BIOACTIVITY OF AQUEOUS CRUDE EXTRACTS OF *Tephrosia vogelii* Hook AGAINST *Helicoverpa armigera* (Lepidoptera: Noctuidae) LARVAE IN CHICKPEA (*Cicer aurientinum*)

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A Thesis Submitted to the Graduate School in Partial Fulfilment of the Requirements for the Conferment of the Degree of Master of Science in Agronomy (Crop Protection Option) of Egerton University.

EGERTON UNIVERSITY

May 2012

DECLARATION AND RECOMMENDATION

Declaration

I declare that this thesis is my original work and has not been presented in this or any other University for any degree.

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Recommendation

This thesis has been submitted with our approval as the candidate's supervisors.

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DEDICATION

I genuinely dedicate the success of this research study to God for helping me to live and become a believing scholar.

To my parents, Frederick and Margaret, who understood the importance of education.

ACKNOWLEDGEMENT

This thesis study was funded by The National Council for Science and Technology (NCST) and Lake Victoria Resaerch Initiative (VicRes).

I wish to gratefully thank my academic advisors, Professor Arop L. Deng and Dr Joshua O. Ogendo for their truly exceptional guidance, critique, mentorship and advice during the entire Master's study. Special thanks go to all Egerton University Biotechnology Laboratory staff and Field 7 staff.

I wish to acknowledge my loving dad and mum, Frederick Wambua Kitema and Margaret Titi Wambua; my sweet daughter Margaret Titi; my sisters Phyllis, Alice, Merrick, Lilian and my brother Solomon for all the encouragement, moral and spiritual support during this difficult phase of life.

Lastly, the support and humour offered by Mr Anderson Ngolo and his entire family is greatly appreciated.

ABSTRACT

The pod borer (*Helicoverpa armigera*) is a major pest of chickpea (*Cicer aurientinum* L) in many areas of the world. In Kenya it causes up to 80% yield losses in chickpea. Laboratory and field studies were conducted to evaluate the bioactivity (contact toxicity, repellence, antifeedant and field efficacy) of aqueous crude extracts of *T. vogelii* Hook against *H. armigera*. Three laboratory bioassay studies (contact toxicity, antifeedant and repellence tests) laid out in Completely Randomized Design (CRD) with 5 replicates per treatment were conducted under controlled conditions of temperature (30 ± 2°C), relative humidity (68 ± 2%) and 12L: 12D at Egerton University's Biotechnology Laboratory. Field experiments were laid out in a Randomized Complete Block Design (RCBD) with three replicates per treatment. Twelve treatments of separate crude extracts obtained from leaves, succulent stems and pods/flowers of *T. vogelii* (0, 5, 10 and 20% w/v), negative control (ordinary water) and positive control (Dimethoate 2% v/v) were evaluated. Data on corrected percent larvae mortality, deterrence coefficient and percent repellence (PR values) were first homogenized using arcsine transformation before being subjected to analysis of variance (ANOVA) and treatment means separated by Least Significance Difference (LSD). Data obtained from various concentration-response bioassays (contact toxicity test) were subjected to probit regression analysis using EPA Probit Analysis Program version 1.4 and LC_{50} values and corresponding 95% fiducial limits obtained from derived regression equations. The LC_{50} values in a column were considered significantly different when 95% fiducial limits do not overlap. Results showed that toxic, antifeedant and repellent effects of aqueous crude extracts of *Tephrosia vogelii* against *H. armigera* larvae were significantly ($P = 0.0001$) influenced by intra-plant variability, concentration applied, exposure time and corresponding factor interaction effects. In the toxicity studies, at higher concentrations (25-40% w/v) of leaf extracts the LC_{50} values decreased with contact duration a manifestation of increased larval mortality. The positive control (Dimethoate at 2% v/v) and 40% w/v *T. vogelii* leaf extract were equally effective. In the antifeedant studies, the leaf extracts (20% w/v) and synthetic insecticide, Dimethoate at 2% v/v, equally had the highest antifeedant (reduced by 89.2%) effects on the *H. armigera* larvae. The results of the choice bioassays showed that, except for leaf and pod/flower extracts at 20% w/v and 1 h exposure time with moderate repellence (PR value: 40%), a strong dose-dependent attraction of *H. armigera* larvae was observed. The number of larvae that visited the chickpea leaves treated with *T.vogelii* extracts was higher compared to the negative control. Use of botanical pesticides will provide a sustainable insect pest control measure and also increase the chickpea yields.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF ACRONYMS

ANOVA	Analysis of Variance
ASAL	Arid and semi arid lands
Bt	*Bacillus thuringiensis*
CRD	Completely Randomized Design
FL	Fiducial Limits for $\mathbf{LC_{50}/LD_{50}}$ values
GC	Gas Chromatography
ICRISAT	International Crops Research Institute for the Semi Arid Tropics
IPM	Intergrated Pest Management
$\mathbf{LC_{50}}$	Lethal Concentration that kills 50% of test larvae or insects
$\mathbf{LD_{50}}$	Lethal Dose that kills 50% of test insects.
PR	Percent Repellence.
RCBD	Randomized Complete Block Design

CHAPTER ONE
INTRODUCTION

1.1 Background information

Chickpea (*Cicer aurientinum* L) is the third most important legume crop in the world after dry beans and peas (Kumar *et al.*, 2005). Currently Kenya produces approximately 40-55,000 tons of chickpea from approximate area of 18-20,000 ha (FAO, 2009; MOA, 2010; Kibe and Kamithi, 2007; ICRISAT, 2008). The national average yield is estimated at 540-1200 kg/ha (KARI, 2009) in farmers fields. However last four years (Njoro, Bomet and Koibatek, Naivasha in Rift valley) report average yields of about 1500-3000 kg/ha (ICRISAT, 2010; Kimurto *et al.*, 2009; Thagana *et al.*, 2010). Data on National demand are not available, but it is estimated to be approximately 70-100,000 tons of chickpea is consumed in Kenya annually (Economic Survey, 2010) and unknown quantity exported through Kenya.

Chickpea is a rich source of essential vitamins, minerals, and important amino acids like lysine and other secondary metabolites (Grusak, 2002). It is also an important component of animal feed. In Eastern and Southern Africa, chickpea is an important legume crop, with Tanzania, Malawi and Sudan being the leading producers (ICRISAT, 2006). In Kenya, it is a relatively new crop grown by few farmers in Eastern and Rift Valley Provinces. Preliminary local investigations show that chickpea is better adapted to varied agroecozones (Kibe and Onyari, 2006). The crop can fix substantial amounts of nitrogen in cereal-legume fallow relay systems, conserve soil moisture through addition of organic matter, act as 'break-crop" that facilitates control of diseases, pests and weeds and also improves the physical characteristics of various soil types (Taa *et al.*, 1997; ICRISAT, 2001; Cheruiyot *et al.*, 2001,2002). In recent years, chickpea has gained importance in Australia, Canada and the USA as a relay and rotational crop with cereals, mainly wheat (ICRISAT, 2008).

Chickpea yields have remained low for the past 2-3 decades due largely to biotic and abiotic stress factors, of which pod borer, *Helicoverpa armigera*, *Fusarium* wilt, *Aschochyta* blight, *Botrytis* mold and low temperatures are the most important (ICRISAT, 2007). Amongst biotic factors, flower and pod feeding Lepidopterans (*H. armigera, Maruca testulalis*, *Etiella zinckenella* and *Lampides* spp) account for up to 85% loss in grain yield in Eastern and Southern Africa (Minja, 2001). In Kenya, it causes average yield losses of 512 kg/ha (Minja, 2001).

Helicoverpa armigera alone causes global loss estimated at $325 million annually (ICRISAT, 1992; Sharma *et al.*, 2005a). Intensification of agriculture has exacerbated the *H. armigera* problem and farmers are resorting to frequent use of toxic insecticides. Due to the widespread use of insecticides to control this pest, particularly on cotton and other high value vegetables and grain legumes, the pest has developed considerable levels of resistance to conventional insecticides (Kranthi *et al.*, 2002). Although the benefits to agriculture from the pesticides cannot be overlooked, there is a greater need to develop alternative technologies, which would allow a rational use of pesticides, which leads to emphasis on integrated pest management (Lewis *et al.*, 1997).

Botanicals possess substances with a wide range of bioactivities principles. For example, extracts from the neem tree *Azadirachta indica* have antifeeding, anti-oviposition, repellent and growth-regulating properties. Studies done by Minja *et al.* (2002) indicated that plots sprayed with *T. vogelii* extracts applied three to four times had acceptable levels of insect control. Similar observations had earlier been reported from Uganda (Kyamanywa *et al.*, 2001). Mugoya and Chinsembu (1995) reported that aqueous fresh-leaf extracts of *T. vogelii* reduced the incidence of the spotted stalk borer *Chilo partellus* Swinhoe (Lepidoptera: Crambidae) in maize in Zambia. There were significant (P= 0.05) increases in grain yield in the sprayed plots and an improvement in grain quality. Kyamanywa *et al.* (2001) observed similar yield increases through the application of *Tephrosia* leaf extract in pigeon pea in Uganda. Similar findings were reported by Smith and Baudoin (2000) whereby plots sprayed by *Tephrosia vogelii* aqueous crude extracts produced an average yield of 671kg/ha slightly lower than those sprayed by the synthetic pesticides (875kg/ha). Preliminary studies have shown that crude leaf extracts of *T. vogelii* has been used to control insects feeding on pigeon pea (Minja *et al.*, 2002). *T. vogelii* has a high potential in controlling field insect pest hence extensive studies on *T. vogelii* to evaluate the bioactivity (contact toxicity, repellence, antifeedant, field efficacy) against field lepidopteran insects should be encouraged. The aim of this research was to evaluate the bioactivity of aqueous crude extracts of *T. vogelii* against *H. armigera* in chickpea, with a view to have an environmentally friendly, cost effective and a safe control method.

1.2 Statement of the problem

Pod borer (*H. armigera*) is an important insect pest that threatens production of chickpea worldwide, especially in countries like India where the crop is one of the staple food crops. In Kenya, Chickpea is an emerging crop with potential to reduce food insecurity in the dry land areas due to its inherent drought tolerance. The current chickpea average yield in Kenya is 1.7 t/ha. The low yield is partially attributed to drought, *Aschochyta* blight and *H. armigera* of the causal agent *H. armigera* limits its productivity substantially causing up to 80% yield losses. Currently, the application of chemical spray insecticides is the common method of controlling *H. armigera* in chickpea. However, chickpea is grown by resource poor farmers who cannot afford the high cost of pesticides. These synthetic pesticides are also of environmental concern and responsible for human health problems such as eye, skin, lung, cardiovascular, immunosuppressive and neurological disorders. Hence, the need to seek alternative strategies to combat the pest attacks in the field. The use of botanicals holds good promise for cost-effective, environment- friendly and sustainable field insect pest management in subsistence agriculture.

1.3 Objectives

1.3.1 Broad Objective

To contribute to increased chickpea production and food security among small scale farmers through improved insect pest management in Kenya.

1.3.2 Specific Objectives

To determine the:

1. Efficacy of aqueous crude extracts of *Tephrosia vogelii* on larval stage of pod borer (*Helicoverpa armigera*).
2. Antifeedant and repellent effects of aqueous crude extracts of *T. vogelii* against the larval stage of pod borer (*H. armigera*).
3. Effects of spraying aqueous crude extracts of *T. vogelii* on the population of *H. armigera* and yield and yield components of chickpea.
4. Intra-plant variability in bioactivity (toxicity, repellence and anti-feeding) against *H. armigera* and effects on the grain yield of chickpea.

1.4 Hypotheses (Ho)

1. Aqueous crude extracts of *T. vogelii* have no toxic effects on larval stage of pod borer (*H. armigera*).
2. Aqueous crude extracts of *T. vogelii* have no antifeedant and repellent effects on larval stage(s) of pod borer (*H. armigera*).
3. Aqueous crude extracts of *T. vogelii* have no effects on the population of *H. armigera* and the yield and yield components of chickpea.
4. Intra-plant variability does not affect the bioactivity (toxicity, repellence and anti-feeding) of aqueous crude extracts of *T. vogelii* against *H. armigera* and the grain yield of chickpea.

1.5 Justification

Chickpea is a hardy crop and grows under low moisture and soil fertility regimes. It also contributes to food security through supply of plant proteins which is a serious problem in the semi-arid tropics leading to human malnutrition, starvation and famine. In addition it fixes atmospheric nitrogen and yields a nutritious grain with a potential yield of 5t/ha, thus improves soil fertility and nutritional status of humans. Although effective control of insect pests in chickpea can be achieved through the use of conventional insecticides, in the semiarid tropics, an estimated loss of more than $328 million is still being reported, despite a $500 million worth of pesticides applied to control this pest worldwide (Sharma, 2001). In Kenya, less than 10% of farmers use chemicals owing to their high costs, toxicity to non-target organisms including man and their adverse effects on the environment. Most of the documented studies show that the majority of farmers use fresh crude extracts of *Tephrosia vogelii* for control of *Helicoverpa armigera* in the field. In addition, *T. vogelii* is locally available and well accepted in the society. Uses of botanical pesticides are known to be cost- effective field insect pest control agents that contribute to increased crop yields. Also chickpea being a legume fixes atmospheric nitrogen which improves the soil fertility. Lastly, propagation of *Tephrosia vogelii* is easy and does not need technical advice since its through seeds. Use of botanical pesticides in *H.armigera* control will reduce the expenses of purchasing synthetic pesticides hence improve the economy of households. Hence, botanical pesticides provide an environmentally sound and sustainable pest management alternative to the synthetic pesticides.

CHAPTER TWO

LITERATURE REVIEW

2.1. Production and Economic importance of Chickpea

Chickpea is a widely distributed crop with its origin in the Mediterranean region around Turkey. It is grown in over 45 countries with average yields of 818 kg/ha (ICRISAT, 2005; 2007). The crop is a rich source of essential vitamins, minerals, and amino acids such as lysine and other secondary metabolites (Grusak, 2002). Chickpeas also contribute to soil fertility replenishment through biological nitrogen fixation (BNF) and the diversification of cereal-based cropping systems. Through their BNF efficiency, chickpeas are a component of sustainable cropping systems which reduce need for extra N-fertilizer applications (Serraj, 2004). In addition, chickpea often attracts higher market prices than other staple legumes, making it an important source of income to small scale farmers.

Nutritionally, chickpeas comprises 20-28% crude protein, 40-45% total carbohydrates, and 4-10% oil (Abbo *et al.,* 2005). Chickpea contain approximately 50% oleic and 40% linoleic acids. It is also an excellent source of folate, vitamins B_6, & Ca, and zinc. Chickpea is rich in fibre and minerals (phosphorus, calcium, magnesium, iron and zinc) and supplies higher levels of carotenoids (β–carotene, cryptoxanthin, lutein and zeaxanthin) than genetically engineered 'golden rice' (Ranga- Rao and Shanower, 1999; Abbo *et al.,* 2005). Chickpea is relatively free from various anti-nutritional factors such as protease inhibitors and polyphenols.

There are two types of chickpea, namely, Desi and Kabuli. The Desi types have angular seeds and are brown to pale tan in colour. The seeds are small with 100-seed weight of 13-25g. Desi chickpeas are mainly grown for the dry seeds, which are usually dehulled, and may be used whole, split or milled. The Kabuli types have large, rounded seeds with 100-seedweight of 55-60g, cream to white in colour and may be consumed as dry seeds or green pods in salads and vegetable mixes (Ranga- Rao and Shanower, 1999).

Chickpea acreage has decreased slightly globally, but has been stable at 9 million hectares (ha) in Asia the leading producer and consumer for the past 25 years. However, production in Asia has increased by 39%. Even then, the current average yield in Asia (0.8 t/ha) is low, and far below the potential yield (5 t/ha) (ICRISAT, 2006). The global demand for chickpea by 2010 is

estimated at 11.1 MT (up from the current 8.6 MT). In East Africa, Tanzania is the leading producer where the crop is grown mainly under conserved moisture after wheat and maize harvests (ICRISAT, 1996). A combination of productivity enhancement through crop improvement using biotechnological tools, integrated crop management and expansion of crop to new niches and production systems are needed to achieve this target of 11.1Metric tonnes (MT) (ICRISAT, 2007).

In Kenya, preliminary investigations have shown that chickpea is highly adapted to varied agroecozones (Kibe and Onyari, 2006) and can fix 140 kg N/ha of nitrogen in cereal-legume fallow relay systems, conserve soil moisture through addition of organic matter and act as 'break-crop" that facilitates control of diseases, pests and weeds and improve soil physical characteristics in Rift valley highlands (Cheruiyot *et al.*, 2001, 2002).

2.2 Botany of Chickpea

According to van der Maesen (1972), the cultivated chickpea has been taxonomically placed in the genus *Cicer*, which belongs to the family *Fabaceae* and its monogeneric tribe *Cicereae* Alef. Presently, the genus consists of 43 species divided into 4 sections, namely *Monocicer*, *Chamaecicer*, *Polycicer* and *Acanthocicer*. This classification is based on morphological characteristics and geographical distribution (Van der Maesen, 1972). Eight of the *Cicer* species share the annual growth habit with chickpea and are of particular interest to breeders. There are two broad groups of chickpea; the *desi* types which have brown coloured seed coats and are usually de-hulled and split to make *dhal* or flour (*besan*), and the *kabuli* types which are white or cream-coloured and are often cooked as whole grains. Chickpeas have the best nutritional compositions of any dry edible legume and are mainly used for human consumption; haulms are used for animal feed. In addition, chickpea improves soil fertility through biological nitrogen fixation up to 140 kg N/ha (ICRISAT, 2005).

Plants are multiple branched, spreading growth habit annuals ranging from 20cm to 100cm tall. Some chickpea varieties have compound leaves (8 to 20 leaflets) and some have simple leaves, which are pubescent (hairy) in appearance. Chickpea leaves exude malic and oxalic acids. Kabuli varieties is generally taller than the desi varieties. Because of its deep tap root system, chickpea can withstand drought conditions by extracting water from deeper in the soil profile. Flowers (self pollinated) which are borne in groups of two or three are 1.27 cm to 2.54 cm long

and come in purple , white pink and blue colour depending upon variety. Each flower produces short pubescent pod which is 1.9 cm to 5.04 cm long and which appears to be inflated. One or two seeds (1.27 cm to 2.54 cm) are present in each pod. The seeds come with either rough or smooth surfaces and can be cream, yellow brown, black or green in colour. There is a definite groove visible between the cotyledons about two-thirds of the seed, with a beak – like structure present (Van Rheenen *et al.,* 1991).

2.3 Major insect pests of chickpea

The major insect pests of chickpea include pod borer or pod feeder (*Helicoverpa armigera*), leaf miners (*Liriomyza cicerina* and *Phytomyza cicerina*), seed beetle (*Callosobruchus spp*), armyworm (*Spodoptera exigua*), and semi looper (*Autographa nigrisigna*) (Ranga- Rao and Shanower, 1999). However, their incidence and pest status are generally restricted to certain regions and cropping systems. Aphids (*Aphis craccivora*), Cutworms (*Agrotis ipsilon*), and termites cause localized problems whereas bruchid infestations in storage are widespread (ICRISAT, 1990; Ranga- Rao and Shanower, 1999).

The pod borer (*Helicoverpa armigera*) is the most important insect pest of chickpea in the world (Sharma, 2001). In addition to feeding on high value crops, it is an extremely versatile pest owing to its high fecundity, host range of over 180 different plant species, natural ability to diapause during adverse conditions and migration over long distances (Ranga- Rao and Shanower, 1999).

2.4 The Economic importance and Biology of *Helicoverpa armigera* (Lepidoptera: Noctuidae)

Helicoverpa armigera is currently placed on Annex IA II of Council Directive 2000/29/EC, indicating that it is considered to be relevant for the entire EU and that phytosanitary measures are required when it is found on any plants or plant products. EU member states, in particular The Netherlands and United Kingdom, frequently intercept *H. armigera* on imported produce (especially Dianthus and Rosa cut flowers, Phaseolus, Pisum and Zea mays) and some ornamental cuttings. These imports often originate from Third Countries. Furthermore, *H.armigera* is capable of migrating over lond distances during summer, leading to transient findings all over Europe.

The pest can attack many species that are of economic importance in the PRA area, such as tomato, maize, beans and ornamental plants such as Chrysanthemum and Pelargonium (Blues *et al.*, 2009; Kurban *et al.*, 2009)

Helicoverpa armigera is a moth belonging to the family Noctuidae. It is a major insect pest because its larvae can feed on a wide range of economically important crops from cereals to horticultural crops. Freshly laid eggs are usually pale white, eventually turn pale brown. Eggs turn dark brown before hatching. Eggs are ridged and clinodome shaped. A female moth lays 150 – 1500 eggs during its life span with an average of 450 eggs. Eggs are usually laid between 9 pm-12 midnight.

Larva undergoes six instars; freshly hatched larva is white and later turns pale. Head, thorax and legs turn brown and faint red markings appear on the dorsal surface. Second instar larva becomes pale white and black spots prominent on the body. First instar larvae measures 1.75 mm in size compared to 3.5-4.0 and 9-10 mm for second and third instar larvae, respectively. As larvae pass through 4^{th}, 5^{th} and 6^{th} instars, their body colour changes according to food and weather conditions. A full-grown larva measures 35 – 42 mm in length. In later instars, stripes (continuous or broken) appear on the dorsal and lateral sides. White hairs also can be seen as soon as larva approaches last instar. Freshly hatched larva gets its food from the broken egg and later on eats part of leaves on which egg was laid. In the last instar of its development, larva consumes 80% of its food and spreads havoc in the crops (Ranga- Rao and Shanower, 1999; Nasreen and Mustafa, 2000; Ali *et al.*, 2009).

Cannibalism is present in the bigger larvae. Bigger larvae often travel on different parts of plant and attack other larvae and eat them. Bigger larvae bore into the fruits and consume the inner contents by inserting their heads into the fruits, the lateral part of their body remains outside the fruit. The life span of larvae depends upon factors like temperatures and humidity. Larval stage is completed within 15-30 days depending upon the weather conditions. It leaves the feeding spot and crawls down on the ground for pupation. It burrows into the soil approximately 2.5-17.5 cm deep depending on the soil texture for pupation. Pupa is brown in colour and measures 14-18 cm in length. Its anterior and posterior, both ends are round and two spikes can be seen on the posterior side. The moth emerges in 5-8 days. Total life span of *H. armigera* takes 20-38 days influenced by temperature and humidity.

2.6 Methods of Pest Control

2.6.1 Chemical control

Pyrethoids such as cypermethrin have been used to control *Helicoverpa armigera* in chickpea (Chandrakar and Srivastava, 2001). Recently, Steward (indoxacarb) was introduced as an insecticide that is more effective than pyrethoids as it acts as antifeedant that destroys the pest's capacity to get food and thus provides immediate protection (Bhagwat, 2001). Tracer, which follows the same mode of action, contains spinosad as its active ingredient - a new class of naturally produced metabolite from a bacterium. In Kenya Dimethoate is commonly used by farmers (ICRISAT, 2002).

The consequence has been in relation to environmental pollution leading to contamination of soil, water and vegetation. Persistent toxic residues have been found to contaminate soils, aquatic sediments in water bodies and agricultural produce (Soon, 1997). The health impairments to humans include eye, skin, and lung, cardiovascular, immunosuppressive and neurological disorders. The second serious problem of unregulated use of chemical pesticides has led to the development of pest resistance due to their non-specificity and effectiveness (Thomas, 1999). Besides, due to their non-specificity, the chemical pesticides also eliminate the other beneficial insects and natural enemies of the pest resulting in a loss of balance in the ecosystem (Soon, 1997).

2.6.2 Biological control

Use of *Bacillus thuringiensis* (Bt) toxin for controlling *H. armigera* infestation is an effective alternative to chemical pesticides. However, the lethal effect of this toxin on insect population has led to a rapid development of resistance against it and hence the need for alternative strategies to combat the pest attacks in the field (Lewis *et al.*, 1997). The unregulated use of *Bt* has the potential to develop resistance in insects in a short period of time, similar to chemical pesticides (Brousseau *et al.*, 1999). One biological insecticide known to control *H. armigera* is nuclear polyhedrosis virus but it is inactivated by UV light (Ranga- Rao and Shanower, 1999).

2.6.3 Use of Plant Extracts for Field Pest Management.

Plants are rich sources of bioactive compounds that can be used to develop environmentally safe pest managing agents. The intergration of botanical pesticides in the management of both storage and field insects offers a more promising alternative control method compared to the use of conventional insecticides. This is because natural products are renewable, readily available, biodegradable, more selective and generally low in toxicity. Documented information has shown that botanicals possess both toxic, antifeedant and repellent activities. Strong contact toxicity of essential oils of *Ostericum sieboldii* (Apiaceae) (13.82 μg/adult) has been reported against *Sitophilus zeamais* and *Tribolium castaneum* (Liu *et al.*, 2011). Also in a study to control Oblique banded leaf roller, powders of *Humulus lupins* (4% w/w) reduced larval survival by 57% (Gokcel *et al.*, 2010). Additionally, studies conducted with ethanol extract (5% v/v) of *Azadirachta indica* against *Pieris brassicae* Linn caused a mortality of 82.5% (Anurag and Rakesh, 2009). Similarly, 4.0 mg/cm^2 acetone extract of *Sterculia foetida* (L) seed extracts caused 100% mortality to *Spodoptera litura* (F) after 24 hours (Usha Rani and Rajasekharreddy, 2009). Botanicals have also shown antifeedant activity towards castor semi-looper, *Achaea janata* L and Asian armyworm, *Spodoptera litura* (Fab). In a laboratory study, *Sterculia foetida* (L) seed extracts at 10% w/v produced 100% feeding deterrent activity against *Achaea janata* (Usha Rani and Rajasekharreddy, 2009). The strong repellenct activity of ethanol extract (5% w/v) of *Azadirachta indica* (PR 94%) was very beneficial in controlling *Pieris brassicae* Linn (Anurag and Rakesh, 2009). Similarly, *Azadirachta indica* at 10% w/v repelled 100% Okra flea beetles, *Podagrica uniforma* (Echereobia *et al.*, 2010). Farmers experience a challenge in formulating the botanicals and knowing the exact concentrations which can give the best results. In addition, knowing the best time for application of the botanicals is also a problem. Also they lack proper knowledge on the right time for harvesting the botanicals and the shelf life of the botanical. Lastly, the extraction of the active compounds in the leaves of the test plants are affected by the solvent used in extraction (Obilo *et al.*, 2005; Matovu and Olila, 2007b)

2.6.4 Aqueous extracts of *Tephrosia vogelii* Hook, and insect control

A plant species with a potential to be used as a natural pesticide is Fish poison bean, *Tephrosia vogelii* Hook (Fabaceae) which is also important in improving soil management especially for the control of *Striga hermonthica* (Mathias, 1997). *T. vogelii* was widely used in pest control before the invention of DDT. The chemical in the leaves is called rotenone, and is classified by the World Health Organisation as a moderately hazardous or class II pesticide. The rotenoids present in its leaves are effective in killing numerous pests. Also, rotenone breaks down within 3 - 5 days after application and is of relatively low mammalian toxicity of most mammals (Ibrahim *et al.,* 2000; Neuwinger, 2004). Additionally to the insecticidal compounds, the leaves of T. vogelii also contain 5- methoxyisolon chocarpin , which is a highly effective antifeedant, active at 10 ppm for some pest species (Simmonds *et al.,* 1990). *T. vogelii* has both acaricidal and larvicidal properties. Studies done using its above ground parts produced promising acaricidal effect causing 100% kill of the exposed nymphs and adult ticks within 24 h. In related studies *T.vogelii* powder caused 100% kill of mosquitoes larvae within 8 minutes (Kambewa *et al.,* 1997; Matovu and Olila, 2007a, b) and the insecticidal effect (rotenone, deguelin and tephrosin) of aqueous extracts of *T. vogelii* (16% w/v) has been used in the management of American boll worm in cotton (Mathias, 1997) Studies have also reported antifeedant effects of *Tephrosia spp* on spotted cereal stem borer (Machocho, 1992). Lastly *T. vogelii* was most effective in controlling insect pests of cowpea in the field (Adebayo, 2007). Little local research efforts have been done regarding use of *T. vogelii* in control of field insects of chickpea in Kenya.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Site Description

The study was conducted at Egerton University (0°20'S, 35°56'E) situated along Nakuru- Mau Narok road, approximately 190 km to the South-West of Nairobi. Egerton University is at an altitude of 2250 metres above sea level (masl) with mean annual precipitation and temperature of 1300 mm and 15°C, respectively. It is situated in the agro ecological zones LH_3 (Jaetzold and Schmidt, 1983). The soils are vintric mollic andosols that are well drained, deep to dark reddish brown friable and silt clay soils with humic top soils. (FAO/UNESCO, 1990).

3.2 Procedures

3.2.1 Collection and Preparation of Crude Aqueous Extracts of *Tephrosia vogelii*

Separate samples of fresh leaves, succulent stems, and pods / flowers of *Tephrosia vogelii* were collected in sufficient quantities from Egerton University's Tatton Farm (Field 15) and transported in labelled bags to a laboratory room. Thereafter the samples were shade-dried at ambient temperatures 18-28°C for 2 weeks and further oven dried at 35°C for 48 h (Ogendo, 2000). Dry samples were ground into fine powder using an electric laboratory hammer mill. Each of the three plant parts was tested at four concentrations (0, 5, 10 and 20% w/v) using weighed samples of dry crude powders dissolved in water for 24 h at room temperature (Garcia-Mateos *et al.,* 2007). Ordinary water and Dimethoate at 2% v/v were used as negative and positive controls, respectively. The powders were stored in an air-tight glass jar in a cool place away from sunlight.

3.2.2 Mass Rearing of *Helicoverpa armigera* Larvae

Heavily infested chickpea pods were obtained from feed stock planted of variety ICCV 97105 (Desi) according to Nasreen and Mustafa (2000) with modifications. Sixth instar larvae from natural infestation were collected and transferred into Petri dishes to avoid cannibalism. Chickpea leaves and pods were used as larval food. Observations were made until the larvae changed to pupa and then to adult moth. Moths were sexed and paired according to Nasreen and Mustafa, (2000). The sex of newly emerged adults was determined by the colour of forewings. In males, the forewings were greenish whilst in female the wings were brown. The pairs were kept in transparent plastic jars (30×15 cm) separately.

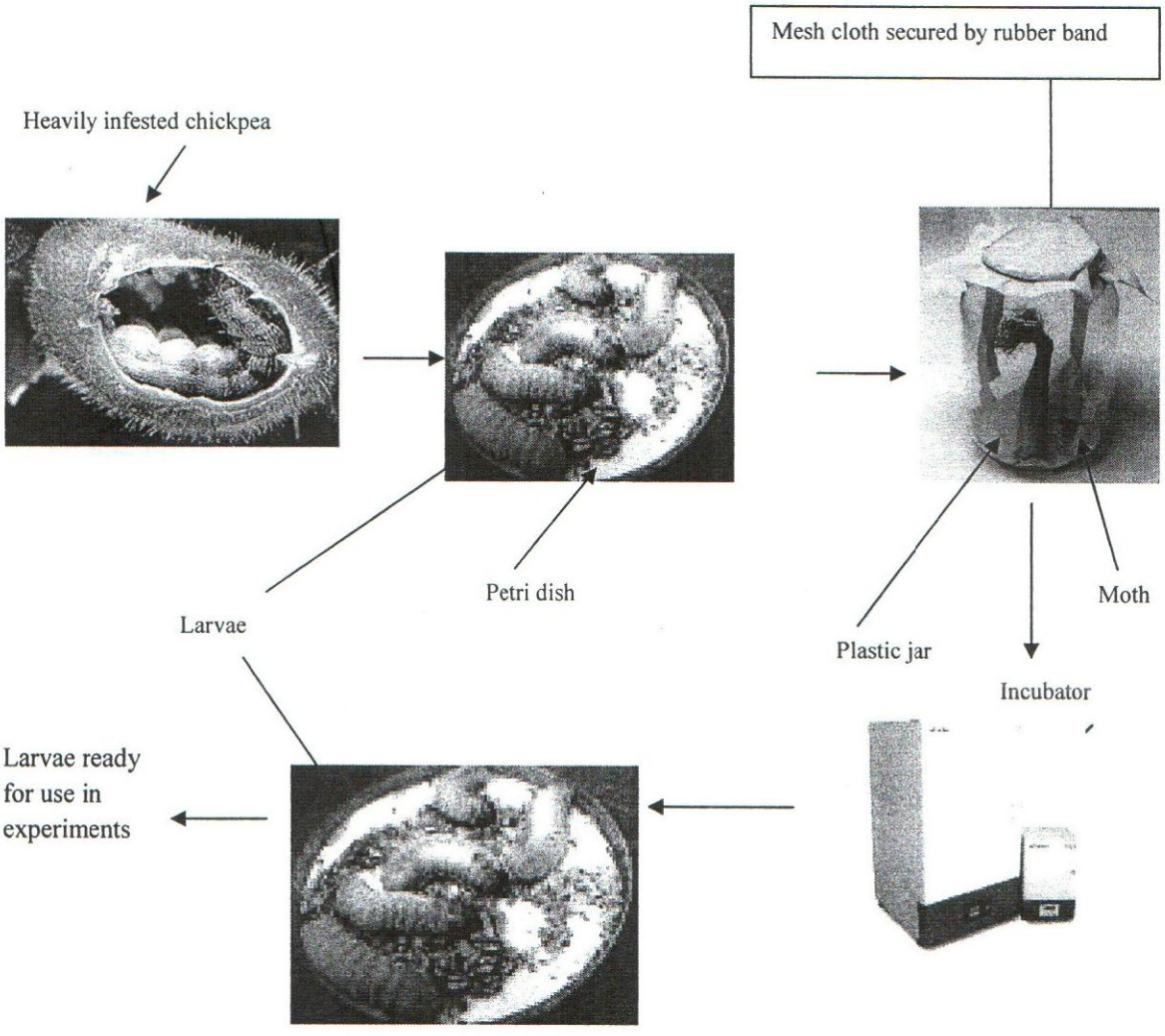


Fig. 1: Diagram showing the mass rearing of test *H. armigera* larvae

The walls and lid of each jar was perforated to allow ventilation. A strip of green cotton cloth towelling (6 cm by 17 cm), to mimic natural vegetation, was kept inside each jar for egg collection. Sucrose-based adult diet containing honey (10%) and water (90% v/v) was provided in a 5 ml plastic vial on cotton wool. The vials containing food were kept at the bottom of the jar. Fresh diet was provided and eggs laid by a pair of *H. armigera* adults were collected every 24 h and kept separately on the towelling in the incubator at 28^0C. After hatching, neonate larvae were transferred into Petri dishes (diameter: 15 cm). Fresh chickpea leaves and pods were used as larval food.

3.3 Data Analysis

The actual and corrected percent larvae mortalities in contact bioassays were computed according to Asawalam` *et al.* (2006) and (Abbott, 1925) in equations 1 and 2, respectively:

$$\text{Actual Mortality (\%)} = \frac{(N_D)}{(N_T)} x100 \qquad \text{Equation 1}$$

$$\text{Corrected mortality}\,(P_T) = \frac{(P_O - P_C)}{(100 - P_C)} X100 \qquad \text{Equation 2}$$

Where P_T, P_O, and P_C represent the corrected, observed and control percent mortalities, respectively. N_D and N_T represent number of dead larvae and total number of larvae used in the experiment, respectively.

Data on corrected percent larvae mortality, deterrence coefficient and percent repellence (PR values) were first homogenized using arcsine transformations before being subjected to analysis of variance (ANOVA) and treatment means separated by Least Significance Difference (LSD) (Talukder and Howse, 1995; Ogendo *et al.*, 2008). Data obtained from various concentration-response bioassays (contact toxicity test) were subjected to probit regression analysis using EPA Probit Analysis Program version 1.4 and LC_{50} /LD_{50} values and corresponding 95% fiducial limits obtained from derived regression equations (Finney, 1971).The LC_{50} values in a column were considered significantly different when 95% fiducial limits do not overlap.

CHAPTER FOUR

CONTACT TOXICITY OF AQUEOUS CRUDE EXTRACTS OF *Tephrosia vogelii* HOOK AGAINST *H. armigera* LARVAE

Abstract

A study was conducted to evaluate the contact toxicity of aqueous crude extracts of *T. vogelii* against *H. armigera* larvae. Aqueous crude extracts obtained from leaves, pods/ flowers and succulent stems of *T. vogelii* were evaluated at four rates (0, 5, 10 and 20% w/v). Ordinary water and synthetic insecticide, Dimethoate (Rogor E40)® 2% v/v, were included as negative and positive controls, respectively. Laboratory bioassay was laid out in a completely randomized design (CRD) with four replications. Field bioassay was laid out in a randomized complete block design (RCBD) with 3 replications. Results showed that contact toxicity significantly (P = 0.0001) depended upon plant part assayed, concentration of the extract applied, exposure time and corresponding factor interactions. The positive control (Dimethoate (Rogor E40)® 2% v/v) caused 100% kill 24 h after treatment compared to 74% kill for aqueous crude *T. vogelii* (40% w/v) leaf extract. At the highest concentration (40% w/v) of *T. vogelii* leaf extract, the end-point *H. armigera* larvae mortality of 100% was attained 72 h after treatment. Results from the field showed that the positive control (Dimethoate (Rogor E40)® 2% v/v) and aqueous crude extracts of *Tephrosia vogelii* 40%w/v were equally high yielding. The relationship between the larval density and the percentage of pod damaged was worked out by correlation coefficient and regression equations. Successful adoption of *T. vogelii* crude extracts for control of *H. armigera* promises an environment -friendly control measure as a substitute to the synthetic chemicals. Management of *Helicoverpa armigera* by use of botanical pesticides will greatly increase chickpea yields and hence increase the household incomes of small scale farmers.

Key words: Contact toxicity, *Tephrosia vogelii, Helicoverpa armigera , Cicer aurentium*

4.1 Introduction

Plants provide an alternative to currently used pesticides for the control of plant pests, as they constitute a rich source of bioactive chemicals (Daoubi *et al.*, 2005; Dawit and Bekelle, 2010). *T.vogelii* is known to have insecticidal properties that are important in the control of both storage and field pests. Currently, a lot of research on toxicity of *T. vogelii* in the control of storage pests has been done and it has shown promising results. Plant powders from *T. vogelii* showed a 93.7%

reduction in insect damage by bruchids (Koona and Dorn, 2005). Essential oils from *T. vogelii* have also shown up to 83% kill of storage insects whereas *T. vogelii* powders caused a 85.0-93.7% mortality of storage insects (Ogendo *et al.*, 2003: Ogendo, 2008). Studies done on larger grain borer showed that *T. vogelii* (5% w/w) was toxic after 96 h (LC_{50}:0.033%) (Mukanga *et al.*, 2010). The objective of this study was therefore to evaluate the intra-plant variability in contact toxicity of aqueous crude extracts obtained from aerial parts of *T. vogelii* against *H. armigera* larvae.

4.2 Materials and Methods

4.2.1 Mass rearing of test larvae

The larvae used for contact toxicity were obtained from the mass reared culture as described in section 3.2.2 above.

4.2.2 Bioassays

(a) Laboratory toxicity studies

The inner walls of 100 ml sample bottles were coated with a solution of aqueous crude extracts of *T. vogelii* according to Brigitte *et al.* (2002) with modifications. Separate samples of leaves, succulent stems and pods/ flowers of *T. vogelii* crude extracts were used instead of stemona alkaloids. Separate samples of leaves, succulent stems and pods/ flowers of *T. vogelii* crude extracts were each evaluated at four rates (0, 5, 10 and 20% w/v) except for leaves in which a maximum concentration of 40% w/v was tested due to rising toxicity in the dose-response in the preliminary studies. Ordinary water and Dimethoate (Rogor E40)® (2% v/v) were used as negative and positive controls, respectively. A total of 12 treatments arranged in a CRD with 3 replicates per treatment were evaluated. Ten (10) second or third according to Sharma *et al.*, 2005 instar larvae of *H .armigera* (N_T) were introduced into separate test bottles. Fresh chickpea leaves were administered after every 24 h. The number of dead larvae (N_D) was recorded 24, 48, 72, 96, 120, 144 and 168 h after setup and actual percent larvae mortality computed according to Asawalam *et al.* (2006) (Eq. 1 in section 3.3). Actual mortality data were corrected for natural mortality using Abbott (1925) formula (Eq. 2 in section 3.3). The data analysis model used is as shown below:

$Y_{ijk} = \mu + P_i + T_j + C_k + PC_{ik} + PT_{ij} + \Sigma_{ijk}$

Where

Y_{ijk}= observation

μ = general mean

P_i=i^{th} replicate in the j^{th} plant part

T_j = j^{th} Treatment

C_k= k^{th} concentration in the i^{th} replicate and the j^{th} plant part

PC_{ik} = is the interaction between the i^{th} replicate and k^{th} concentration

PT_{ij} = is the interaction between i^{th} replicate and the j^{th} plant part

Table 4.1: Cummulative percent mortality (Mean±SE,n=3) of *H.armigera* larvae as affected by aqueous extracts obtained from aerial parts of *T.vogelii*.

		Cumulative percent larval mortality (Mean±SE; n=3) CONTACT TIME (HRS)						
plant part/Conc (%w/v)	N	24	48	72	96	120	144	168
Pods/flowers								
0	10	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
5	10	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
10	10	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
20	10	11.11±0.59	11.11±0.59	11.11±0.59	11.11±0.59	25.92±3.71	51.85±7.54	62.97±3.71
$LSD_{0.05}$		1.10	1.217	1.32	1.37	7.42	15.02	6.35
Leaves								
0	10	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	25.92±3.72
5	10	11.11±0.59	11.11±0.59	11.11±0.59	25.92±3.71	48.85±3.71	62.97±3.71	100.00±0.00
10	10	11.11±0.59	11.11±0.59	11.11±0.59	29.63±3.71	51.85±7.54	74.08±3.71	100.00±0.00
20	10	25.92±3.71	29.63±3.71	48.15±3.71	74.08±3.71	100.00±0.00	100.00±0.00	100.00±0.00
25	10	29.63±3.71	37.03±3.71	51.88±7.54	81.48±7.54	100.00±0.00	100.00±0.00	100.00±0.00
30	10	40.74±3.71	48.15±3.71	62.97±3.71	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
35	10	59.26±3.71	62.97±3.71	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
40	10	74.08±3.71	70.37±3.71	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00	100.00±0.00
$LSD_{0.05}$		7.35	7.49	15.09	15.03	14.99	9.35	
LC_{50} Value		30.95	29.95	22.78	7.01	6.40	4.56	
95%FL		(29.30,32.88)	(27.90,31.47)	(15.67,26.94)	(0.87,12.07)	(2.50,9.58)	(2.08,6.44)	

Dimethoate(Rogor E40) ® (2% v/v) caused 100% mortality within 24 h whereas no mortality was recorded in ordinary water treatments after 168 h.
No mortality was observed in the treatments with crude aqueous extracts obtained from the succulent stems of *T.vogelii.*
Aqueous crude *T. vogelii* leaf extracts were evaluated at four rates (25, 30, 35 & 40% w/v) beyond rates for other plant parts based on laboratory efficacy results

(b) Field toxicity studies

Field experiments were carried out according to Zahid *et al.* (2008) with modifications in which bamboo sticks were used instead of iron wires. The experiment was laid out in a Randomised Complete Block Design with three replications. Each plot measuring 1.0 x1.6 m was planted with four rows of Desi chickpea variety ICCV97105 at inter- and intra-row spacing of 0.3 m and 0.1 m, respectively.

Seedbed preparation was carried out before the onset of the rains for Season 1, and immediately after harvesting the first season crop, in readiness for planting Season 2 crops. Two seeds of chickpea were sown per hole at inter- and intra-row spacing of 0.3 m and 0.1 m, respectively, in the furrow. They were later thinned to one plant per hill a week after emergence. Manual weeding was first done four weeks after emergence in Season 1 and 2 and again after flowering.

Aqueous crude extracts of *T. vogelii* leaves were evaluated at six concentrations (0, 20, 25, 30, 35 and 40% w/v) based on laboratory bioassay results. Ordinary water and synthetic insecticide, Dimethoate at 2% v/v, were included as negative and positive controls, respectively. Each experimental plot consisted of four rows of chickpea variety ICCV 97105 (Desi), planted at a spacing of 40 cm and 10 cm inter- and intra-row spacing, respectively. The plot size was 1.6 m by 1.0 m. Three plants were sampled at the middle of each of the two centre rows giving a total of six plants sampled per plot. Ten 2nd or 3rd instar larvae, most active feeding stages, were used for infestation. per row according to Sharma *et al.* (2005). Plants in each plot were covered with nylon mesh cages (2.0 x 0.7 x 2.0 m size) before flowering to avoid natural infestation. The cages were designed in such a way that they did not interrupt ventilation and aeration to the growing plants inside. The entire perimeters of the bottom edges of the cages were inserted into the ground to deter escape or entry of larvae.

The nylon mesh cages were erected on bamboo sticks fixed in four corners. The larvae were released once at the time of flowering. First spraying was done at flower bud expansion stage and four subsequent sprays at 15-day intervals. The survival rate of *H. armigera* larvae and the number of total and damaged pods were monitored. Data on larval density and percent pod damage were used to calculate correlation coefficient and regression equations. Stand count, yield components [(number of pods per plant, number of damaged pods per plant, dry matter

(kg), 100 seed weight (g)] and grain yield (kg/plot) was also recorded data at harvest. Data on plot yield and plot area were used to compute grain yield (kg/ha) (Forbes and Watson, 1992). The data analysis model used is as shown below:

$$Y_{ijk} = \mu + R_i + S_j + C_k + SC_{jk} + \Sigma_{ijk}$$

Where

Y_{ijk}= observation

μ = general mean

R_i= i^{th} replicate in the j^{th} season

S_j = j^{th} Season

C_k=k^{th} concentration in the i^{th} replicate and j^{th} season

SC_{jk} = is the interaction between j^{th} season and k^{th} concentration

4.3 Results

Results showed that the contact toxicity of aqueous crude extracts of *T.vogelii* against *H. armigera* larvae were significantly (P = 0.0001) influenced by intra-plant variability, concentration applied, contact duration (hours) and corresponding factor interactions. At 20% w/v and 24 h, the aqueous crude extracts obtained from the leaves and pods/flowers of *T.vogelii* were weakly toxic against *H. armigera* larvae causing 22 and 11% mortality, respectively, whereas the succulent stem extracts were non- toxic (LC_{50} values were insignificant) (Table 4.1). The positive control, Dimethoate (Rogor E40)® at 2% v/v, was the most toxic achieving 100% kill within 24 h. The LC_{50} values decreased with contact duration a manifestation of increased larval mortality. The positive control, Dimethoate (Rogor E40)® at 2% v/v and *T. vogelii* leaf extract at 40% w/v were equally effective. End-point (100%) larval mortality were recorded 24 and 72 h after treatment with synthetic insecticide, Dimethoate at 2% v/v and aqueous crude *T. vogelii* (40% w/v) leaf extracts respectively (Table 4.1).

A dose depended decrease in larval survival was recorded in plots sprayed with graded levels of aqueous crude extracts obtained from *T.vogelii* leaves (Table 4.2). At the highest concentration (40%w/v), 97% reduction in survival rate of *H.armigera* above the untreated control was recorded 90 days after planting. Similar results were recorded in plots sprayed with Dimethoate 2%v/v above the untreated control in the first season (Table 4.2).

A dose – dependent increase in dry matter, yield and yield components of chickpea was recorded in plots sprayed with graded levels of aqueous crude extracts obtained from *T. vogelii* leaves. At the highest concentration (40%w/v), a 634% increase in chickpea grain yield above the untreated control was recorded during the first season which was comparable to synthetic insecticide, Dimethoate (Rogor E40)® 2%v/v (642%). Similar result trends were observed during season 2 with 582% and 628% increases in chickpea grain yield in plots treated with aqueous extracts of *T. vogelii* (40% w/v) and Dimethoate (Rogor E40)® (2% v/v), respectively (Table 4.3). In the same experiment, a dose-dependent reduction in pod damage by *H. armigera* larvae in plots treated with varying concentrations of aqueous crude extracts of *T. vogelii* leaves was observed (Fig. 4.1). Correlation analysis revealed a strong positive correlation (r = 0.904) during Aug – Dec, 2009 and (r = 0.988) during Jan – April, 2010, respectively between larval density and pod damage (Fig. 4.2 and 4.3). The regression equations derived were Y= 47.82X + 9.736 during Aug – Dec, 2009 and Y = 50.97X + 12.56 during , Jan – April, 2010 (fig 4.2 and 4.3), respectively.

Table 4.2: ***H.armigera*** **larvae counts per plant (Mean ±SE, n=3) as influenced by concentration of aqueous crude extracts of *T. vogelii* leaves and time after spraying in the field**

Conc (w/v%)	Number of *H. armigera* larvae per plant (Mean ±SE, n=3)				
	Days after planting (DAP)				
	N	45	60	75	90
Season 1 (Aug - Dec 2009)					
Dimethoate(2%/v/v)	3	0.16±0.00	0.16±0.00	0.16±0.00	0.16±0.00
Ordinary water(100 ml)	3	1.00±0.16	1.50±0.29	1.50±0.29	1.00±0.16
0	3	1.50±0.29	1.50±0.29	1.50±0.29	1.33±0.17
20	3	0.83±0.20	0.60±0.08	0.60±0.08	0.50±0.09
25	3	0.60±0.08	0.50±0.09	0.60±0.08	0.40±0.09
30	3	0.33±0.00	0.60±0.08	0.30±0.00	0.33±0.00
35	3	0.16±0.00	0.16±0.00	0.16±0.00	0.16±0.00
40	3	0.16±0.00	0.16±0.00	0.16±0.00	0.16±0.00
$LSD_{0.05}$		0.58	0.58	0.58	0.34
Season 2 (Jan-Apri 2010)					
Dimethoate(2% v/v)	3	0.05±0.00	0.11±0.05	0.05±0.00	0.05±0.00
Ordinary water(100 ml)	3	1.33±0.17	1.17±0.17	1.33±0.17	1.33±0.17
0	3	1.50±0.00	1.50±0.00	1.50±0.00	1.33±0.17
20	3	0.72±0.10	0.68±0.08	0.57±0.04	0.44±0.05
25	3	0.64±0.10	0.44±0.05	0.60±0.00	0.38±0.02
30	3	0.39±0.05	0.42±0.09	0.33±0.00	0.33±0.00
35	3	0.16±0.00	0.11±0.05	0.16±0.00	0.11±0.00
40	3	0.05±0.00	0.05±0.00	0.05±0.00	0.05±0.00
$LSD_{0.05}$		0.34	0.34	0.34	0.34

Table 4. 3: Effect of spraying aqueous crude extracts obtained from *T. vogelii* leaves on dry matter, yield and yield components of chickpea in Njoro, Kenya

Conc (%w/v)	Pods/plant	100-seed wt(g)	Dry matter (kg/ha)	yield(kg /ha)
Season 1 (Aug - Dec 2009)				
Dimethoate	40.3±1.1	21.1±0.0	3050.03±16.52	1796±473.0[a]
Ordinary water	8.0±0.0	19.5±0.0	639.60±30.81	273±133.7[d]
0.0	10.0±0.0	19.7±0.0	614.60±30.81	242±96.9[d]
20.0	29.3±0.6	20.2±0.3	2218.77±218.72	1192±260.4[c]
25.0	36.0±0.0	20.3±0.0	2483.37±78.12	1411±163.1[b]
30.0	40.5±0.9	20.5±0.0	2637.53±124.87	1542±258.8[b]
35.0	40.0±1.0	20.7±0.5	2752.10±52.40	1654±371.7[ab]
40.0	41.0±1.0	21.3±0.1	2966.70±67.42	1777±492.3[a]
Season 2 (Jan-Apri 2010)				
Dimethoate	33.7±1.2	21.0±0.1	3041.67±295.36	1777±84.9[a]
Ordinary water	5.3±0.6	19.2±0.3	645.83±219.49	254±37.7[d]
0.0	8.3±0.6	19.6±0.3	666.70±254.54	244±16.6[d]
20.0	25.7±1.6	20.2±0.2	2312.50±437.50	952±150.0[c]
25.0	30.3±0.6	20.4±0.3	2514.60±617.05	1198±32.1[b]
30.0	33.0±1.7	20.3±0.5	2722.93±254.36	1321±61.4[b]
35.0	34.0±1.7	20.7±0.2	2760.43±296.99	1552±56.4[ab]
40.0	35.0±1.3	21.0±0.2	2993.77±495.39	1665±128.2[a]

Dimethoate was applied at 2% v/v whereas ordinary water was tested at 100 ml per treatment.

Aqueous crude *T. vogelii* leaf extracts at 5% w/v and 10% w/v were not included in the field studies based on efficacy results obtained from laboratory bioassays.

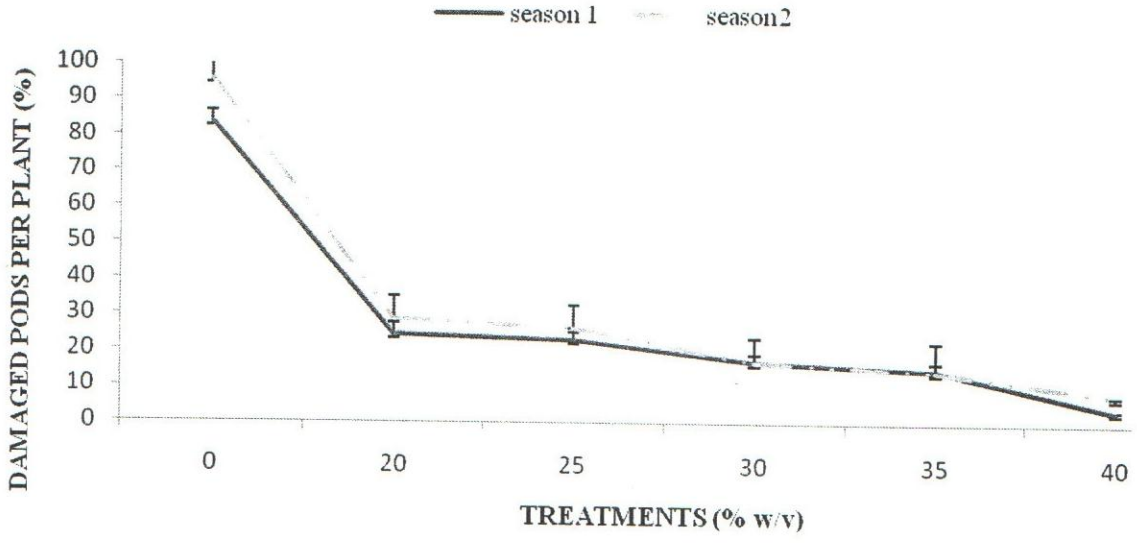


Fig. 4. 1: **Percent pod damage per plant (Mean±SE, n=3) as influenced by concentration of crude aqueous *T.vogelii* leaf extract sprayed**

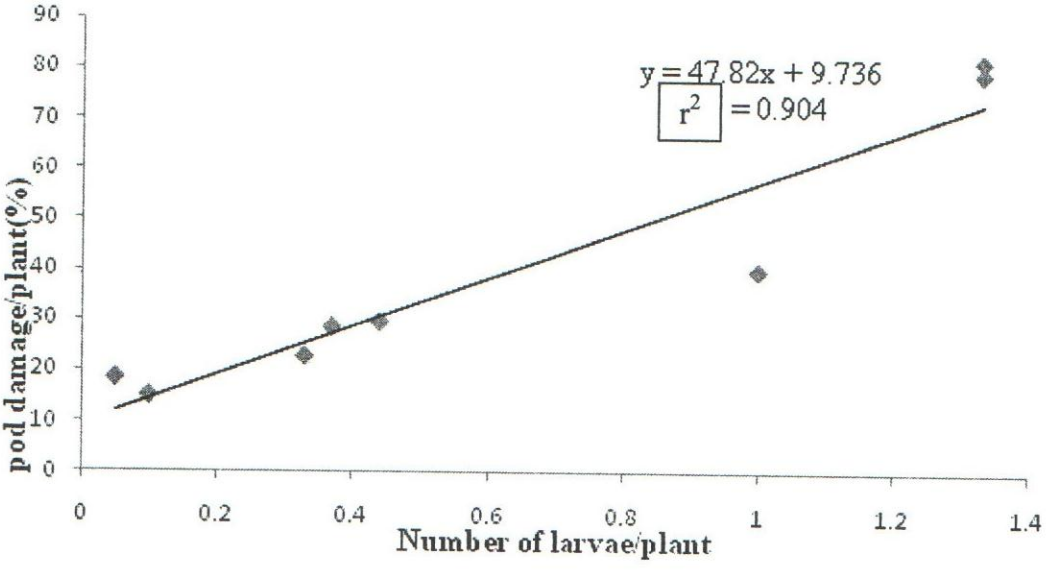


Fig. 4. 2: **Relationship between larvae population and pod damage by *H. armigera* In chickpea (Aug-Dec 2009)**

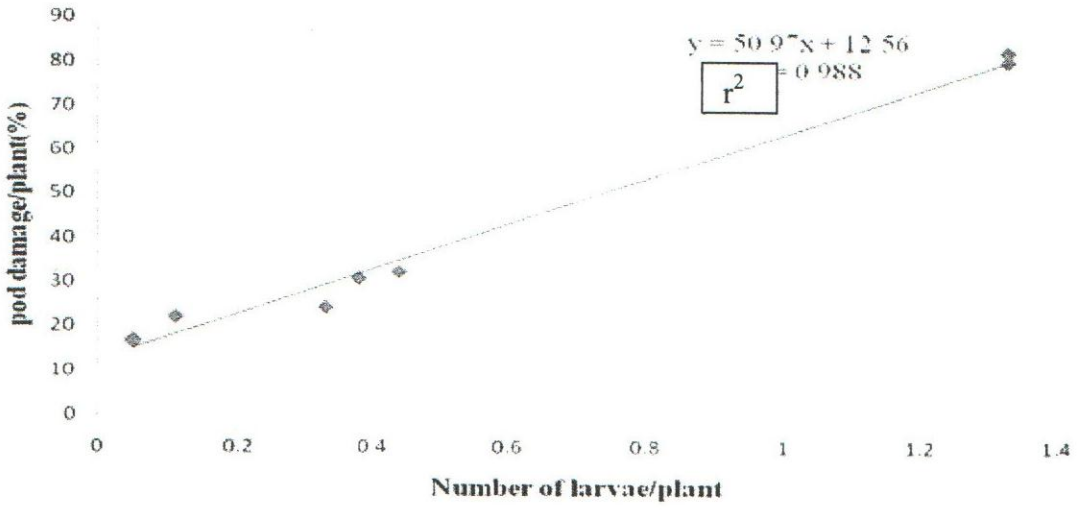


Fig.4. 3 : Relationship between larvae population and pod damage of *Helicoverpa armigera* in chickpea (Jan-Apr 2010)

4.4 Discussion

Results have demonstrated that aqueous extracts of *T. vogelii* leaves had strong dose- dependent contact toxicity (Simmonds *et al.*, 1990; Machocho, 1992; Morris, 1999). Additionally, recent local laboratory studies showed that *Tephrosia vogelii* essential oils produced strong contact toxicity (up to 83% kill) against four coleopteran pests of stored cereal and legume grains including insects feeding on pigeon pea and chickpea (Minja *et al.*, 2002; Ogendo *et al.*, 2008). Also, a collaborative study done by scientists from Malawi, Zambia and Zimbabwe showed that *T.vogelii* methanol extracts caused 55% mortality of *Spodoptera littoralis* (Stevenson *et al.*, 2007).

Pesticides, both synthetic and botanical, no doubt markedly reduce pest infestations and increase seed yield of crops. The fact that aqueous crude *T. vogelii* extract 40% w/v produced yield response comparable to synthetic insecticide Dimethoate (Rogor E40)® (2% v/v) is a welcome scientific delight. These results are at variance with with Agona *et al.* (2001, 2002) and Opolot *et al.* (2006) who reported that synthetic insecticides were more effective than the botanical pesticides. Many crude extracts of plants are known to be effective in controlling insect pests of various crops (Stoll, 2001; Adebayo and Olaifa, 2004; Owolade *et al.*, 2004). In addition, plant

extracts applied at flowering and pod formation stages reduced the level of infestation of *Maruca vitrata* and thrips and increased yield of plants (Panhwar, 2002; Ahmed *et al.*, 2009). The results also supported the views of Stoll (1988) and Panhwar (2002) who independently reported that the effect of plant extracts on crop yield and yield component were dependent on the effectiveness of the individual plant extracts. However, the efficacy of plant-based insecticidal application may be enhanced if it is sprayed either in early morning or in late evening (Oparaeke *et al.*, 2003; Ahmed *et al.*, 2009). *Tephrosia vogelii* leaves and seeds have been reported to contain tephrosine (Adebayo *et al.*, 2007; Ogendo, 2008). Documented information has shown very promising results on the use of *Tephrosia vogelii* in the control of field pests and they concur with results achieved from this study. Studies done on pigeon pea indicated that plots sprayed with *T.vogelii* extracts, applied 3-4 times, had acceptable levels of insect control (Kyamanywa *et al.*, 2001; Minja *et al* ., 2002). Additionally, Mugoya and Chinsembu (1995) reported that aqueous fresh-leaf extracts of *T. vogelii* reduced the incidence of the spotted stalk borer *Chilo partellus* Swinhoe (Lepidoptera: Crambidae) and increased yield and quality of maize grains. Results from this study are a great achievement and the scientific world since the use of synthetic chemicals which has raised a lot of environmental concerns will be minimized. From the results of this study it can be concluded that the aqueous crude extracts of *Tephrosia vogelii* had strong contact toxicity against the larval stage of Helicoverpa armigera with total (100%) kill achieved within 72h post-application.

CHAPTER FIVE

ANTIFEEDANT EFFECTS OF AQUEOUS CRUDE EXTRACTS OF *Tephrosia vogelii* HOOK AGAINST *Helicoverpa armigera* LARVAE

Abstract

A study was conducted to evaluate the antifeedant effects of aqueous crude extracts of *T. vogelii* against *H. armigera* larvae. Aqueous crude extracts obtained from leaves, pods/ flowers and succulent stems were evaluated at four rates (0, 5, 10 and 20% w/v). Ordinary water (100 ml) and Dimethoate (Rogor E40)® (2% v/v) were included as negative and positive controls, respectively. Laboratory bioassays were arranged in a CRD replicated five times per treatment. Field bioassays were laid out in a RCBD with 3 replicates per treatment. Results showed that the larval weight, amount of food consumed and feeding deterrence coefficients were significantly ($P = 0.0001$) influenced by intra-plant variability, feeding duration (hours), concentration of extract applied and corresponding factor interactions. Except for ordinary water treatment, strong dose- and plant part-dependent decreases in larval weights and amount food of consumed were recorded. At the highest concentration (20% w/v) and 168 h, leaf and pod/flower extracts reduced the larval weights by 75 and 78.7%, respectively compared to ordinary water and untreated control. Similar result trends were recorded in which the leaf, pod/flower and stem extracts suppressed the larval food consumption by 89.2, 67.6 and 35.1%, respectively, after 8 days of feeding. The leaf extracts and synthetic insecticide, Dimethoate at 2% v/v, equally had the highest antifeedant (reduced by 89.2%) effects on the *H. armigera* larvae. Synthetic insecticide, Dimethoate (Rogor E40)® at 2% v/v and *T. vogelii* leaf extract at 20% w/v, after 8 days of feeding, had the highest deterrence coefficients of 90 and 70, respectively, followed by pod/flower (57) and stem (5) extracts in order of decreasing deterrence whereas ordinary water (1.5-4.3) and untreated control (0.0) treatments had the lowest deterrence values. Results of this study provide strong scientific backing for a rationalized exploitation of *Tephrosia* based botanical pesticides for increased chickpea production in smallholder agriculture.

Key words: Antifeedant, *Helicoverpa armigera*, *Cicer aurentium L*, *Tephrosia vogelii*, aqueous extracts

5.1 Introduction

Plants represent a vast storehouse of potentially useful natural products, and indeed, many laboratories worldwide have screened thousands of species of higher plants in search of pharmaceuticals and pest control (Arnason *et al.,* 1989; Van Beek and Breteler, 1993; Sarmah *et al.,* 1999; 2006; 2009). During the last 50 years, use of synthetic insecticides to control insect pests has led to both insecticide resistance and environmental persistence (Roush and Tabashnik, 1990). Phytochemicals derived from plants are advantageous over the synthetic pesticides in that they are eco-friendly, non-toxic to non-target organisms and non persistent in nature. (Liu *et al.,* 2000; Choudhary *et al.,* 2001; Ahmad, 2007; Ramya *et al.,* 2008). Application of bio-pesticides has been reported to have reduced bollworm population (Ge and Ding 1996; Ramya *et al.,* 2008).

Although *H. armigera* in chickpea has been controlled using synthetic insecticides, insect resistance to such chemicals has been reported (Sharma *et al.*, 2005). Other control measures against *H. armigera* include the use of *Bacillus thuringiensis* (Bt) toxin and nuclear polyhedrosis virus which also are ineffective (Lewis *et al.,* 1997; Brousseau *et al.*, 1999). (Ranga- Rao and Shanower, 1999). Botanical pesticides offer a viable cost-effective and eco-friendly alternative to synthetic pesticides in chickpea production.

A number of plants have been shown to have antifeedant activity against *H. armigera*, of which neem has been subjected to extensive investigation (Koul, 1985; Chopra *et al.,* 1994; Jaglan *et al.,* 1997; Koul *et al.,* 2000). Sundararajan and Kumuthakalavalli (2001) evaluated antifeedant activity of aqueous extract of *Gnidia glauca* and *Toddalia asiatica* against *H. armigera* and a strong antifeedant effect was recorded. *T.vogelii* also showed a high feeding deterrence effect against the larger grain borer, *Prostephanus truncatus* (Mukanga *et al.,* 2010). Botanicals obtained from various indigenous plant species have shown strong antifeedant activity against *H. armigera* (Kamaraj *et al.,* 2008; Ramya *et al.*, 2009). A strong antifeedant effect of 76.13% was recorded when isolates of Rhein from the flowers of *Cassia fistula* L. were evaluated against *H. armigera* (Pavunraj *et al.*, 2011). Literature review has revealed that little or no local research intervention focusing on the antifeeding effects of *T. vogelii* extracts against *H. armigera* has been conducted. With this background, a study was conducted to evaluate the antifeedant effects of aqueous crude extracts obtained from the aerial parts of *T.vogelii* against *H. armigera* larvae.

5.2 MATERIAL AND METHODS

5.2.1 Mass rearing of test *H. armigera* larvae

The larvae used for antifeedant test were obtained from the mass reared larvae as described in section 3.2.2 above.

5.2.2 Bioassays

(a) Laboratory antifeedant studies

In order to determine the amount of food consumed, 10 chickpea leaves of the same dimensions were immersed in test *T. vogelii* aqueous extracts and control treatments for 30 minutes as described in Section 4.2.2 above. Separately, treated leaves were removed using forceps, placed inside plastic Petri dishes (15 cm in diameter) lined with filter paper (Whatman No. 1) at the base and were then weighed according to Erturk (2006) with some modifications where *Tephrosia vogelii* leaves were used instead of flour. Ten 2nd and 3rd instar larvae, most active feeding larval stages, were weighed and introduced into each of the treated diets. A total of 12 treatments were arranged in a CRD with five replicates per treatment. Data on the amount of food consumed and weight of larvae were recorded. The larvae in each experimental unit received fresh treated diet every 24 h. Based on the amount of food consumed, the absolute deterrence coefficient (DC) was calculated using Kielczewski and Nawrot (1979) formula as follows:

$$\text{Deterrence Coefficient (DC)} = \frac{(C-T)}{(C+T)} \times 100 \qquad \text{Equation 3}$$

Where T represented the weight of food consumed by larvae in the experimental unit and C represented the weight of food consumed in the control unit. The data analysis model used is as shown below:

$$Y_{ijk} = \mu + P_i + T_j + C_k + PC_{ik} + PT_{ij} + \Sigma_{ijk}$$

Where Y_{ijk}= observation

μ = general mean

P_i=ith replicate in the jth plant part

T_j = jth Treatment

C_k= kth concentration in the ith replicate and the jth plant part

PC_{ik} = is the interaction between the ith replicate and kth concentration

PT_{ij} = is the interaction between ith replicate and the jth plant part

(b) Field antifeedant studies

Field experiments were carried out according to Zahid *et al.* (2008) with modifications in which bamboo sticks were used instead of iron wires. The experiment was laid out in a Randomised Complete Block Design with three replicates. Each plot measuring 1.0m x1.6m was planted with four rows of Desi chickpea variety ICCV97105 at inter- and intra-row spacing of 0.3 m and 0.1 m, respectively. Seedbed preparation was carried out before the onset of the rains for Season 1, and immediately after harvesting the first season crop, in readiness for planting Season 2 crops. Two seeds of chickpea were sown per hole at a spacing of 0.3 m x 0.1 m in the furrow. They were later thinned to one plant per hill a week after emergence. Manual weeding was first done four weeks after emergence in Season 1 and 2 and again after flowering.

The treatments, as described in section 4.2.2 above, were laid out in a RCBD with three replicates per treatment. Each plot consisted of four rows of chickpea variety ICCV 97105 (Desi) planted at spacing of 0.4 m by 0.1 m inter- and intra-spacing, respectively. Three plants were randomly sampled from each of the two centre rows giving a total of six plants sampled per plot. Ten 2^{nd} or 3^{rd} instars larvae, most active feeding larval stages, per row were used for infestation. Plants in each plot were covered with nylon mesh cages (2.0 x 0.7 x 2.0 m size) before flowering to avoid natural infestation. The cages were designed in such a way that they did not interrupt ventilation and aeration to the growing plants inside. The entire perimeters of the bottom edges of the cages were inserted into the ground to deter escape or entry of larvae. The nylon net cages were erected on bamboo sticks fixed in four corners. The larvae were released once at the time of flowering. First spraying was done at flower bud expansion stage and four subsequent sprays at 15-day intervals. The weights of 10 leaves and 10 pods from the covered plants of each cage were separately recorded with respect to the spraying intervals. The leaves and pods were sampled from the centre two rows. The data analysis model used is as shown below:

$$Y_{ijk} = \mu + R_i + S_j + C_k + SC_{jk} + \Sigma_{ijk}$$

Where

Y_{ijk}= observation

μ = general mean

R_i=i^{th} replicate in the j^{th} season

S_j = j^{th} Season

C_k=k^{th} concentration in the i^{th} replicate and j^{th} season

SC_{jk} = is the interaction between j^{th} season and k^{th} concentration

5.3 Results

5.3.1 Laboratory bioassays

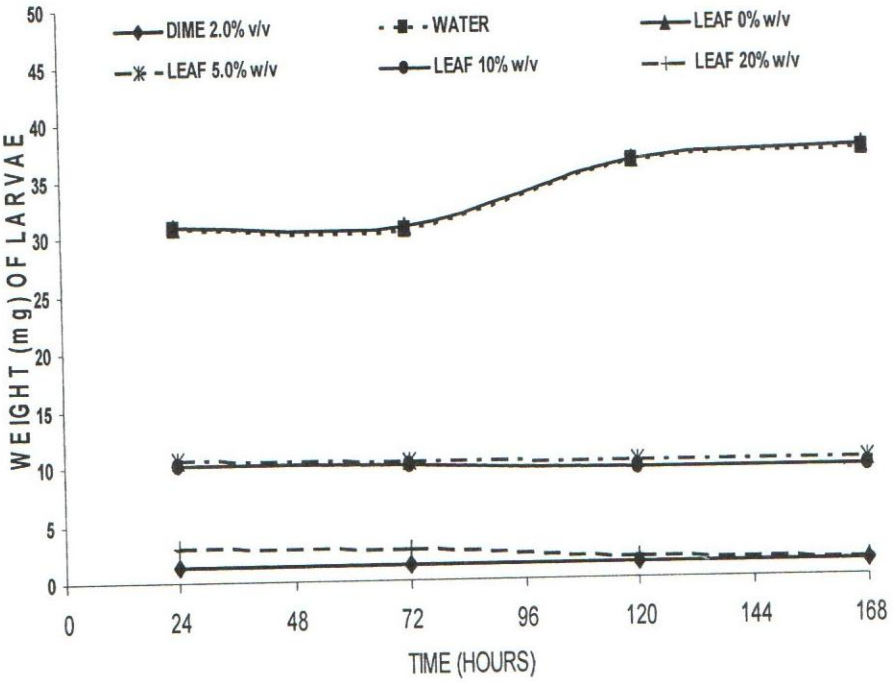
Results showed that the larval weight, amount of food consumed and feeding deterrence coefficients were significantly (P = 0.0001) influenced by intra-plant variability, feeding duration (hours), concentration of aqueous crude extract applied and the corresponding factor interactions. Except for ordinary water and untreated control treatments, strong dose- and plant part-dependent decrease in larval weights and amount of food consumed were recorded (Fig. 5.1-5.2). At the highest concentration (20% w/v) and 168 h, leaf and pod/flower extracts reduced the larval weights by 75 and 78.7%, respectively (Fig 5.1) compared to ordinary water and untreated control. Ordinary water and untreated control treatments, on the other hand, had the lowest or negligible deterrence coefficient values of 1.5-4.3 and 0.0, respectively (Table 5.1). Similar result trends were recorded in which the aqueous crude extracts obtained from leaf, pod/flower and succulent stems suppressed the larval food consumption by 89.2, 67.6 and 35.1%, respectively, after 8 days of feeding (Fig 5.2). The leaf extracts and synthetic insecticide, Dimethoate at 2% v/v, equally had the highest antifeedant (reduced by 89.2%) effects on the *H. armigera* larvae. Synthetic insecticide, Dimethoate at 2% v/v and aqueous crude *T. vogelii* extract at 20% w/v, had the highest deterrence coefficients of 90 and 70, respectively, after 8 days of feeding followed by pod/flower (57) and stem (5) extracts in order of decreasing deterrence.

Table 5.1: Deterrence coefficient (Mean±SE, n=4) of aqueous *T. vogelii* extracts against *Helicoverpa armigera* larvae as influenced by plant part, concentration and contact duration

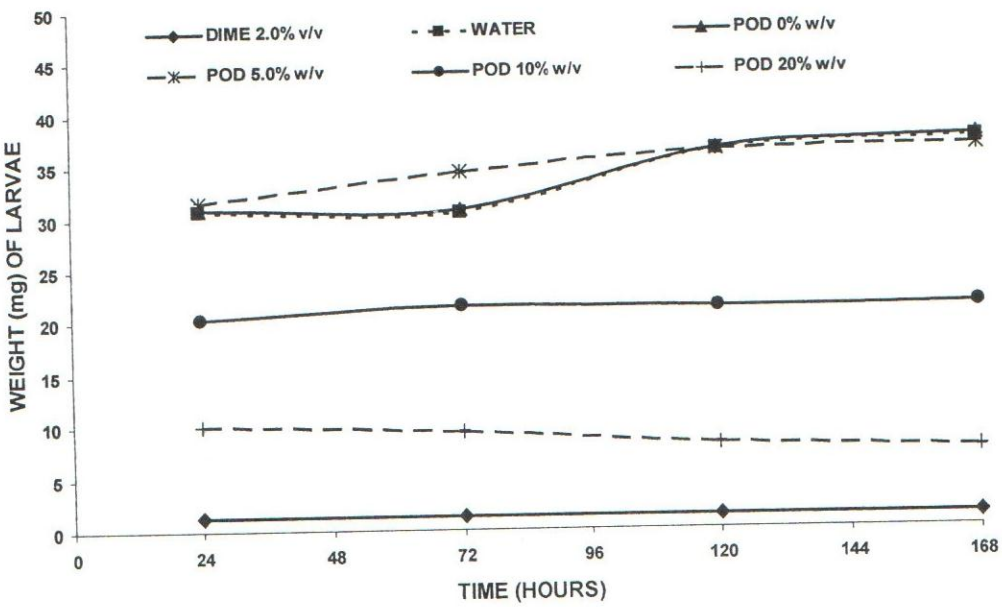
		CONTACT TIME (HOURS)						
Plant part/Conc(% w/v)	N	24	48	72	96	120	144	168
Leaves								
Dimethoate(2.0% v/v)	5	86.9±1.4	84.0±1.6	86±1.2	84.5±1.6	85.9±1.2	83.5±1.7	87.0±1.1
Ordinary water(100 ml)	5	4.3±0.9	3.2±0.9	1.9±0.3	3.6±0.0	1.6±0.0	3.6±0.0	1.5±0.0
0.0	5	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
5.0	5	32.5±1.3	38.0±1.3	29.0±0.3	42.3±0.5	28.2±0.1	23.2±0.3	32.8±0.3
10.0	5	55.5±1.7	48.4±0.6	50.5±0.5	51.4±1.7	46.8±0.5	39.3±0.5	44.6±1.2
20.0	5	71.0±1.7	75.9±2.0	75.6±0.9	73.3±1.5	74.4±1.4	71.6±1.6	68.7±0.8
$LSD_{0.05}$		3.4	3.9	2.9	3.3	2.7	3.3	2.2
Pods/flowers								
Dimethoate(2.0% v/v)	5	86.9±1.4	84.0±1.6	86.0±1.2	84.5±1.6	85.9±1.2	83.5±1.7	87.0±1.1
Ordinary water(100 ml)	5	4.3±0.9	3.2±0.9	1.9±0.3	3.6±0.0	1.6±0.0	3.6±0.0	1.5±0.0
0.0	5	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
5.0	5	0.9±0.3	1.4±0.1	2.0±0.1	1.8±0.6	3.0±0.8	1.9±0.0	1.5±0.7
10.0	5	8.9±0.3	11.9±0.0	12.4±1.1	10.8±0.8	14.6±0.1	10.4±0.4	18.6±0.8
20.0	5	59.9±0.5	56.9±0.8	50.5±0.5	53.8±0.7	53.2±1.2	43.8±1.3	52.9±1.0
$LSD_{0.05}$		2.8	3.2	2.9	3.1	2.4	3.3	2.2
Succulent stems								
Dimethoate(2.0% v/v)	5	86.9±1.4	84.0±1.6	86.0±1.2	84.5±1.6	85.9±1.2	83.5±1.7	87.0±1.1
Ordinary water(100 ml)	5	4.3±0.9	3.2±0.9	1.9±0.3	3.6±0.0	1.6±0.0	3.6±0.0	1.5±0.0
0.0	5	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
5.0	5	0.6±0.4	1.8±0.0	1.9±0.3	1.8±0.6	3.0±0.8	1.9±0.0	1.5±0.7
10.0	5	3.9±0.3	1.9±0.0	2.0±0.5	1.9±0.1	1.6±0.1	1.4±0.4	1.6±0.1
20.0	5	5.5±0.3	3.8±0.0	6.0±0.7	1.8±0.0	8.7±0.1	1.9±0.0	4.7±0.6
$LSD_{0.05}$		2.8	3.2	2.9	2.4	2.4	3.3	2.2

Leaf and pod/flower extracts reduced the larval weights by 75 and 78.7%, respectively
Ordinary water and Dimethoate 2%v/v was used as negative and positive controls respectively
Ordinary water and untreated control treatments, had the lowest or negligible deterrence coefficient values of 1.5-4.3 and 0.0, respectively

(a) Leaf extract



(b) Pod/flower extract



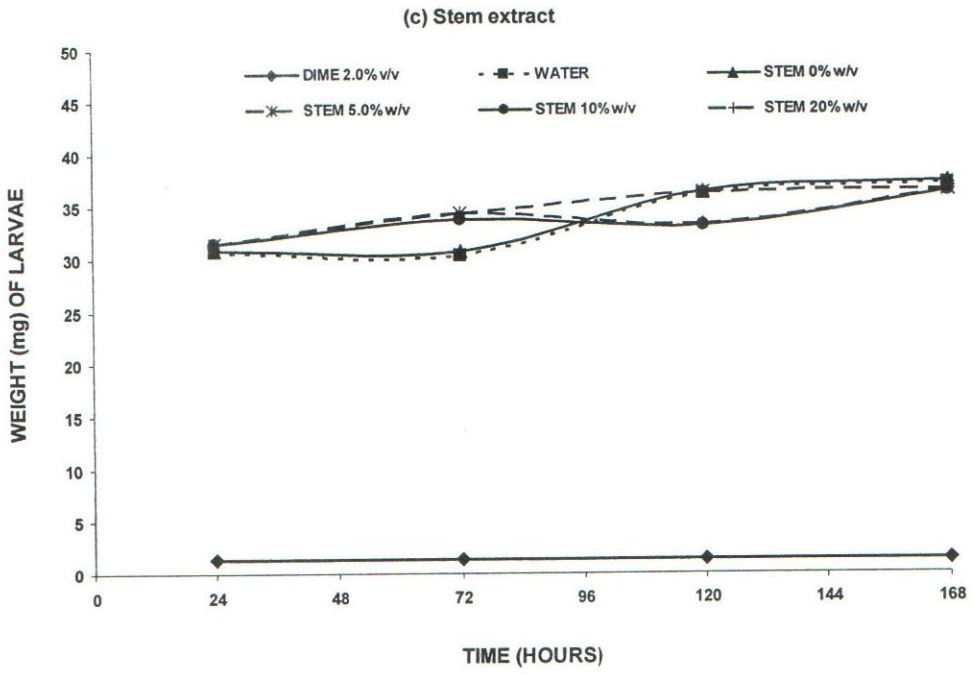
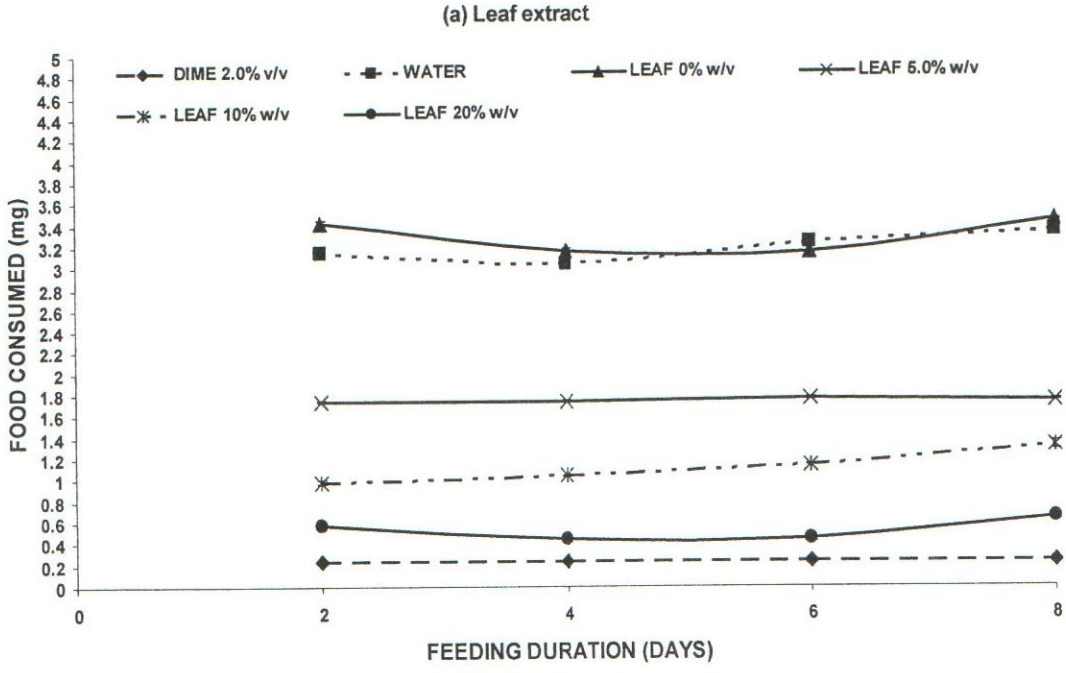


Fig. 5. 1: **Weight (Mean±SE,n=5) of *H. armigera* larvae as influenced by plant part, concentration a exposure duration to aqueous extracts obtained from aerial parts of *Tephrosia vogelii.***



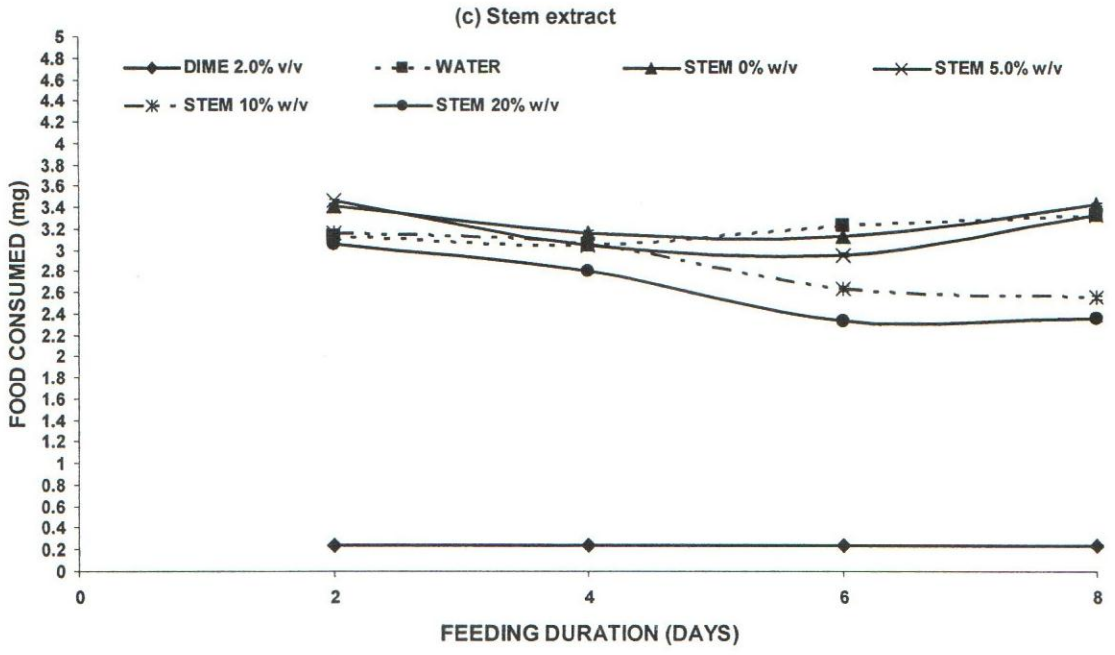


Fig.5. 2: Weight of food consumed (Mean±SE, n=5) by *H. armigera* larvae as influenced by plant part, concentration and exposure duration to aqueous extracts of obtained from aerial parts of *T. vogelii.*

5.3.2 Field Bioassays

Results showed that the weight of leaves and pods were significantly (P = 0.0001) influenced by the stage of the crop and concentration of aqueous crude extract applied. At the highest concentration (40%w/v) a 96% and a 92% increase in the weight of leaves above the untreated control was recorded during the first an second season respectively. Similar results were recorded in plots sprayed with Dimethoate (Rogor E40)® 2%v/v above the untreated control (Table 5.2)

Table 5. 2: Effect of spraying aqueous crude extracts obtained from *T. vogelii* leaves on the weight of leaves and pods sampled from treated chickpea plots.

Treatment	Weight(g) of leaves and pods			
Season 1 Aug-Dec 2009	**Leaves**		**Pods**	
	DAYS AFTER SOWING (DAS)			
T.vogelii leaves (% w/v)	N 45	60	75	90
Dimethoate (2.0% v/v)	3 2.67±0.09	3.30±0.20	7.23±0.20	8.47±0.26
Ordinary water(100ml)	3 0.20±0.00	0.20±0.00	3.07±0.04	3.00±0.06
0.0	3 0.10±0.00	0.30±0.00	3.20±0.00	2.93±0.10
20.0	3 1.80±0.06	2.47±0.10	3.70±0.00	5.30±0.70
25.0	3 1.83±0.09	2.63±0.10	5.03±0.34	5.53±0.60
30.0	3 2.03±0.09	2.73±0.20	6.40±0.10	6.73±0.10
35.0	3 2.63±0.10	3.37±0.20	6.70±0.40	7.70±0.20
40.0	3 2.77±0.10	3.73±0.10	7.63±0.18	8.57±0.30
$LSD_{0.05}$	0.20	0.40	0.80	1.40
Season 2 Jan-Apr 2010				
Dimethoate (2.0%v/v)	3 2.90±0.20	3.10±0.04	7.40±0.20	9.00±0.30
Ordinary water(100ml)	3 0.30±0.00	0.40±0.00	3.30±0.04	3.20±0.20
0.0	3 0.20±0.00	0.30±0.00	3.10±0.00	3.40±0.10
20.0	3 2.10±0.06	2.40±0.10	3.90±0.00	5.00±0.34
25.0	3 2.00±0.09	2.60±0.10	5.30±0.70	5.30±0.70
30.0	3 2.10±0.06	2.74±0.20	6.50±0.10	6.90±0.40
35.0	3 2.60±0.10	3.30±0.20	6.80±0.40	7.90±0.20
40.0	3 2.60±0.10	3.54±0.10	7.50±0.18	8.50±0.30
$LSD_{0.05}$	0.40	0.40	1.40	1.40

5.4 Discussion

The results of this study have shown that aqueous extracts obtained from leaf and pods/flowers of *T. vogelii* caused a dose-dependent reduction on weight of larvae and amount of food consumed which are clear antifeedant indicators. The converse was true for resultant deterrence coefficients (DC) in which a dose-dependent increase in DC values as the amount of food

consumed and subsequent larval weights decreased. These findings concur with past studies in which *T. vogelii* leaves and seeds have been reported as a source of rotenoids, including rotenone, tephrosin, and deguelin, known to possess strong feeding deterrent activities (Arnason *et al.,* 1987; Adebayo *et al.,* 2007). In other bioassays against the larger grain borer, *Prostephanus truncatus, T. vogelii* extracts showed a high feeding deterrence effect (Mukanga *et al.,* 2010). Additionally, anti-feeding effects of *Tephrosia* have also been reported on spotted cereal stem borer (*Chilo partellus*) (Machocho, 1992). In addition to the insecticidal compounds, the leaves of *T. vogelii* also contain 5-methoxyisolonchocarpin, a highly effective antifeedant, active even at 10 ppm for some lepidopteran species (Simmonds *et al.*, 1990). Hence, the antifeedant activity principle in the aqueous extracts obtained from aerial parts of *T. vogelii* could partially be attributed to the presence of known chemical constituents. Results from this study indicate that *T. vogelii* crude extracts have a potential as an antifeedant to control *Helicoverpa armigera* in chickpea and hence lead to an increased chickpea production. From the results of this study it can be concluded that the aqueous crude extracts of *Tephrosia vogelii* had strong antifeedant activity, as manifested in high deterrence coefficients, however the level of deterrence was inferior to that of the positive control, Dimethoate (Rogor E40)® 2%v/v.

CHAPTER SIX

REPELLENT EFFECTS OF AQUEOUS CRUDE EXTRACTS OF *Tephrosia vogelii* HOOK AGAINST *Helicoverpa armigera* LARVAE

Abstract

A laboratory study was conducted to evaluate the repellent activity of aqueous crude extracts of *Tephrosia vogelii* against *Helicoverpa armigera* larvae in a choice bioassay. Aqueous crude extracts obtained from leaves, pods/ flowers and succulent stems of *T. vogelii* were evaluated at four rates (0, 5, 10 and 20% w/v). Ordinary water and synthetic insecticide, Dimethoate at 2% v/v, were included as negative and positive controls, respectively. The laboratory bioassay was laid out in a CRD with five replicates per treatment. Results showed that the repellent activity of aqueous crude extracts of *T. vogelii* against *H. armigera* larvae was significantly ($P = 0.0001$) influenced by intra-plant variability, concentration applied, exposure time and corresponding factor interactions. Except for leaf and pod/flower extracts at 20% w/v and 1 h exposure time with moderate repellence (PR value: 40%), a strong dose-dependent attraction of *H. armigera* larvae was observed. The number of larvae that visited the chickpea leaves treated with *T. vogelii* extracts was higher compared to that of negative control. The number of larvae that were attracted to the untreated chickpea leaves (food) also increased with exposure time. The repellent activity of aqueous crude extracts against *Helicoverpa armigera* larvae in the field, does not give hope for their potential use in the field.

Key words: Repellent, *Tephrosia vogelii, Helicoverpa armigera, Cicer aurentium L.*

6.1 Introduction

Ever since man started cultivating the crop plants, many protective measures have been used against insect pests and diseases. In recent years, documented information on hazardous effects of synthetic insecticides on plant and animal health and the increasing cases of pest resistance has re-ignited scientific search for alternative eco-friendly non-chemical options (Gupta et al., 2005; Sharma and Gupta, 2009).

Naturally occurring substances, have a broad spectrum bioactivity due to the presence of several active ingredients with varied modes of action. In the recent years, aqueous crude extracts have

attracted enormous attention as an alternative pest control option particularly due to their specificity to pests, biodegradable nature, potential for commercial application and ecosocio compatibility (Ramya *et al.,* 2008). An insect repellent is a chemical that acts in the vapour phase and prevents an insect from reaching a target which it would otherwise be attracted (Murugan *et al.*, 2007). Repellents from plant origins are considered safe pest control agents due to their minimal pesticide residues, relative safety to man, environment and wildlife (Talukder and Howse, 1995; Talukder, 2006). Various plant extracts, powders and essential oils have been reported as repellents against economically important insect pests of field and stored food commodities. (Ogendo *et al.*, 2003; Isman, 2006; Liu *et al.*, 2006; Ogendo *et al.*, 2008). Little or no local scientific interventions have pursued the use of aqueous crude extracts as repellents of field insect pests. The objective of this study was to evaluate the repellent properties of aqueous crude extracts obtained from the aerial parts of *T.vogelii* Hook, against *H. armigera* larvae.

6.2Materials and Methods

6.2.1 Mass Rearing of test insects

The larvae used for repellent activity were obtained from larvae reared as described in chapter 3.2.2 above.

6.2.2 Bioassays

(a) Choice Bioassay (larvae)

Choice bioassay tests using 2nd and 3rd instar larvae were conducted in a circular flat bottomed plastic basin (45cm in diameter by 30 cm high) whose base was divided into four equal portions as described by Ogendo *et al.* (2004) (Plate 1). Separate aqueous crude extracts obtained from leaves, pods/ flowers and succulent stems of *T. vogelii* were evaluated against *H. armigera* larvae at four rates (0, 5, 10 and 20% w/v). Ordinary water and Dimethoate (Rogor E40) ® at 2% v/v were included as negative and positive controls, respectively. A total of 12 treatments were arranged in a CRD with three replicates per treatment. Alternate treated and untreated 10 chickpea leaves were placed equidistant from the centre of the circular base. This was repeated for all treatments including a no-choice control with untreated chickpea leaves in all four portions. The top of the basin was covered with a nylon mesh to prevent the larvae from escaping. In each treatment, twenty 2nd or 3rd instar larvae were released at the centre of the

basin. The number of larvae that settled on the control and treated chickpea leaves was recorded after 1, 12, and 24 h of exposure. Percent repellence (PR) was calculated as described by Ogendo *et al.* (2003) as follows:

$$PR = 2(C - 50) \qquad \text{Equation 4}$$

C is the percent of larvae that settled on the untreated chickpea leaves.

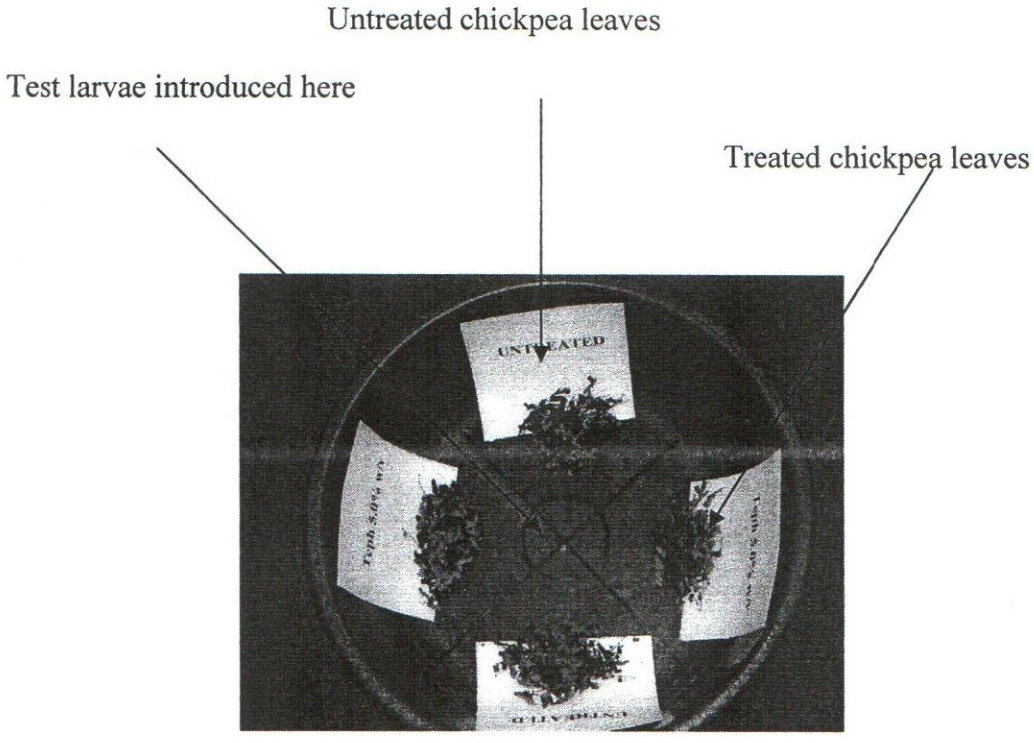


Plate 1: Repellence test showing the four portions of the circular base with alternate treated-untreated control chickpea leaves

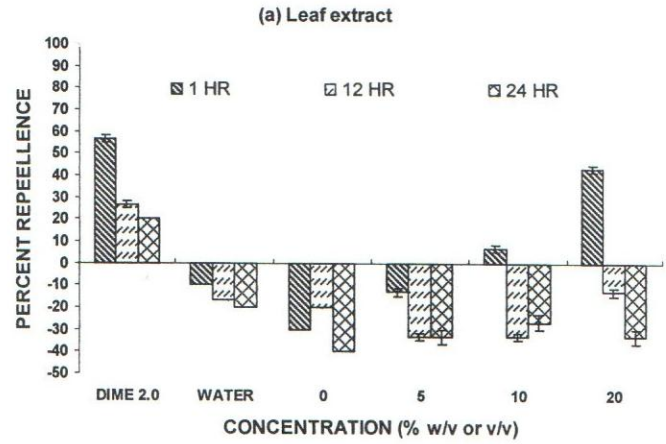
6.3 Results

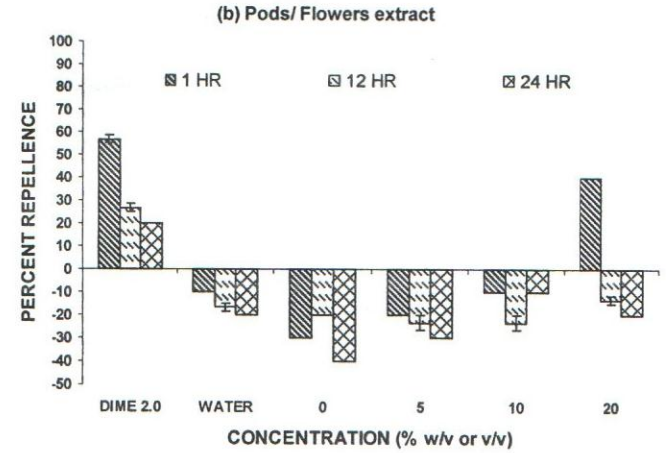
Results showed that the repellent activity of aqueous crude extracts of *T. vogelii* against *H. armigera* larvae was significantly (P = 0.0001) influenced by intra-plant variability, concentration applied, exposure time and corresponding factor interactions. Results showed that, except for leaf and pod/flower extracts at 20% w/v and 1 h exposure time with moderate repellence (PR value: 40%), a strong dose-dependent attraction of *H. armigera* larvae was

observed (Fig. 6.1). Attraction was noted in the other treatments. The response in the untreated control and the Separate samples of leaves. Pods/flowers and succulent stems was equally the same. The number of larvae that visited the chickpea leaves treated with *T. vogelii* extracts was higher compared to the negative control. The number of larvae recorded on the untreated chickpea leaves (food) increased with exposure time (Fig. 6.1).

6.4 Discussion

In the choice bioassay studies, dose- and exposure time-dependent attractions (negative PR values) of *H. armigera* larvae to chickpea leaves (food) treated with aqueous extracts of *T. vogelii* were observed. The differential (PR values) could be attributed to intra-plant variations in the amounts of chemical compounds / principles associated with repellent activity against insects. These results are in contrast with previous local studies in which *T. vogelii* powders, 10% w/w and 0.11w/w were strongly repellent (PR value: 87.5% after 24 h exposure) against adult *S. zeamais* and (PR value: 80%) against *Prostephanus truncates* Horn (Kirui *et al.*, 2009; Ogendo, 2008; Ogendo *et al.*, 2003). More over the rotenone compounds in *Tephrosia vogelii* are useful in the control of mosquito larvae where they attract them kill causing up to 100% kill (Matovu and Olila,2007b). The compound responsible for attraction or repellence in *Tephrosia vogelii* is rotenone as compared to the other rotenoids in *Tephrosia* example tephrosin and deguelin. In addition, *Tephrosia vogelii* is rich in terpenes (either macaroni or acumeni) which are responsible for its strong repellence effect. From the results of this study, it can be concluded thatthe aqueous crude extracts of *Tephrosia vogelii* were weakly repellent against *Helicoverpa armigera* larvae.





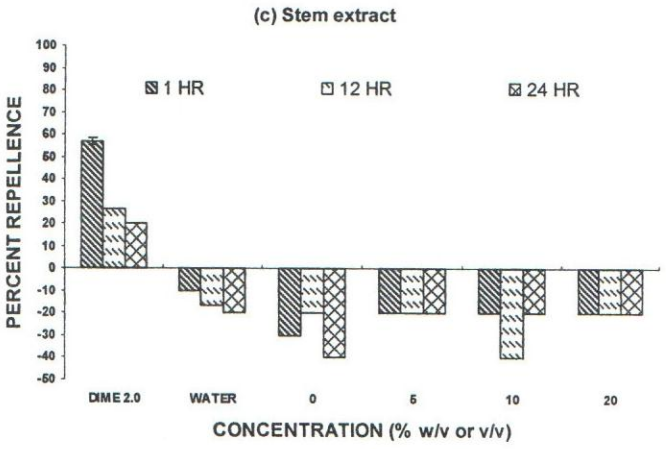


Fig. 6. 1: **Percent repellence (Mean± SE; n=4) of *H. armigera* larvae to aqueous extracts obtained from (a) leaves, (b) pods/flowers and (c) succulent stems of *T. vogelii* in a choice bioassay**

CHAPTER SEVEN

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

7.1 General Discussion

The use of plant and plant derived products to control pests in the developing world is an age-old practice. Plants are rich sources of natural substances that can be utilized in the development of environmentally safe products for insect pest control (Sedek, 2003; Sujatha *et al.*, 2010). Past scientific endeavours have showed that plant extracts often consist of complex mixtures of active compounds with greater overall bioactivity compared to the individual constituents. The deleterious effects of crude plant extracts on insects are manifested in several ways, including toxicity (Hiremath *et al.,* 1997) and feeding inhibition (Wheeler and Isman, 2001). However, there has been over–emphasis on the use of synthetic pesticides at the expense of botanicals in agricultural production (Isman, 2006). Despite their efficacies in the control of insect pests, extensive use of synthetic chemical insecticides in agriculture is currently under threat owing to their negative effects such as toxicity to non-target organisms and adverse effects to the environment (Thomas, 1999; Isman, 2006; Negahban *et al.,* 2006). The use of synthetic insecticides, Bt toxins and NPV, although effective, have been rendered largely incompatible with smallholder agriculture owing to documented negative effects, pest resistance or instability of control protocol (Lewis *et al.,* 1997; Ranga- Rao and Shanower, 1999; Sharma, 2001). In conformity with international trade and biosafety standards, the scientific rationalization process must ensure that newly developed botanical pesticides have high efficacy against target insects, leave no harzardous residues, have no adverse effects on the nutritional, flavour of field crops and are simple and convenient (Ogendo *et al.,*2008).

This study has shown that aqueous extracts obtained from aerial parts of *Tephrosia vogelii* Hook cannot be relied upon as repellents of *Helicoverpa armigera* larvae. However, *T. vogelii* extracts have potential use as toxicant antifeedant and attractant plant products as manifested in mortality and depressed feeding and growth and negative repellence (weight) of *H. armigera* larvae. This is welcome scientific hope for rationalized use of these extracts in the control of *H. armigera* in chickpea. The fact that botanical pesticides are locally available, biodegradable, cost effective and environmentally benign pest management technologies offer a good alternative to synthetic

pesticide. If followed to a logical conclusion, the results of such studies will contribute to improved national, regional and global food security and livelihoods of farming communities

7.2 Conclusions

On the basis of the finding of this study, the following conclusions can be made:

1. The aqueous crude extracts of *T.vogelii* had strong contact toxicity against the larval stage of *Helicoverpa armigera* with total (100%) kill achieved within 72h post application.
2. The aqueous crude extracts of *T.vogelii* had strong antifeeding activity, as manifested in high deterrence coefficients, but weakly repellent against *Helicoverpa armigera* larvae. However, the level of feeding deterrence was lower than to that of the positive control, Dimethoate 2%v/v.
3. The aqueous crude extracts of *T.vogelii* reduced the population of *H.armigera* by 37% similar to the positive control although the yield and yield components of chickpea in the positive control, Dimethoate (2%v/v) were slightly higher.
4. There was a strong intra-plant variability in bioactivity (toxicity,repellence and anti-feeding) in the three plant parts i.e leaves, pods and flowers in succulent stems. *T.vogelii* leaves had a stronger toxicity and antifeedant properties as compared to the pods / flowers and the succulent stems.

7.3 Recommendations

From the study it is recommended the following studies can further be carried out:

1) Reproduction inhibition of aqueous crude extracts of *Tephrosia vogelii* against *H. armigera.*
2) Effects of aqueous crude extracts of *Tephrosia vogelii* on the oviposition of *H. armigera.*
3) Growth and development on the larval and pupal stages of *H. armigera* when exposed *to* aqueous crude extracts of *Tephrosia vogelii*

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APPENDICES

Appendix 1: ANOVA-Antifeedant test of aqueous crude extracts of *Tephrosia vogelii* against *Helicoverpa armigera.*

Source	DF	Sum of Squares	F RATIO	Prob>F
Weight of larvae				
Plant part	2	19010.19289	1656.50**	0.0001
Days	6	4662.711746	135.43**	0.0001
Treatment	11	75416.02579	2628.63**	0.0001
Plant part*Days	12	6898.024444	100.18**	0.0001
Plant part*Treatment	10	22715.06521	395.87**	0.0001
Days*Treatment	30	1919.455873	11.15**	0.0001
Error	560			
Weight of food				
Source	2	84.84155556	3409.99**	0.0001
Plant part	6	10.06453968	134.84**	0.0001
Days	5	626.1288889	10066.27**	0.0001
Treatment	11	1.37488889	9.21**	0.0001
Plant part*Days	10	119.1664444	57.92**	0.0001
Plant part*Treatment	30	11.23355556	30.1**	0.0001
Days*Treatment	560			
Error				
Deterrence coefficient				
Plant part	2	59510.69999	3495.8**	0.0001
Days	6	1982.37994	38.82**	0.0001
Treatment	11	569260.8867	13375.89**	0.0001
Plant part*Days	12	1001.633788	9.81**	0.0001
Plant part*Treatment	10	85744.73983	1007.37**	0.0001
Days*Treatment	30	2449.001114	9.59**	0.0001
Error	560			
Mortality				
Plant part	2	507.7317897	9.39**	0.0001
Days	6	384.0641986	2.37*	0.0287
Treatment	11	846269.9614	6261.32**	0.0001
Plant part*Days	12	1773.398979	5.47**	0.0001
Plant part*Treatment	10	3018.710829	11.17**	0.0001
Days*Treatment	30	1567.570254	1.93**	0.0024
Error	560			

Appendix 2: ANOVA-Contact toxicity of aqueous crude extracts of *T.vogelii* against *H. armigera*

Source	DF	Sum of Squares	F RATIO	Prob>F
Aerial parts				
Plant part	2	0.00686478	3.88*	0.0231
Time	2	0.09901811	56.03**	0.0001
Treatment	11	23.10613294	5229.86**	0.0001
Plant part*Treatment	10	0.18598922	21.05**	0.0001
Plant part*Time	4	0.20899956	59.13**	0.0001
Time*Treatment	10	0.03472789	3.93**	0.0001
Error	34			
Leaves only				
Plant part	0	0.00000	.	-
Time	6	51617.91995	557.53**	0.0001
Treatment	11	27582.26457	255.36**	0.0001
Plant part*Treatment	0	0.00000	.	-
Plant part*Time	0	0.00000	.	-
Time*Treatment	42	21892.99404	33.78**	0.0001
Error	110			

Appendix 3: ANOVA-Repellent effects of aqueous crude extracts of *T. vogelii* against *H. armigera.*

Source	DF	Sum of Squares	F RATIO	Prob>F
Aerial parts				
Plant part	2	52.77777778	0.67^{NS}	0.514
Treatment	11	13816.66667	70.06**	0.0001
Time	2	3567.592593	45.23**	0.0001
Plant part*Treatment	10	1413.888889	3.58**	0.0003
Plant part*Time	4	1351.851852	8.57**	0.0001
Time*Treatment	10	1393.518519	3.53**	0.0004
Error	127			

Appendix 4: ANOVA-Toxicity studies in the field bioassays.

Source	DF	Sum of Squares	F RATIO	Prob>F
Season 1				
Stand count				
Treatment	7	3477.958333	3629.17**	0.0001
Error	14			
Total pods/plant				
Treatment	7	4066.6058	1225.97**	0.0001
Error	14			
Percent damaged pods/plant				
Treatment	7	14581.90292	437.79**	0.0001
	14			
Dry matter Kg/ha				
Treatment	7	20410975.84	91.28**	0.0001
Error	14			
Seed weight				
Treatment	7	8.47291667	24.35**	0.0001
Error	14			
Yield Kg/ha				
Treatment	7	8469037.258	43.73**	0.0001
Error	14			
Season 2				
Stand count				
Treatment	7	3453.166667	559.97**	0.0001
Error	14			
Total pods/plant				
Treatment	7	3030	272.36**	0.0001
Error	14			
Percent damaged pods/plant				
Treatment	7	15626.38153	37.04**	0.0001
Error	14			
Dry matter Kg/ha				
Treatment	7	20485759.95	425.81**	0.0001
Error	14			
Seed weight				
Treatment	7	8.54958333	18.11**	0.0001
Error	14			
Yield Kg/ha				
Treatment	7	7521341.41	189.16**	0.0001

Appendix 5: ANOVA- Antifeedant studies in the field bioassays

Source	DF	Sum of Squares	F RATIO	Prob>F
Season 1				
Weight of leaves and pods				
Stages	3	1.36622529	534.2**	0.0001
Treatment	7	0.0275736	16.17**	0.0038
Stages*Treatment	21	0.04085052	9.58**	0.0079
Error	62			
Mean plant per plant				
Stages	3	0.30624812	961.28**	0.0001
Treatment	7	0.00716871	33.75**	0.0005
Stages*Treatment	5	0.01216417	22.91**	0.0008
Error	62			
Season 2				
Weight of leaves and pods				
Stages	3	329.4478125	844.19**	0.0001
Treatment	7	215.1340625	236.26**	0.0001
Stages*Treatment	21	25.85635417	9.47**	0.0001
Error	62			
Mean plant per plant				
Stages	3	0.13345833	2.62^{NS}	0.0589
Treatment	7	24.76841667	208.11**	0.0001
Stages*Treatment	21	0.30845833	0.86**	0.6342
Error	62			

Appendix 6: ANOVA-Combined across seasons analysis for field bioassays

Source	DF	Sum of Squares	F RATIO	Prob>F
Mean larvae per plant				
Season	1	1754.46	93.38**	0.0001
Treatment	3	106631.32	1891.84**	0.0001
Season*Treatment	3	2481.953333	44.03**	0.0001
Error	80			
Stand count				
Season	1	28.16666667	68.28**	0.0001
Treatment	3	14416.5	11649.7**	0.0001
Season*Treatment	3	1.83333333	1.48 NS	0.2259
Error	80			
Total pods per plant				
Season	1	599.2002667	861.06**	0.0001
Treatment	3	12926.13413	6191.67**	0.0001
Season*Treatment	3	91.73413333	43.94**	0.0001
Error	80			
Percent damaged pods /plant				
Season	1	1880.094017	89.82**	0.0001
Treatment	3	53087.38258	845.42**	0.0001
Season*Treatment	3	2464.719917	39.25**	0.0001
Error	80			
Dry matter Kg/ha				
Season	1	266.6666667	0.26 NS	0.6115
Treatment	3	2175502.667	707.26**	0.0001
Season*Treatment	3	321.3333333	0.1 NS	0.9572
Error	80			
Seedweight				
Season	1	0.02666667	0.69 NS	0.4095
Treatment	3	25.8	221.7**	0.0001
Season*Treatment	3	0.12	1.03 NS	0.3834
Error	80			
weight Kg/ha				
Season	1	4213.5	6.01 NS	0.0164
Treatment	3	878005.8333	417.65**	0.0001
Season*Treatment	3	4377.833333	2.08**	0.0001
Error	80			

Appendix 7: Author's Publications

7.1 Refereed Journal Publications

1. **L.M. Wambua**, A.L. Deng, J.O. Ogendo, J. Owuoche, P.K. Bett (2011) Toxic, antifeedant and repellent activity of aqueous crude extracts of *Tephrosia vogelii* Hook on the larval stages of *Helicoverpa armigera* Hubner. Baraton Interdisciplinary Research Journal 1(1): 19-29. March 2011 (ISSN 2079-4711)

Baraton Interdisciplinary Research Journal (2011)/ (1), 19-29

TOXIC, ANTIFEEDANT AND REPELLENT ACTIVITY OF AQUEOUS CRUDE EXTRACTS OF *Tephrosia vogelii* HOOK ON THE LARVAL STAGES OF *Helicoverpa armigera* HÜBNER

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Abstract

Laboratory bioassays were conducted to evaluate the bioactivity of aqueous crude extracts of *Tephrosia vogelii* Hook against *Helicoverpa armigera* Hübner larvae. Fresh chickpea leaves, immersed in aqueous crude extracts of *Tephrosia vogelii* at four rates (0, 5, 10 and 20% w/v), were assayed for toxic, antifeedant and repellent effects against 2nd and 3rd instar larvae of *H. armigera* in a completely randomized design (CRD) with 3-5 replicates per treatment. Ordinary water and Dimethoate (Rogor E40) ® at 2% v/v were included as negative and positive controls, respectively. Data on corrected percent mortality, repellence and deterrence coefficient were first homogenized using angular transformations before being subjected to analysis of variance (ANOVA) and means separated by Tukey's HSD test. Results showed that the toxic, antifeedant and repellent effects of crude aqueous extracts of *T.vogelii* against *H. armigera* larvae were significantly ($P<0.0001$) influenced by intraplant variability, concentration applied, duration (hours) and corresponding factor interactions. At the highest concentration of 20% w/v, the aqueous crude extracts obtained from the leaves (22%) and pods/flowers (av. 11%) of *T.vogelii* were weakly toxic. In the antifeedant bioassay, leaf extracts caused the highest reduction (96%) in weight of larvae followed by pods/flowers (79%) and succulent stems (2.5%), respectively. There were corresponding reductions in larval feeding as the concentration of aqueous crude extracts increased. In the repellence test, except for leaf and pod/flower extracts at 20% w/v and 1 h exposure that produced moderate percent repellence (41.67%) against the larvae, there was a dose- and exposure time-dependent attraction of *H. armigera* larvae to chickpea leaves (food) treated with aqueous extracts of *T. vogelii*. The plant offers hope as a potential cost-effective and environmentally benign antifeedant for *H. armigera* control in chickpea.

Key words: *Tephrosia vogelii*, *Helicoverpa armigera*, toxicity, antifeedant, repellence.
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7.2 Manuscripts under review

1. **L.M. Wambua**, A.L. Deng, J.O. Ogendo, J. Owuoche, P.K. Bett (2012) Evaluation of *Tephrosia vogelii* leaf extract on *Helicoverpa armigera* larval survival and yield of chickpea. Submitted to Journal of Tropical Agriculture ; March 2012.

Evaluation of *Tephrosia vogelii* leaf extract on *Helicoverpa armigera* larval survival and yield of chickpea

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Abstract

Studies were conducted to evaluate contact toxicity and field efficacy of aqueous *Tephrosia vogelii* leaf extract against *Helicoverpa armigera* larvae and effect on yield of chickpea. Aqueous *T. vogelii* leaf extract at six rates (0, 20, 25, 30, 35 and 40% w/v), ordinary water and Dimethoate (Rogor E40)® at 2% v/v were assayed for toxicity against 2nd or 3rd instar *H. armigera* larvae in completely randomized design (CRD) with 3 replicates per treatment. In the field trials, same treatments were arranged in randomized complete block design (RCBD) with 3 replicates per treatment. Data were collected on mortality and survival of larvae, yield and yield components of chickpea. Results showed that contact toxicity and yield significantly ($P<0.0001$) depended upon concentration of extract applied and contact duration. At 20% w/v and 24 h contact duration, *T. vogelii* leaf extract caused 25% larval mortality compared to 100% kill for Dimethoate and 100% kill when contact duration increased to 120 h. A dose-dependent decrease in larval survival and pod damage by *H. armigera* larvae was observed. At 40% w/v and 90 DAP, 88-96 and 90-97% reductions in larvae survival and pod damage per plant, respectively, were recorded over the two seasons. Aqueous *T. vogelii* leaf extract at 40% w/v (av. Yield: 1721 kg/ha) and Dimethoate (av. Yield: 1787 kg/ha) were equally high yielding. Our findings have demonstrated potential of *T. vogelii* as an effective toxicant for *H. armigera* control in chickpea.

Key words: Aqueous extract, botanical insesticide, toxicity, *Cicer aurentium*

Conference Proceedings

1. **L.M. Wambua**, A. L. Deng, J.O. Ogendo, J.O. Owuoche, P.K. Bett (2010) Bioefficacy of aqueous crude extracts of *Tephrosia vogeli* on the larval stages of *Helicoverpa armigera*. Paper presented at 6th Research Week and International Conf., ARC Hotel, Egerton Univ. 22-24 Sept. 2010.