 1, 2, 4, 6, 8, 24 and 30 hours after spraying the aphids and mites and numbers of dead pests on plants were sampled and recorded at each observation.

3.7 Data analysis

The mortality data derived from this experiment was analyzed using descriptive statistics software MINITAB version 14. Mortality of aphids and mites was summarized in a table as percent mortality. Modeling was done using logarithmic curve graphs for the number of *T. urticae* and *A. fabae* dying at different times for 24 hours of exposure to different concentration of *T. minuta* and *T. vogelii* crude leaf extract. R^2 in the graphs represents the relationships between different concentrations of the two botanicals and the two pests used in the experiment.

The *in vitro* repellency bioassay was calculated using the method described by Thorsel *et al.*, (2005);

R=a/n x 100

Where: **R** is the repellency

a is the number of adults of each pest species avoiding treated area

n is the total number of aphids or red spider mites put in the centre of filter paper

The hypothesis for mortality was tested using MINITAB 14 mood's median test since this is a non-parametric count data while that of repellency bioassay was calculated using Fisher's Least Significant difference (LSD) test ($p<0.05$)

CHAPTER FOUR

RESULTS

4.1Treatment of black bean aphid and red spider mites with Tephrosia vogelii Hook extract in the laboratory

The percent mortality of bean aphids and red spider mites after being sprayed with *T. vogelii* extract at three concentrations of 10 g/L, 30 g/L soaked for 12 hours and 60 g/L when soaked for 12 hours and 48 hours respectively and exposed for 24 hours were as shown in table 4.1. The percent mortality of aphids and mites increased with increasing concentration and also with increasing soak time *of T. vogelii* as follows; after one hour of exposure to the extract, concentration of 10 g/L had killed 12 % aphid, while same concentration killed 4 % of mites, the percent mortality increased to 30 % in aphid at concentration 30 g/L and it was 7 % for mites. At 60 g/L soaked 12hours, percent mortality for aphids after one hour of exposure to the extract was 38 %, and it was 9 % in mites. However the same concentration of 60 g/L soaked 48hours showed that percent mortality for aphids was 58 % while in mites it was 22 %. An identical phenomenon of percent mortalities was observed for exposure period of 2-24 hours for both aphids and mites.

At the end of 24 hour exposure of bean aphid and red spider mites to *T. vogelii* extract, the extract with a concentration of 60 g/L under a 48 hour soaking in distilled water recorded the highest percent mortalities of 98 % in aphids and 92 % in mites, while concentration of 10 g/L soaked 12 hours recorded the lowest percent mortalities of 44 % in aphids and 16 % in mites. Concentration of 60 g/L soaked 12 hours had percent mortality of 94 % in aphids and 60 % in mites; 30 g/L soaked 12 hours had killed 76 % of aphids and 22 % of mites after 24 hour of exposure to the extract.

Alpha cymber insecticide 10 EC was used on aphids while Disect 10 EC Bifenthrin insecticide was used on mites as positive controls. These insecticides killed many pests within a very short period of exposure. At the end of one hour of exposure to the spray, Alpha cymber had killed 86 % aphids while Disect 10 EC had killed 45 % mites. However the percent mortality increased cumulatively and at the end of 24 hour exposure to insecticide, 100 % of aphids were dead while percent mortality for mites was 98 %.

Table 4.1- Percent mortalities of Aphids and mites when sprayed with *T. vogelii* extract at different concentrations and soak period over 24 hour exposure in the laboratory

Hours	**1**		**2**		**4**		**6**		**8**		**10**		**24**	
Extract Concentration	Aphids	Mites	Aphids	Mites	Aphids	Mites	Aphids	Mites	Aphids	Mites	Aphids	Mites	Aphids	Mites
10g/l-12hours	12	4	22	6	26	8	32	10	34	12	36	13	44	16
30g/l-12hours	30	7	42	9	50	11	58	13	66	15	70	17	76	22
60g/l-12hours	38	9	50	16	58	27	68	34	80	45	84	48	94	60
60g/l-48hours	58	22	68	31	76	48	82	61	88	72	92	79	98	92
+Ve Control	86	45	86	56	90	67	94	76	98	86	100	92	100	98

Key

Where,

+Ve control = Alpha cymber insecticide for aphids and Disect 10 EC insecticide for red spider mites

Figure 4.1 shows the model that best fitted the number of black bean aphids dying at different concentrations and different soak times. The models were logarithmic curves. Increase in concentration and soak time resulted in increase in the number of aphids that died when sprayed using *T. vogelii* crude leaf extracts. At a concentration 10 g/L and soaked for 12 hours, the initial number of aphids that died was ≈ 6 with R^2=0.9884 , at concentration 30 g/L and soaked for 12 hours, the initial death recorded was ≈ 15 aphids with R^2=0.9728 , at concentration 60 g/L and soaked for 12 hours, ≈ 18 aphids were recorded to have died at the first count with R^2=0.9709 , while at the same concentration and at 48 hours soak time, ≈29 aphids had died at the first count with R^2=0.9826.

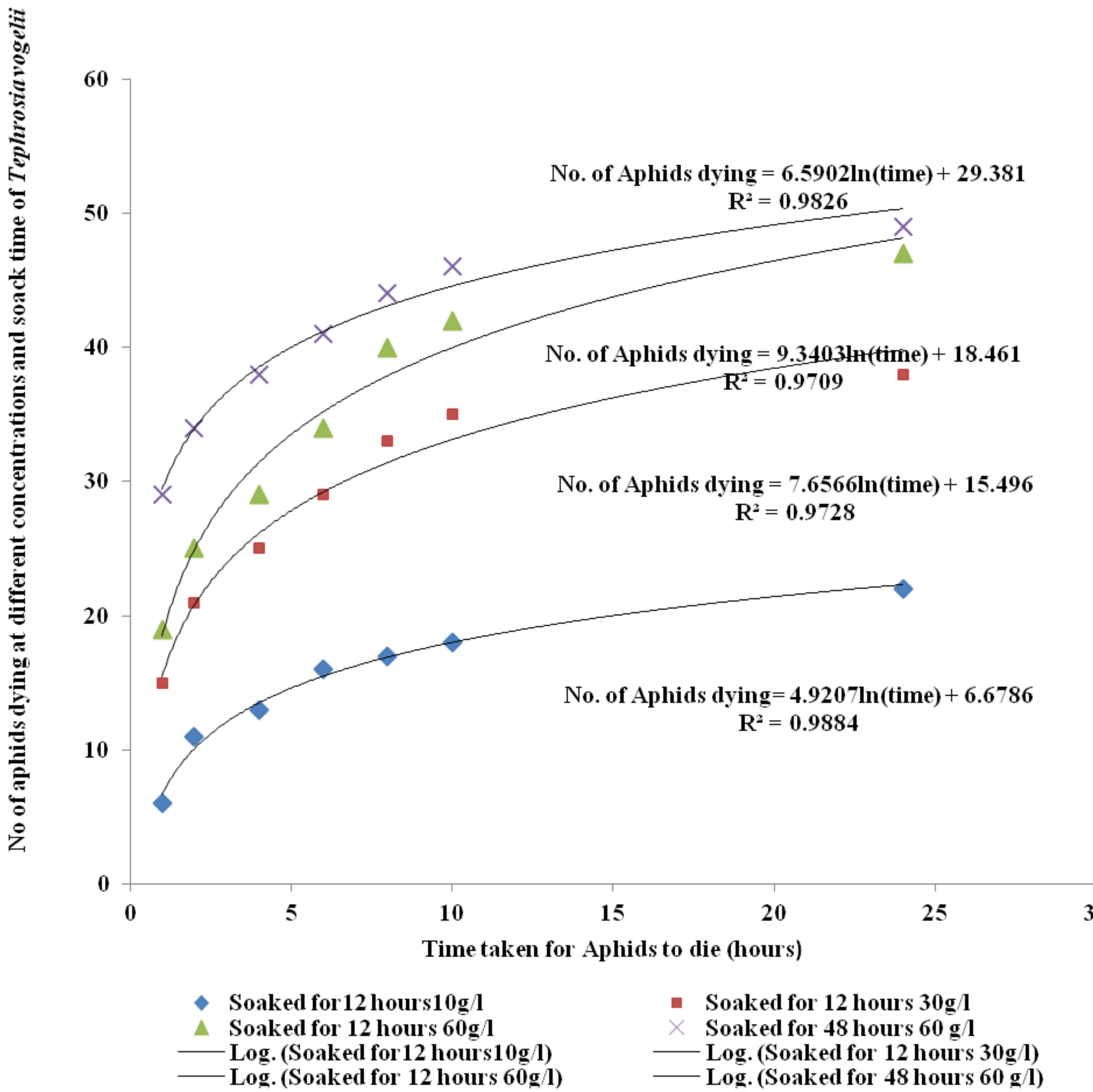

Figure 4.1: Model equation that relates the number of aphids dying when sprayed with different concentrations and soak period of *Tephrosia vogelii* crude leaf extract

The number of mites that died after 24 hours of exposure when sprayed with different concentrations and soak period of *T. vogelii* were as in the figure 4.2. The initial death recorded at concentration 10 g/L and soaked for 12 hours was ≈ 3 mites with value of R^2=0.983, at concentration 30 g/l and the same soak period, ≈6 mites were recorded dead at the initial count with R^2=0.9569, at concentration 60 g/L soaked 12 hours had initial death of ≈6 mites with R^2=0.9773. High death was however recorded with spray of concentration 60 g/L at 48 hours soak time with an initial death record of ≈18 mites and R^2=0.9767.

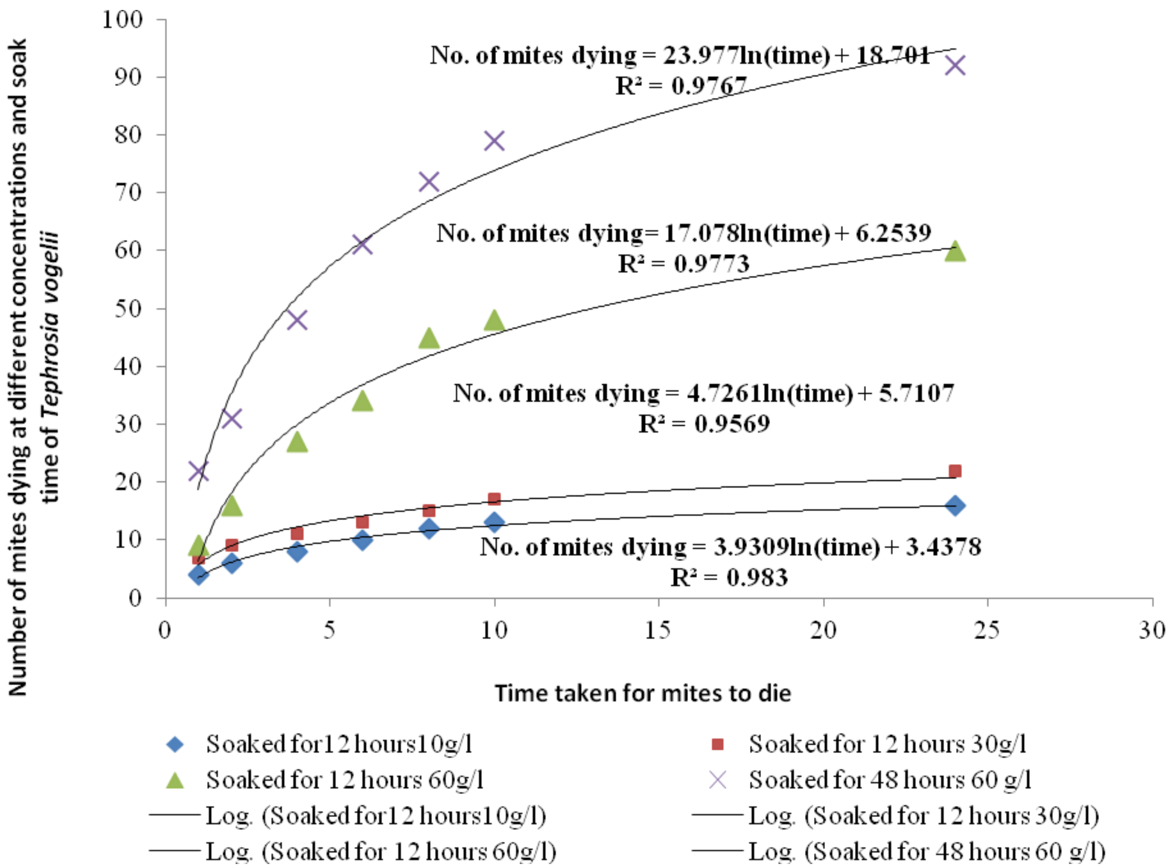

Figure 4.2: Model equation that relates the number of mites dying when sprayed with different concentrations and soak period of *Tephrosia vogelii* Hook crude leaf extract

4.2 Treatment of black bean aphids and red spider mites with *Tagetes minuta* L crude leaf extract in the laboratory

T. minuta crude leaf extract were also used to spray on the pest. The percent mortality of black bean aphids and red spider mites after being sprayed with different concentration of *T. minuta* at different soak period and exposure of the pest for 24 hours were as in Table 4.2. After one hour of exposure to *T.minuta* extract, concentration of 50 g/L had killed 26 % aphids and 7 % mites, 100 g/L had killed 42 % aphids and 11 % mites, and 150 g/L soaked 24 hours had killed 54 % aphids and 14 % mites. The percent mortality increased to 58 % in aphids when 150 g/L soaked 48 hours was used while in mites, the percent mortality was slightly lower at 30 % after one hour of exposure to the spray.

The concentration of 150 g/L soaked for 48 hours had a high percent mortality of 90 % in aphids and a slightly lower percent in mites (69 %) at the end of 24 hour exposure to the extract while the same concentration soaked for 24 hours had percent mortality of 84 % in aphids and 35 % in mites followed by that of 100 g/L soaked for 24 hours which had percent mortality of 80 % in aphids and 25 % in mites at the end of 24 hour exposure. Concentration 50 g/L killed 62 % of aphids and 22 % of mites at the end of 24 hour exposure to the extract.

Table 4.2- Percent mortalities of Aphids and mites when sprayed with *T. minuta* extract at different concentrations and soak period over a 24 hour exposure in the laboratory

Hours	**1**		**2**		**4**		**6**		**8**		**10**		**24**	
Extract Concentration	Aphids	Mites	Aphids	Mites	Aphids	Mites	Aphids	Mites	Aphids	Mites	Aphids	Mites	Aphids	Mites
50g/l-24hours	26	7	32	9	44	13	48	15	52	18	56	21	62	22
100g/l24hours	42	11	52	13	60	16	66	19	70	20	72	22	80	25
150g/l24hours	54	14	62	21	68	26	72	29	74	30	76	31	84	35
150g/l48hours	58	30	66	35	70	40	76	47	78	56	82	60	90	69
+Ve Control	86	45	86	56	90	67	94	76	98	86	100	92	100	98

Efficacy of *T.minuta* on bean aphids was as shown in Figure 4.3.The initial death recorded was ≈13 aphids when sprayed with 50 g/L *T.minuta* and soaked for 24 hours ($R^{2=}$ 0.9813), ≈22 aphids at concentration 100 g/L and soaked for 24 hours ($R^{2=}$0.9911), ≈27 aphids at concentration 150 g/L soaked for 24 hours (R^2=0.9969) and finally, ≈29 aphids at concentration 150 g/L and soaked for 48 hours (R^2 =0.9908). The graphs were however very close to each other indicating that the concentrations killed relatively close to the same number of the aphids when compared.

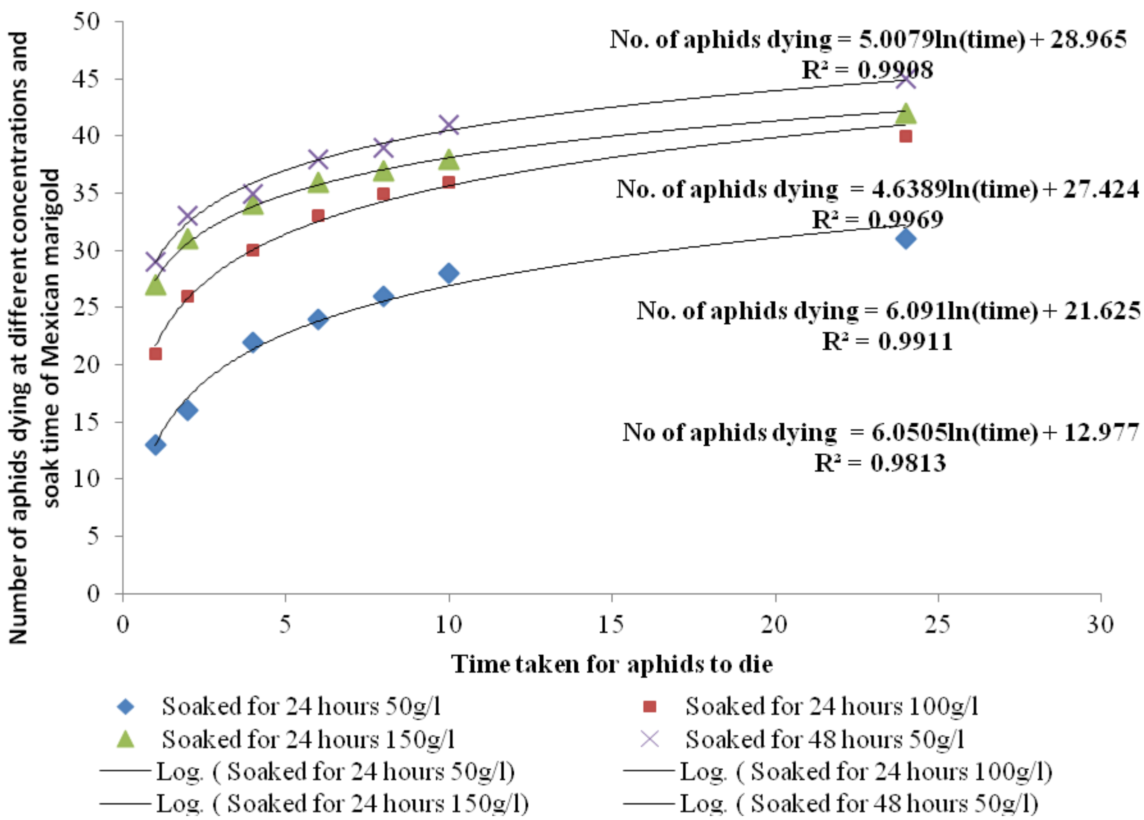

Figure 4.3: Model equation that relates the number of aphids dying when sprayed with different concentrations and soak period of *Tagetes minuta* crude leaf extract.

Modeling for the number of red spider mites dying at different concentrations and soak period for Mexican marigold were as in Figure 4.4. More mites died when *T.minuta* was soaked for 48 hours and at concentration 150 g/L, the initial death record being ≈27 mites with an R^2 of 0. 9505. When the same concentration was used but at shortened soak period (24 hours), the spray killed less mites, the initial death record being ≈ 16 mites and R^2 of 0.9664. The death record of mites became less and less when lower concentrations were used; 100 g/l soaked 24 hours had an initial death record of ≈10 mites with an R^2 of 0.984 while 50 g/L soaked 24 hours initially killed ≈6 mites, R^2 being 0.9784.

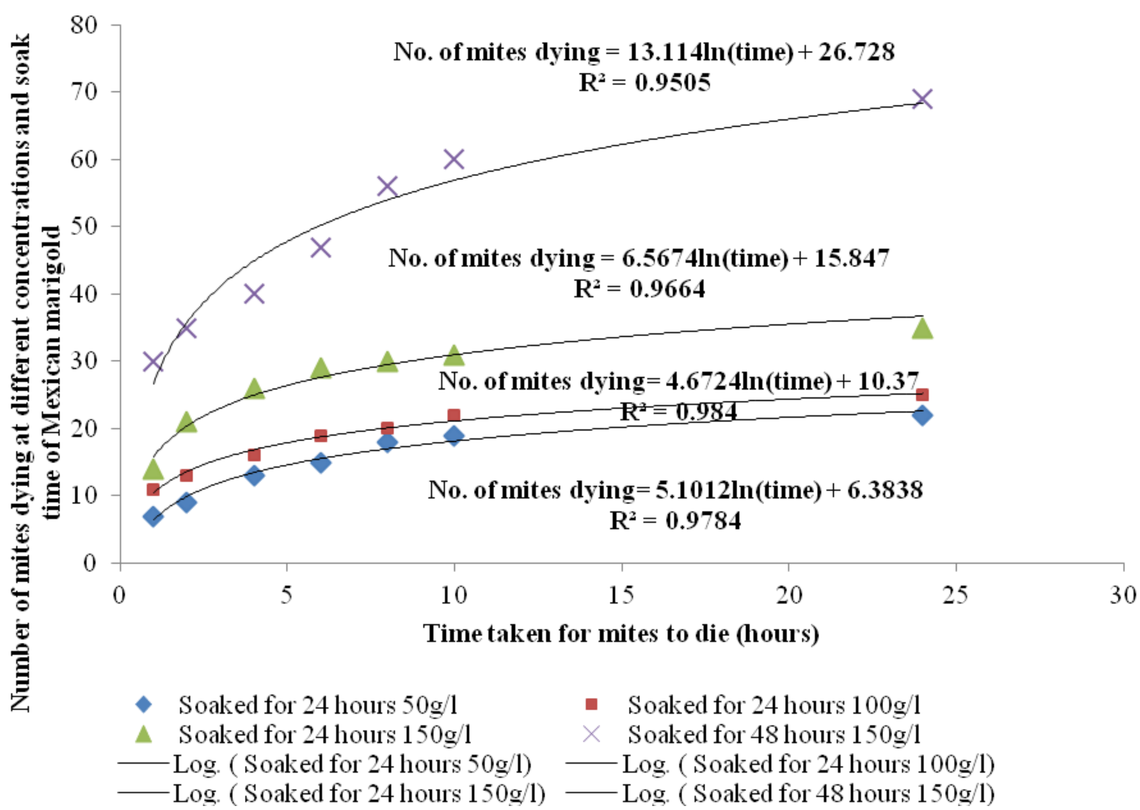

Figure 4.4: Model equation that relates the number of red spider mites dying when sprayed with different concentrations and soak period of *Tagetes minuta* crude leaf extract.

4.3 In vitro repellence bioassay

Table 4.3 shows the repelling activity of red spider mites to *T.minuta* and *T. vogelii* crude leaf extracts at different concentration. The repellency duration was 45 minutes for *T.minuta* at 150 g/L soaked for 48 hours, while same concentration at soaking period of 24 hours repelled the mites up to 15 minutes only. *T. vogelii* at concentration 60 g/L soaked for 48 hours repelled the mites up to 15 minutes while the same concentration at 12 hours soak period repelled up to 10 minutes. Concentration of 30 g/L soaked for 12 hours repelled up to 5 minutes which was the lowest. Black bean aphids when tested for repellency response did not show any repellency to the botanicals that were being used.

Table 4.3: Red spider mites repelling activity to*T.minuta* and *T.vogelii* at different concentration and different soak period

		Mite Repellency (%) at;										
Name of extract	Soaking time	5 min	10 mins	15 mins	20 mins	25 mins	30 mins	35 mins	40 mins	45 mins	Mean Repellency (%)	Class repellency
T.minuta (150g/l)	48 hours	100	97	77	63	57	47	30	17	3	55	3b
T. minuta (150g/l)	24 hours	60	40	7	0	0	0	0	0	0	12	1a
T. vogelii (60g/l)	48 hours	97	40	20	0	0	0	0	0	0	17	1a
T. vogelii (60g/l)	12 hours	50	20	0	0	0	0	0	0	0	8	1a
T. vogelii (30g/l)	12 hours	20	0	0	0	0	0	0	0	0	2	1a

Values followed by the same letter are not significantly different according to the Fisher’s LSD test (P<0.05)

4.4 Testing for the Hypothesis on the botanicals used on pests

4.4.1 Fish poison bean (*Tephrosia vogelii* Hook) crude leaf extract

Table 4.4 shows moods median test for significant difference in the number of black bean aphids dying after being sprayed with *T. vogelii* crude leaf extract. The p value being 0.017 indicating significant difference in the number of black bean aphids dying when sprayed with *T. vogelii* at concentration 10 g/L, 30 g/L and 60 g/L. Simultaneous test for similarity depicted 10 g/L to be the significantly different concentration in killing the aphids. The overall median of the number of aphids that died was 25 for *T. Vogelii*

Table 4.4: Mood's median test for significant difference in number of aphids dying verses *T. vogelii* extract

Mood Median Test: Number of Aphids dying versus Concentration

Mood median test for Number of Aphids dying
Chi-Square = 7.25   DF = 2   P = 0.027

```
                               Individual 95.0% CIs
Concentration  N<=  N>  Median  Q3-Q1   +---------+---------+---------+------
10g/l           7   0    16.0    7.0   (-----*--)
30g/l           3   4    29.0   14.0         (---------*------)
60g/l           2   5    34.0   17.0            (----------*--------)
                                +---------+---------+---------+------
                               10        20        30        40
Overall median = 25.0
```

Table 4.5 shows the number of red spider mites that were killed by *T. vogelii* crude leaf extract. The p value was 0.027, indicating a significant difference in the death recorded for the different concentrations of the herb. This was as a result of concentration 60 g/L being significantly different from concentration 10 g/L, concentration 30 g/L was intermediate of the two and so was not significantly different from the other two. The overall median of the number of mites that died was 13. This also depicted that the herb killed many aphids than mites at the same concentration and soak time.

Table 4.5: Mood's median test for the number of red spider mites dying verses different concentration of *T. vogelii*

Mood Median Test: Number of mites dying versus Concentration

Mood median test for Number of mites dying
Chi-Square = 8.32   DF = 2   P = 0.017

```
Individual 95.0% CIs
Concentration  N<=  N>  Median  Q3-Q1  ------+---------+---------+---------+
10g/l            6   1    10.0    7.0  (--*-)
30g/l            4   3    13.0    8.0   (--*--)
60g/l            1   6    34.0   32.0       (-------------*----------)
                                       ------+---------+---------+---------+
                                            15        30        45        60
Overall median = 13.0
```

4.4.2 Mexican marigold (*Tagetes minuta* L) crude leaf extract

Moods median test was also done to test any significant difference in the number of aphids that died after being sprayed by *T.minuta,* Table 4.6. The p value was 0.017 for aphids indicating that there was significant difference in the number of aphids that were killed by the different concentrations of the herb. The similarity test showed that, the significant difference came from comparison between 150 g/L concentration and 50 g/L. The overall median for the number of aphids that were killed by this herb was 31.

Table 4.6: Mood's median test for the number of aphids dying verses different concentration of *Tagetes minuta* L

Mood Median Test: Number of Aphids dying versus Concentration

Mood median test for Number of Aphids dying
Chi-Square = 8.47 DF = 2 P = 0.014

Concentration	**N<=**	**N>**	**Median**	**Q3-Q1**	**Individual 95.0% CIs** --------+---------+---------+--------
100g/l	**3**	**4**	**33.0**	**10.0**	(-----------*-----)
150g/l	**2**	**5**	**36.0**	**7.0**	(-------*----)
50g/l	**7**	**0**	**24.0**	**12.0**	(-----------*------)
					--------+---------+---------+--------
					21.0 28.0 35.0

Overall median = 31.0

The test was also conducted on the number of mites that died after being sprayed with *T. minuta,* Table 4.7; the p value was 0.027 again indicating a significant difference in the number of mites dying after being sprayed with the herb at different concentrations. Here too, concentration 150 g/L and 50 g/L were the results of the significant difference observed. The overall number of mites that were killed by *T. minuta* was 19. This again gave the same impression that as the previous (*Tephrosia vogelii*), generally, fewer mites than aphids were killed using *T.minuta*.

Table 4.7: Mood's median test for the number of mites dying verses different concentration of *Tagetes minuta* L

Mood Median Test: Number of mites dying versus Concentration

```
Mood median test for Number of mites dying
Chi-Square = 7.25    DF = 2    P = 0.027

                              Individual 95.0% CIs
Concentration  N<=  N>  Median  Q3-Q1  --------+---------+---------+--------
100g/l          4   3    19.0    9.0      (--------*-----)
150g/l          1   6    29.0   10.0             (-------------*----)
50g/l           6   1    15.0   10.0  (--------*------)
                              --------+---------+---------+--------
                                  14.0      21.0      28.0
Overall median = 19.0
```

4.5 Field Results

When *T. Urticae* and *A.Fabae* were subjected to the same field treatment of botanicals as was in the laboratory experiment, the mean mortalities was not very much different from that of the laboratory results. However, the mean mortalities in the field was slightly lower as compared to that of the laboratory, the positive controls (standard insecticides) registered quite a higher mean mortality.

4.5.1 Mean mortality of *Aphis fabae* after treatment with *Tephrosia vogelii* Hook crude leaf extract

Modeling for the number of *Aphis fabae* dying at different concentrations and soak period for *T. vogelii* were as in Figure 4.5. More aphids died when sprayed with *Tephrosia vogelii* soaked for 48 hours and at concentration 60 g/L, the initial death record being ≈353 mites with an R^2 of 0.9704, which was closely similar to that of 60 g/L soaked for 12 hours. The two graphs were very close to each other indicating that the concentrations killed relatively close to the same number of the aphids when compared. The death record of aphids became less when 10 g/L was sprayed on aphids and the initial death record of ≈84 aphids with an R^2 of 0.9773 was recorded.

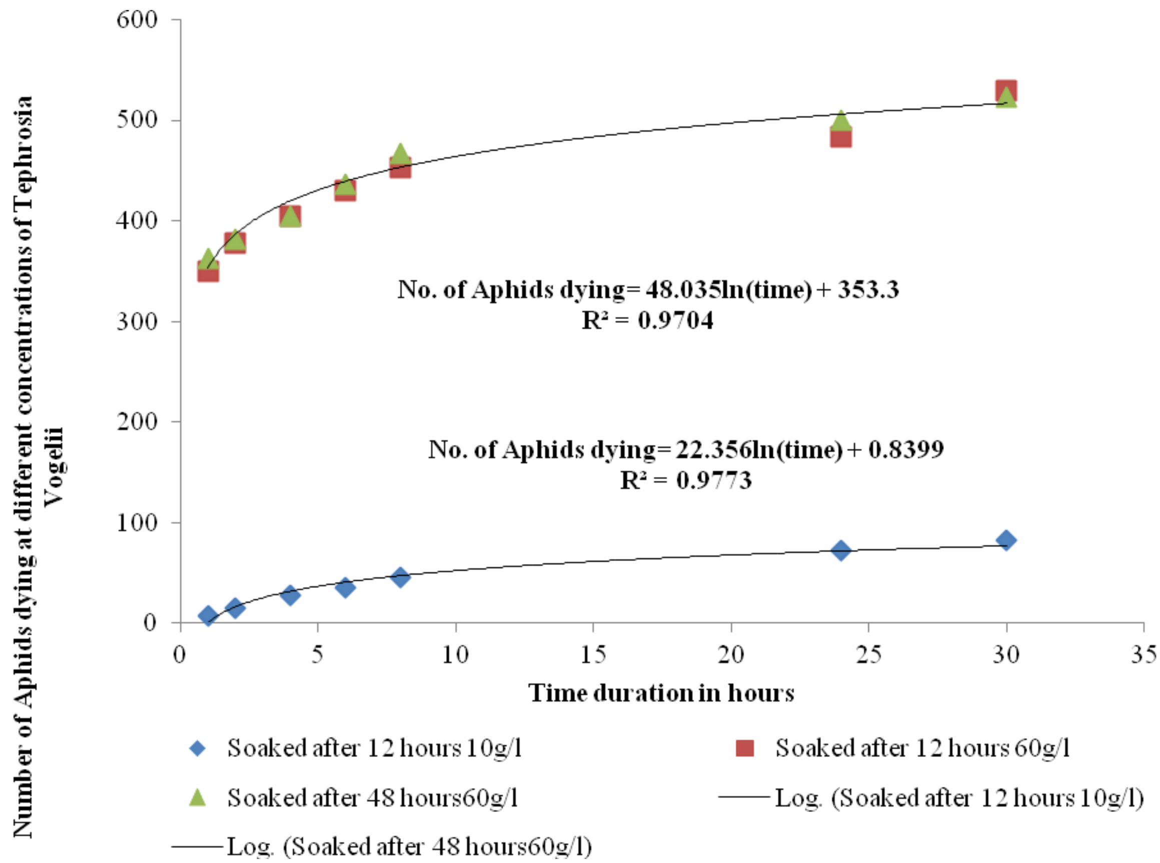

Fig 4.5: Model equation that relates the number of *A. fabae* dying when sprayed with different concentrations and soak period of *T. vogelii* in the field experiment.

4.5.2 Mean mortality of *Tetranychus urticae* Koch after treatment with *Tephrosia vogelii* Hook crude leaf extract

The model that predicted the death rates at different concentrations and soak hours of *T. vogelii* were as in Figure 4.6.The initial death recorded was ≈62 mites when sprayed with 60 g/L *T. vogelii* and soaked for 48 hours (R^2 =0.9803), while the same concentration soaked for 12 hours recorded the lowest mean mortalities with an initial death of ≈6 mites (R^2 =0.9818). Concentration of 10 g/L soaked for 12 hours recorded the very least mortalities with initial death being ≈3 mites (R^2 =0.9589).

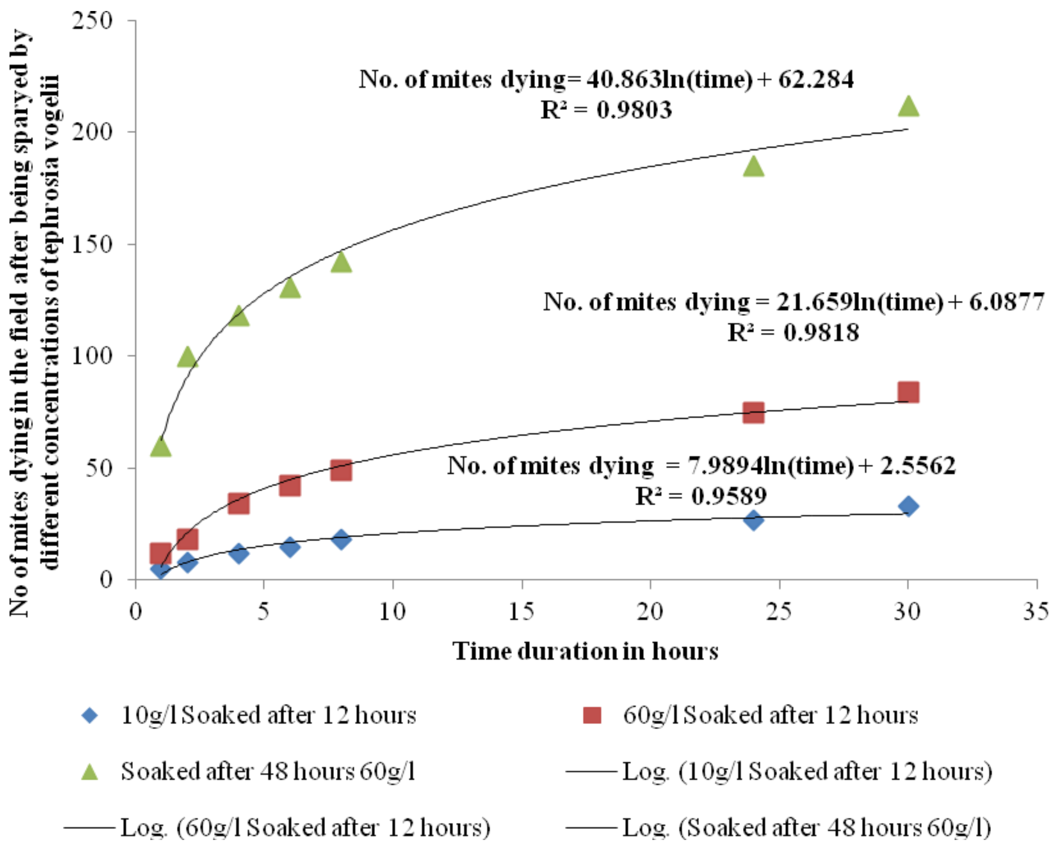

Fig 4.6: Model equation that relates the number of *T. urticae* dying when sprayed with different concentrations and soak period of *Tephrosia vogelii* in the field experiment

4.5.3 Mean mortality of Aphis fabae Scopoli after treatment with T.minuta L Plant extract in the field experiment

The number of aphids that died after 30 hours of exposure when sprayed with different concentrations and soak period of *T. minuta* were as in the Figure 4.7. The initial death recorded at concentration 50 g/L and soaked for 24 hours was ≈ 37 aphids (R^2=0.9841), at concentration 150 g/L and soak period of 24 hours, ≈147 aphids were recorded dead at the initial count ((R^2=0.9691), while the same concentration soaked 48 hours had a higher initial death of ≈ 215 aphids (R^2=0.9728).

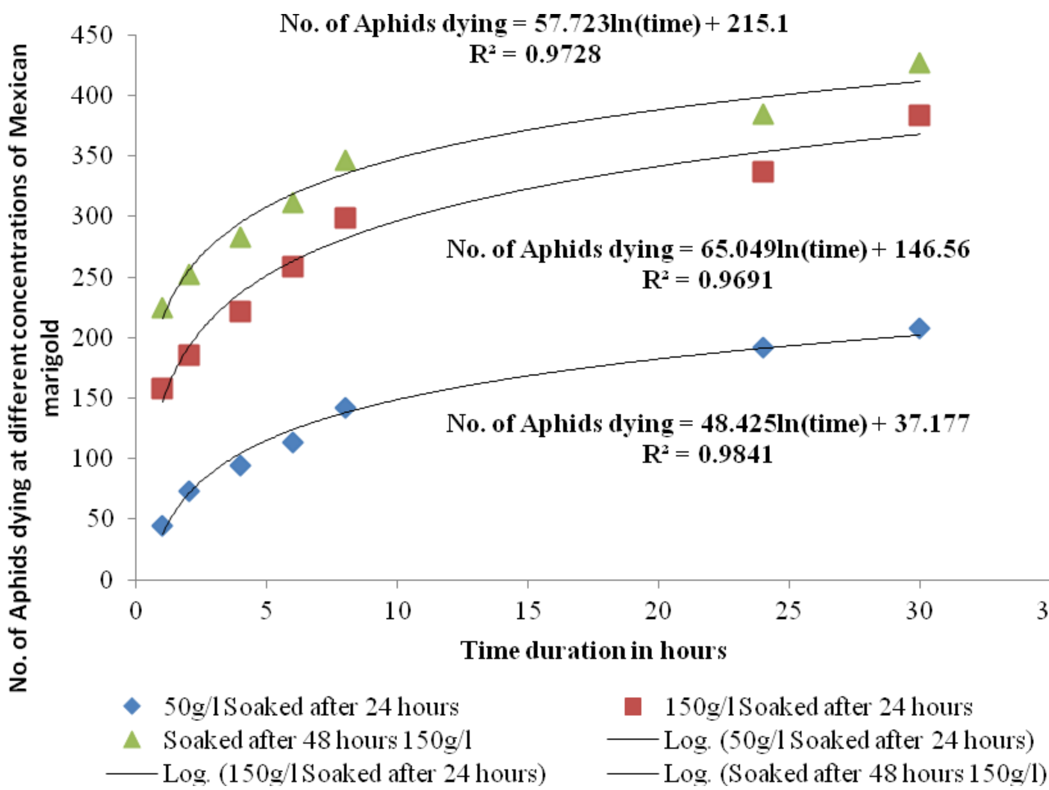

Fig 4.7: Model equation that relates the number of *A. fabae* dying when sprayed with different concentrations and soak period of *T. minuta* in the field experiment

4.5.4 Mean mortality of *T. urticae* Koch after treatment with *T.minuta* L Plant extract in the field experiment

Figure 4.8 shows the number of mites that died after 30 hours of exposure when sprayed with different concentrations and soak period of *T. minuta*. The initial death recorded at concentration 150 g/L and soaked for 48hours was ≈ 15 mites (R^2=0.9752), while the same concentration and soak period of 24 hours recorded lower mortality of ≈10 mites as initial count (R^2=0.9409). The concentration of 50 g/L at soaking period of 24 hours recorded initial death of ≈ 4 mites (R^2=0.9531).

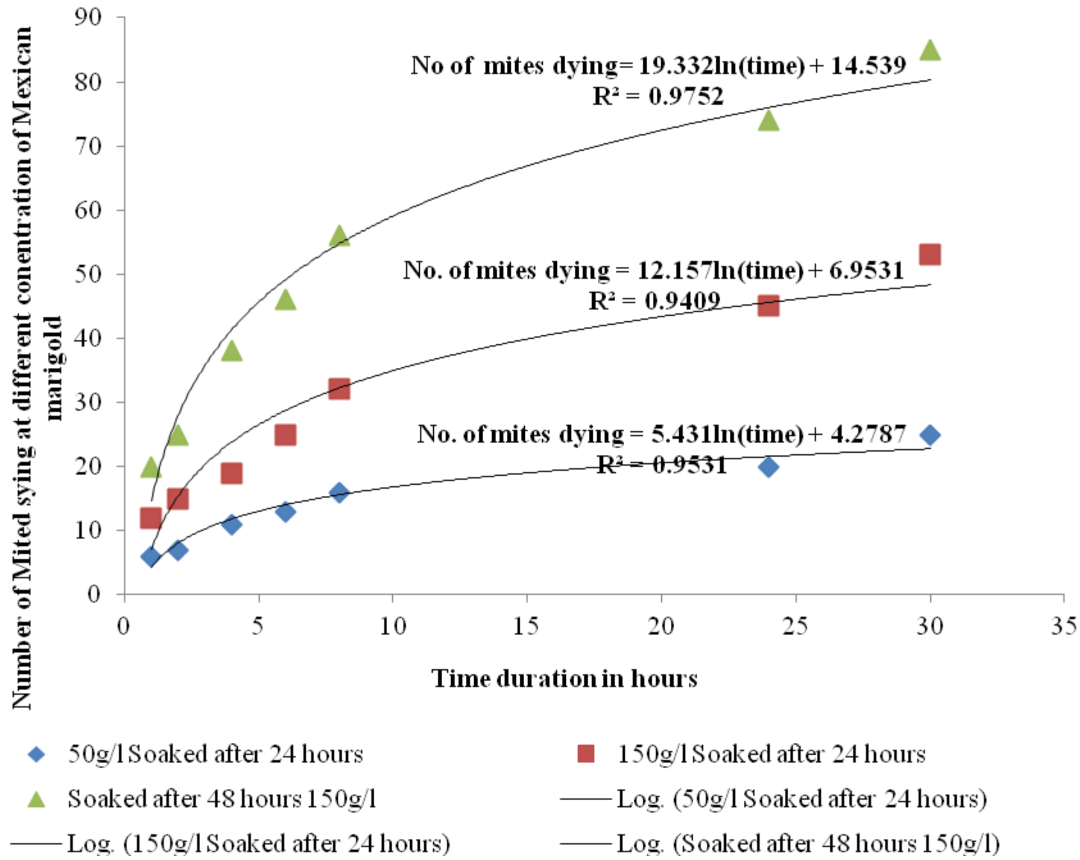

Fig 4.8: Model equation that relates the number of *T. urticae* dying when sprayed with different concentrations and soak period of *T. minuta* in the field experiment

4.6 Yield of bean seeds

At harvest (three months after planting bean seeds) of bean seeds from the experimental plots, good seeds were separated from bad seeds and both were weighed in grams. The seeds that had a shriveled coat, unusually small or rotten was considered as bad seeds while the seeds that had the right size and smooth coat was considered as good seeds.

From Figure 4.9, more good seeds were harvested from the plots that had been sprayed with a synthetic pesticide (positive control) and 60 g/L of *T. vogelii* soaked 48 hours (5000 g & 4985 g respectively). The plot that had not been treated (negative control) registered quite a lower yield of good seeds (3758 g) and slightly more bad seeds. The plot treated with 60 g/L *T. vogelii* soaked for 12 hours had yield of 4260 g good seeds while that of 10 g/L had 3980 g of good seeds at harvest.

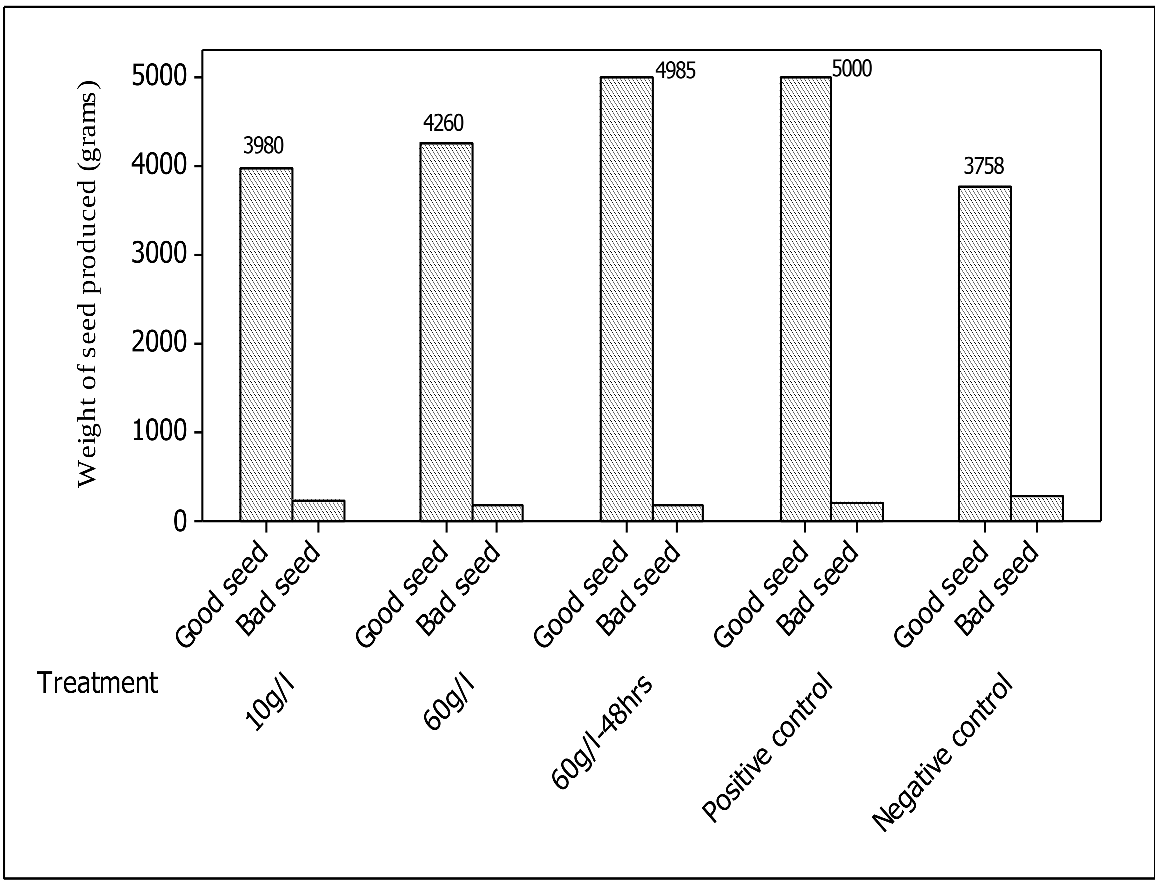

Figure 4.9: Bar graphs showing the weights of good and bad seeds produced from an *Aphis fabae* infested plot sprayed with *T. vogelii* extract

The untreated plot (negative control) infested with mites from Figure 4.10 had low yield of good bean seeds (3671 g) and slightly more bad seeds as compared to the other plots infested with mites that were treated. The standard insecticide (positive control) had the highest yield of good seeds (5080 g) followed by the plot treated with 60 g/L *T. vogelii* soaked 48 hours (4480 g), 60 g/L soaked 12 hours sprayed plot had 4160 g of good seeds while 10 g/L sprayed plot had 3911 g good seeds at harvest.

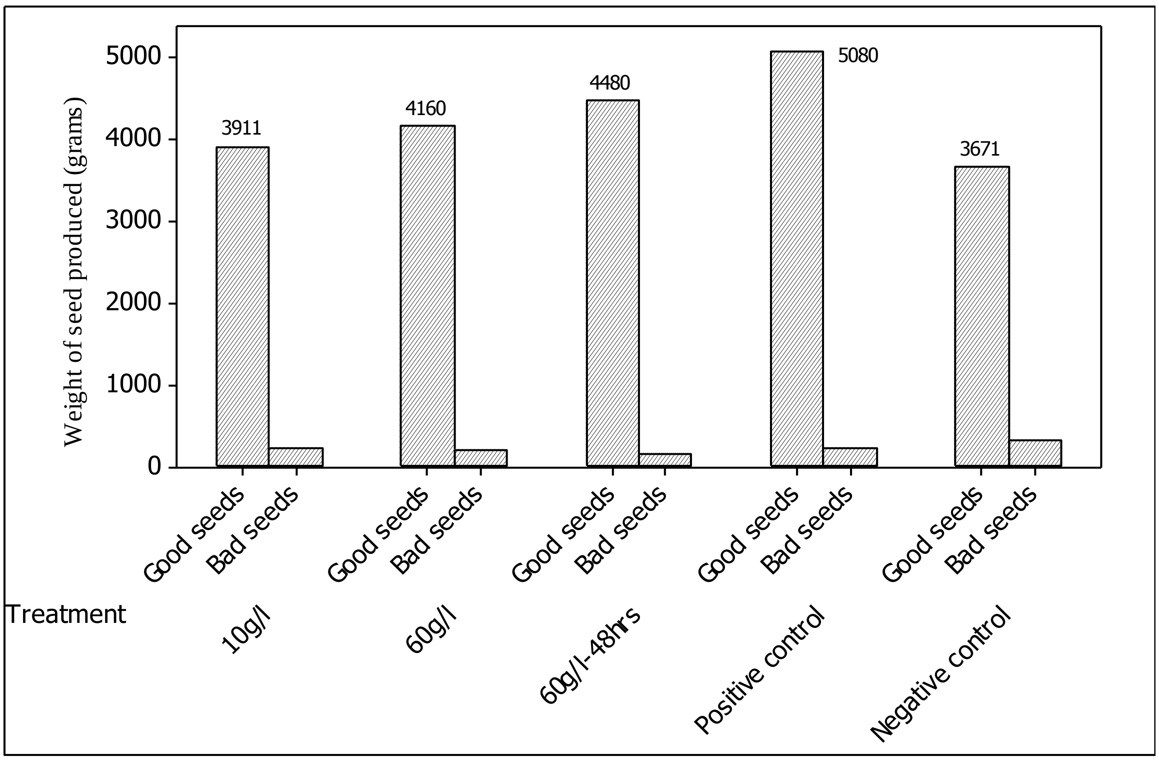

Figure 4.10: Bar graphs showing the weights of good and bad seeds produced from *T. urticae* infested plots sprayed with *T. vogelii* extract

Figure 4.11 shows that *T. minuta* at concentration 50 g/L soaked 24 hours sprayed on bean aphid infested field had the lowest yield of bean seeds (4100 g), followed by plot sprayed with 150 g/L that had 4390 g of good seed as compared to concentration 150 g/L soaked 48 hours that produced 4800 g of good bean seeds. The plots that were not treated recorded the least yield of 3758 g of good bean seeds.

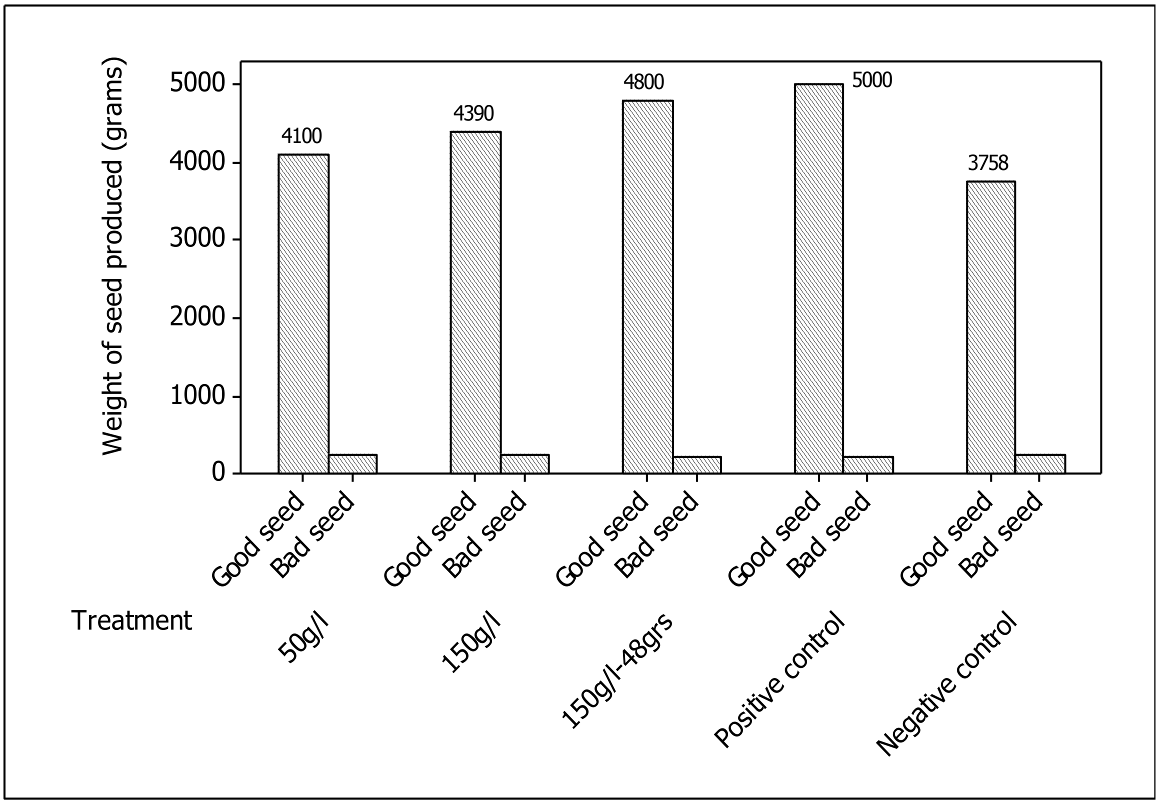

Figure 4.11: Bar graphs showing the weights of good and bad seeds produced from an *Aphis fabae* infested plot sprayed with *T.minuta* extract

Figure 4.12, shows the yield results of beans when *T.minuta* was sprayed on mites. Plots infested with mites and treated with Disect 10 EC insecticide (positive control) had the highest yield of good seeds (5080 g) followed with plots treated with *T.minuta*150 g/L under 48 hour soaking (4500 g). The untreated plot and the plot that had been treated with 50 g/L *T.minuta* soaked 24 hours had the same yield of good seeds 3900 g, while that of 150 g/L under 24hour soaking produced 4100 g of good bean seeds.

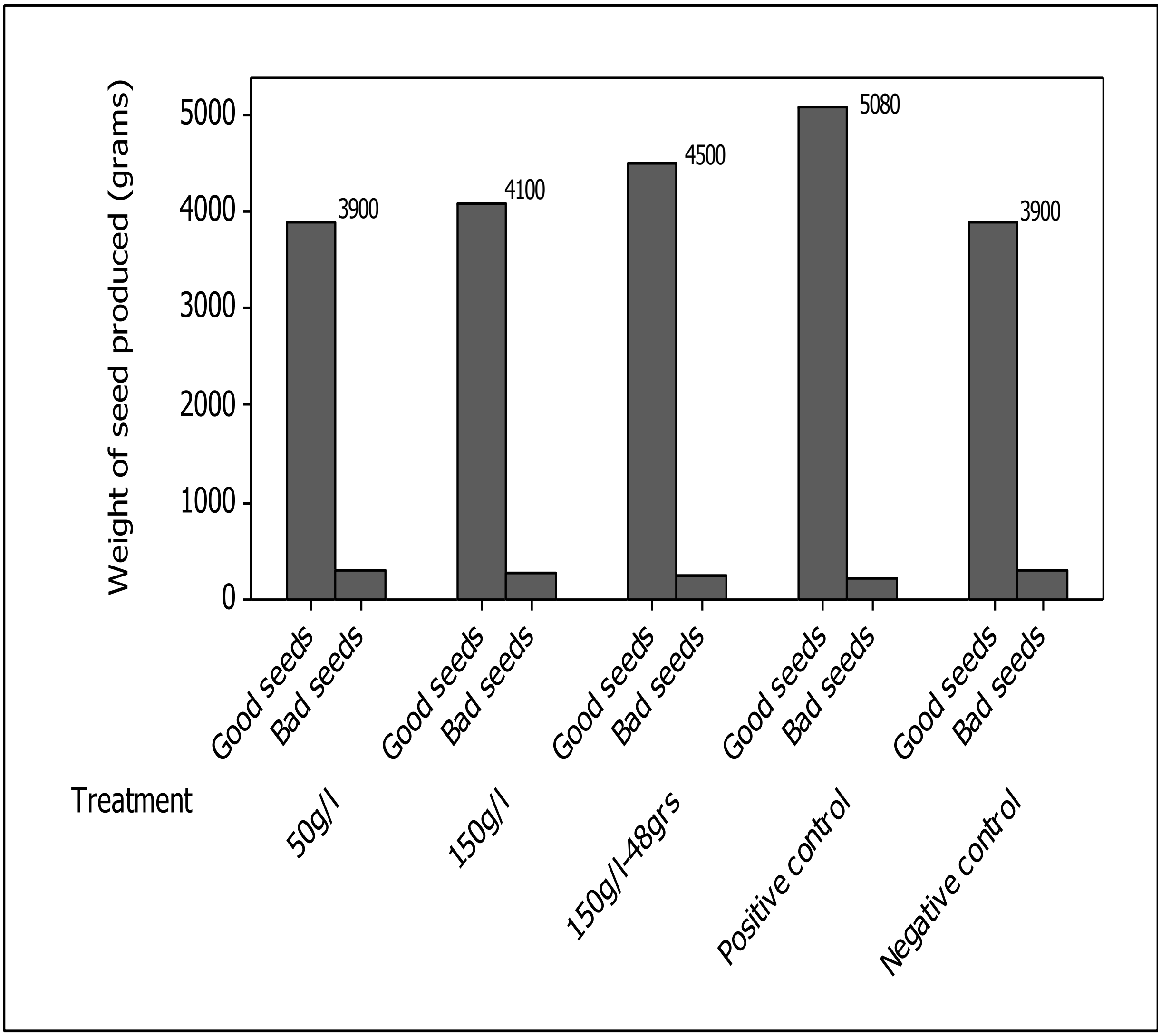

Figure 4.12: Bar graphs showing the weights of good and bad seeds produced from *T. urticae* infested plots sprayed with *T.minuta* extract

CHAPTER FIVE

DISCUSSION

5.1 Effects of different concentrations of *T. minuta* and *T. vogelii* on black bean aphids and red spider mites

Plant-derived products are increasingly being used to combat crop pests because they are natural and often assured to be safe to the environment, (Kumar *et al.,* 2000). Katsvanga and Chigwaza (2004) described these natural herbs as botanical insecticides, naturally derived insect toxins from plants. They have usually been regarded as a part of the plant's defense against plant feeding insects and other herbivores (Rosental and Janzen, 1979). Mugisha-Kamatenesi *et al*., (2008) have documented a survey of botanical extracts used as insecticides within the Victoria basin. Their study has demonstrated that usage of botanical pesticides pest management by the subsistence farmers is normal around Lake Victoria and among the plants used here are *Tagetes spp* and *Tephrosia vogelii*. In the current study, *T. vogelii* at a concentration of 60 g/L soaked for 48 hours gave the highest percent mortalities both in aphids and red spider mites in the lab environment than the same concentration soaked for 12 hours. This was followed by 30 g/L and 10 g/L soaked for 12 hours as the lowest recorded percent mortality in that order especially on red spider mites. This could be that as the concentration of the extract is reduced, the pesticidal property in the leaves become less effective.

According to Gaskin *et al.,*(1972), Lungu (1987), *T. vogelii* has a pesticidal property that is capable of killing vegetable pests, due to the isoflavonoid rotenone present in its leaves, explaining its traditional use as a fish poison as well as its efficacy against a range of insect pests (Ogendo *et al*., 2003). It is probably this pesticidal property that could have been responsible for the mortality of both mites and aphids in this experiment. *T. vogelii* at high concentration and longer soak period resulted to higher percent mortality on black bean aphids and red spider mites whereas lower concentration of *T. vogelii* resulted in lower percent mortalities recorded in red spider mites than in black bean aphids meaning that the efficacies component needed long soaking of botanicals to be fully released.

Several studies have been written on the use of extracts of *T.minuta* (Keita *et al*., 2000; Boeke, 2004), and it is reported to have a high seasonable level of effectiveness. In the present study, crude leave extract of *T. minuta* was used, since this is an easy mode of application for farmers to adopt. Trials were conducted in the laboratory and in the field. Laboratory results showed that, Mexican marigold extract at a concentration of 150g/l soaked 48hours had a higher percent mortality of aphids than red spider mites at the end of 24 hours exposure to the extract in the laboratory experiment than same concentration soaked for 24 hours which recorded very low percent mortality in red spider mites than in aphids. This was followed by 100g/l and 50g/l as having caused lowest percent mortalities in the laboratory environment in that order. Obongoya *et al.,* (2010) discovered that the bioactive extracts from *Tagetes* species have been shown to have insecticidal, nematicidal, and fungicidal properties. According to Devine *et al.*, (2001), red spider mites can highly tolerate several acaricides. This is due to its phytophagous nature, high reproductive potential and short life cycle. It is probably due to these characteristics that red spider mites seem to appear not to be drastically affected by especially *T. minuta* at lower concentration and then followed by *T. vogelii* also at a lower concentration and shorter soaking period of the botanicals tested.

The filter paper treated with *T.minuta* and *T. vogelii* was significantly repellent against red spider mites but not black bean aphids. The degree of repellence was greatly influenced by the plant species, the concentration of crude leaf extract, the soaking period of the plant species and the exposure time. *T.minuta* crude leaf extract at 150 g/L and soaked for 48 hours was more repellent on red spider mites (mean PR value=55 %) than same concentration soaked for 24 hours (mean PR value=12 %). *T. vogelii* at concentration 60 g/L soaked for 48 hours had a mean PR of 17% while the same concentration had a lower mean PR of 8 % and 30 g/L soaked for 12 hours had the lowest mean PR of 2 %. Studies done in the laboratory by Marta Ferreita Maja and Sarah (2011), shows that *T.minuta* repellence % protection can be up to 86.4% protection against *An. Stepehensi* for 6 hours, 84.2 % protection against *C. quinquefasciatus* for 6 hours and 7.5 % protection against *Ae. Aegypti* for 6hours. Ogendo *et al*., (2003), in their study, found out that maize treated with *T. vogelii* had higher percent repulsion (PR) values of up to 87 %.

The pest mortality and repellency results of the current study suggest that there exists good potential for the two local plant species, *T.minuta* and *T. vogelii* to be effectively used as insecticide in the traditional resource poor farming communities in Kenya and further a field in sub- Saharan Africa. Although many studies on botanicals have been conducted in the past (Bekele *et al*., 1996), sustainable use of botanical pesticides will boost the food security in those environments where investment in the synthetic pest control is uneconomical.

5.2 Effects of *T. minuta* and *T. vogelii* crude leaf extracts on aphids and mites in the experimental plots

Field results showed that *T. vogelii* extract concentration of 60 g/L that had been soaked for 48 hours gave the highest mortalities on aphids followed by red spider mites when sprayed and exposed for over 30 hours. The concentration of 10 g/L soaked for 12 hours recorded lowest mortalities in both bean aphids and mites.

When *T. minuta* was used on the pests species, a concentration of 50 g/L soaked 24 hours caused the lowest mean mortalities in especially mites, followed by aphids, while that of 150 g\L soaked 48 hours significantly killed many aphids followed by mites compared to the same concentration soaked at 24 hours which killed less. Many factors could have led to low mortality of pests in the field as compared to the laboratory experiment. Some of these factors could have been, at the time of spraying, it used to rain early in the day and this could have washed the extract before killing many pests, and also there could have been movement of pests in the field and thus not getting in contact with the extract at the time of spraying. From this study, it shows that these concentrations can be very effective to control red spider mites and also aphids in the field and in the green house, if the spraying can be done early before the level of infestation of the pests in the field reaches an economic threshold. The synthetic insecticides that were used for both aphids and mites respectively had killed quite many of them at the end of 30 hours of exposure.

Although *T. minuta* did not significantly reduce red spider mites' populations in comparison to *T. vogelii,* it has been used as an insecticide, and nematicide (Perich *et al*., 1994) and fungicide (Welty and Prestbye, 1993).

Whole plant extracts of *T. minuta* contain a phototoxin alphaterthienyl, which is even highly insecticidal (Philogene *et al*., 1985). However the reduced effectiveness of *T.minuta* on mites compared to *T. vogelii* could be explained by seasonal variation in activeness of essential oils being more active and concentrated in the plant in September compared to May per se (Kumar *et al*., 2000).

5.3 Yield harvest of bean seeds

The length of time that *Aphis fabae* is present on *Vicia faba* has been correlated with yield loss in a field study in South America. The study showed that high infestations at early stage (before flowering) decreased yields (total weight of seeds) by up to 42 % (Shannag and Ababner, 2007). The synthetic pesticide used on aphid infested plots of beans and red spider mites in the current study registered significantly high yields of good beans and low bad seeds. This was followed by plots sprayed with 60 g/L of *T. vogelii* soaked 48 hours and 12 hours respectively. A concentration of 10 g/L *T. vogelii* and under soaking of 12 hours sprayed on both aphids and mites infested plots registered a slightly lower yield of good seed and slightly higher yield of bad seeds in grams. The plots that were not treated registered slightly low yields with slightly higher number of bad seeds at harvest. Minja *et al.*, (2002) observed that application of *T. vogelii* leaf extract has shown beneficial effects on grain yield and quality when used appropriately. These applications have to be effected either very early in the morning or late in the evening to avoid degradation of the bio-pesticide due to exposure to light and air.

T. minuta concentration of 150 g/L that had been soaked for 48 hours when sprayed on both aphid and mite infested plots recorded high yield of good bean seeds as compared to the untreated plots. The bad seeds were fewer as compared to the plots that had been sprayed with a lower concentration; 50 g/L soaked 24 hours. This shows that *T.minuta* and *T. vogelii* seem to be a promising insecticide especially to the Kenyan subsistence farmers who can't afford synthetic pesticides. The application of botanical pesticide however needs to be done early after planting immediately the farmers notices the emergence of the pests in order to avoid multiplication and therefore destroy the flowers and pods (Minja *et al.,* 2002)

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusion

Based on the results of the present study, the following conclusions can be stated.

First and foremost the two botanicals can be used effectively to reduce the population of aphids and red spider mites on beans and other vegetables both in the field and in the green house although more frequent applications may be necessary.

Secondly both *T. minuta* and *T. vogelii* respectively can compare very well with the synthetic pesticides used in this research especially if the botanicals are collected during a favorable season.

6.2 Recommendations

Although findings from the current study have shown that *T. minuta* and *T. vogelii* have pesticidal properties, research efforts should focus not only on their efficacy, but also on mammalian toxicity, mode of action in insects, effects on nutritional quality, and stability of the compound for enhancement prudence in their usage.

Secondly, the efficacy seemed to be best at concentration 60 g/L for *T. vogelii* and 150 g/L for *T. minuta* hence can be considered ideal for control of both aphids and mites affecting crops.

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APPENDICES

Appendix I

Friedman test is a non-parametric test; an alternative of two-way ANOVA

The two factors being concentration and the pests

Friedman test for different concentrations of *T. vogelii* for aphids and mites

Estimated media			
Concentration	Aphids	Mites	Sum of Ranks
+ Ve Control	94.4	75.6	35
10g/l-12hours	31.6	10	7
30g/l-12hours	58	13	14
60g/l-12hours	68	34.4	21
60g/l-48hours	82	61	28
	Grand median = 66.80	Grand median = 38.80	
P = 0.000			

Statistical test is significant at p-value < 0.05

Friedman test for different concentrations of *T. minuta* for aphids and mites

Estimated media			
Concentration	Aphids	Mites	Ranks
+ Ve Control	94.8	76	35
100g/l24hours	66	18.8	14
150g/l24hours	71.6	28.6	21
150g/l48hours	75.6	47	28
50g/l-24hours	48	15.6	7
	Grand median = 71.20	Grand median = 37.20	
P = 0.000			

Statistical test is significant at p-value < 0.05