BIOLOGICAL ACTIVITY OF *Tephrosia vogelii* Hook. AND *Lantana camara* L. AQUEOUS CRUDE EXTRACTS AGAINST GOLDEN FLEA BEETLE (*Aphthona whitfieldi* Bryant) IN JATROPHA (*Jatropha curcas L.*)

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A Thesis submitted to the Graduate School in partial fulfilment for the Requirements of the Degree of Master of Science in Agronomy (Crop Protection) of Egerton University

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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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APPROVAL

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DEDICATION

To my dad and mentor, David Igogo Mukui who has always supported, motivated and inspired my post graduate studies despite many ups and downs.

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ABSTRACT

In an attempt to address the insect pest menace in Jatropha (*Jatropha curcas* L.), a study was conducted to document the major insect pests of Jatropha in Kenya and evaluate the bioactivity of aqueous *T. vogelii* and *L. camara* extracts against adult golden flea beetle (GFB). A stratified simple random sampling procedure was used to gather information on the major insect pests of Jatropha in Bondo and Kibwezi districts. In the laboratory and field studies, aqueous crude extracts, at four rates (0, 2.5, 5.0 and 10.0% w/v) and a synthetic insecticide, Karate (2.5% w/v), were evaluated for bioactivity against adult GFB. The laboratory experiments were laid out in completely randomized design (CRD) with 4-5 replicates per treatment. In the field experiments, treatments were laid out in a randomized complete block design (RCBD) with three replicates per site. Data collected on the major insect pests were analyzed using GLM of SPSS version 12. Data on percent mortality, pest reduction, feeding deterrence and repellence were to analysis of variance and means separated by Tukey's HSD test. Contact toxicity data were subjected to probit regression analysis using EPA Probit Analysis Program version 1.4 and LC_{50} values obtained. Field survey results showed that GFB was the most important insect pest of Jatropha. In the laboratory bioassays, the results showed that aqueous crude extracts of *T.vogelii* and *L. camara* had significant ($P<0.0001$) inter-plant-, dose-, and contact duration –dependent toxic, anti-feedant and repellent effects against GFB adults. At the dose range tested and 8 days of contact, *L. camara* and *T. vogelii* extracts caused 18–56% and 50.0–62% insect mortality *T. vogelii* extract produced the highest deterrence coefficient of 100%, at 5-10% w/v and 168 h after treatment, compared to 28-36% for *L. camara* extract. Irrespective of concentration and exposure time, *L. camara* and *T. vogelii* produced the same percent repellence values except the end-point repellence after 24 h in which *L. camara* was more repellent than *T. Vogelii*. Results from field bioassays have showed that percent pest reduction, leaf damage per plant, number of feeding holes and chlorophyll content per leaf were significantly ($P<0.0001$) influenced by inter-plant, concentration applied, exposure time and site. Irrespective of the plant assayed, exposure time, and site, *T. vogelii* (29-50%) caused higher percent pest reduction than *L. camara* (11-22%) at the concentration range tested. The results of this study show that the test botanical plants, *Tephrosia vogelii* and *Lantana camara,* have moderate to strong bioactivity (toxicity, repellence and anti-feeding) against the golden flea beetle and hold good promise as eco-friendly and cost-effective alternatives to synthetic pesticides for field insect pest control.

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ANOVA	Analysis of Variance
ASALs	Arid and Semi-Arid Lands
CETRAD	Centre for Training and Integrated Research in Arid and Semi Arid Lands Development (CETRAD)
CRD	Complete Randomized Design
EPA	Environmental Protection Agency.
FAO	Food and Agriculture Organization
GDP	Gross Domestic Product (*Aphthona whitfieldi* Bryant)
GFB	Golden Flea Beetle
GTZ	German Technical Cooperation
HSD	Honest Significant Difference.
KIHBS	Kenya Integrated Board of Statistics
LC_{50}	Lethal concentration that kill 50% of test insects
LD_{50}	Lethal dose that kills 50% of test insects.
LSD	Least Significance Difference
PR	Percent Repellence
RCBD	Randomized Complete Block Design.
RH	Relative Humidity

CHAPTER ONE
GENERAL INTRODUCTION

1.1 Background information

As the world focuses on global climate change, biofuels have assumed importance as the most practical alternative to petroleum fuels in efforts to reduce carbon emissions in the transport sector (GOK, 2004). *Jatropha curcas* whose seeds are rich in oil (19-59% oil) and can be extracted for biofuel purposes (Heller, 1996; Jones and Miller, 1991; Sujatha, 2007). Currently, approximately 3,860 acres of Kenyan land has been covered by the plant in eastern, coast, Nyanza, and rift valley provinces (Muok and Källbäck, 2008). (Muok and Källbäck, 2008 ; GTZ, 2009; Swallow and Tomomatsi, 2007). However, Kenya is said to be well ahead of other African countries in research on the same biofuel (Muok and Källbäck, 2008).

Jatropha curcas is currently producing low yields throughout Kenya (GTZ, 2009). The low yields could be attributed to inherent low soil fertility, moisture stress, inappropriate crop husbandry (agronomic) practices, insect pests and diseases among other limiting factors known to affect plant establishment (Muok and Källbäck, 2008). In a recent farm survey, GTZ (2009) reported that only 3-5 % of farmers apply chemical inorganic fertilizers and 61% never apply any pest and disease control measures owing to poverty (Achten *et al*., 2007; GTZ, 2009).

The golden flea beetle (*Aphthona whitfieldi* Bryant) has been reported as a major pest of Jatropha in Zimbabwe and Kenya (Zulu and Nielsen, 2007). The GFB adult is known to cause leaf damage leading to severe defoliation (Orwa *et al.,* 2009). Although initial feeding by adults does not penetrate the leaf completely, tissues below the injury eventually dry up and break or fall out giving a shot-hole appearance (Gavloski *et al.,* 2000). Under conditions of severe infestation, the pest can cause 100% plant damage (Nielsen and Jongh, 2009). In Kenya alone, a 42% GFB incidence in Jatropha farms has been reported (GTZ, 2009).

Although effective synthetic insecticides are available, the Jatropha farmers in Kenya are yet to integrate them into their production systems owing to rising costs, emergence of resistant pests and perceived toxicity to non-target organisms including human beings (Sharma and Gupta, 2009). There is, therefore, an urgent need for alternative non-chemical pest control measures which are affordable to resource poor farmers, offer acceptable level of persistence, non-toxic to

non-target organisms including man and readily biodegradable. The use of botanical pesticides to protect crop plants from pests is promising as they offer the following distinct advantages; namely: they are generally safer than synthetic pesticides; are part of nature for millions of years without any ill or adverse effects on the ecosystem; are renewable and cheaper and often have more than one chemical as an active principle responsible for their biological properties (Sharma and Gupta, 2009). Many plant species, in families such as Myrtaceae, Asteraceae, Piperaceae, Meliaceae and Annonaceae, are known to possess various chemical compounds which act as antifeedant, repellents, insecticides or growth inhibitors to many insect species (Formisano *et al.,* 2008; Odeyemi *et al*., 2008). A study was therefore instituted to evaluate locally available plants, *Tephrosia vogelii* Hook. and *Lantana camara* L. to determine their bioactivity against golden flea beetle on Jatropha. The results of this study will inform the future of botanical pesticide for control of GFB and other pests of Jatropha plants in Kenya and beyond.

1.2 Statement of the Problem

Evidence from the field shows that Jatropha is susceptible to many pests and diseases. Among the insect pests of Jatropha include; golden flea beetle (*Aphthona whitfieldi* Bryant), termites (*Agriotes spp*), leaf boring worms (*Salebria morosalis*), red spider mite (*Tetranychus* sp), mealy bug (*Ferrisia virgata*), shield backed bug *(Scutellera nobilis)* and scarabeid grub (*Scarabaeidae spp*). Of the above mentioned insect pests, the golden flea beetle (*Aphthona whitfieldi* Bryant) is the major pest of Jatropha in Kenya and is known to cause crop losses of up to 100% in severe infestation. Although synthetic insecticides are available, 61% of Jatropha farmers never apply any pest control measures owing to the high cost of synthetic insecticides. The use of synthetic insecticides has been associated with rising cases of resistant pests, destruction of natural enemies, turning formerly innocuous species into pests and toxicity to non-target organisms including man. These necessitate the need for evaluation of locally available botanicals pesticides which are cost effective and affordable.

1.3 Objectives

1.3.1 Broad objective

To contribute to improved *Jatropha curcas* production through reduced insect pest incidences.

1.3.2 Specific objectives

The Specific objectives of this study were to:

i. To determine the major insect pest species affecting *J. curcas* in Kenya.
ii. To evaluate differential toxicity, repellence and antifeedant effects of aqueous crude *Tephrosia vogelii* Hook and *Lantana camara* L. extracts against adult golden flea beetle (*Aphthona whitfieldi* Bryant)
iii. To evaluate the field bio-efficacy of aqueous crude *T. vogelii* Hook and *L. camara* L. extracts against golden flea beetle *A. whitfieldi* Bryant in *J. curcas* crop in Kenya.

1.4 Research questions.

i. Does *J. curcas* suffer from insect pests attack in Kenya?
ii. Do aqueous crude *T. vogelii* and *L. camara* extracts differ in their toxicity, repellence and antifeedant effects against adult golden flea beetles?
iii. Do aqueous crude *T. vogelii* and *L.camara* extracts differ in their field efficacy against adult golden flea beetles in *J. curcas* crop?

1.5 Justification

Jatropha can be cultivated in arid and semi-arid lands (ASALs) that are characterized by harsh weather conditions and soils of relatively low physical and chemical quality (Muok and Källbäck, 2008). *Jatropha curcas*, whose seeds are rich in oil (19-59%) (Heller, 1996), can be extracted for biofuel purposes and this can reduce the amount fossil fuel imports resulting in foreign exchange savings. For example, oil imports for the year 2005/06 accounted for 7.4% of GDP (Ksh.95.2 billion) and 25% of the total annual foreign exchange earnings (GOK, 2007). Also promoting Jatropha cultivation in Kenya is expected to contribute to reductions in greenhouse gas emissions and offer employment opportunities to the rural populations. Hence, *Jatropha curcas* whose cultivation is being encouraged in marginal areas of Kenya may help in poverty alleviation and rural development. High production of Jatropha through use of inorganic

inputs predisposes the crop to pests, whose incidences are currently low in Kenya. Unless cost effective control measures are instituted, these pests will become a major constraint to Jatropha production. Currently there is no control measure despite Golden Flea beetle causing 100% crop loss in severe infestation. Botanicals if proven effective will reduce infestation of GFB leading to increased yield hence more income to farmer through increased sale. More Jatropha yields means more biofuel production resulting to reduction of net importation of fuel and hence less of foreign exchange used.

CHAPTER TWO
GENERAL LITERATURE REVIEW

2.1 Jatropha Botany

Jatropha *(Jatropha curcas* L.; physic nut), belongs to the Euphorbiaceae family and has good potential as a biofuel crop in tropical and subtropical countries (Heller, 1996). Among the oil-bearing tree species, *Jatropha* is desired due to its drought hardiness, rapid growth ease of propagation, high oil content, wide adaptation, ability to grow in degraded soils and the optimum plant size that makes the seeds harvesting more convenient (Jones and Miller, 1991; Sujatha, 2007; GTZ, 2009). Jatropha is a large shrub, which can reach a height of 3 to 5 meters under normal conditions, and as much as 8 to 10 m under favourable conditions. The plant can be monoecious, or hermaphroditic, with the terminal inflorescences bearing unisexual flowers (Kumar and Sharma, 2008).

2.2 Importance of Jatropha

Among various alternative sources, Jatropha is one of the best alternatives for bio-diesel production (Jones and Miller, 1991). It has the desired physio-chemical and performance characteristic comparable to petro-diesel. Jatropha oil has higher cetone number which is comparable to diesel (46 to 50) and makes it an ideal alternative fuel and requires no modification in the engine (Punia, 2008). By 2030, the world is projected to experience net deficits of petroleum supplies as new oil discoveries are offset by depletions. Furthermore, as the world focuses on global climate change, biofuels have assumed importance as the most practical alternative to petroleum fuels in efforts to reduce carbon emissions in the transport sector (GOK, 2004).

2.3 Constraints of Jatropha production

Though the outlined benefits of Jatropha especially for biofuel purposes, the market supply of the marketable products have remained low because of various factors (Punia, 2008). First, is prevalent low yield associated with the most growing Jatropha farmer's fields. Low yields are attributed to overreliance by farmers on over-age plants, which increase the cost of production especially during harvesting which is characterized by irregular picking seasons (Nielsen and Jongh, 2009). In addition, the genotypes adopted by most farmers lack the desirable market qualities in term of yield, berry size, oil content, oil shelf life and resistance to field insect pests

(Muok and Källbäck, 2008). Secondly, pest and diseases also contribute to loss of yield and quality of the marketable yield (Orwa *et al.,* 2009).

For most farmers, the high cost of crop protection has greatly continued to contribute to worsening of the insect pest menace (Achten *et al*., 2007). A survey conducted in Kenya by GTZ showed that only about 3-5 % of farmers apply chemical fertilizer and 61% don't do pest and disease control which is mostly because of poverty (GTZ, 2009). The controversy surrounded by the cultivation of Jatropha has demotivated the adoption of this plant. For example, The leaves and nuts of Jatropha are considered toxic (containing Phorbol esters and curcin, a highly toxic protein similar to ricin in Castor) (Benge, 2006).The expanding use of land for the Jatropha production, even though it is marginal land, could mean that people living in the area would have to find other places for collecting firewood, herbs and fields for pasture land etc. Finally, a survey conducted in Tanzania, East Africa indicated that most farmers were discouraged by the lack of reliable market for their produce (Benge, 2006). For this reason, the farmers neglected their field in terms of crop protection and production practices.

2.4 Field insect pests of Jatropha

In its native range, more than 40 species of insects infest Jatropha with the shield-backed bug (*Pachycoris klugii*), leaf-footed bug (*Leptoglossus zonatus*), millipede (*Julus* sp.), locust (*Oedaleus senegalensis*), cushion scales (*Pinnaspis strachani*), the woody mealybug (*Ferrisia virgata*), blue bug (*Calidea dregei*) and green vegetable bug are among the major insect pests causing economic damage to Jatropha in the Asaian continent (Heller, 1996; Grimm and Fuhrer, 1998; Grimm and Somarriba, 1999; Donaldson and Tsang, 2006; Manoharan *et al*., 2006). Literature survey indicates that two insects have recently emerged as important pests of Jatropha in India. The first is a scutellarid bug (*Scutellera nobilis* Fabr.) which causes flower fall, fruit abortion and malformation of seeds (Shanker and Dhyani, 2006). The second is an inflorescence and capsule-borer (*Pempelia morosalis*), which causes economic damage by feeding on inflorescences and in later stages boring into the capsules (Shanker and Dhyani, 2006). In northern parts of Australia, the larvae of the polyphagous tobacco cut worm, *Spodoptera litura* (Fabricius), are a major pest that feeds on Jatropha leaves (Herbison-Evans and Crossley, 2006).

In Africa, Flea beetles (*Aphthona spp*.) are the main insect pests of Jatropha known to cause severe leaf defoliation and up to 100% plant death (Jongh, 2006; Nielsen, 2007; Gagnaux, 2008; Nielsen and Jongh, 2009). The golden flea beetle (*Aphthona whitfieldi* Bryant.) has been reported as a major insect pest of Jatropha in Zimbabwe and Kenya with its pest status more severe in younger host plants and in less fertile soils (Zulu and Nielsen, 2007).

2.5 Biology of golden flea beetle (Aphthona whitfieldi Bryant)

The golden flea beetle (*Aphthona whitfieldi* Bryant) belongs to the family Chrysomelidae. Mating occurs on plant shoots, after which adult females lay eggs at the soil surface or in the soil, on or near the base of stem. Generally, *Aphthona spp* females lay a total of 100-300 eggs during their lifetime, in a series of small groups after every 6 to 7 days of intensive feeding (Gassmann, 1990; Volkovitsh *et al.,* 2000). Normally, the embryonic development stage takes an average of 18 days at 22^{o}c (Volkovitsh *et al.,* 2000) Newly hatched larvae burrow in the soil and begin feeding on very small roots and feed on progressively larger roots as they develop (Skinner *et al.,* 2004). The pest overwinters as a diapausing larva for 3-4 months in the soil and on or near the roots when the temperature goes below 3^{o}c. Overwintered larvae resume development in the spring when temperature begins to rise to 26^{o}c and pupation occurs within a soil cell from late spring to early summer after 1 month of feeding (Skinner *et al.,* 2004; Volkovitsh *et al.,* 2000). Adult beetles emerge from the soil throughout the summer, and begin feeding on leaves and flowering structures. Adults are about 3 mm long; they rarely fly under field conditions and instead move about by hopping in typical flea beetle fashion. Adults are relatively long-lived beetles, capable of surviving up to 4 months (Jackson*,* 1998). Adult flea beetles damage plants by feeding on the surface of leaves and stems, resulting in numerous small holes over the surface of the leaf. Intense feeding damage can kill plants, especially young seedlings (Orwa *et al.,* 2009). The pest uses many sensory cues (odours, taste, and vision) to locate their host plants (Hazzard *et al.,* 2002).

2.6 Methods of pest control

2.6.1 Chemical control

Garden insecticides containing carbaryl (Sevin), spinosad, bifenthrin and permethrin can provide fairly good control for about a week. Diatomaceous earth is one of the more effective repellents,

applied as a dry powder to the plants. Horticultural oils and neem-based insecticides also have repellent effects against this insect (Cranshaw, 2006). Under conditions of heavy golden flea beetle infestations in the field, foliar sprays are recommended as soon as possible, since these beetles can cause substantial damage quickly (MAFR, 2010).

2.6.2 Biological control

Predators, parasites and diseases can be important in regulating insect populations. To date the effect of biological control agents seems to be limited but several insects have been reported attack adult flea beetles (Cranshaw, 2006). Lacewing larvae (*Chrysopa carnea*), big-eyed bugs (*Geocoris bullatus*), the two-lined collops (*Collops vittatus*), the western damsel bug (*Nabis alternatus*) and the northern field cricket (*Gryllus pennsylvanicus*) are a few of the insects known to prey on flea beetles. The native braconid wasp (*Microctonus vittatae*), parasitizes adult golden flea beetles and sterilizes the female GFB (Kuepper, 2003). Unfortunately, GFB populations emerge in large numbers during a relatively short period of time and tend to overwhelm the parasites and predators (Cranshaw, 2006). Commercial formulations of entomopathogenic nematodes are effective agents for controlling flea beetles. Applied to the soil, the nematodes attack the beetle's larval stage, reducing root feeding and helping to prevent the next cycle of adults from emerging (Kuepper, 2003).

2.6.3 Botanical Pest Control

The concerns of the public on the problems associated with the continuous use of conventional insecticides have necessitated the search for possible alternatives (Hassanali *et al*., 1990). Plants in general are able to produce secondary metabolites that are physiologically active in insects and other organisms and that provide the plants with one of the most important defense mechanisms (Strauss and Zangerl, 2002). Although botanical insecticides comprise only a very small portion of the total volume of insecticides used annually, they remain an important component in insect pest management owing to their perceived efficacy against insect pests that have become resistant to synthetic insecticides (Weinzierl, 2000). Plant-derived insecticides are short-lived in the environment, thus pose less risk to non-target organisms. (Isman, 2000; Weinzierl, 2000). Phytochemicals such as rotenone, nicotine and pyrethrum were all used as pesticides before the advent of synthetic insecticides (Odeyemi *et al*., 2008). Many members of families such as Lamiaceae, Myrtaceae, Asteraceae, Piperaceae, Meliaceae and Annonaceae are known to possess

various chemical compounds which act as toxicants, antifeedants, repellents or growth inhibitors to many insect species (Formisano *et al.,* 2008; Hillock, 2008; Odeyemi *et al*., 2008; Ogendo *et al*., 2008).

2.7 Effects of botanical pesticides on flea beetles (Coleoptera: Chrysomelidae)

In their field evaluations, Oladimeji and Kannike (2009) reported that *Azadirachta indica* and *Ocimum basilicum* leaf extracts, at 2.0% w/v, were not phytotoxic and achieved 54 and 43% reductions, respectively, in okra leaf damage by flea beetle, *Podagrica* spp. In related studies, aqueous extracts obtained from six different botanical plants, at 5-20% w/v dose range, showed clear dose- and contact duration-dependent efficacy (toxicity) against adult flea beetles (*Phyllotreta nemorum*) (Subedi and Vaidya, 2003)

2.8 Aqueous extracts of *Lantana camara* L.

Lantana camara L. (Verbenaceae) is a hardy, evergreen, shrub with characteristic odour, it grows up to 3 m height, with or without minute prickles on the branches. It is a perennial shrub found growing up to 2000 m above sea level (masl) in tropical, sub tropical and temperate parts of the world (Dua *et al.,* 2008). Several tri- terpenoids, flavonoids, alkaloids, and glycosides isolated from this plant are known to exert diverse biological activities (Prasad and Purohit, 2009). Extract from the leaves of *L. camara* possess larvicidal activity (Chavan and Nikam, 1982) while extract from flowers of the plant show repellent activity (Prasad and Purohit, 2009). Insecticidal action of aerial parts of *L. camara* against *Callosobruchus chinensis* (Coleoptera: Bruchidae) produce 10-43% mortality, complete feeding deterrent and loss of fecundity (Prasad and Purohit, 2009*)*. In their laboratory studies, Ogendo *et al*. (2003) reported that *Lantana camara* L. powder, at 10% w/w, caused 90% kill of adult *Sitophilus zeamais* 21 days after treatment. Other documented information indicate that aqueous *Lantana* extracts, at 1% w/v, caused complete feeding inhibition of first instar larvae of *P. brassicae* and also reduced the infestation of tea leaves by the tea mosquito bug (Sharma and Gupta, 2009).

2.9 Aqueous extracts of Tephrosia vogelii Hook.

Fish poison bean, *Tephrosia vogelii* Hook (Fabaceae), is a semi perennial, shrubby plant indigenous to Africa and grows best on depleted light soils where it is a valuable fallow improvement species particularly for the control of *Striga hermonthica* (Mathias, 1997).

Tephrosia species contain complex mixtures of rotenoids and other flavonoids (Go´mez-Garibay *et al.,* 2002). *T. vogelii* only contain compounds such as rotenone, tephrosin, and deguelin which can be economically and commercially exploited as a phytochemical in the pesticide industry (Gaskins *et al.,* 1972). The toxic principle compound in the plant is the presence of rotenoids known to be mitochondrial chain inhibitors, inhibiting cellular respiration in almost every living organism including insect and mammals. These compounds block the enzymes glutamate and succino dehydrogenase and thus H^+transport (Neuwinger, 2004). Anti-feeding effects of *Tephrosia* have also been reported on spotted cereal stem borer (*Chilo partellus*) There were significant (P = 0.05) increases in grain yield in the sprayed plots and a concomitant improvement in grain quality (Kyamanywa *et al*., 2001).

CHAPTER THREE
MATERIALS AND METHODS.

3.1. Field survey

3.1.1 Sampling procedure

Stratified simple random sampling was administered in two administrative districts of Bondo and Kibwezi (Deng *et al.,* 2009; Ogendo *et al.,* 2004). Kibwezi is a dry and hot area with little rainfall (550-670 mm) and high temperatures of 24^0C. The district receives scarce rainfall which varies with altitude and experiences high temperatures during the day and low temperatures at night. Most parts of the region are semi-arid although areas below 670 meters in elevation are generally arid. Soil types vary between sand, loam and clay. Bondo region falls in agro-climatic zones ranging from humid in high altitude areas (1,400-2,000 meters), sub-humid (1,200 to 1,400 meters) and semi-humid (1,100 to 1,300 meters). This region has diverse soil types, though mainly loamy and black cotton soils. Vegetation type is mostly bush land and dry woodland. The district experiences a bimodal rainfall with an average of 1100 to 1350mm and means temperatures of 22^0 C. Each of the two districts was considered as homogenous sampling block/stratum and administrative divisions, locations, sub-locations and villages randomly represented. A total of 85 Jatropha farmers, 44 and 41 in Bondo and Kibwezi districts, respectively, were randomly selected and interviewed on Jatropha cultivation, major insect pests, indigenous knowledge and practices of insect pest control using semi-structured questionnaire and complemented by additional observations.

3.1.2 Socio-economic traits of Jatropha farmers

Information was collected on the farmer's sex, age, education level and primary occupation of respondents. These variables were considered to have influence on the decision-making and crop- pest management at the farm level (Deng *et al.,* 2009).

3.1.3 Major Field insect pests of Jatropha in Kenya.

The major insect pest species and their infestation status in Jatropha fields were investigated. On-the-spot identification of insect pests mentioned by respondents was carried out by the researchers on the basis of expertise, pictorial aids and available literature materials during the survey re (Jongh, 2006; Mutaquin *et al*., 2010). Identification also relied on farmers' description

and ability to recognize the said pests from own knowledge. Preserved voucher specimens of insect samples were forwarded to the National Museums of Kenya for authentic identification.

3.1.4 Cultural characteristics of Jatropha fields.

Data were collected on the scale of Jatropha cultivation (number of trees per farmer), source of planting materials (propagation method), age of plantation (year of planting and cropping system information.

3.2 Laboratory bioassays

3. 2.1 Collection and preparation of test plant materials

Two locally available indigenous plants, *T. vogelii* Hook (Fabaceae; fish poison bean) and *L. camara* L (Verbenaceae; wild sage) were selected as test botanical plants based on their local availability and perceived insecticidal properties (Ogendo *et al.,* 2003; Ogendo, 2008). Fresh leaves were harvested and then transported in separately well labelled bags. Thereafter the samples were shade-dried at ambient temperatures (18-28^{o}C) for 2 weeks and further oven dried at 35^{o}C for 48 hours (Ogendo *et al*., 2003). Dry samples were ground into fine powders using an electric laboratory hammer mill. The resulting powders were then stored separately in glass containers with screw cap and stored at room temperature prior to use.

3.2.2 Preparation of aqueous crude extracts

Aqueous concentrations (0, 2.5, 5.0 and 10.0% w/v) were prepared using the crude powders of *T. vogelii* and *L. camara* described in section 3.2.1 above. Three (3) ml of an emulsifier, Teepol, was then added to 1 L of test extract prior to application (Oladimeji and Kannike, 2009). The emulsifier helps the extracts to stick well to the leaf surface (Agboka *et al.,* 2009). A synthetic insecticide, lambda-cyhalothrin (Karate), at 2.5% v/v, was included as a positive control.

3.2.3 Collection and laboratory maintenance of golden flea beetle.

Adult golden flea beetle, *Aphthona whitfieldi* Bryant was collected by sweep nets from pesticide-free Jatropha farmers' field. The test insects were then maintained in cages in a growth room under constant temperature (23±1^{o}C), relative humidity (RH) (65 ±5 %), and a 16:8-h (light: dark) regime at Egerton University (0^{o}20'S, 35^{o}56'E) Biotechnology Laboratory. Adults were fed with young Jatropha seedlings leaves to ensure consistency of response in the bioassay. Water was supplied daily by spraying small droplets onto the cage screen. Before each test,

beetles were starved for six hours with only access to the honey solution (Gavloski *et al.,* 2000; Subedi and Vaidya, 2003). Only healthy-looking beetles were randomly assigned to separate treatment units within 2 days of field collections (Wang *et al*., 2005).

3.2.4 Contact toxicity studies

The inner walls of experimental cages (transparent plastic bottles: 7.5 cm high by 6 cm in diameter) (Subedi and Vaidya, 2003) were coated with separate crude extracts obtained from *T. vogelii* and *L. camara* (0, 2.5, 5.0 and 10.0 % w /v) and a positive control (Karate) at 2.5% v/v according to Brigitte *et al*. (2002) with modifications. Coating was done by filling the cages with separate crude extracts for 5 minutes and then emptied. Ten beetles were introduced in each cage bottle immediately and exposed for 30 min (Wang *et al.,* 2005) before introducing untreated fresh leaves. The mouths of the bottles were covered with muslin cloth for aeration and to prevent insect from escaping. A total of eight treatments were arranged in a completely randomized design (CRD) with four replicates per treatment. Fresh Jatropha leaves were administered after every 24 h. The number of dead adult beetles (N_D) were recorded 24, 48, 72 , 120 and 168 h after setup (Subedi and Vaidya, 2003) and actual percent mortality computed according to Asawalam *et al*. (2006) (Equation 1). The insect mortality data were corrected for natural mortality using Abbott (1925) formula (Equation 2).

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

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

Where P_T, P_O, and P_C represent the corrected, observed and control percent mortalities, respectively (Abbott, 1925).

3.2.5 Antifeedant tests

To determine the amount of food that adult can feed, Jatropha leaf discs (30 mm by 30mm) immersed in aqueous extracts at 2.5, 5, 10 % w /v and controls (30 minutes) were air dried and then weighed. Three leaf discs were then placed in each experimental cages (Transparent plastic bottles: 7.5 cm high by 6 cm in diameter.) for each plant extract according to Erturk (2006) with some modifications. Ten adults were placed on the diet (leaf discs) in each container for each

assay. A total of eight treatments, each replicated 5 times, were arranged in a CRD in the laboratory under controlled conditions of temperature, relative humidity and light : dark regime. Based on the amount of food consumed, the absolute deterrence coefficient (DC) then calculated using the formula (Equation 3) according to Kielczewski and Nawrot (1979):

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

Where T is weight of food eaten by the adult in the experimental unit and C is weight of food consumed by the adult in the control unit. The adults in each experimental received freshly treated diet every 24 h and data recorded 24, 48, 72, 120 and 168 h after setup.

3.2.6 Repellence test (Choice bioassay)

Choice bioassay tests were conducted in circular flat bottomed plastic basins (45cm in diameter by 30cm high) with their bases divided into four equal portions as described by Ogendo *et al*. (2004). For each treatment, alternate treated and untreated five (5) Jatropha leaf discs (50 mm by 50 mm) were placed equidistant from the centre of the circular base and this arrangement was repeated for all treatments, including a standard repellent, DEET (N,N-diethyl-meta-toluamide) and a no-choice control with untreated leaves in all four portions. The top of the basin was covered with a nylon mesh to prevent the insects from escaping while allowing aeration. For each treatment, 20 adults were released at the centre of the basin. The experiment was laid out in a CRD with four replications per treatment. The total number of insects that settled on the control and the treated leaves were then recorded 1, 3, 5 and 24 hours after setup.

Percentage repellence (PR) values were computed according to Echereobia *et al.* (2010) using the following formula (Equation 4):

$$PR = \frac{NC - NT}{NC + NT} \times 100 \qquad \text{Eq. 4}$$

Where PR= Percentage Repellency, NC= Number of pests on control portion, NT= Number of Pests on treated portion. Positive (+) and negative (-) PR values represent repellence and attractance, respectively.

3.3 Field experiments

3.3.1 Field layout

Two field experiments were initiated during the 2010/2011 growing season at two geographically distinct sites namely Kibwezi and an experimental farm at the Egerton University's Chemeron Field Station. The Egerton University Chemeron field station is located in Baringo (latitude: 00°47'N and longitude: 36°16'E) at an altitude of 1,080 m above sea level. The station falls in the Lower Midland agro-ecological zone (LM5), with an annual mean annual temperature and rainfall of 23°C and 700 to 950 mm, respectively. The rainfall peaks in the April/May and July/August rainy seasons (Ondiek *et al.,* 2010). The other site, Kibwezi (latitude: 02°27'S and longitude: 37°57'E) is a dry and hot area with little rainfall (550-670mm) with peaks in the March/May and October/November rainy seasons and high temperatures of 24^0C during the dry season. Most parts of the region are semi-arid although areas below 670 meters in elevation are generally arid (Mwangangi *et al.,* 2009). Jatropha seeds were planted on 1st September 2010 on polybags measuring 15cm diameter and 30 cm height on a raised seedbed. After 1 month, nine uniform (in height, leaf number and size) Jatropha seedlings were selected and grouped together to form a treatment. A total of 8 treatments including the untreated control were laid out in RCBD with three replicates per treatment per site. A spacing of 0.6 and 1.7 metres was used intra- and inter-row, respectively. All the non-experimental agronomic practices were applied as and when needed and kept constant for the whole experiment.

3.3.2 Treatment application

The population of GFB insects in the experimental field monitored weekly and botanical treatments applied (spraying) when pest symptoms, shot-hole appearance on leaves, were observed. Before each spray, the volume of spray solution was calibrated by spraying measured volume of water on the check plots. Each treatment was repeated after 15 days giving a total of two spray applications. The treated Jatropha plants, in groups, were laid out in a RCBD with three replicates per treatment per site. All the other agronomic practices were uniformly applied as and when needed in all experimental units.

3.3.3 Golden Flea beetle, *Aphthona whitfieldi* Bryant populations

Leaves of three randomly selected Jatropha plants, in each experimental plot, were assessed early in the morning for GFB insects (Subedi and Vaidya, 2003; Oladimeji and Kannike, 2009; Shanna

and Srivastava, 2010). Adult GFB counts were recorded 24 h before application of botanical treatment and 1, 3 and 7 days after each spray. The percent pest reduction (PPR) was computed according to Sharma and Srivastava (2010) as follows (Equation 5):

$$PPR\,(\%) = [1 - (\frac{PTi}{PTo} \times \frac{PCo}{PCi})] \times 100 \qquad \text{Equation 5}$$

PPR=Percent pest reduction, PTi= Population after treatments, PTo= Population before treatment, PCo = Population in the control before spray, PCi= Population in the control after spray

3.3.4. Plant damage assessment

For estimating the effect of various treatments on plant damage, percent leaf damage per plant, number of (feeding) shot holes and leaf chlorophyll content per leaf were recorded. For percent leaf damage per plant, the number of leaves showing evidence of defoliation was recorded from three randomly selected plants in each plot. Each leaf was carefully assessed for skeletonization with aid of a magnifying glass**.** For each tree that was assessed for leaf damaged, number of (feeding) shot holes were counted and leaf chlorophyll content (μg $cm^{-}2$) measured with SPAD-502 Chlorophyll meter (Minolta) three hours after sunrise **(**Firdaus *et al.,* 2010**)** on three fully expanded mature leaf from each tree. These parameters from each plot were recorded after a growth period of 8 months.

3.4 Data analysis.

Data obtained from the survey was analyzed using descriptive statistics (frequencies and percentages) done using SPSS version 17. Data on corrected percent mortality was first homogenized using arcsine transformations before being subjected to ANOVA using the SAS package (SAS, 2001) and means separated by Tukey's HSD test (Ogendo *et al*., 2008). Corrected percent mortality data obtained from contact toxicity bioassay 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 (Finney, 1971).The LC_{50} values in a column were considered significantly different when 95% fiducial limits did not overlap. Deterrence coefficient data were first homogenized using arcsine transformations before being subjected to ANOVA using the SAS package (SAS, 2001) and

means separated by Tukey's HSD test (Ogendo *et al*., 2008). Repellence data were first homogenized using arcsine transformations before being subjected to ANOVA using the SAS package (SAS, 2001) and means separated by Tukey's HSD test (Ogendo *et al*., 2008). Data on actual pest population and percent pest reduction (PPR) were first homogenized using square root and arcsine transformations, respectively, before being subjected to ANOVA using the SAS package (SAS, 2001) and means separated by Tukey's HSD test (Mead *et al*., 1994; Ogendo *et al*., 2008). Plant damage was directly subjected to ANOVA using the SAS package. The PPR values in various treatments were used as indirect indicators of botanical pesticide efficacy in which the higher the PPR, the higher the pesticide efficacy and vice versa (Khattak, *et al.,* 2004).

CHAPTER FOUR.

RESULTS AND DISCUSSION

4.1 Results.

4.1.1. Field survey

4.1.1.1 Socio-economic traits of respondents

The results showed no differences ($P<0.05$) in gender, age and educational levels of Jatropha farmers in Bondo and Kibwezi districts. However, there were more males (56.8%) than females (43.2%) in Bondo whereas the converse was true for females (53.7%) (Table 1). Seventy two percent of the respondents were aged ≥41 years. Education status, on the other hand, was similar across the districts with majority (87%) of respondents having at least primary level education . Conversely, primary occupation varied considerablely across the districts ($P < 0.05$), with most of the farmers involved in Farming 68.2%. (81.8%) and (53.7%) respondents were involved in farming in Bondo and Kibwezi respectively.

4.1.1.2 Cultural characteristics of Jatropha fields

Results showed that Jatropha farming started in Kibwezi and Bondo in 2006 and 2008 respectively (Table 2a). There were more Jatropha trees in Kibwezi (580 ± 137) than in Bondo (181 ± 28). Forty and 37.6% of the farmers practiced monocropping and intercropping systems, respectively (Table 2b). Propagation method for Jatropha was predominated by use of seeds (57.6%) and seedlings (35.3%).

Table 1: Socio-economic characteristics of respondent farmers in Bondo and Kibwezi districts

Socio-economic characteristics		Percentage of Respondents (Mean)		
		Bondo	Kibwezi	Mean (%)
Age	<25	6.8	4.9	5.9
	26-40	13.6	29.3	21.2
	41-55	29.5	31.7	30.6
	≥56	50	34.1	42.4
Sex	Female	43.2	53.7	48.2
	Male	56.8	46.3	51.8
Education	No formal education	9.1	12.2	10.6
	Primary education	68.2	70.7	69.4
	Secondary education	20.5	14.6	17.6
	Tertially education	2.3	2.4	2.4
Primary Occupation	Farming	81.8	53.7	68.2
	Employed	9.1	9.8	9.4
	Small business	6.8	14.6	10.6
	Agri. Worker	0	14.6	7.1
	Pension	2.3	7.3	4.7

Table 2: Cultural practices in Jatropha fields in Bondo and Kibwezi districts, Kenya.

a)

	mean ± SE		
Source	**Bondo**	**Kibwezi**	**total**
No of trees	181 ± 28	580 ± 137	374 ± 71
year of planting	2008 ± 0.142	2006 ± 1.147	2007 ± 0.572

b)

		Percentages		
Source		**Bondo**	**Kibwezi**	**Mean**
Cropping system	Monocropping	43.2	36.6	40.0
	Iintercropping	40.9	34.1	37.6
	Fence	15.9	29.3	22.4
Propagation method	Seeds	36.4	80.5	57.6
	Seedling	61.4	7.3	35.3
	Cutting	2.3	12.2	7.1

4.1.1.3 Insect pests and control methods

Majority (87.1%) of the farmers were aware of pests in their farms with the golden flea beetle, *Aphthona whitfieldi* Bryant as the major field insect pest of Jatropha in Bondo (93.2%) and Kibwezi (63.4%) (Table 3). The other insect pests reported were the leaf boring worms (*Salebria morosalis*), soil grubs (*Scarabaeidae spp*) and the shield-backed bug *(Scutellera nobilis)*. Although, majority (87.1%) of the farmers were aware of insect pests, 80% of farmers never applied any pest control measures in Jatropha mostly due to the high cost of synthetic insecticides (Table3).

Table 3: Knowledge of insect pests and pesticides control method among farmers of Bondo and Kibwezi

	Percentage (%) occurrence		
Farmers' knowledge	Bondo	Kibwezi	Mean (%)
Pest awareness:			
aware	97.7	75.6	87.1
Not aware	2.3	24.4	12.9
Major insect pests:			
Golden flea beetle	93.2	63.4	78.8
Leaf boring worms	4.5	2.4	3.5
Blue bug	0	7.3	3.5
Soil grub	0	2.4	1.2
None	2.3	24.4	12.9
Use of chemicals:			
No Chemical control	75	85.4	80
chemical control	25	14.6	20
Why didn't apply pesticides:			
High cost	72.7	70.7	71.8
Advised not to	25	12.2	18.8
No pests	2.3	17.1	9.4

4.1.2 Laboratory bioassays

4.1.2.1 Contact toxicity studies

Results of contact toxicity studies showed that adult GFB mortality was significantly ($p<0.0001$) influenced by plant species, concentration of extract applied, exposure time and corresponding factor interaction effects. Although differentially toxic against adult GFB, both plants produced weak dose- and exposure time-dependent toxicity when topically applied. *Lantana camara* registered less than 50% mortality (LC_{50} value = 8.54 w/v %) compared to 50-62 % mortality (LC_{50} values = 1.9 w/v %) for *T. vogelii* 168 h after treatment (Fig.1; Table 4).The LC_{50} values for both test plants decreased with exposure time, a manifestation of increasing mortality of adult GFB (Table 4).

Table 4: Mean percent mortality (±SE, n=4) of golden flea beetle, *Aphthona whitfieldi* Bryant over 168 hrs in contact with aqueous crude extracts of *L. camara* and *T. vogelii* leaves.

			Percent Adult Mortality (Mean±SE) Expousure time (hours)				
plant	Treatments (% w/v)	N	24	48	72	96	168
L. camara							
	karate 2.5	10	90 ± 0.00	100 ± 0.00	100 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
	0.00 (untreated control)	10	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	2.50	10	5.56 ± 3.21	5.56 ± 3.21	5.56 ± 3.21	6.25 ± 3.61	18.75 ± 3.62
	5.00	10	8.33 ± 2.78	8.33 ± 2.78	11.11 ± 0.00	18.75 ± 3.61	31.25 ± 3.61
	10.00	10	16.67 ± 3.21	16.67 ± 3.21	19.44 ± 2.78	31.25 ± 3.62	56.25 ± 3.62
	$LSD_{0.05}$		6.36	6.36	6.36	7.15	7.15
	LC_{50} Value 95%FL		83.56 (15.84,80.76)	83.56 (15.84,80.76)	51.29 (20.74, 3404.51)	18.77 (12.53, 48.38)	8.54 (6.88, 12.16)
T.vogelii							
	karate 2.5	10	90 ± 0.00	100 ± 0.00	100 ± 0.00	100.00 ± 0.00	100.00 ± 0.00
	0.00 (untreated control)	10	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	2.50	10	11.11 ± 0.00	27.78 ± 3.21	33.00 ± 0.00	40.63 ± 3.13	50.00 ± 0.00
	5.00	10	25.00 ± 2.89	50.00 ± 3.21	50.00 ± 3.21	56.25 ± 3.61	62.50 ± 0.00
	10.00	10	37.50 ± 2.50	50.00 ± 3.22	55.56 ± 3.21	62.50 ± 5.10	62.50 ± 3.13
	$LSD_{0.05}$		5.72	6.36	6.36	10.01	6.2
	LC_{50} Value 95%FL		15.75 (10.72, 39.29)	7.94 (5.59, 19.31)	6.34 (4.45, 11.87)	4.09 (2.17, 5.96)	1.99 (0, 0)

Any two means in a column whose SE overlaps or their difference is smaller than LSD value are not significantly different at α=0.05.

LC_{50} values were considered significantly different when 95% fiducial limits (FL) didn't overlap

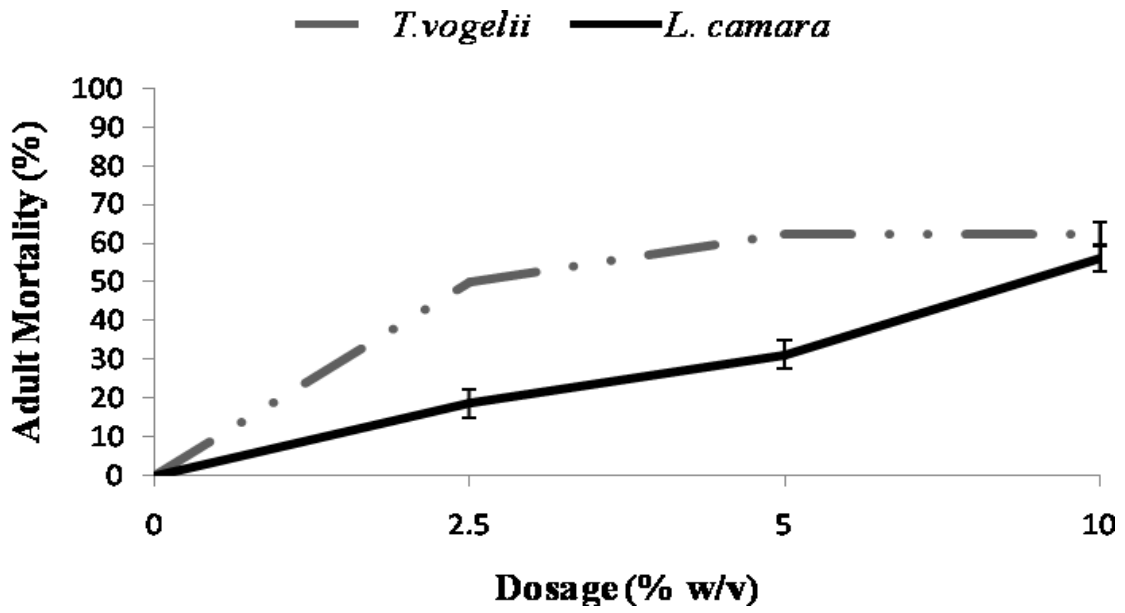


Figure 1: Endpoint adult mortality of golden flea beetle, *Aphthona whitfieldi* Bryant in contact toxicity studies.

4.1.2.2 Antifeedant test.

Results showed that the amount of food consumed and feeding deterrence against adult GFB were significantly ($P<0.001$) influenced by inter-plant, concentration of extract applied, exposure time and corresponding factor interaction effects (Tables 5-6; Fig. 2). A dose- and exposure time-dependent decrease in amount of food eaten by adult GFB was recorded (Table 5) for both test plants. At the dose range tested (2.5-10.0% w/v), *T. vogelii* extract had a higher feeding inhibition (32.0- 100%) than *L. camara* extract (28.7-35.7%) (Table 6). Similarly, a dose- and exposure time-dependent increase in antifeedant activity in which a DC of 100% was obtained for *T. vogelii* extract (5-10% w/v) 168 h after treatment compared to 35.7% for *L. camara* extract (10% w/v) 168 h after treatment. The cumulative percent deterrence coefficient values were highest 168 h after treatment compared to 24 h (Fig. 2).

Table 5: Cumulative food consumed (mean± SE; n=4) of flea beetles feeding on Jatropha leaf diet treated with aqueous extracts obtained from *L. camara* and *T. vogelii*.

			Cumulative food consumed (Mean ± SE) Exposure time (hrs).				
plant	Trt (w/v)	N	24	48	72	120	168
L. camara							
	untreated control	10	0.317 ± 0.00	0.632 ± 0.01	0.967 ± 0.01	0.968 ± 0.01	1.555 ± 0.01
	Karate 2.5%	10	0.193 ± 0.00	0.193 ± 0.01	0.193 ± 0.01	0.193 ± 0.01	0.193 ± 0.01
	2.50%	10	0.290 ± 0.01	0.549 ± 0.02	0.764 ± 0.02	0.947 ± 0.02	1.112 ± 0.02
	5%	10	0.249 ± 0.01	0.512 ± 0.01	0.759 ± 0.01	0.913 ± 0.02	1.066 ± 0.02
	10%	10	0.261 ± 0.02	0.499 ± 0.02	0.697 ± 0.03	0.843 ± 0.03	0.984 ± 0.03
	$LSD_{0.05}$		0.0396	0.0198	0.0396	0.0396	0.0396
T.vogelii							
	untreated control	10	0.317 ± 0.00	0.632 ± 0.01	0.968 ± 0.01	0.968 ± 0.01	1.555 ± 0.01
	Karate 2.5%	10	0.193 ± 0.00	0.193 ± 0.01	0.193 ± 0.01	0.193 ± 0.01	0.193 ± 0.01
	2.50%	10	0.308 ± 0.03	0.562 ± 0.02	0.799 ± 0.03	0.968 ± 0.02	1.21 ± 0.02
	5%	10	0.245 ± 0.01	0.487 ± 0.01	0.709 ± 0.01	0.847 ± 0.02	0.847 ± 0.02
	10%	10	0.206 ± 0.01	0.435 ± 0.01	0.671 ± 0.01	0.787 ± 0.04	0.787 ± 0.04
	$LSD_{0.05}$		0.0396	0.0198	0.0396	0.0594	0.0594

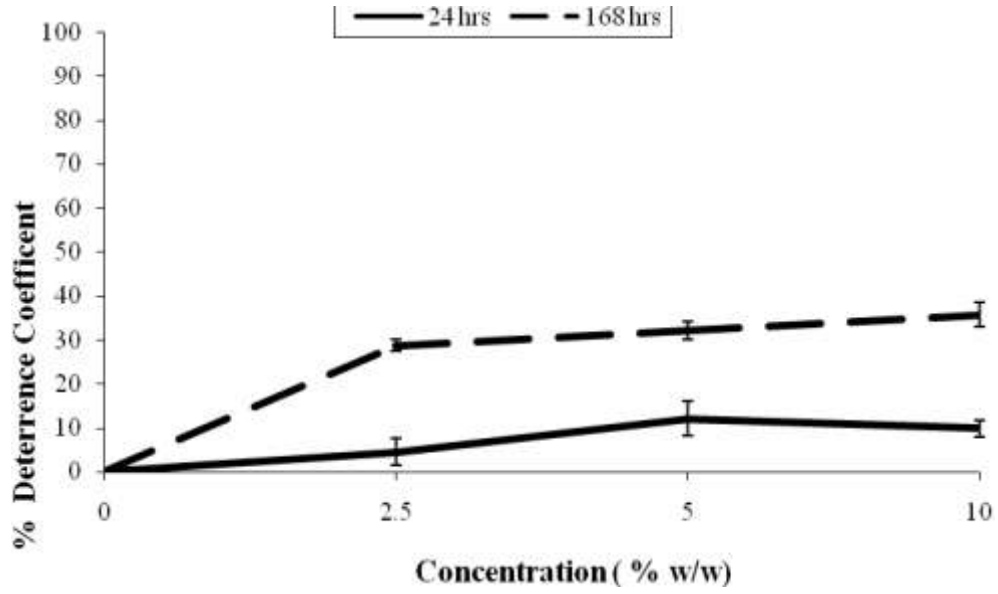
Any two means in a column whose SE overlaps or their difference is smaller than LSD value are not significantly different.

Table 6: Percent deterrence (mean± SE; n=4) of flea beetles feeding on Jatropha leaf diet treated with aqueous extracts obtained from *L. camara* and *T. vogelii*.

Deterrence Coefficient (%)(Mean ± SE)							
			Exposure time (hrs)				
Plant	Treatments (w/v)	N	24	48	72	120	168
L. camara							
	untreated control	10	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Karate 2.5%	10	24.28 ± 2.04	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00
	2.50%	10	4.48 ± 1.34	9.89 ± 1.67	21 ± 2.93	23.09 ± 2.13	28.73 ± 3.07
	5.0%	10	12.10 ± 2.00	9.19 ± 2.93	15.33 ± 2.93	30.87 ± 1.66	32.03 ± 3.93
	10.0%	10	9.81 ± 2.72	14.23 ± 4.14	26.18 ± 4.14	33.23 ± 2.07	35.7 ± 1.88
	$LSD_{0.05}$		5.39	5.80	5.80	4.22	7.78
T.vogelii							
	untreated control	10	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Karate 2.5%	10	24.28 ± 2.04	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00
	2.50%	10	4.44 ± 1.10	15.09 ± 2.18	17.15 ± 3.06	27.11 ± 2.79	32.01 ± 3.73
	5.0%	10	12.88 ± 1.63	13.23 ± 1.55	20.37 ± 2.02	36.12 ± 4.29	100 ± 0.00
	10.0%	10	21.16 ± 1.90	15.93 ± 1.36	17.30 ± 1.63	47.59 ± 3.73	100 ± 0.00
	$LSD_{0.05}$		8.12	4.32	6.06	8.49	7.39

Any two means in a column whose SE overlaps or their difference is smaller than LSD value are not significantly different.

A)



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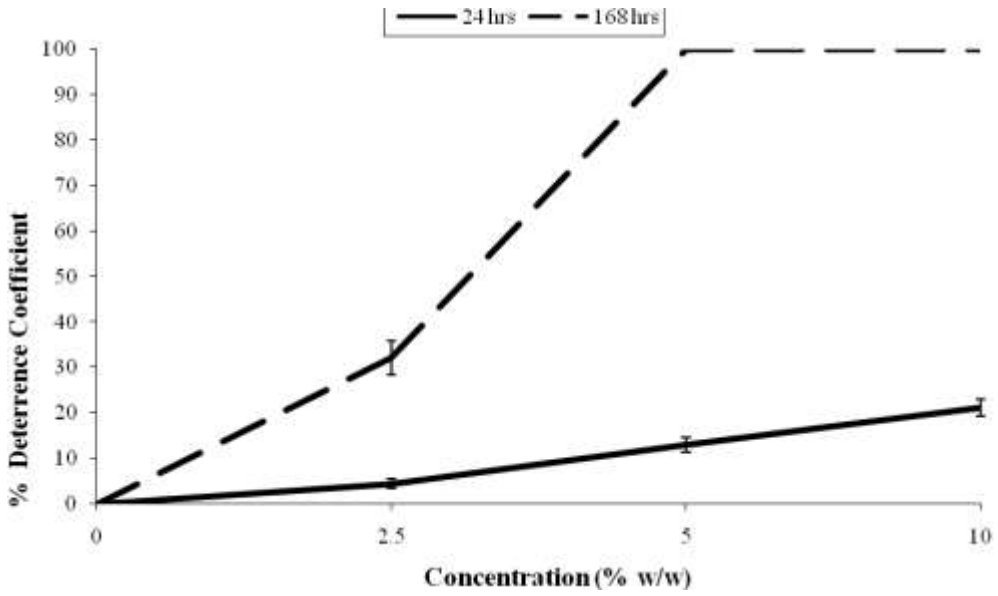
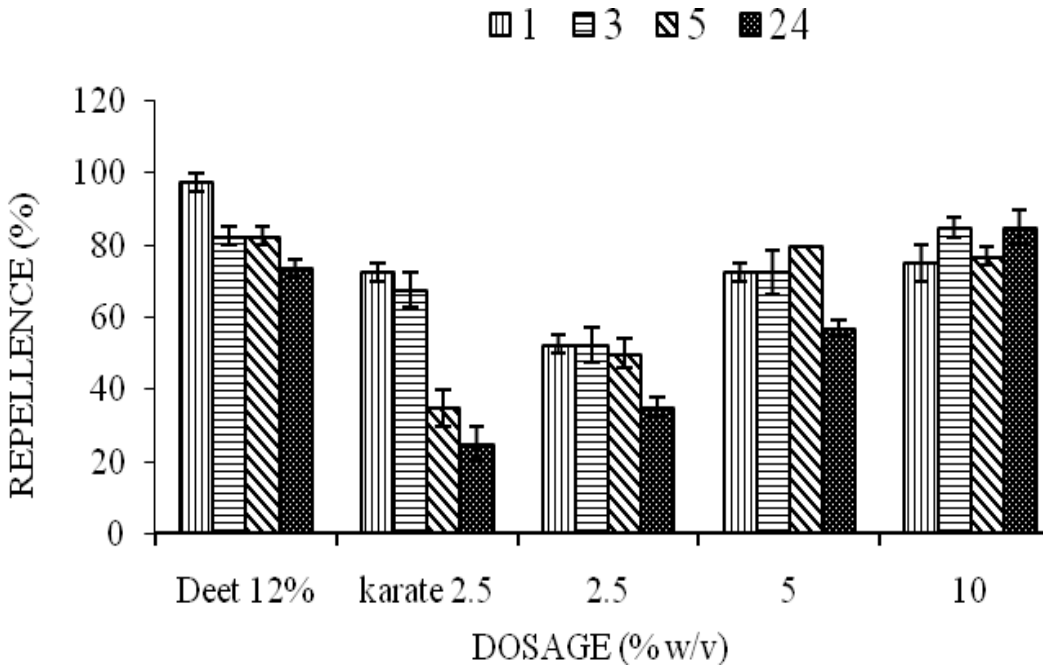


Figure 2: Percent deterrence (mean± SE; n=4) of flea beetles feeding on Jatropha leaf diet treated with aqueous extracts obtained from (A) *L. camara* and (B) *T. vogelii.*

4.1.2.3. Repellence (PR) test.

The percent repellence (PR) values obtained from the choice bioassay system are presented (Fig. 3). Results showed that repellence was significantly ($p<0.0001$) influenced by inter-plant, different concentration of extract applied and exposure time. The PR values for both test botanical plants increased with concentration of extract applied but decreased with exposure time. Irrespective of the plant assayed, concentration of extract applied and exposure time, *L. camara* and *T. vogelii* extracts were equally repellent except 24 h after setup when *L. camara* produced a higher repellence (mean PR value = 55) than *T. vogelii* (mean PR value = 43) (Fig. 3). The results further showed significant differences in PR values between DEET and the two plant extracts at all rates except *L. camara* at 10.0% (w/v) after 24 h exposure. At the highest concentration (10% w/v) evaluated, L. *camara* (PR value: 85%) was more repellent that *T. vogelii* (PR value: 62%) against adult GFB 24 h after setup.

a*). L. camara*



b. *T. vogelii*

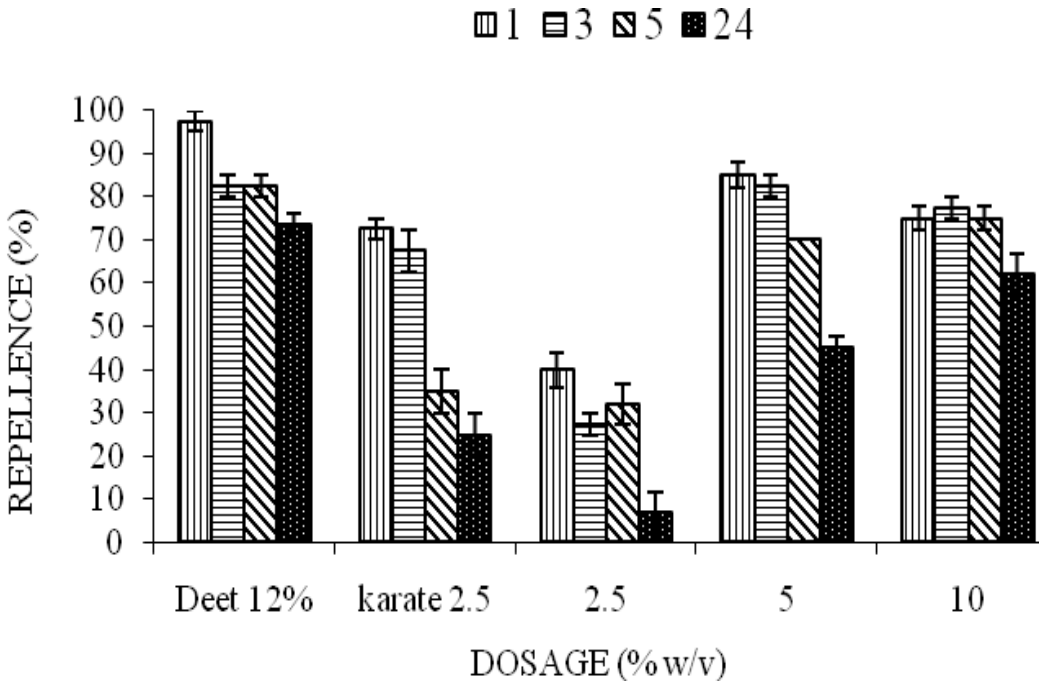


Figure 3: Percent repellence (mean± SE; n=4) of golden flea beetles to aqueous extracts obtained from (a) *L. camara* (b) *T. vogelii* in a choice bioassay system.

4.1.3 Field experiments.

4.1.3.1. Percent pest reduction of Adult *Aphthona whitfieldi* Bryant adult's population

GFB populations and percent pest reduction were significantly ($P<0.0001$) influenced by inter-plant concentration of extract applied, expousure time and experimental site (Tables 7-10; Fig 4). Though GFB population was significantly higher in Kibwezi than Chemeron (Fig. 4), the effect of site on PPR was insignificant. The results further showed a clear dose- and exposure time-dependent reductions in GFB populations (Tables 7-8). Irrespective of the, concentration of extract applied, exposure time, and experimental site, *Tephrosia vogelii* (29-50%) produced higher efficacy than *Lantana camara* (11-22%) at the dose range tested (Tables 10-11). Both plants were weakly toxic against adult golden flea beetle, *Aphthona whitfieldi* Bryant when field applied. At the highest concentration (10% w/v) tested, *Tephrosia vogelii* extract was as effective as synthetic insecticide, Karate, with PPR values ≥50% (Tables 9-10).

Table 7: Effects of *L. camara and T. vogelii* aqueous crude extracts of aqueous crude extracts on populations per plant (Mean ± SE) of Golden Flea beetle, *Aphthona whitfieldi* Bryant populations in Kibwezi.

		Insect counts per plant(Mean ± SE)							
		First spray				Second spray			
Plant	Treatments (% w/v)	1 DBT	1 DAT	3 DAT	7 DAT	1 DBT	1 DAT	3 DAT	7 DAT
L. camara	karate 2.5	14.67 ± 0.33	5.78 ± 0.78	7.00 ± 2.00	5.78 ± 0.79	6.00 ± 1.00	1.78 ± 0.40	0.96 ± 0.55	1.78 ± 0.22
	0.00 (untreated control)	14.67 ± 0.88	10.11 ± 0.59	12.67 ± 0.33	10.11 ± 0.59	6.00 ± 0.58	5.11 ± 0.11	5.78 ± 0.40	5.44 ± 0.29
	2.50	13.33 ± 0.33	12.56 ± 1.39	10.67 ± 1.67	7.67 ± 2.90	6.33 ± 0.66	5.33 ± 0.33	5.11 ± 0.59	5.00 ± 0.58
	5.00	14.00 ± 0.58	12.67 ± 1.45	10.78 ± 1.18	8.78 ± 0.77	6.00 ± 0.00	4.67 ± 0.33	4.44 ± 0.44	4.33 ± 0.19
	10.00	14.67 ± 0.66	12.11 ± 1.95	10.78 ± 1.61	7.33 ± 1.20	6.33 ± 0.33	3.67 ± 0.88	3.78 ± 0.78	4.22 ± 0.23
	LSD0.05	1.08	2.67	3.27	4.52	1.96	1.51	0.74	0.71
T.vogelii	karate 2.5	14.67 ± 0.33	5.78 ± 0.78	7.00 ± 2.00	5.78 ± 0.79	6.00 ± 1.00	1.78 ± 0.40	0.96 ± 0.55	1.78 ± 0.22
	0.00 (untreated control)	14.67 ± 0.88	10.11 ± 0.59	12.67 ± 0.33	10.11 ± 0.59	6.00 ± 0.58	5.11 ± 0.11	5.78 ± 0.40	5.44 ± 0.29
	2.50	14.67 ± 0.66	7.67 ± 2.90	10.33 ± 2.85	7.89 ± 0.44	7.00 ± 0.00	4.33 ± 0.33	4.22 ± 0.77	4.11 ± 0.48
	5.00	14.33 ± 1.2	8.78 ± 0.78	9.22 ± 1.60	6.11 ± 0.11	6.67 ± 0.33	3.44 ± 0.29	3.11 ± 0.68	3.22 ± 0.11
	10.00	13.67 ± 0.66	7.33 ± 1.20	8.00 ± 2.52	5.11 ± 1.06	6.33 ± 0.33	3.00 ± 0.00	2.78 ± 0.22	0.88 ± 0.51
	LSD0.05	1.71	4.52	3.27	1.86	1.96	0.78	1.08	1.25

Any two means in a column whose SE values overlap are not significantly different at $\alpha = 0.05$ using LSD.

Key: DBT means days before treatment while DAT stands for days after treatment.

Table 8: Effects of *L. camara and T. vogelii* aqueous crude extracts of aqueous crude extracts on Populations per plant (Mean ± SE) of Golden Flea beetle, *Aphthona whitfieldi* Bryant populations in Chemeron.

		Pest population (Mean ±SE) per plant							
		First spray				Second spray			
Plant	Treatments (% w/v)	1 DBT	1 DAT	3 DAT	7 DAT	1 DBT	1 DAT	3 DAT	7 DAT
L. camara	karate 2.5	10.00 ± 0.58	4.11 ± 0.59	4.11 ± 0.33	2.76 ± 0.40	3.67 ± 0.33	1.11 ± 0.22	1.00 ± 0.33	1.00 ± 0.33
	0.00 (untreated control)	9.67 ± 1.77	10.22 ± 0.68	8.22 ± 1.15	8.33 ± 0.51	5.33 ± 0.33	3.56 ± 0.80	4.11 ± 0.29	4.67 ± 0.51
	2.50	9.67 ± 0.33	9.89 ± 1.68	8.11 ± 1.06	8.22 ± 1.11	4.33 ± 0.33	2.76 ± 0.11	2.67 ± 0.19	2.89 ± 0.11
	5.00	10.33 ± 1.77	10.00 ± 1.95	7.56 ± 1.79	7.89 ± 1.95	6.33 ± 0.33	4.22 ± 0.29	3.67 ± 0.33	4.11 ± 0.29
	10.00	10.00 ± 1.00	9.44 ± 1.75	7.44 ± 1.05	5.89 ± 1.06	6.00 ± 0.58	3.00 ± 0.58	3.44 ± 0.55	3.89 ± 0.48
	LSD0.05	1.71	4.52	3.27	1.86	1.96	0.78	1.08	1.25
T.vogelii	karate 2.5	10.00 ± 0.58	4.11 ± 0.59	4.11 ± 0.33	2.76 ± 0.40	3.67 ± 0.33	1.11 ± 0.22	1.00 ± 0.33	1.00 ± 0.33
	0.00 (untreated control)	9.67 ± 1.77	10.22 ± 0.68	8.22 ± 1.15	8.33 ± 0.51	5.33 ± 0.33	3.56 ± 0.80	4.11 ± 0.29	4.67 ± 0.51
	2.50	10.33 ± 0.33	7.89 ± 0.48	7.89 ± 0.87	6.67 ± 1.07	4.00 ± 0.58	2.33 ±0.33	1.44 ± 0.40	2.78 ± 0.29
	5.00	11.00 ± 1.15	7.00 ± 1.73	7.00 ± 1.07	6.44 ± 1.36	4.66 ± 0.88	2.22 ± 0.59	1.78 ± 0.22	1.78 ± 0.22
	10.00	10.33 ± 1.85	6.11 ± 1.16	6.11 ± 0.87	5.11 ± 1.06	4.66 ± 0.66	2.11 ± 0.59	1.89 ± 0.44	1.78 ± 0.91
	LSD0.05	1.08	2.67	3.27	4.52	1.96	1.51	0.74	0.71

Any two means in a column whose SE values overlap are not significantly different at $\alpha = 0.05$ using LSD

Key: DBT means days before treatment while DAT stands for days after treatment

Table 9: Percent reduction (Means, n=3) in *A. Whitfield* population in Jatropha plants treated with aqueous *T.vogelii* and *L. camara* extracts in Kibwezi

		Percent pest reduction (Mean ± SE) over untreated control					
		First spray			Second spray		
Plant	Treatments (% w/v)	1 DAT	3 DAT	7 DAT	1 DAT	3 DAT	7 DAT
L. camara	karate 2.5	61.21 ± 0.38	71.31 ± 3.05	69.96 ± 1.05	67.42 ± 1.71	71.03 ± 14.68	69.33 ± 1.81
	0.00 (untreated control)	0.00 ±0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	2.50	6.90 ± 2.01	11.8 ± 2.36	8.33 ± 7.39	3.65 ± 11.85	17.00 ± 1.66	11.65 ± 3.89
	5.00	8.40 ± 8.19	22.36 ± 5.33	16.00 ± 2.60	13.15 ± 5.27	24.07 ± 4.90	19.63 ± 1.62
	10.00	16.00 ± 3.48	25.43 ± 2.55	20.39 ± 14.01	17.78 ± 1.11	27.78 ± 5.55	28.89 ± 4.45
	LSD0.05	16.13	10.5	27.6	23.34	28.92	8.77
T.vogelii	karate 2.5	61.21 ± 0.38	71.31 ± 3.05	69.96 ± 1.05	67.42 ± 1.71	71.03 ± 14.68	69.33 ± 1.81
	0.00 (untreated control)	0.00 ±0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	2.50	26.72 ± 11.70	34.06 ± 2.31	23.22 ± 3.27	30.32 ± 7.58	35.71 ± 7.14	36.83 ± 6.03
	5.00	38.31 ± 5.10	38.16 ± 4.03	37.74 ± 5.22	44.29 ± 2.97	53.17 ± 7.05	49.05 ± 4.97
	10.00	46.04 ± 7.33	50.90 ± 0.56	46.42 ± 10.62	46.67 ± 3.33	55.56 ± 5.55	53.33 ±10.18
	LSD0.05	23.05	7.94	20.9	14.93	28.92	20.05

Any two means in a column whose SE values overlap are not significantly different at $\alpha = 0.05$ using LSD.

Key: DBT means days before treatment while DAT stands for days after treatment

Table 10: Percent reduction (Mean±SE, n=3) in *A. Whitfield* population in Jatropha plants treated with aqueous *T.vogelii* and *L. camara* extracts at Chemeron Field Station

Mean percent pest reduction (Mean ± SE) over untreated control							
		First spray			Second spray		
Plant	Treatments (% w/v)	1 DAT	3 DAT	7 DAT	1 DAT	3 DAT	7 DAT
L. camara	karate 2.50	63.06 ± 1.53	72.27 ± 2.87	69.92 ± 3.98	63.06 ± 1.53	72.27 ± 2.87	69.92 ± 3.98
	0.00 (untreated control)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	2.50	5.25 ± 2.17	14.86 ± 3.34	7.90 ± 6.82	27.60 ± 1.52	33.64 ± 3.64	27.70 ± 8.94
	5.00	10.31 ± 6.79	25.73 ± 1.54	17.13 ± 2.03	43.46 ± 2.14	47.64 ± 3.64	37.95 ± 1.10
	10.00	12.17 ± 1.54	24.81 ± 3.04	21.42 ± 7.86	45.88 ± 2.49	53.26 ± 1.94	46.43 ± 2.94
	LSD0.05	13.38	6.58	15.48	4.91	7.17	17.61
T.vogelii	karate 2.50	63.06 ± 1.53	72.27 ± 2.87	69.92 ± 3.98	65.28 ± 5.01	73.61 ± 13.25	79.58 ± 10.21
	0.00 (untreated control)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	2.50	27.60 ± 1.52	33.64 ± 3.64	27.70 ± 8.94	26.94 ± 8.56	31.39 ± 7.40	23.22 ± 3.39
	5.00	43.46 ± 2.14	47.64 ± 3.64	37.95 ± 1.10	49.44 ± 5.30	52.78 ± 2.78	45.56 ± 13.65
	10.00	45.88 ± 2.49	53.26 ± 1.94	46.43 ± 2.94	50.00 ± 7.23	55.91 ± 10.00	57.64 ± 27.43
	LSD0.05	4.91	7.17	17.61	16.86	26.1	54.04

Any two means in a column whose SE values overlap are not significantly different at $\alpha = 0.05$ using LSD.

Key: DBT means days before treatment while DAT stands for days after treatment

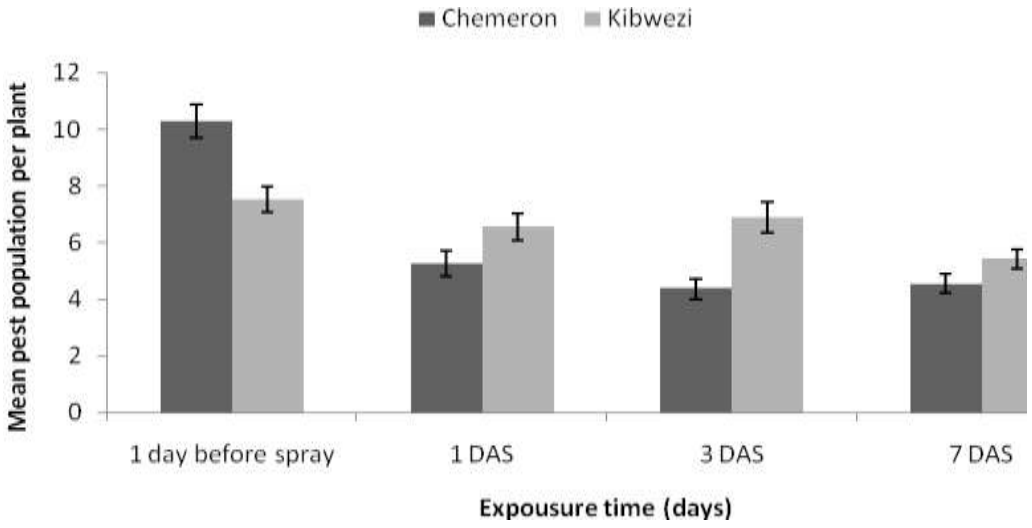


Figure 4: Variation in golden flea (Mean ± SE) across experimental sites.

4.1.3.2. Plant damage assessment

Results showed that percent leaf damage per plant, number of feeding holes and chlorophyll content per leaf (Table 11) were significantly ($p<0.0001$) influenced by inter-plant, different concentration of extract applied and experimental site. The chlorophyll content per leaf increased with increase in concentration of aqueous extract for both plants. The percent leaf damage per plant and number of feeding holes per leaf, on the other hand, were inversely correlated to concentration of extract applied for both plants (Table 11). Irrespective of the plant assayed, concentration of extract applied and experimental site, plants treated with *Tephrosia vogelii* suffered the least damage to their leaves by adult GFB in terms of Percent leaf damage per plant (60-53%), number of feeding holes (21-13) and chlorophyll content (19-22 µg cm−2) than in *Lantana camara* where insects caused 71-59 percent leaf damage per plant, 37-22 feeding holes and 17-18 chlorophyll content (Table 11). Leaves damaged per plant and number of feeding holes was higher in Kibwezi than Chemeron. However, chlorophyll content per leaf was higher in Chemeron than in Kibwezi (Table 11). Consequently, percent leaf damage per plant and number of feeding holes in all treated plants were significantly lower than in untreated control in both plants (Table 11). A strong linear inverse relationship was observed ($r = 0.770$) between number of feeding holes per leaf and chlorophyll content per leaf (Fig. 5).

Table 11: Effects of *L. camara and T.vogelii* crude extracts treatments on *Jatropha curcas* plant damage (Mean ± SE) by golden flea beetle adults and chlorophyll content (µg cm−2)

Effect of various treatments on plant damage (Mean ± SE) by golden flea beetle adults							
		% leaf damage per plant		Feeding holes per leaf		Chlorophyll content per leaf	
Plant	Treatments (% w/v)	Kibwezi	Chemeron	Kibwezi	Chemeron	Kibwezi	Chemeron
L. camara	Karate 2.5	27.38 ± 1.19	21.67 ± 1.67	12.16 ± 0.58	9.81 ± 1.22	22.23 ± 0.56	26.20 ± 0.99
	0.00 (untreated control)	91.53 ± 4.33	93.33 ± 6.67	50.81 ± 1.19	47.07 ± 2.28	15.85 ± 0.80	18.03 ± 0.74
	2.50	76.67 ± 1.67	67.22 ± 4.34	37.80 ± 1.17	36.26 ± 7.34	16.50 ± 0.62	18.65 ± 0.95
	5.00	75.00 ± 8.33	56.67 ± 3.33	28.34 ± 1.20	25.41 ± 0.80	17.44 ± 0.46	18.87 ± 0.95
	10.00	61.38 ± 8.74	56.67 ± 3.34	23.88 ± 1.42	20.30 ± 1.16	17.04 ± 0.07	20.03 ± 0.32
	LSD0.05	15.25	10.1	1.7	14.67	1.47	1.35
T.vogelii	Karate 2.5	27.38 ± 1.19	21.67 ± 1.67	12.16 ± 0.58	9.81 ± 1.22	22.23 ± 0.56	26.20 ± 0.99
	0.00 (untreated control)	91.53 ± 4.33	93.33 ± 6.67	50.81 ± 1.19	47.07 ± 2.28	15.85 ± 0.80	18.03 ± 0.74
	2.50	71.11 ± 4.44	50.00 ± 0.00	21.78 ± 0.82	21.48 ± 1.38	18.08 ± 0.62	20.24 ± 0.67
	5.00	54.76 ± 2.38	53.33 ± 3.33	19.46 ± 0.34	15.30 ± 1.42	18.83 ± 1.32	22.06 ± 1.02
	10.00	54.76 ± 2.39	52.22 ± 7.78	14.41 ± 0.81	11.67 ± 1.71	21.27 ± 0.63	24.20 ± 0.51
	LSD0.05	6.57	15.72	1.72	2.14	1.54	1.03

Any two means in a column whose SE values overlap are not significantly different at $\alpha = 0.05$ using LSD.

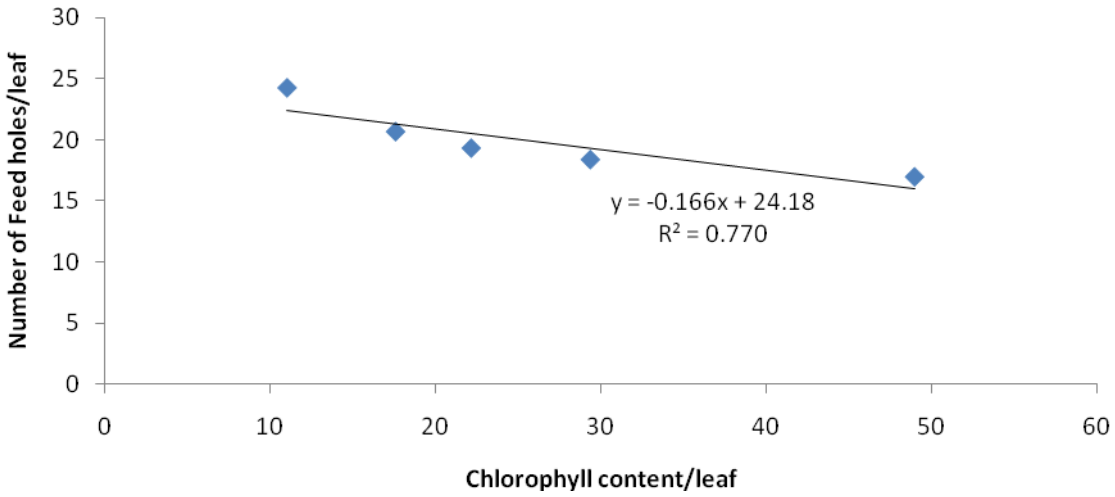


Figure 5: Relationship between number of feeding holes per leaf and chlorophyll content per leaf (µg cm^{-2}) on *J. curcas* plant

4.2 Discussion.

4.2.1 Field survey.

The fact that farming is the main source of livelihood would engender willingness to carry out practices that would enhance their productivity and by extension derivable income (Okunlola and Ofuya, 2010). Most farmers have gone through primary and secondary education .This implied that most of them were sufficiently literate to understand some of the important research findings thereby enhancing adoption of new technologies and improving the farmers' access to information on relevant research findings (Okunlola and Ofuya, 2010).

The large difference in number of Jatropha trees per farmer between Bondo and Kibwezi districts can be attributed to history of the crops introduction in Kenya. According to Swallow and Tomomatsi (2007), Jatropha farming was first introduced in Kenya in 2005 in Kitui, Malindi and Makueni districts and that organized cultivation was initiated in 2006 by organizations such as Green Africa Foundation, Vanilla-Jatropha Development Foundation, in parts of Eastern Province (GTZ, 2009). Hence more Jatropha cultivation is done in Kibwezi than Bondo. Jatropha has generated a lot of excitement in the country owing to the growing global interest in biofuel and demand for reduced greenhouse gas emissions (GTZ, 2009). This has stimulated Jatropha

farming with farmers using different planting materials and cropping systems for better yields and incomes. The use of seeds and seedlings by most farmers could be attributed to farmers' knowledge of good crop establishment and productivity compared to cuttings. Plants resulting from seeds and seedlings are known to develop deep taproots, and thus have better access to nutrients and moisture from deeper soil layers (Achten *et al.,* 2008). Intensive production of Jatropha involve mono-culture and intercropping systems which encourages use of inorganic inputs (regular irrigation and fertilization). For these reason, the crop is predisposed more to insect pests (Mutaquin, *et al*., 2010; Achten *et al*., 2007).

The golden flea beetle*, Aphthona whitfiendi* Bryant, has emerged as the most important field insect pest of Jatropha (Jatropha curcas L) in both Bondo and Kibwezi districts of Kenya. These results concur with Zulu and Nielsen (2007) who also reported the same pest as the most economically important on Jatropha in Zimbabwe. High level of pest awareness may be attributed to level of education among respondents hence farmers are aware of the problem that pests can cause if unattended hence resulted to the only alternative available. Most of respondents (69%) have gone through primary education hence are aware of problems posed by pests if not controlled. Although insect pests can be managed through use of chemical pesticides (Okunlola and Ofuya, 2010) majority of farmers responded that high cost of insecticides was the major reason for not using synthetic pesticides against insect pests. This makes it apparent that controlling field insect pests is not an easy job although synthetic chemicals are apparently available for use. For these reason, it's necessary to develop alternative strategy for sustainable pest management in Jatropha.

4.2.2. Laboratory bioassays

4.2.2.1 Contact toxicity studies

The results showed species-, dose- and expousure time- dependent efficacy of *Tephrosia vogelii* and *Lantana camara* treatments against golden flea beetle adults. The increased efficacy with exposure time could be attributed to cumulative toxicity against adult beetles. The differential efficacy of *Tephrosia vogelii* and *Lantana camara* could be attributed to the varied quantity and quality of chemical compounds / principles responsible for the observed toxic effects against insects (Gökçe *et al.,* 2010). The Jatropha leaves are known to contain most of chemicals that are mostly found in *Lantana camara* like saponin, flavonoids, terpenoids (Nayak, and Patel, 2010**;** Kumar and Maneemegalai*,* 2008) which explains the weak efficacy of *Lantana camara* extract

against the beetle. These results concur with Subedi and Vaidya (2003) who reported significant 71-75% reduction in flea beetle populations in Radish (*Rhaphanus sativus*) fields treated with different animal urines. Our study has shown that pest mortality rate was significantly higher on treated leaves than untreated but less effective than karate at all rates. Similar result trends have been reported for aqueous plant extracts against various field insect pests (Ogunwolu and Ameh, 1999). In related investigations, Oladimeji and Kannike, (2010) reported that *A. indica* and *O. basilicum* leaf extracts effectively controlled *Podagrica* spp. on okra but were comparatively less effective than lambda-cyhalothrin. Results observed in the study cleary shows the potential of these plants as toxicants of against field insect pests. However, differential contact toxicities observed shows the existence of intra-species variation between these two crude extracts

4.2.2.2. Antifeedant tests

In the antifeedant studies, results showed strong species – specific, dose- and exposure time-dependent feeding inhibition effects (DC values) of Jatropha leaves (food) treated with aqueous extracts of *T. vogelii and l. camara* against GFB adults. Mechanism of antifeedant action is based on fact that the choice of food is primarily based upon contact chemoreception of various allelochemicals (Opender, 2008). The differential antifeedant responses could be attributed to variations in the amounts of chemical compounds / principles associated with antifeedant activity against insects (Gökçe *et al.,* 2010). The observed complete feeding inhibition of *T. vogelii* could possibly be due to the presence of rotenoids, highly toxic compounds, known to be mitochondrial chain inhibitors, inhibiting cellular respiration in almost every living organism including insects and mammals. These compounds block the enzymes glutamate and succino dehydrogenase and thus H^+transport (Neuwinger, 2004, Lapointe *et al.,* 2003, Ogendo, 2008). In unrelated studies, Sharma and Gupta (2009) reported a complete feeding inhibition of cabbage leaves treated with *L. camara* at 1% v/v against first instars larvae of *P. brassicae.* Insecticidal action of aerial parts of *L. Camara* ground powder produced a complete feeding deterrent against *Callosobruchus chinensis* (Prasad and Purohit, 2009*)*. Strong antifeeding effects of botanical pesticides has been proven to contribute to insect mortality due to dose-dependent reduction in daily consumption of treated plants (Wang *et al.,* 2005). In addition to toxicity *via* contact or ingestion, plant extracts and allelochemicals have been screened for activity as insect antifeedants. In some instances, the bioactivity mechanism of crude plant extracts on insects is explained by their toxic and antifeedant effects (Gökçe *et al.,* 2010). The complete feeding inhibition and insect mortality of

T. vogelii (100% DC values), at 5% w/v, was comparable to the feeding deterrence obtained from synthetic chemical (karate) 168 h after treatment. The results of this study provide good scientific promise for rationalized adoption of aqueous extracts of *T. vogelii* as cost-effective and environment friendly biocontrol measure of golden flea beetles and related insect species.

4.2.2.3. Repellence (PR) test.

From the choice bioassay tests, the magnitude of repellence was greatly influenced by the plant species, dosage of plant crude extract and the exposure time. The differential repellence (PR values) responses could partly be attributed to variations in the amounts of chemical compounds / principles (aromatic volatile essential oils and terpenoid constituents) associated with repellent activity against insects. *L. camara* was more repellent (PR value = 85%) than *Tephrosia vogelii* (PR value = 62%) 24 h after setup. These findings are in agreement with Ogendo (2008) who reported PR values of 79 and 75% for *L. camara* and *Tephrosia vogelii* essential oils against adult *Sitophilus oryzae* in stored treated wheat grains. Similarly, Echereobia *et al.* (2010) reported total repulsion (PR value: 100 %) of aqueous *Piper guineense* and *Azadirachta indica*, at 10% w/v, against Okra flea beetles (*Podagrica* species) after 12 h expousure. In their earlier laboratory studies, Ogendo *et al.* (2003) reported that *Tephrosia vogelii* powder was more repellent (PR value: 87.5%) *than L. Camara* (PR value: 62.5%) against adult *S. zeamais*. The fact that the two botanical extracts, *L. camara* (5% w/v) and *T. Vogelii* (10% w/v), produced PR values comparable to the universally recognized insect repellent, DEET, provides good promise for a rationalized exploitation of these botanicals as cost-effective, socially acceptable and environment friendly alternative control measures against GFB and other important field insect pests in tropical agriculture.

4.2.3 Field experiment

The results of field evaluations have demonstrated clear plant species-, dose- and exposure duration-dependent efficacy of *T. vogelii* and *L. camara* aqueous crude extracts against adult golden flea beetles. The inverse relationship between concentration of extract applied / exposure time and adult GFB population may be attributed to slow contact toxicity and feeding deterrent effects of the extracts. The observed effects of test botanical extracts could be attributed to the presence of chemical compounds / principles associated with ingestion and contact toxicity activity against insects (Gökçe *et al.,* 2010). The fact that Jatropha leaves contain most of

chemicals that are mostly found in *Lantana camara* like saponins, flavonoids and terpenoids (Kumar and Maneemegalai, 2008) partially explains the low efficacy of *L. camara* extract against GFB. However, the presence of rotenone, tephrosin, and deguelin in *T. vogelii (*Lapointe *et al*., 2003) explains why its aqueous extract was more efficacious than that of *L. camara*. In related field studies, Subedi and Vaidya (2003) reported 70-75% reduction in flea beetle populations treated with buffalo and cow urines and *Spilanthes acmella*. Although inferior to synthetic insecticide, Karate, the botanical extracts significantly reduced GFB population in treated Jatropha plants. These findings concur with other studies in which various plant extracts, either aqueous or ethanolic, have been found effective against some insect pests of crops but less effective than the synthetic insecticides (Ogunwolu and Ameh, 1999; Oladimeji and Kannike, 2010).

Results have shown that Jatropha plant damage by GFB was significantly influenced by plant species, concentration of aqueous extract applied, exposure time and site. Plant damage, expressed as percent leaf damage per plant and number of feeding holes, was significantly lower in Jatropha plants treated with *L. camara* and *T. vogelii* aqueous extracts and synthetic insecticide, Karate, than untreated control. The observed decrease in Jatropha leaf damage by the golden flea beetle, *Aphthona whitfieldi*, has revealed that the test insect is a major Jatropha leaf feeder thereby causing substantial loss in crop productivity resulting from reduced photosynthetic capacity. Plants treated with *T. vogelii* suffered the least damage to their leaves by GFB in terms of percent leaf damage per plant, number of feeding holes and chlorophyll content. This can be attributed to the presence of toxic constituents such as tephrosin (Lapointe *et al*., 2003). In their recent field studies, Oladimeji and Kannike (2009) reported that *Azadirachta indica* and *Ocimum basilicum* aqueous extracts, at 20 ml/L, caused 54 and 43% reductions, respectively, in okra leaf damage by flea beetles, *Podagrica* spp. Our findings with *T. vogelii* leaf extract, at 10% w/v, in which a 53% reduction in Jatropha leaf damage was recorded, are comparable to the level of pigeon pea seed damage reported by Minja, *et al.* (2002). The Jatropha plant damage, expressed as leaf damage per plant and number of feeding holes, was higher in Kibwezi than Chemeron due to site variations in GFB populations. The observed strong inverse linear relationship (r =0.770) between number of feeding holes per leaf and leaf chlorophyll content is an indication that GFB feeding directly affects the crop yield and productivity. Literature survey indicates herbivory reduces leaf area, disrupts tissues and reduces

photosynthetic surface area (Stone *et al.,* 2001; Nabity *et al*., 2009). The above field results have undisputedly shown a certain degree of efficacy by *Tephrosia vogelii* Hook. and *Lantana camara* L. against golden flea beetle (*Aphthona whitfieldi* Bryant) in Jatropha (*Jatropha curcas L.*). The fact that *Tephrosia vogelii* Hook. Produced an up to 57% pest reduction (efficacy) shows a substantial hope for the production and use of natural pesticide as alternative to the widely used synthetic chemicals like Karate.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 CONCLUSIONS

From the results of this study, the following conclusion can be made.

1. The main pest that causes most damage on Jatropha was the Golden Flea beetle, *Aphthona whitfiedi*. Additionally, Jatropha was affected by other minor insect pests which included leaf boring worms (*Salebria morosalis*), soil grubs (*Scarabaeidae spp*) and the shield-backed bug *(Scutellera nobilis)*.
2. Aqueous crude extracts of *Tephrosia vogelii* Hook. and *Lantana camara* L. had moderate to strong toxic, antifeedant and repellent effects against adult golden flea beetle. The levels of toxicity and repellence were comparable to synthetic insecticide, Karate and universal repellent, DEET respectively.
3. Aqueous crude extracts of *T. vogelii* Hook. and *L. camara* L. had moderate field efficacy against GFB adults in Jatropha crops. This was manifested through significant reductions in pest population and crop damage and consequent increases chlorophyll content.

5.2 RECOMMENDATIONS.

From my studies on *J. curcas L,* the following recommendation can be made.

1. Results of this study should be validated in relation to small holder farmer environments before adoption.
2. For effective broad spectrum control of the GFB, its life cycle needs to be fully studied, both in- and ex-situ.

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APPENDICES

Appendix 1: ANOVA- Variations of Socio-economic characteristics of respondents, cultural characteristics of Jatropha fields and pest awareness among farmers across the two survey districts

Variables	Source	df	SS	F value	Pr>F
gender	Between districts	1	0.233	0.921	0.340 NS
Age	Between districts	1	1.617	1.874	0.175 NS
Education	Between districts	1	0.157	0.424	0.517 NS
Occupation	Between districts	1	15.486	9.569	0.003**
plot type	Between districts	1	0.153	0.942	0.335 NS
total land size	Between districts	1	2897.778	16.398	0.00**
plot size range	Between districts	1	7.259	2.796	0.098 NS
year of planting	Between districts	1	140.586	5.314	0.024*
pesticide status	Between districts	1	0.228	1.416	0.213 NS
reasons for no application	Between districts	1	1.118	0.696	0.196 NS
pest awareness	Between districts	1	1.038	10.093	0.002**
major insect pest	Between districts	1	0	0.00	0.991 NS

* Means significant at 0.05, ** Means highly significant at 0.01, NS means not significant

Appendix 2: ANOVA-contact toxicity test of aqueous crude extracts of aqueous crude extracts of *L. camara* and *T. vogelii* leaves against golden flea beetle, *Aphthona whitfieldi* Bryant.

SOURCE	DF	SS MEAN	F VALUE	Pr>F
plants	1	7948.2093	402.01**	**0.0001**
Time (Hrs)	4	1003.0149	50.73**	**0.0001**
Treatments (% w/v)	4	42066.4239	2127.68**	**0.0001**
rep	3	2.6027	0.13 ^{NS}	0.9411
Plants* Time (Hrs)	4	157.7695	7.98**	**0.0001**
plants * Treatments	4	1763.8066	89.21**	**0.0001**
Time (Hrs)* Treatment	16	119.6117	6.05**	**0.0001**
Error	147	19.771		

* Means significant at 0.05, ** Means highly significant at 0.01, NS means not significant

Appendix 3: ANOVA- Antifeedant test (amount of food consumed) of aqueous crude extracts of aqueous crude extracts of *L. camara* and *T. vogelii* leaves

SOURCE	DF	SS MEAN	F VALUE	Pr>F
plants	1	0.0165	32.02**	**0.0001**
Time (days)	4	0.1501	292.13**	**0.0001**
Treatments % w/v)	4	0.4754	925.32**	**0.0001**
rep	4	0.0007	1.31 ^{NS}	0.2665
Plants* Time (days)	4	0.0086	16.64**	**0.0001**
plants * Treatments	4	0.0068	13.16**	**0.0001**
plants * Treatments* time	16	0.0033	6.45**	**0.0001**
Time (days)* Treatment	16	0.0204	39.67**	**0.0001**
Error	196	0.0005		

* Means significant at 0.05, ** Means highly significant at 0.01, NS means not significant

Appendix 4: ANOVA- Antifeedant test (deterrence coefficient) of aqueous crude extracts of *L. camara* and *Tephrosia vogelii* leaves

SOURCE	DF	SS MEAN	FVALUE	Pr>F
plants	1	1616.9157	66.91**	**0.0001**
Time (days)	4	6683.1265	276.57**	**0.0001**
Treatments (% w/v)	4	30887.6854	1278.22**	**0.0001**
rep	4	40.0831	1.66 NS	0.1612
Plants* Time (days)	4	920.9626	38.11**	**0.0001**
plants * Treatments	4	613.0935	25.37**	**0.0001**
plants * Treatments* trt	16	328.8653	13.61**	**0.0001**
Time (days)* Treatment	16	1521.4679	62.96**	**0.0001**
Error	196	24.1647		

* Means significant at 0.05, ** Means highly significant at 0.01, NS means not significant

Appendix 5: ANOVA- Repellency test of aqueous crude extracts of aqueous crude extracts of *L. camara* and *Tephrosia* vogelii leaves

SOURCE	DF	SS MEAN	F VALUE	Pr>F
plants	1	648.9108	20.06**	**0.0001**
Time (Hrs)	3	2351.0357	72.69**	**0.0001**
Treatments (% w/v)	4	5477.6598	169.36**	**0.0001**
rep	3	56.7393	1.75 NS	0.1598
Plants* Time (Hrs)	3	179.1477	5.54**	**0.0014**
plants * Treatments	4	322.8845	9.98**	**0.0001**
Time (Hrs)* Treatment	12	343.1744	10.61**	**0.0001**
Error	117	32.3437		

* Means significant at $\alpha = 0.05$, ** Means highly significant at $\alpha = 0.01$, NS means not significant

Appendix 6: ANOVA- Effects of *L. camara* and *T. vogelii* aqueous crude extracts on populations per plant (Mean ± SE) of golden flea beetle, in Jatropha plants in Chemeron and Kibwezi**.**

Source	DF	Type IV SS	Mean Square	F Value	Pr > F
SITE	1	11.16392480	11.16392480	88.18**	**<.0001**
SPRAY	1	86.10983122	86.10983122	680.14**	<.0001
PLANT	1	4.96963532	4.96963532	39.25**	<.0001
TIME	2	1.74328136	0.87164068	6.88**	0.0012
TRT	4	46.77618486	11.69404621	92.37**	<.0001
REP	2	0.56654037	0.28327018	2.24NS	0.1090
SITE*SPRAY	1	0.00059533	0.00059533	0.00NS	0.9454
SITE*TIME	2	1.35329260	0.67664630	5.34**	0.0054
SITE*TRT	4	0.23656073	0.05914018	0.47NS	0.7598
SPRAY*TIME	2	2.18344177	1.09172089	8.62**	0.0002
SPRAY*PLANT	1	0.01838790	0.01838790	0.15NS	0.7035
PLANT*TRT	4	3.70386908	0.92596727	7.31**	<.0001
SPRAY*TRT	4	0.58483062	0.14620766	1.15NS	0.3315
SIT*SPR*PLA*TIME*TRT	74	3.96351626	0.05356103	0.42NS	1.0000
PLANT*TIME*TRT	10	0.21966081	0.02196608	0.17NS	0.9979
TIME*TRT	8	0.86551234	0.10818904	0.85NS	0.5557

* Means significant at $\alpha = 0.05$, ** Means highly significant at $\alpha = 0.01$, NS means not significant

Appendix 7: ANOVA- Mean percent pest reduction (PPR) in GFB population on Jatropha leaves treated with aqueous *L. camara* and *T. vogelii* extracts in Kibwezi and Chemeron.

SOURCE	DF	SS MEAN	F VALUE	Pr>F
Sites	1	128.5667	1.12 ^{NS}	0.2914
Sprays	1	1129.6893	9.83**	0.0019
Plants	1	18909.8615	164.48**	0.0001
Time	2	1204.8151	10.48**	0.0001
Treatments (% w/v)	4	46425.4967	403.81**	0.0001
rep	2	6.0766	0.05^{NS}	0.9485
site*Spray	1	1.8884	0.02 ^{NS}	0.8981
site*time	2	1.3136	0.01 ^{NS}	0.9886
site*treatment	4	27.142	0.24 ^{NS}	0.9178
Spray*time	2	47.9135	0.42 ^{NS}	0.6597
spray*plant	1	2.4191	0.02 ^{NS}	0.8848
Plant*treatment	4	3399.2071	29.57**	0.0001
spray*treatment	4	100.8817	0.88**	0.0001
Time*trt	8	120.2558	1.05 ^{NS}	0.4022
Error	359	114.9701		

* Means significant at $\alpha = 0.05$, ** Means highly significant at $\alpha = 0.01$, NS means not significant

Appendix 8: ANOVA-Percent leaf damage per plant by golden flea beetle adults on *Jatropha curcas* plants treated with *L. camara* and *T.vogelii* aqueous crude extracts

SOURCE	DF	SS MEAN	F VALUE	Pr>F
Sites	1	641.51172	18.38**	0.0001
Plants	1	494.34156	14.17**	0.0006
Treatments (% w/v)	4	7090.57094	203.2**	0.0001
Rep	2	326.80556	9.37 ^{NS}	0.0511
Sites*plants	1	8.23045	0.24 ^{NS}	0.63
sites*Treatments	4	124.67367	3.57**	0.0144
Sites*plants*Trts	4	77.92764	2.23 ^{NS}	0.0836
Plants*treatment	4	100.80021	2.89**	0.035
Error	38	34.89522		

* Means significant at $\alpha = 0.05$, ** Means highly significant at $\alpha = 0.01$, NS means not significant

Appendix 9: ANOVA-Feeding holes per leaf caused by by golden flea beetle adults on *Jatropha curcas* plants treated with *L. camara* and *T.vogelii* aqueous crude extracts

SOURCE	DF	SS MEAN	F VALUE	Pr>F
plants	1	648.9108	20.06**	0.0001
Time (Hrs)	3	2351.0357	72.69**	0.0001
Treatments (% w/v)	4	5477.6598	169.36**	0.0001
rep	3	56.7393	1.75 NS	0.1598
Plants* Time (Hrs)	3	179.1477	5.54**	0.0014
plants * Trts	4	322.8845	9.98**	0.0001
Time * Trts	12	343.1744	10.61**	0.0001
Error	117	32.3437		

* Means significant at α = 0.05, ** Means highly significant at α = 0.01, NS means not significant

Appendix 10: ANOVA-Chlorophyll content per leaf on *Jatropha curcas* plants treated with *L. camara* and *T.vogelii* aqueous crude extracts against golden flea beetle adults.

SOURCE	DF	SS MEAN	F VALUE	Pr>F
Sites	1	110.2519	65.83**	0.0001
Plants	1	39.0783	23.33**	0.0001
Treatments (% w/v)	4	91.6121	54.7**	0.0001
Rep	2	3.3187	1.98 NS	0.1518
Sites*plants	1	0.4623	0.28 NS	0.6024
sites*Treatments	4	1.8452	1.1 NS	0.3698
Sites*plants*Trts	4	0.4925	0.29 NS	0.88
Plants*treatment	4	9.2581	5.53**	0.0013
Error	38	1.6748		

* Means significant at α = 0.05, ** Means highly significant at α = 0.01, NS means not significant

Appendix 11: Author's Publications

a) Refereed journal publication

Igogo, J.M., Ogendo, J.O., Kariuki, S.T and Otaye, D. O. 2011 Insecticidal, antifeedant and repellent effects of *Tephrosia vogelii* Hook. and *Lantana camara* L. aqueous crude extracts against Golden Flea Beetle, *Aphthona whitfieldi* Bryant in Jatropha, *Jatropha curcas* L. *Biopesticide international* **7(2):** 93-103

Abstract.

Toxic, antifeedant and repellent activity of aqueous crude extracts of *Lantana camara* and *Tephrosia vogelii* were evaluated for toxic, antifeedant and repellent effects against golden flea beetle (GFB), *Aphthona whitfieldi* Bryant (Coleoptera: Chrysomelidae) in Jatropha (*Jatropha curcas L.*). Four treatment rates (0.0, 2.5, 5.0 and 10.0% w/v) of each aqueous crude extracts plant material, and a synthetic insecticide (Karate 2.5 % v/v) were laid out in a completely randomized design (CRD) with 4-5 replicates per treatment. Results showed that the toxic, antifeedant and repellent effects of crude aqueous extracts of *L. camara* and *T. vogelii* against GFB adults were significantly ($P<0.0001$) influenced by plant species, concentration applied and duration (hours) of contact. After 8 days, *L. camara* and *T. vogelii* caused 18–56 and 50.0–62% insect mortality, respectively. LC_{50} values for *Tephrosia vogelii* (15-1.9% w/v) were lower than *Lantana camara* (83-46% w/v) for any time period in the contact bioassay. A high (100%) deterrence coefficient (DC) was obtained for *T. vogelii* 168 hours (h) after treatment at 5% and 10% rates. *L. camara*, on the other hand, produced a weaker DC values of 28.7, 32.0 and 35.7% at the same concentrations 168 h after treatment. Irrespective of the plant assayed, concentration applied and exposure time, *L. camara* and *T. vogelii* were equally repellent except 24 h after treatment when *L. camara* produced higher PR values than *T. vogelii*. The results of this study provides useful scientific mileage in the exploitation of botanical pesticides, *L. camara* and *T. vogelii* extracts, as potential eco-friendly and cost-effective substitutes for synthetic pesticides in tropical agriculture in Kenya

Key words: *Tephrosia vogelii, Lantana camara*, Golden Flea Beetle, *Aphthona whitfieldi* Bryant, contact toxicity, antifeedant, repellence

b) Manuscripts submitted for publication

Igogo, J.M., Ogendo, J.O., Kariuki, S.T and Otaye, D. O. 2012 Field efficacy of *Tephrosia vogelii* Hook and *Lantana camara* L. aqueous crude extracts against golden flea beetle, *Aphthona whitfieldi* Bryant, in Jatropha (*Jatropha curcas* L). Submitted to AJAR

Abstract

Aqueous extracts of *Lantana camara* and *Tephrosia vogelii* were evaluated against *Aphthona whitfieldi* Bryant in *Jatropha curcas* L., each at four rates (0.0, 2.5, 5.0 and 10.0% w/v) and a synthetic insecticide, Karate at 2.5 % w/v, in a Randomized Complete Block Design (RCBD) with three replicates per treatment per site. Results showed that insect pest population, plant damage and chlorophyll content were significantly ($P<0.0001$) influenced by inter-plant, concentration, exposure time, and corresponding factor interaction effects. Irrespective of the plant assayed, concentration, exposure time and site, *T. vogelii* extract was the most efficacious with 29-50% pest reduction at the dosage range tested. Plants treated with *T. vogelii* extract, at increasing dosage, suffered the least damage by *A. whitfieldi* with respect to leaf damage per plant (60-53%), number of feeding holes (21-13) and chlorophyll content (19-22). In the same study, jatropha plants treated with L. *camara* extracts recorded 71-59% leaf damage per plant, 37-22 feeding holes and 17-18 mg/m^3 chlorophyll content. There was significant positive inverse linear relationship ($r = 0.770$) between number of feeding holes and chlorophyll content. The results of this study indicate that *L. camara* and *T. vogelii* extracts hold good potential for control of golden flea beetle.

Key words: Botanical pesticides, Biocontrol, pest population, leaf damage, chlorophyll content

c) Conference proceedings.

Igogo, J.M., Ogendo, J.O., Kariuki, S.T and Otaye, D. O. 2011. Potential of *Tephrosia vogelii* Hook and *Lantana camara* L. aqueous crude extracts for control of golden flea beetle, *Aphthona whitfieldi* Bryant, in Jatropha in Kenya. 6th Egerton university international conference: research and expo. Pp 27-28

Abstract

Laboratory studies were conducted to determine the biological activity of aqueous crude extracts of *Tephrosia vogelii* and *Lantana camara* against golden flea beetle, *Aphthona whitfieldi* Bryant (Coleoptera: Chrysomelidae) in Jatropha (*Jatropha curcas* L.). Each botanical extract, at five rates (0.0, 2.5, 5.0, 7.5 and 10.0% w/v), and a synthetic insecticide, Karate at 2.5 % w/v, were evaluated for toxic, anti-feeding and repellent effects against adult *A. whitfieldi* in a completely randomized design (CRD) with 4-5 replicates per treatment. Results showed that the toxic, anti-feeding and repellent effects of test crude aqueous extracts against adult *A. whitfieldi* were significantly ($P<0.0001$) influenced by inter-plant variability, concentration applied, duration of contact and corresponding factor interactions. At the dose range tested and 8 days of contact, *L. camara* and *T. vogelii* extracts caused 18–56 and 50–62% mortality of adult *A. whitfieldi*, respectively. In the feeding deterrence bioassay, *T. vogelii* extracts, at 5-10% w/v, had 100% deterrence coefficient 7 DAT whereas *L. camara* extracts produced weaker deterrence coefficients of 29-36% for dose range tested. Results of choice bioassay tests showed that aqueous crude extracts obtained from *L. camara* (PR: 55) were more repellent than those of *T. vogelii* (PR: 43).The results of this study provides good scientific promise for targeted exploitation of *T. vogelii* and *L. camara* extracts as eco-friendly and cost-effective alternatives to synthetic insecticides for insect pest management in Jatropha in Kenya and beyond. Further in-depth scientific investigations are recommended on dose-responses and bioactivity spectrum of other extracts against major insect pests of Jatropha including the golden flea beetle.

Key words: *Tephrosia vogelii, Lantana camara, Aphthona whitfieldi , Jatropha curcas* L., contact toxicity, anti-feeding, repellence