KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI, GHANA

EFFICACY OF ETHANOLIC EXTRACT OF *THEVETIA PERUVIANA* (PERS.) K. SCHUM. (MILK BUSH) ROOT IN THE CONTROL OF MAJOR INSECT PESTS OF COWPEA

A THESIS SUBMITTED TO THE DEPARTMENT OF CROP AND SOIL SCIENCES, COLLEGE OF AGRICULTURE AND NATURAL RESOURCES, KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI, GHANA, IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE DEGREE IN CROP PROTECTION (ENTOMOLOGY OPTION)

BY

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CERTIFICATION

This thesis entitled: **'Efficacy of ethanolic extract of *Thevetia peruviana* (Pers.) K. Schum. (milk bush) root in the control of cowpea major insect pests'** was composed by me and this is based on my own work. Acknowledgement has been made in the text and in caption where other peopleôs works have been used.

I certify that this work has not been accepted in substance for any degree, and is not concurrently being submitted for any other degree than that of the Master of Science (MSc) Crop Protection (Entomology) of the Kwame Nkrumah University of Science and Technology.

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DEDICATION

To him who inspires me, who tells me I can make it, who wants the best for me, to him I dedicate this thesis with love. God bless you, ***Agyapong Kwaku Kumanyini Gyamfi****.*

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***'…..God of [my] weary years, God of [my] silent tears, Thou Who hast brought [me] thus far on the way……. '* (Johnson, 1899).**

Great is Thy faithfulness to me, Lord, and I will forever be grateful to You.

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ABSTRACT

Insect pests are a serious constraint to cowpea production in sub-Saharan Africa. Every phenological stage in the life cycle of the crop has at least one major insect pest. Aphids, *Aphis craccivora* Koch (Hemiptera: Aphididae) attack cowpea especially at the seedling stage, flower thrips, *Megalurothrips sjostedti* (Trybom) (Thysanoptera: Thripidae) at flowering, the pod borer, *Maruca vitrata* Fabricius (Lepidoptera: Pyralidae) at flowering and pod formation stages, and, a complex of pod sucking bugs at pod formation stage. The cowpea bruchid, *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae) is the major storage pest. The attack of one or more of these pests may result in total crop loss. Their successful management is therefore critical to ensuring food security. Reports show that these pests are developing resistance to some of the synthetic pesticides commonly used for their control. This and many other reasons have led to the quest for extracts from pesticidal plants to control these pests. The efficacy of ethanolic extract of the roots of milk bush, *Thevetia peruviana* (Pers.) K. Schum, was therefore assessed for the control of the major field insect pests of cowpea in the 2008 minor season and 2009 major season. The extract was prepared from the dry coarse powder using 77% v/v ethanol in a soxhlet apparatus for four hours. The concentrations of the extract used were 0.1%, 0.5% and 1% in the first experiment but changed to 5%, 10% and 15% in the second experiment. These concentrations were compared with the recommended insecticide, cymethoate 25 EC (cypermethrin + dimethoate). The root extract at 15% was superior in reducing insect populations and compared favourably with the synthetic insecticide for many of the parameters measured. Pod density was also enhanced on cowpea plants treated with 10% and 15%

of the extract compared with the control plots (PÒ0.05). *M. vitrata* was the most susceptible to the extract whilst *M. sjostedtii* was not much affected. Cymethoate was consistently superior to all the root extract treatments in substantially reducing the population densities of all the insect pests. But the results of this study indicate that, root extract of *T. peruviana* has the potential to replace synthetic products for the control of the major insect pests of cowpea.

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LIST OF ABBREVIATIONS

AATF	African Agricultural Technology Foundation
ANOVA	Analysis of Variance
cm	Centimetre(s)
CRI	Crop Research Institute
CSIR	Council for Scientific and Industrial Research
DAP	Days after planting
DAS	Days after spraying
DBS	Days before spraying
FAO	Food and Agriculture Organization
g	Gram(s)
IITA	International Institute of Agriculture
IPM	Integrated Pest Management
kg	Kilogram(s)
KNUST	Kwame Nkrumah University of Science and Technology
L	Litre(s)
LC_{50}	Concentration of chemical that kills 50% of sample population
LC_{90}	Concentration of chemical that kills 90% of sample population
LSD	Least Significant Difference
ml	Millilitre(s)
ml/ l	Millilitres/litre
RCBD	Randomised Completely Block Design
TP_5	Ethanolic root extract of *Thevetia peruviana* at 5%
TP_{10}	Ethanolic root extract of *Thevetia peruviana* at 10%
TP_{15}	Ethanolic root extract of *Thevetia peruviana* at 15%
v/v	Volume by volume

CHAPTER ONE

INTRODUCTION

1.1. General Introduction

Cowpea, *Vigna unguiculata* (L.) Walp. (Fabales: Fabaceae), is a grain legume grown mainly in the Savanna regions of the tropics and subtropics in Africa, Asia, and South America (IITA, 2007). Grain yield varies with variety and the method of field insect pest control. An average yield of 1.5 t/ha is obtainable on farmersô fields (Sokoto and Singh, 2008), whereas between 1.8 and 2.5 t/ha has been obtained on researchersô plots (Adu-Dapaah *et al*., 2005). In 2000, out of the worldôs total production of 3.3 million tonnes, Nigeria produced 2.1 million tonnes making it the worldôs largest producer (FAO, 2000). In 2007, IITA reported that Sub-Saharan Africa accounted for about 70% of total world production of 7.56 million tonnes.

Cowpea is a very important pulse in Africa. The young leaves, immature pods and shoots are used as vegetables. All parts of the plant used for food are nutritious, providing protein, vitamins and minerals. The cowpea grain contains an average of 23-25% protein and 50-67% carbohydrates (Singh *et al*., 1997). It has fat content of 1.3%, fibre content of 1.8% and 8-9% of water. Because of its high protein content, cowpea is extremely valuable where many people cannot afford high protein foods such as meat and fish (IITA, 2007).

The usefulness of cowpea as a fodder plant for hay, silage or pasture has led to more research into its improvement for such use (Singh *et al*., 2003). It also provides ground cover that suppresses weeds in sole and intercropped plots and offers some protection ï especially the spreading types of the crop ï against soil erosion (Lawson *et al*., 2006). Because of its ability to fix atmospheric nitrogen through symbiosis with nodule bacteria (*Bradyrhizobium* species) (Singh *et al*., 1997), farmers usually do not apply fertilizers to their cowpea fields, although it has been reported that cowpea responds significantly to fertilizers including poultry manure and mineral fertilizers (Agyenim Boateng *et al*., 2006; Sokoto and Singh, 2008). As a result of its nitrogen- fixing ability, the crop does not deplete the natural (low) reserves of soil nitrogen and many experimental findings illustrate that soil nitrogen increases following cowpea (Singh *et al*., 1997). It therefore becomes an integral component of rotational programmes due to this attribute.

In addition to the above benefits, cowpea provides a reliable source of livelihood to several people particularly women in both rural and urban communities in the production areas and even beyond, through the trading of fresh produce and processed foods from the crop (Singh *et al*., 1997).

1.2. Problem Statement

Cowpea is particularly attractive to insect pests whose attack and damage limit its production (Dugje *et al*., 2009). In the West African sub region, low levels of cowpea yield (200-350 kg/ha) obtained by some farmers are directly attributed to insect pest damage on the field (IITA, 2007).

In order to control these insect pests, farmers adopt the use of conventional insecticides which aside from being sometimes wrongly applied may have devastating effect on the environment (Singh *et al.*, 2004).

The indiscriminate use of chemicals has given rise to a number of problems including genetic resistance of the insect pest species, accumulation of toxic residues in treated grains (Singh *et al.*, 2004) which may result in acute and chronic poisoning of consumers, health hazards to homoeotherms, environmental contamination resulting in problems such as destruction of fish, birds, and other wildlife and the disruption of natural biological control and pollination. Other environmental problems include extensive groundwater contamination. Chemical insecticides are also expensive and their use results in increased cost of production (Pretty and Waibel, 2005). These problems have necessitated the search for alternative and effective biodegradable insecticides, which have greater selectivity. The re-evaluation and use of traditional botanical pest control agents (powder, water extracts, oil and wood ash) that local farmers have been using over the past decades, though without much success, seem to provide a clue to local sourcing of pest control strategies. Plant extracts, as biopesticides, are gaining more popularity because they are inherently less harmful than conventional pesticides (Oparaeke *et al.,* 2000; Mitchell *et al.,* 2004). Biopesticides are often effective in very small quantities and they decompose quickly, thereby resulting in lower persistence and largely without the pollution problems caused by conventional pesticides (Mitchell *et al.,* 2004). When used as a component of Integrated Pest

Management (IPM) programs, biopesticides can greatly decrease the use of conventional pesticides, while crop yields remain high (Banwo and Adamu, 2003).

Extracts of seeds, leaves, barks and roots, and powders and wood ash of various plant species have been used by resource-limited farmers in traditional agriculture to protect crops from insect pest infestation. Literature has shown that most of the studies conducted by scientists throughout the world on the use of plant materials for insecticidal purposes were storage-based (Oparaeke *et al.*, 2000). Research on the potential use of plant extracts as biopesticides for field pests control is also currently receiving attention worldwide. Plant extracts act as mortality agents, repellents, antifeedants, attractants, oviposition deterrents and sterility agents (Lale, 2002).
Ghana is endowed with plant varieties which have insecticidal properties that may be used for pest control on arable crops across the different ecological zones (Dokosi, 1998). One of such plants is the milk bush, *Thevetia peruviana* (Pers.) K. Schum.

Several *Thevetia* spp, including *T. peruviana*, have been found to have insecticidal properties (Oji and Okafor, 2000; Bai and Koshy, 2004; Mollah and Islam, 2007) and their root extracts have the potential of being insecticidal to cowpea insects. In view of the problems associated with the use of heavy doses of conventional pesticides particularly in cowpea production and the increasing interest in the use of botanicals, it has become imperative that a study is carried out for an effective and a safer alternative for controlling these pests.

1.3. Objectives of Study

The main objective of this work was to study the effect of various concentrations of ethanolic extract of *T. peruviana* roots on the major insect pests of cowpea namely *Aphis craccivora* Koch (Hemiptera: Aphididae), *Megalurothrips sjostedti* (Trybom) (Thysanoptera: Thripidae), *Maruca vitrata* Fabricius (Lepidoptera: Pyralidae), a complex of pod sucking bugs and *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae). Although some plant pesticides have proven effective in controlling these pests, the identification of new sources of plant pesticides is necessary, as these plants are all not cosmopolitan in distribution. The new sources will also offer local farmers more options in their desire to control these pests. The reduction in the use of synthetic chemicals on cowpea will go a long way to reduce the associated negative environmental impacts especially in less developed countries where pesticide pollution is on the increase (Wilson and Tisdell, 2001).

Much research has not been conducted so far to evaluate the toxic effects of extracts of the various parts of the *T. peruviana* plant (Mollah and Islam, 2007). This study was therefore carried out to assess the toxic effect of ethanolic extract of *T. peruviana* root against insect pests of cowpea. The result of this project is aimed at optimizing the use of *T. peruviana* in cowpea insect pest management by finding out the concentrations of the extract that may be effective on selected cowpea pests. This will help develop approaches that optimize their use (Stevenson *et al*., 2009).

1.3.1. Specific Objectives

The specific objectives of this study were to:

1. determine the effect of milk bush root extract on cowpea pests.
2. determine which of the pests is most susceptible to the milk bush root extract.
3. determine which of the pests is least susceptible to the milk bush root extract.
4. make recommendations based on the results obtained.

CHAPTER TWO

LITERATURE REVIEW

2.1. Origin and Distribution of Cowpea

The wild *Vigna* species is widespread in tropical Africa. It seems reasonable, therefore, to assume that the crop was domesticated in Africa and that it spread from there to other areas (Faris, 1965). It was subsequently introduced into India, the West Indies and the USA (Purseglove, 1968). Three known varieties common in West Africa are *V. unguiculata var unguiculata, V. unguiculata var sinensis and V. unguiculata var sequipedalis*, with *V. unguiculata var unguiculata* being the commonest (Faris, 1965). The name ócowpeaô probably came about as a result of the crop being an important source of hay for cows in south-eastern United States and some other parts of the world (Timko *et al*., 2007). Although a single crop species, cowpea has a wide diversity in terms of seed morphology ï shape, size and colour (Figure 2.1). Some varieties such as the black-eyed peas and the crowder peas are indigenous to specific regions of the world (Timko *et al*., 2007).

Figure 2.1: Diversity of seed types in cowpea - seed shape, color and texture

Source: Timko *et al*. (2007)

2.2. Biology and Damage caused by Cowpea Insect Pests

Every phenological stage in the life cycle of cowpea has at least one major insect pest. Aphids (*Aphis craccivora*) attack cowpea especially at the seedling stage, flower thrips (*Megalurothrips sjostedti*) at flowering, legume pod borer (*Maruca vitrata*) at flowering and pod formation stages and a complex of pod sucking bugs at pod stage. The cowpea bruchid (*Callosobruchus maculatus*) is the major storage pest. These are the insect pests of economic importance although other insects do attack cowpea. Attack from any of these pests has been reported to cause reduction of the yield of the crop. Because of the economic importance of these pests, it is important that efforts are channeled into their control.

2.3.1. Cowpea Aphids

There are about 4,000 known species of aphids of which about 250 are serious pests. Scientists believe that the number of species remained relatively small until the angiosperms (flowering plants) became more common, then as the aphids adapted to these new and rapidly speciating plants, their species numbers increased also (Dixon, 1990). About 25% of all plant species are infested with aphids, and though it is believed that the speciation of aphids has followed that of plants, not all groups of plants are equally infested (Moran and Baumann, 1994). The cowpea aphid, *A. craccivora* attacks cowpea at the pre-flowering stage. A general feeder, the cowpea aphid infests a large number of crops such as carrot, cotton, peanut and many other legumes.

Cowpea aphids are medium-sized, shiny black insects whose biology varies depending on climate and soil. Adult aphids are generally wingless (apterae), but there are also winged forms (alates). Wingless female adults produce about 80 nymphs in about 14 days (Powell *et al*., 2006). Due to this high reproductive rate, large colonies quickly form at the feeding sites ï mostly under the leaf and on young shoots (Figure 2.2). At low temperatures, the developmental period of nymphs into adults may take up to 22 days. However, at warmer temperatures of about 29°C, development takes only five days (CIPM, 2010). Most nymphs mature into wingless females, but periodically, winged females develop and migrate to new host plants (Powell *et al*., 2006). The ability to produce winged individuals provides the insect with a way to migrate to other new host plants when the food source gets depleted or scarce. Many generations are produced in a year.

Figure 2.2: Cowpea plant heavily infested by aphids

Source: http://www.infonet-biovision.org

When present in large numbers, they cause direct feeding damage where the plants become stunted, leading to leaf distortion, premature defoliation and seedling death. The aphids excrete copious amounts of honeydew which leads to growth of sooty mould mostly on the upper receptive surface of leaves. The aphids do not only cause direct damage to their host, but also transmit the cowpea aphid-borne mosaic virus (Adu-Dapaah *et al.,* 2005). Some cultivars are however resistant to this pest (IITA, 1979).

2.3.2. Flower Thrips

The flower thrips are among the most important pests of cowpea. In West Africa, they are frequently responsible for total crop loss (Singh and Allen, 1979). Adult thrips (Figure 2.3) are shiny black, minute (usually 1 mm to 1.5 mm), slender and usually winged insects found in flower buds and flowers.

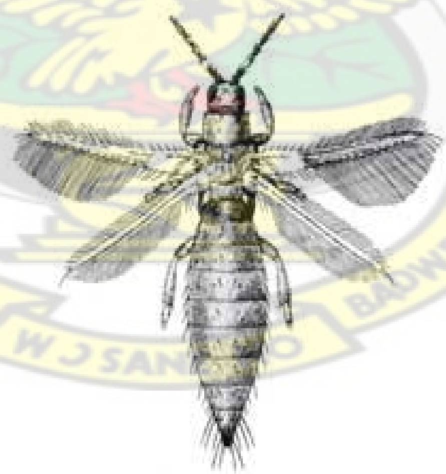


Figure 2.3: Adult flower thrips (*M. sjostedti*)

Source: http://www.infonet-biovision.org

The wings are long, narrow and fringed with long hairs, and at rest, are tied on the back along the body. Eggs are laid in the flower buds and nymphs feed and do extensive damage. Pupae develop in the soil. The entire life cycle takes 14-18 days under warm conditions, and this gives them an enormous capacity for increase (Gitonga *et al.*, 2002). During the pre-flowering period, nymphs and adults may damage the terminal buds, petioles and leaves. However, the main damage is on the flower buds and flowers. Attacked petioles and leaves have tiny patches surrounded by discoloured areas. When the thrips population is very high, open flowers are distorted and discoloured (Singh and Allen, 1979). Flowers drop prematurely with the result that few pods are formed. Characteristically, peduncles also fail to elongate. Severely infested plants do not produce any flowers (IITA, 1979). Thrips also feed on pollen leading to decrease in pollination and seed set. Thrips migrate actively between different hosts for better food sources.

At least two other species of thrips are found on cowpea in Africa: *Frankliniella schultzei* (Trybom) (Thysanoptera: Thripidae) (Figure 2.4) and *Sericothrips occipitalis* Hood (Thysanoptera: Thripidae). These are known to be minor pests of cowpea (Singh and Allen, 1979).

Figure 2.4: Western flower thrips, *Frankliniella schultzei*

Source: http://www.insects.tamu.edu

2.3.3. *Maruca* Pod Borer

Maruca vitrata is a common species known in Africa and south East Asia. It is widely distributed throughout the tropics and sub tropics where they may cause extreme damage (Adati *et al*., 2007). The adult (Figure 2.5) is a nocturnal moth, light brown with whitish markings on its forewings (Usua and Singh, 1979). It can live for about 10 to 12 days. During this period, mating occurs and the female lays up to 150 eggs on leaf buds, flower buds and in flowers. Eggs hatch in about three days at 24-27 ºC (Ramasubramanian and SundaraBabu, 1989). There are five larval instars (Odebiyi, 1981) which together last 8-14 days before pupae develop in the soil (Okeyo-Owuor and Ochieng, 1981). The pupal stage lasts five to seven days before adults emerge.

Figure 2.5: *Maruca vitrata* adult

Source: http://www.mcknight.ccrp.cornell.edu

The larval stage of *M. vitrata* is the stage of economic importance. The larva (or caterpillar) (Figure 2.6) is dull to yellowish and often reaches a length of 18 mm. Each segment has dark spots that form a distinct series along the length of the body. The head is dark brown to black. The larva moves from one flower to another and can consume four to six flowers during the larval development period (Taylor, 1967). Their feeding habits usually result in flower abscission (Jackai *et al*., 1992). *Maruca* larvae also feed on tender parts of the stem, peduncles, flower buds and pods (Zhu-Salzman and Murdock, 2007). The characteristic signs of larval feeding are webbing of flowers, pods and leaves and production of frass on pods. Flowers attacked may be discoloured and have damaged or missing reproductive parts (Atachi, 1998). Damage by this pest also results in flower bud shedding and reduced pod production. Damaged pods have small, darkened entry holes on the surface. The adults do not feed on cowpea as the larvae; they are pollen feeders.

Figure 2.6: *M. vitrata* larva in split cowpea pod

Source: http://www.infonet-biovision.org

Cowpea varieties in which pods on the same peduncle are close together are especially prone to damage, resulting in considerable losses in grain yield and quality. Damage may amount to 80-100% where good control measures are absent, especially on cowpea in West Africa (Singh *et al*., 1990).

2.3.4. Pod Sucking Bugs (PSBs)

A complex of pod sucking bugs attack cowpea at the pod formation stage and cause similar damage to the cowpea pods (Singh and Allen, 1979). They suck sap from pods and seeds and cause various levels of damage depending on the stage of growth of seeds at the time of attack. Feeding may cause necrosis at points of stylet insertion, pod malformation, premature shriveling and drying of pods and seeds, loss of germinability, and empty pods (Karungi *et al*., 2000). Bugs are difficult to control since they usually feed on a wide range of crops and are very mobile (Clementine *et al*., 2005). The bugs of economic interest on cowpea include:

a. *Anoplocnemis curvipes* (Fab.) (Hemiptera: Coreidae)

Commonly known as the giant coreid bug, this insect is a major pest which can cause yield losses varying from 30 to 70% (Karungi *et al*., 2000). Eggs, which are usually laid on leguminous trees or weeds but seldom on cowpea plants, are laid in chains and are grey to black in colour. They hatch in about 7-11 days and go through five nymphal instars. The total nymphal period varies from 29-54 days; the life of an adult varying from 24-84 days depending on the weather. Full grown bugs (Figure 2.7) are about 2.5 cm long (http://www.infonet-biovision.org). Adults are strong fliers and seek refuge in trees when disturbed (IITA, 1979).

Figure 2.7: *A. curvipes* adult

Source: http://www.infonet-biovision.org

b. *Riptortus dentipes* Fab. (Hemiptera: Coreidae)

R. dentipes is another serious pest of cowpea in tropical Africa. The adult bug is cylindrical, light brown with characteristic white or yellow lines on the side of the body (Figure 2.8). Eggs are laid either in short rows or are scattered and are mostly laid on leguminous trees and weeds but few are found on cowpea plants. There are five nymphal instars.

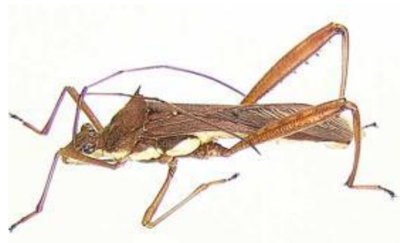


Figure 2.8: Adult *Riptortus* bug

Source: http://www.infonet-biovision.org

c. *Clavigralla* spp. (Hemiptera: Coreidae)

C. tomentosicollis and *C. shadabi* are the two most common species in tropical Africa. The adult measures about 1 cm in length (http://www.infonet-biovision.org). Both species cause extensive damage and can cause yield losses of up to 90% (Tanzubil, 2000). Eggs are laid on cowpea and both nymphs and adults feed on pods by sucking the sap. They are not easily disturbed, and large numbers are found feeding together on a single pod (Karungi *et al*., 2000).

Figure 2.9: *Clavigralla* spp

Source: http://www.ikisan.com

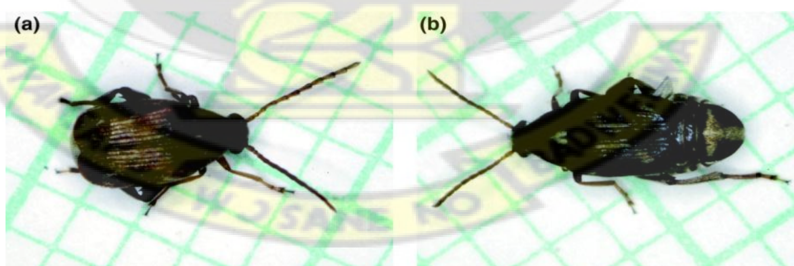
d. *Nezara viridula* (Linnaeus) (Hemiptera: Pentatomidae)

Commonly known as the green stink bug, it is widespread in the tropics and sub tropics. Batches of 30 to 80 eggs are laid on the underside of leaves. A single female may lay from 100 to 250 eggs in four to six batches. Nymphs are shiny with bright spots and there are five nymphal instars. Adult are green and roughly 5-sided in shape (Figure 2.10). The entire life cycle may take 30-60 days (Singh and Allen, 1979).

Figure 2.10: The southern green stink bug

Source: http://www.ipmworld.umn.edu

2.3.5. Cowpea Storage Bruchids



Male   Female

Figure 2.11: Male (a) and female (b) adults of *C. maculatus*

Source: Tower and Arbeitman (2009)

Several species of *Callosobruchus* (Coleoptera: Bruchidae) cause damage to cowpea during storage but the two most important ones are *Callosobruchus maculatus* (Figure 2.11) and *Callosobruchus chinensis* Linnaeus (Coleoptera: Bruchidae) (Singh and Jackai, 1985). *C. maculatus* (the cowpea bruchid) is a field-to-store pest with a West African origin, from where it spread throughout the tropical and subtropical world (Singh *et al.,* 1997). Infestation begins in the field but serious damage is done to the pods or seeds during storage (Warui, 1984). Females attach eggs to the surface of seeds or pods. In the field, gravid females deposit eggs on the surfaces of pods still attached to the plant. Females prefer mature green pods but will oviposit on dry mature pods as well (Singh *et al*., 1997). Females oviposit more readily on exposed grains in cowpea lines that dehisce easily. The egg hatches in about three to five days and the larvae bore into the seeds where they complete their entire development, including pupation. Fully matured adults emerge from the seeds. *C. maculatus* takes about 22-25 days to develop from egg to adult at 30 ^{0}C, and emerged adults are ready to mate and oviposit almost immediately (Mery and Tadeusz*,* 2003). The adult is a small square-shouldered beetle with dark markings on the wing cases. The adult does not feed and therefore survives on the food reserves obtained as a larva.

Each female can produce about 21 female offspring that survive to adulthood in susceptible grains, the population of bruchids in a cowpea store, therefore, can grow exponentially in few months (Singh *et al*., 1997). All the pre-adult stages of *C. maculatus* are found within the grain, where the larva feeds entirely, and while doing so, reduces the quality and quantity, and hence the nutritional and commercial value of stored cowpea (Figure 2.12). Control of this pest, therefore, is either preventive or curative, and directed at the grain (Caswell, 1981).

Figure 2.12: Cowpea heavily infested with *C. maculatus*

Source: http://www.springhalen.dk

2.4. Common Insect Pest Management Practices for Cowpea Production

All cowpea pest control measures commonly used have their own merits. No single component is effective when used alone but various research works have shown that specific combinations can work synergistically (Adipala *et al*., 2000). This is because the synergistic effect of the various control options is often better than the effect of a single control strategy.

The small-scale farmer rarely considers initiating crop protection until the crop is attacked. Therefore, guidance should be focussed on pest management, which requires an understanding of pest biology. In any event, it is necessary to put measures in place to help keep the populations of pests below the economic threshold. This makes the issue of monitoring important to ensure that pests are controlled when there is the need

(Afun *et al.,* 1991). The common pest control options include cultural control, the use of resistant varieties, biological control, insecticide use and the use of biopesticides and plant extracts.

2.4.1. Cultural Control

Cultural control of cowpea pests is useful, but only as a component of IPM. This is because, as a stand-alone strategy, cultural control options are usually not capable of effectively controlling the insects. While certain cultural practices may help control some pests, other pests may be encouraged to multiply. For instance, Asiwe *et al*. (2005) found that increased spacing resulted in an increase in the severity of aphid infestation, but damage caused by thrips and *M. vitrata* was reduced. Other cultural practices that have been employed include crop rotation, mixed cropping, zero tillage and early harvesting of cowpea pods (Adipala *et al*., 2000).

Cowpea aphids are brought under control through many cultural practices. Farmers are advised to plant early to reduce if not avoid aphid infestation. This way, the natural enemies are also conserved to naturally control the pest. The practice of zero tillage where the field is left unploughed but herbicides applied also help avoid early colonization of aphids on farmersô fields (Adipala *et al*., 2000). Studies have shown that high levels of nitrogen fertilizers favour aphid reproduction (http://www.ipm.ucdavis.edu). Because cowpea is capable of fixing its own nitrogen, it is thus important to reduce the amount of chemical fertilizers so as to reduce the build-up of nitrogen in the soil which is likely to favour multiplication of aphids. Proper timing of planting of cowpea just at the beginning of the rains has been reported to be helpful in keeping the density of *A. craccivora* below the economic threshold (Jackai *et al*., 1985). This is because the aphids are easily washed off the cowpea plants by heavy rains. Varying the planting time of the crop ensures that the most vulnerable crop growth stage does not coincide with the highest density of the pest (Dent, 1991). Although zero tillage has proved to help control aphids, ploughing and harrowing before planting help control thrips (http://www.infonet-biovision.org). Moreover there are reports that thrips populations are reduced by up to 50% when cowpeas are intercropped with maize or sorghum (Parella and Lewis, 1997). According to Asiwe *et al*. (2005), increased spacing resulted in the reduction of damage caused by thrips. The cultural control of *M. vitrata* has been mainly through frequent monitoring of cowpea crops for pod borers as there is only a brief period between the hatching of the larva to the time it enters buds or pods. Reports from studies carried out by Asiwe *et al*. (2005) indicated that increased spacing resulted in the reduction of damage caused by *M. vitrata*. Pod sucking bugs can be collected by hand regularly and killed, especially during flowering and pod formation and when populations are still very small. *C. maculatus* is a well known field-to-store pest. A viable pair of adults from the field can result in serious infestation within three months if the grains are not treated prior to storage. Timely harvesting, therefore, reduces the chances of the crop being infested in the field. When the harvested grain is sun-dried before storage, most of the bruchidôs life stages are killed. However, although advantageous, sun drying does not provide high enough temperature to kill all the different stages of the insect. According to Murdock and Shade (1991), exposing the grain to a temperature of 65 ºC for five minutes kills all life stages, while a lower temperature of 57 ºC will kill all stages after an hour of exposure.

The use of other techniques or practices associated with crop production to control *C. maculatus* in the field has been effective. For example, Karel *et al*. (1982) reported that intercropping maize with cowpea reduced infestation of cowpea by *C. maculatus*. Singh and Jackai (1985) noted that low infestation of *C. maculatus* in a cowpea/maize intercrop was due to restriction of movement (barrier effect) of the pest, increased canopy closure leading to increased humidity, reduction in temperature and creation of shade for shelter and increased natural enemy population. It has been reported that, intercropping cowpea with sorghum helped to reduce the number of insecticide sprays required to three, which also resulted in 51% increase over farmersô cowpea yield in Uganda. The increase in yield was as a result of the reduction in the density of the legume pod borers, aphids, thrips and pod sucking bugs (Nabirye *et al*., 2003).

2.4.2. Use of Resistant Varieties

Host Plant Resistance (HPR) has been identified as probably the most ideal means of controlling insects on cowpea and considered a major component of every IPM approach (Rubiales *et al*., 2006). The development and use of resistant varieties of cowpea however has limitations in pest control. With at least five different major insects attacking the cowpea crop at different growth stages, it is difficult to breed varieties that are resistant to all these insects and still maintain all the positive agricultural qualities of the crop (Jackai and Adalla, 1997). Nonetheless, there are some lines developed by the International Institute of Tropical Agriculture (IITA) that are resistant to aphids and flower thrips. The lines "IT90K-277-2", "KVx404-8-1", "TVx3236-01G", and "IT91K-180" are reported to show varying levels of resistance to the cowpea flower thrips in West Africa (IITA, 2007). An ongoing *Maruca*-resistant cowpea project by the African Agricultural Technology Foundation (AATF) is also working to develop transgenic cowpea lines that confer *Maruca* resistance in cowpea (Anonymous, 2009). Researchers at IITA have screened the entire germplasm collection of cultivated *V. unguiculata* (12,000 accessions) and have identified three lines (TVu 2027, TVu 11952 and TVu 11953) that exhibit seed resistance to *C. maculatus* (IITA, 2007).

The use of cowpea varieties resistant to some of the insect pests helps reduce the number of synthetic insecticide sprays that may be required during the production of the crop (Ehlers and Hall, 1997).

2.4.3. Biological Control

Natural enemies of cowpea pests exist as options for pest control. For example, *A. craccivora* is attacked by many predators including coccinellid adults and larvae (Ofuya, 1995) and syrphid flies (ipm.ucdavis.edu). Natural enemies, particularly, predators are also important for the natural control of thrips. These include predatory bugs such as *Orius albidipennis* (Reuter) (Hemiptera: Anthocoridae) which has been found to naturally control the populations of thrips larvae and adults in Kenya (Gitonga *et al*., 2002). Others include *Anthocoris* spp. (Hemiptera: Anthocoridae) and predatory thrips (infonet-biovision.org).

M. vitrata is reportedly controlled by *Trichogrammatoidea eldanae* Viggiani (Hymenoptera: Trichogrammatidae). Parasitism of *M. vitrata* by *T. eldanae* on cowpea can be up to 50% in West Africa (Tamò *et al*., 1997). The level of parasitism on *M. vitrata* is influenced by the host plant the insect is feeding on and the agroecological zone. Other parasitoids of *M. vitrata* on cowpea include *Braunsia kriegeri* Enderlein (Hymenoptera: Braconidae) and *Tetrastichus* spp. (Hymenoptera: Eulophidae) (Tamò *et al*., 2000). The mantid *Polyspilota aeruginosa* Goeze (Mantodea: Mantidae) is an important predator of *M. vitrata* (Okwapam, 1977).

Populations of pod sucking bugs are also controlled by natural enemies such as assassin bugs, spiders, praying mantids and ants (http://www.infonet-biovision.org). A specific example is *Gyron gnidus* Nixon (Hymenoptera: Scelionidae), which has been found to attack the eggs of *Clavigralla tomentosicollis* with an average parasitism of 38% (Egwatu and Taylor, 1983). Okwapam (1977) stated that the activities of predators, parasitoids and pathogens can control *C. maculatus* to some extent. *Uscana lariophaga* Steffan (Hymenoptera: Trichogrammatidae) parasitizes a maximum of 50% of the eggs of the bruchid beetle laid on pods in the field (Pedigo, 1989).

The presence of these natural enemies in cowpea fields, therefore, should be encouraged by avoiding the application of broad spectrum insecticides. However, it should be noted that the impact of natural enemies is often low and erratic, and like HPR, they serve as important components of IPM strategies.

2.4.4. Insecticide Use

The use of synthetic insecticides is by far the most common means of controlling insect pests of cowpea (Jackai and Adalla, 1997). Some of the insecticides that have been recommended for the control of insect pests of cowpea include synthetic pyrethroids such as cypermethrin. Having a rapid knock down effect, it works as contact and stomach poison with some repellent properties and has been used in the control of field insect pests of cowpea. Other insecticides such as dimethoate are used to control post flowering pests such as the pod sucking bugs and *C. maculatus*. Where available, cymethoate, a combination of cypermethrin and dimethoate, has been recommended for all the insect pests of cowpea on the field (Kawuki *et al*., 2005).

For control of *C. maculatus* in storage, Actellic (pirimiphos methyl), both as dust and emulsifiable concentrate, has proven effective over the years (Adu-Dapaah *et al*., 2005; Swella and Mushobozy, 2007), although adulteration of the product is simple and common throughout Africa (P. Stevenson, personal communication). The use of insecticides to protect cowpea grain in storage is more controversial than their use on the field crop, because chemical residues are erroneously feared to persist in the bean after cooking. However, if the right insecticides are used in the appropriate manner, there should be little or no concern about residues in cooked food (Singh *et al*., 1997). Cowpea treated with Actellic can be stored up to six months without any significant bruchid damage.

Recent studies have however shown that various pests have developed or are developing resistance to the synthetic chemicals employed. *M. vitrata* has been found to be resistant to cypermethrin, dimethoate and endosulfan; three of the most commonly used synthetic insecticides for control of insect pests in cowpea (Ekesi, 1999). The cowpea bruchid has also been reported to be developing resistance to pirimiphos methyl (Odeyemi *et al*., 2006).

2.4.5. Use of Biopesticides and Plant Extracts

Biopesticides (also known as biological pesticides) are derived from such natural materials as animals, plants (botanicals), bacteria, and certain minerals. The use of these biopesticides is gaining much popularity because of the numerous problems associated with the use of synthetic chemicals. These biopesticides have also proven effective for the control of several insects of economic interest. They break down readily in soil and are not stored in animal and plant tissue, hence their promotion for use in insect pest management.

Crude extracts of West African black pepper, *Piper guineense* were used by Oparaeke *et al.* (2000) and Oparaeke (2007) on cowpea insect pests. A systemic effect was observed on thrips and *M. vitrata* larvae in cowpea flowers while direct contact with *Maruca* larvae and pod sucking bugs (adults and nymphs) was found to be highly lethal. Pod damage was significantly reduced and grain yields consequently increased in treated plots compared with the untreated control. These combined properties of West

African black pepper were found useful for it to be considered as a potential candidate in the effective management of noxious insect pests of arable crops (Oparaeke, 2007).

Neem extracts have also been found to effectively control aphids, thrips, pod borers and pod sucking bugs on cowpea. Crude extract mixtures of chilli pepper with neem leaf have been reported to be effective in reducing the numbers of thrips (<0.5 thrips/flower) in cowpea flowers and subsequently ensuring higher pod load per plant on treated plots (Oparaeke, 2007). Work done using botanicals in controlling *M. vitrata* include the use of tobacco extracts (Opolot *et al*., 2006) and *Tephrosia* aqueous extracts (Kawuki *et al*., 2005). A number of plants (garlic, oleander, African marigold, goat weed, among others) are reported as effective repellent crops against various species of pod sucking bugs (Elwell and Maas, 1995). Small quantities of cowpea seeds can also be protected from *C. maculatus* by admixing with edible oil (e.g. groundnut oil mixed at the rate of 5 ml/ kg seed). The oil coats the testa, acting as an ovicide by plugging the egg microphyle, thus hindering oxygen supply to the embryo. In some cases the oil may deter oviposition or cause mortality of the adult bruchid (Singh *et al*., 1997). Studies by Keita *et al*. (2000) have shown that the cowpea weevil can be controlled with essential oils particularly those derived from the genus *Ocimum,* the basils, making plant extracts a good alternative to synthetic insecticides. Some naturally occurring botanical materials have a definite ovipositional deterrence against the cowpea bruchid. These botanicals, including neem seed extracts deter oviposition and also act as antifeedants (Elhag, 2000).

Botanical insecticides are prepared in the form of the crude plant material, extracts or resins. The crude plant material is usually ground into powder and may be diluted with a carrier before use. Pyrethrum flowers and neem seeds among others are often ground into powdered form and used (Singh *et al.,* 2004). Plant essential oils are produced commercially from several botanical sources, many of which are members of the mint family (Lamiaceae). Examples are rosemary (*Rosmarinus officinale*) and eucalyptus (*Eucalyptus globus*). A number of the source plants have been traditionally used for protection of stored commodities, especially in the Mediterranean region and in southern Asia, but interest in the oils was renewed with emerging demonstration of their fumigant and contact insecticidal activities to a wide range of pests in the 1990s. The rapid action against some pests is indicative of a neurotoxic mode of action (Isman, 2006).

Potential new botanicals include annonaceous acetogenins that have been traditionally prepared from the seeds of tropical *Annona* species such as the sweetsop (*A. squamosa*) and soursop (*A. muricata*) (Isman, 2006). These contain compounds that are slow acting stomach poisons, particularly effective against chewing insects such as lepidopterans and the Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae). Another group of plants which is gaining popularity is the *Melia* spp., a closely related genus to *Azadirachta.* Seeds from the chinaberry tree, *M. azedarach*, have been found to contain a number of triterpenoids, the meliacarpins that are similar but not identical to the azadirachtins, and these too have insect growth regulating bioactivities (Kraus, 2002).

2.5. Choice of Pesticidal Plant - *Thevetia peruviana*

Thevetia peruviana is otherwise known as ómilk bushô, ôbe still treeô, ôlucky nutô or óyellow oleanderô. It is locally called ñnnye me nnyere meò in the Akan language and this literally means ñdo not hold me downò. It belongs to the order Apocynales and family Apocynaceae. Although a native of tropical America especially Mexico and West Indies, it has established in tropical regions worldwide through its distribution by man, occurring in the West African region as a garden shrub. It grows up to about six metres high (Shepherd, 2004). The plant (Figure 2.13) is cultivated and remains an ornamental shrub in spite of the high oil content (63%) and favourable protein content (37%) of the seed. The defatted seed cake however has a high level of toxicity (Sticker, 1970). It is likely that the attention given to toxins has distracted interest from proper research of the oil and protein that would have promoted its industrial and domestic potentials. Several feeding experiments (Atteh *et al.,* 1995) and thermal studies (Ibiyemi *et al.,* 1995) have shown that the oil has a very good replacement value for orthodox domestic vegetable oils if its plantations would be developed.

Figure 2.13: *T. peruviana* in flower

Source: http://www.wellgrowhorti.com

This plant has been reported to be insecticidal to some extent (Oji and Okafor, 2000). There is however limited research of its use as an insecticide. Finding its insecticidal properties on cowpea pests may give impetus for proper research into the plant. Pot experiments show that the plant responds well to nitrogenous fertilizer and its response to calcium and phosphorus follows the normal pattern for most plants (Ibiyemi and Popoola, 1991). Cultivation of plantations of the plant may therefore not be so much of a problem. Promotion of its cultivation will not only serve as an insecticidal plant but also as a source of oil.

T. peruviana grows abundantly in the wild and as an ornamental flower hedge in the wetter regions of West Africa (Ibiyemi *et al*., 2002). The plant can produce about 400-800 fruits each year depending on the rainfall and plant age. *T. peruviana* is perennial and widely distributed, which makes it an ideal insecticidal plant if it proves efficient on cowpea pests.

The foliage is grazed by livestock especially goats. The fruit pulp is sometimes eaten in Ghana without any apparent ill-effect (Adjei, 2003). Its use as a pesticidal plant may therefore pose little or no danger to man especially if used in the right concentrations. The oil is used externally to treat skin diseases. In regulated doses, it is employed as an emetic and febrifuge, being said to be effective in intermittent fevers. The kernels are sometimes chewed for a purgative effect (Schmelzer, 2006). Thus, because of its medicinal (Mantu and Sharma, 1980) and insecticidal (Mollah and Islam, 2007) properties, it is worth researching on to determine its effects on insect pests of cowpea.

2.6. Choice of Solvent - Ethanol

Several solvents, including water, ethanol, acetone and petroleum ether have been used in the preparation of plant extracts for testing their toxicity to insects. Although each of these solvents has its own merits, ethanol is an accepted solvent for contact application because of its broad solubility properties and low toxicity. It is highly volatile and therefore evaporates from an organismôs surface, food or oviposition site being used as a test medium, leaving the test material deposit of pure compound or extract on the surface without damaging the test organism. Ethanolic extracts are noted to be generally more effective compared with aqueous extracts of the same plant, due to higher solubility of organic compounds in ethanol (Parekh *et al.,* 2005; Koffi *et al*., 2010).

2.7. Choice of Cowpea Variety – 'Asetenapa'

Several cowpea varieties have been released by IITA and CSIR. óAsetenapaô which literally mean ógood livingô is one of such varieties released by the Crops Research Institute (CRI) of the CSIR for cultivation in Ghana.

According to Adu- Dapaah *et al.* (2005), Asetenapa (IT81D-1951) is a medium-maturing variety (63ï 70 days) with an erect growth habit. It has purple pigmentation on the joints connecting the petiole with the main stem as well as on the standard and wing petals. The plant has narrow leaves. The seeds are cream in colour with a smooth seed coat and are of medium size (about 15 g per 100 seeds). Asetenapa is resistant to the major cowpea diseases such as anthracnose, brown blotch, cercospora leaf spot, bacterial blight, cowpea yellow mosaic and cowpea aphid-borne mosaic. It is however susceptible to many of the major insect pests of cowpea (Adu- Dapaah *et al*., 2005).

Asetenapa is a variety that has a good eye appeal and it is preferred by farmers and consumers to other varieties. Because of its acceptance by consumers, farmers tend to grow this variety more than several other varieties. More research therefore is required to ensure the successful cultivation of this crop variety, hence the choice of the crop variety for the present study. The varietyøs susceptibility to major insect pests of cowpea therefore makes it a good material for such a research work.

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study Environment

The project was carried out at two experimental sites: the Plantation Section and the Entomology laboratories of the Department of Crop and Soil Sciences of the Faculty of Agriculture, Kwame Nkrumah University of Science and Technology, Ghana between September 2008 and August 2009. The average temperature and relative humidity were 26 °C and 70%, respectively.

3.2. Cowpea Variety Used

The variety used was óAsetenapaô which literally mean ógood livingö. It has a good eye appeal and is preferred by farmers and consumers to other varieties but susceptible to major insect pests of cowpea. The seeds were obtained from the Seed Production Unit of the Crop Research Institute (CRI), Kumasi, Ghana.

3.3. Source of Test Plant and Preparation of Extract

Fresh *T. peruviana* roots were collected from various sites within the Kumasi metropolis. They were gently washed under running tap water to remove soil and other debris after which they were chopped and air-dried. The dried roots were then milled in a hammer-mill to obtain a coarse powder.

The plant extract was prepared using the procedure employed by Owiredu *et al*. (2007). Every 5 kg of powder was extracted with 77% v/v ethanol in a soxhlet apparatus for four hours (Figure 3.1).

Figure 3.1: The extraction soxhlet apparatus

A small quantity of the powder was put in small calico sacks measuring about 20 cm X 10 cm. The sack containing the powder was placed inside the thimble of the soxhlet extractor which was then placed onto the flask containing the extraction solvent, ethanol. The soxhlet was equipped with a condenser. The solvent was heated to reflux. The solvent vapour travelled up the distillation arm and flooded into the chamber housing the thimble. As the vapour cooled, the solvent dripped back into the thimble containing the powder. As the thimble was slowly filled with the solvent, some of the compounds were dissolved. When the chamber was almost full, it was automatically emptied by the siphon side arm, with the solvent running down to the distillation flask. The process was allowed to repeat many times for four hours.

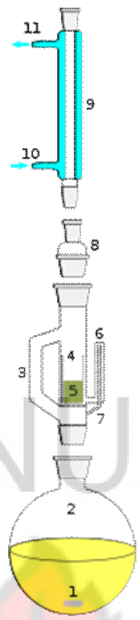


Figure 3.2: A schematic diagram of a soxhlet extractor

(**1:** Stirrer bar; **2:** Distillation flask; **3:** Distillation path; **4:** Thimble; **5:** Coarse powder; **6:** Siphon top; **7:** Siphon exit; **8:** Expansion adapter; **9:** Condenser; **10:** Cooling water in; **11:** Cooling water out).

The hydro-alcoholic filtrate obtained after the extraction was then concentrated under pressure using the rotary evaporator (Figure 3.3) to obtain a yellowish brown syrupy mass which was then air dried (Owiredu *et al*., 2007). The evaporator separated the ethanol from the plant extract which was then diluted to obtain different concentrations for the tests. To reduce duration of exposure to the environment before use, the extract was prepared as and when needed for use.

Figure 3.3: Rotary evaporator for concentrating the extract

3.4. Establishment of Field Experiments

Two field experiments were carried out at the Plantation Section of the Department. The first experiment was conducted during the second rainy (minor) season (September-December) of 2008. A second experiment was carried out during the first rainy (major) season (May to August) of 2009. In both experiments, the treatments were arranged in a Randomised Complete Block Design (RCBD). Each treatment was replicated four times.

The land was ploughed and harrowed approximately two weeks prior to planting. The field was subsequently lined and pegged into 20 plots before sowing. Each plot measured 6 m x 4 m, and separated by one metre alleys. Sowing was done at 60 cm between rows and 20 cm within rows with 2-3 plants per hill. After sowing, a pre-emergence herbicide was sprayed to help control weeds and delay first weeding. Thinning was carried out to maintain the number of plants at two plants per hill at 10 days after sowing. Application of treatments was done once a week. Data were collected twice a week: a day before spraying (DBS) of chemicals and two days after spraying (DAS). This method of data collection was to help find out pest populations and or damage to determine the effect of the chemicals on the various pests.

3.4.1. Field Experiment One – Preliminary Testing of Ethanolic Root Extract of *T. Peruviana* on Insect Pests of Cowpea

This experiment was a preliminary study to help determine the concentrations of *T. peruviana* extracts that may be effective in controlling the cowpea pests. The treatments were:

T_1 (0.1% extract), T_2 (0.5% extract), T_3 (1% extract), T_4 (Cymethoate 25 EC at the rate of 4 ml/l water) and T_5 (control = water). The selection of low concentrations was based on reports that most plant extracts are effective at low concentrations (Mitchell *et al.,* 2004). Results from this experiment gave an idea of the concentrations to use for the actual experiment.

3.4.2. Field Experiment Two - Effects of *T. peruviana* Root Extract on Cowpea Insect Pests

Following the results obtained in experiment one, a second experiment was carried out. The concentration of *T. peruviana* extract was increased to validate or otherwise the results obtained in the first experiment.

The seeds were planted on 12th May 2009. Application of treatments began 17 DAP and once a week thereafter. The treatments, which were applied using a knapsack sprayer, were:

T_1 ï 5% extract (TP_5)

T_2 ï 10% extract (TP_{10})

T_3 ï 15% extract (TP_{15})

T_4 ï Cymethoate 25 EC at the rate of 4 ml/l water

T_5 ï water

Data collection began 21 DAP and they included:

a. Insect population counts two times a week from 21 DAP
b. Damage caused by the various insects
c. Pest infestation
d. Grain damage

Only one weeding was required. This was done 26 DAP. All destructive samplings were carried out on the two third rows from each side of each plot. This was to avoid damage to the middle rows that were used for yield assessment.

3.5. Insect Population Counts and Damage Determination

3.5.1. Aphid Infestation Monitoring

Aphid infestation was monitored twice a week. Scoring for aphids was done three times at 21, 23 and 26 DAP. Visual infestation rating was done on a tagged row of cowpea plants for each plot and average scores per plant calculated. A rating scale of 0 ï 4 (Table 3.1) was used (Litsinger *et al*., 1977).

Table 3.1: Aphid scoring chart

SCORE	SYMPTOMS
0	No attack
1	1-10 aphids
2	11-20 aphids
3	21-30 aphids
4	> 30 aphids

Data collected included:

a. Number of plants with aphids

b. Average aphids score

c. Percent aphid infestation

3.5.2. Flower Thrips Damage Assessment

All the plants in the two third rows from each side of each plot were assessed for thrips damage Thrips damage scoring was done at 30, 33, 37 and 40 DAP. The scoring concentrated on symptoms on leaves, petioles and flower buds. A visual rating scale of 1 ï 5 (Table 3.2) was used (Jackai and Singh, 1988).

Table 3.2: Flower Thrips Scoring Chart

SCORE	SYMPTOMS
1	No browning/drying of stipules, leaf or flower buds; no bud abscission
2	Initiation of browning of stipules, leaf or flower buds; no bud abscission
3	Distinct browning/drying of stipules and leaf or flower buds; some bud abscission
4	Serious bud abscission accompanied by browning/ drying of stipules and buds
5	Very severe browning/drying of stipules and buds; distinct non elongation of most of all peduncles

A second assessment of thrips was carried out at 43, 47 and 50 DAP. At least five flowers were picked from the two rows set aside for data collection. The flowers were placed in vials containing 30% alcohol and carried to the laboratory where they were dissected under a microscope and the thrips counted.

Data collected included:

a. Number of plants with thrips damage symptoms
b. Percent Thrips infestation
c. Average Thrips damage score
d. Average number of Thrips per flower

3.5.3. Legume Pod Borer (LPB) Assessment

Pod borer infestation assessment was done twice a week between 43 DAP and 50 DAP. At least five flowers were picked from the two rows set aside for destructive data collection and used to estimate LPB larval damage. Oparaeke (2006) suggested that at least 20 be picked from plants in each plot for LPB larval damage assessment. This was not possible as some plots had less than 20 flowers at the time of sampling. The flowers were placed in vials containing 30% alcohol and carried to the laboratory where they were dissected under the microscope and the number of *Maruca* larvae present recorded. Damage caused by the larvae as a result of feeding on the reproductive parts was also noted.

Data collected included:

a. Number of flowers with *Maruca* damage
b. Number of *Maruca* larvae/flower

3.5.4. Assessment of Pod Sucking Bugs Infestation

Assessment of pod sucking bugs infestation was done twice a week from 49 DAP to 63 DAP. This was based on visual observation of the two rows tagged for this data collection. Counting of PSBs was done and both nymphs and adults were recorded.

3.5.5. Monitoring of *C. maculatus* Infestation

All mature pods in three one-metre rows demarcated on each plot were harvested, air-dried for five days and threshed. One hundred grains from each lot were counted and placed in kilner jars and observed for a period of four weeks for the emergence of *C. maculatus* adults.

3.6. Yield Assessments

Three one metre rows were demarcated on each plot and used to assess yield. Flowers in these demarcated areas were counted at 43, 47 and 50 DAP. Pod density (a measure of efficacy of chemical against pest infestation) was assessed by counting the pods at 50, 57 and 64 DAP.

3.7. Statistical Analysis

Data were pooled from the various experiments and then subjected to one-way analysis of variance (ANOVA) (Steel *et al*., 1997). For treatments showing significant differences, means were separated using the Least Significant Difference (LSD) Test at a significance level of 5%.

CHAPTER FOUR

RESULTS

4.1. Experiment One – Preliminary Testing of Ethanolic Root Extract of *T. Peruviana* on Insect Pests of Cowpea

Low concentrations (0.1%, 0.5% and 1%) of ethanolic extract of *T. peruviana* were applied to cowpea plants. The incidences of the various pests on the crop were high but effects of the extracts were not significantly different from the control (Tables 4.1 ï 4.3).

Table 4.1: Effect of *Thevetia peruviana* root extract and cymethoate on aphid infestation on cowpea plants at 26 DAP, Kumasi, minor season, 2008

Treatment	Percent aphid infestation at 26 DAP
T. peruviana (0.1%)	12.86±3.63 **a**
T. peruviana (0.5%)	9.33±1.72 **a**
T. peruviana (1%)	10.50±1.72 **a**
Cymethoate	2.83±2.06 **b**
Control	10.93±1.43 **a**

All treatment means within the columns followed by the same letter are not significantly different from each other (PÓ0.05) by LSD test.

Table 4.2: Effect of *Thevetia peruviana* root extract and cymethoate on thrips damage of cowpea plants, Kumasi, minor season, 2008.

Treatment	Percent thrips infestation at 40 DAP
T. peruviana (0.1%)	19.38±1.80 **a**
T. peruviana (0.5%)	21.04±1.80 **a**
T. peruviana (1%)	22.18±2.90 **a**
Cymethoate	12.57±1.53 **b**
Control	19.89±2.76 **a**

All treatment means within the columns followed by the same letter are not significantly different from each other (PÓ0.05) by LSD test.

Table 4.3: Effect of *T. peruviana* root extract and cymethoate on percent *Maruca* damaged cowpea flowers, Kumasi, minor season, 2008

Treatment	Mean flowers with *Maruca* damage at 50 DAP
T. peruviana (0.1%)	4.25±0.25 **a**
T. peruviana (0.5%)	4.25±0.25 **a**
T. peruviana (1%)	3.75±0.25 **a**
Cymethoate	2.00±0.41 **b**
Control	4.25±0.25 **a**

All treatment means within the columns followed by the same letter are not significantly different from each other (PÓ0.05) by LSD test.

Due to the presence of very few pods on most of the plots, other parameters could not be measured. Due to the apparent ineffectiveness of the concentrations applied, higher concentrations were used for the subsequent experiment.

4.2. Experiment Two – Effects of *T. peruviana* Root Extract on Cowpea Insect Pests

The higher concentrations (5%, 10% and 15%) showed varying effects on the various pests. Comparisons were also made between the synthetic chemical and the plant extract. The results are recorded below.

4.2.1. Aphid Infestation Monitoring

None of the treatments was significantly different from the control in terms of the level aphid infestation (Table 4.4).

Table 4.4: Effect of *Thevetia peruviana* root extract and cymethoate on average aphid score on cowpea plants at 26 DAP, Kumasi, major season, 2009

Treatment	Average aphids score at 26 DAP
T. peruviana (5%)	1.00±0.00 **a**
T. peruviana (10%)	1.53±0.32 **a**
T. peruviana (15%)	1.03±0.03 **a**
Cymethoate	0.75±0.25 **a**
Control	1.08±0.08 **a**

All treatment means within the columns followed by the same letter are not significantly different from each other (PÓ0.05) by LSD test.

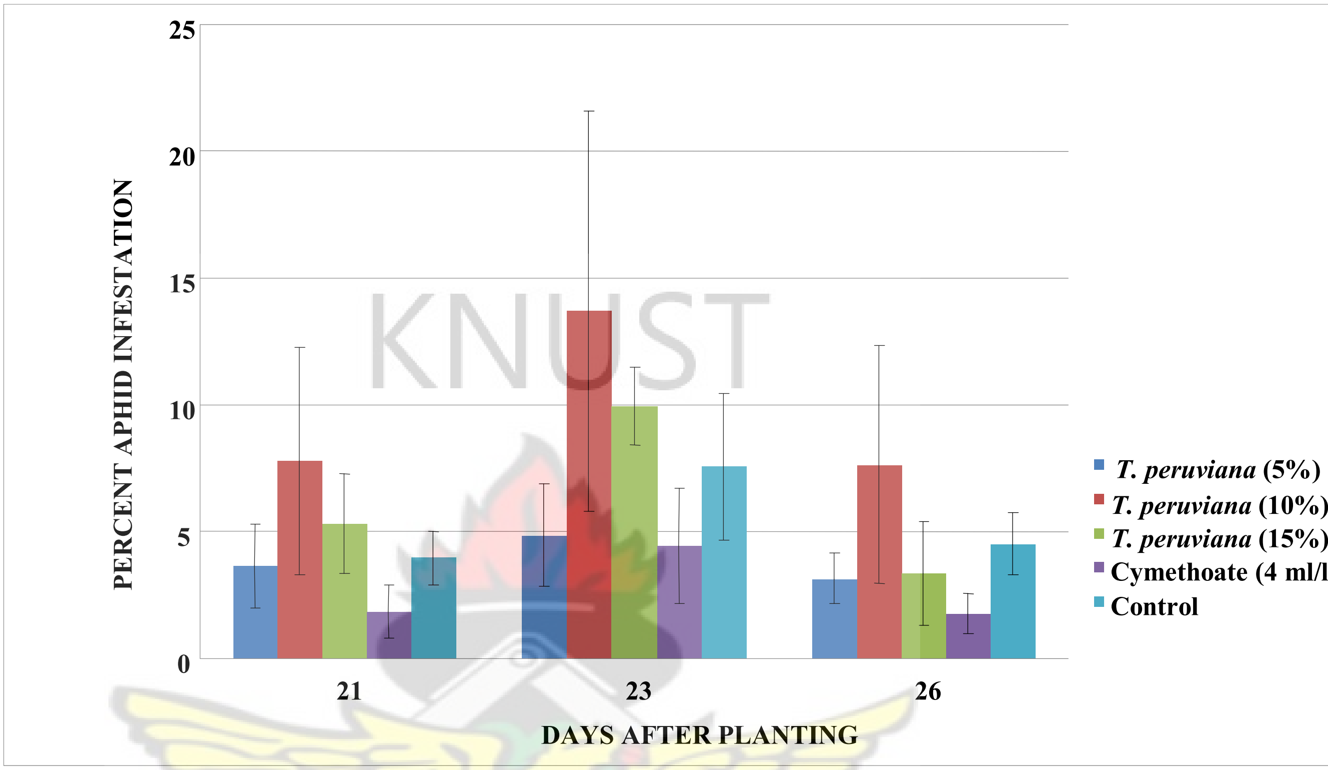


Figure 4.1: Effect of *T. peruviana* root extract and cymethoate on percent aphid infestation on cowpea plants at three different dates in Kumasi, major season, 2009

Figure 4.1 shows the relative efficacies of the extracts and the synthetic chemical on percent aphid infestation. For the three sampling days, the cymethoate treated plots generally recorded the lowest percentage aphid infestations. The root extracts appeared to have increased the aphid infestation.

4.2.2. Flower Thrips Damage Assessment

From Figure 4.2, cymethoate performed better than all concentrations of the root extract as plots sprayed with this chemical recorded significantly lower density of thrips compared with the other treatments. There was no significant difference between the control and all the levels of *T. peruviana* root extract treatments.

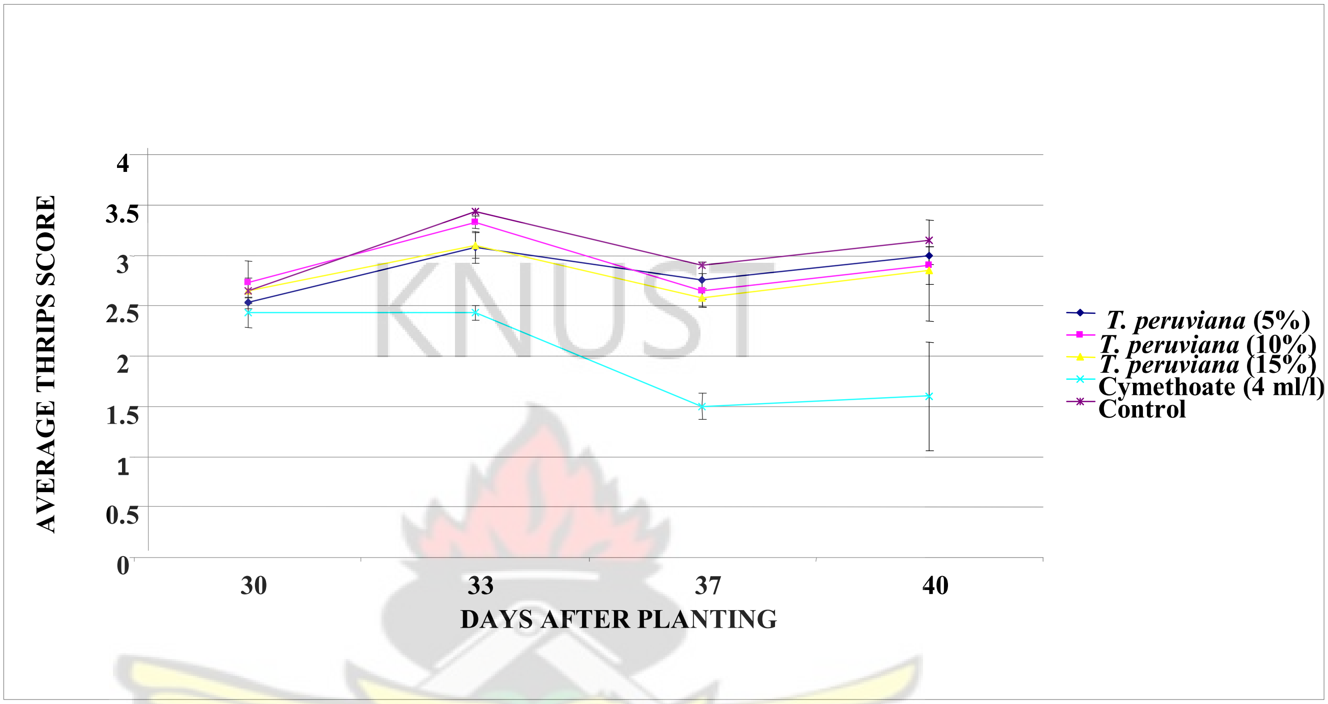


Figure 4.2: Effect of *Thevetia peruviana* root extract and cymethoate on thrips damage of cowpea plants, Kumasi, major season, 2009

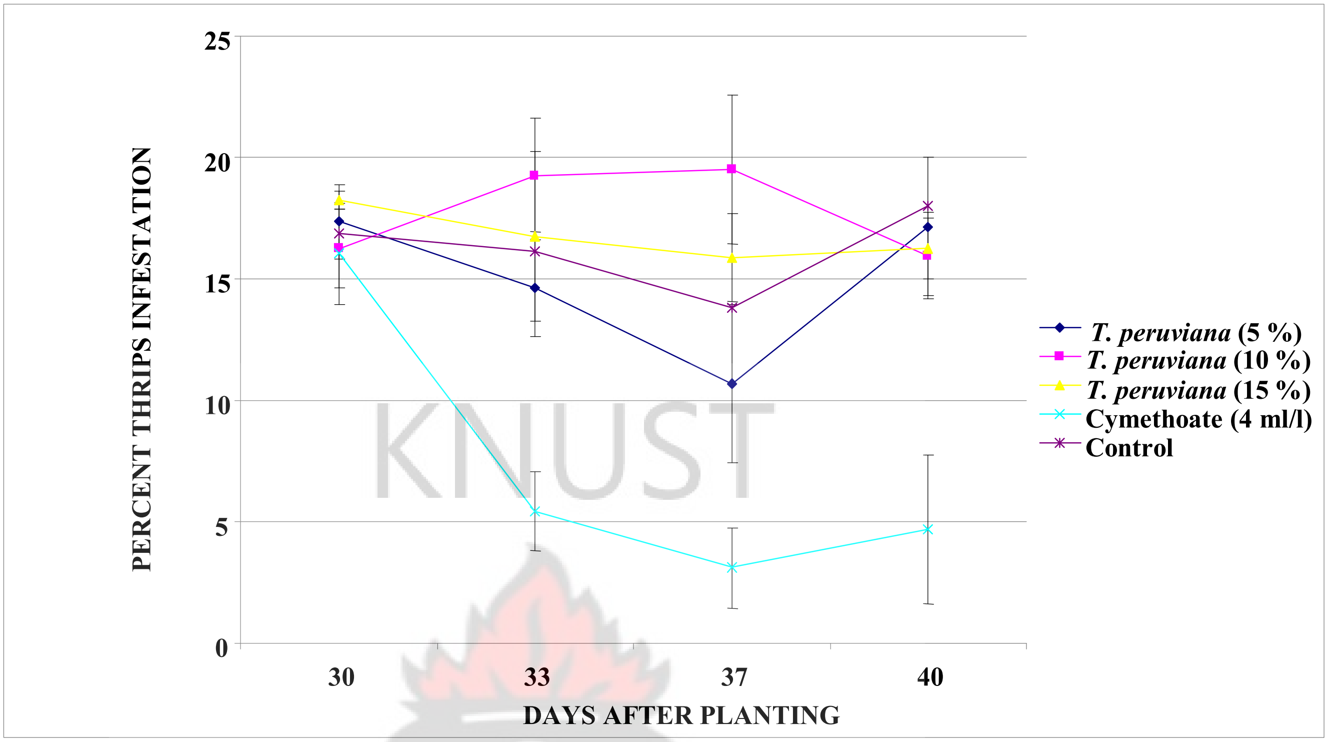


Figure 4.3: Effect of *Thevetia peruviana* root extract and cymethoate on percent thrips infestation of cowpea plants, Kumasi, major season, 2009

The performance of the various treatments followed a general trend as there was a reduction in infestation on the second and third dates of data collection except on plots treated with 10% *T. peruviana* root extract where the opposite was observed. However, the infestation level rose slightly in the cymethoate-treated plots but significantly in the control and the 5% *T. peruviana*-treated plots by the fourth date (Figure 4.3).

4.2.3. Legume Pod Borer (LPB) Assessment

Although the average number of *Maruca* per flower obtained at 50 DAP were smallest in plots sprayed with 15% root extract and cymethoate, they were not significantly different from the average numbers found in the control plots (Figure 4.4). Nonetheless, both the cymethoate and 15% extract treatments performed significantly better than the 5% and 10% root extract. It was also noticed that the control plots were less infested with *Maruca* than the plots treated with 10% extract.

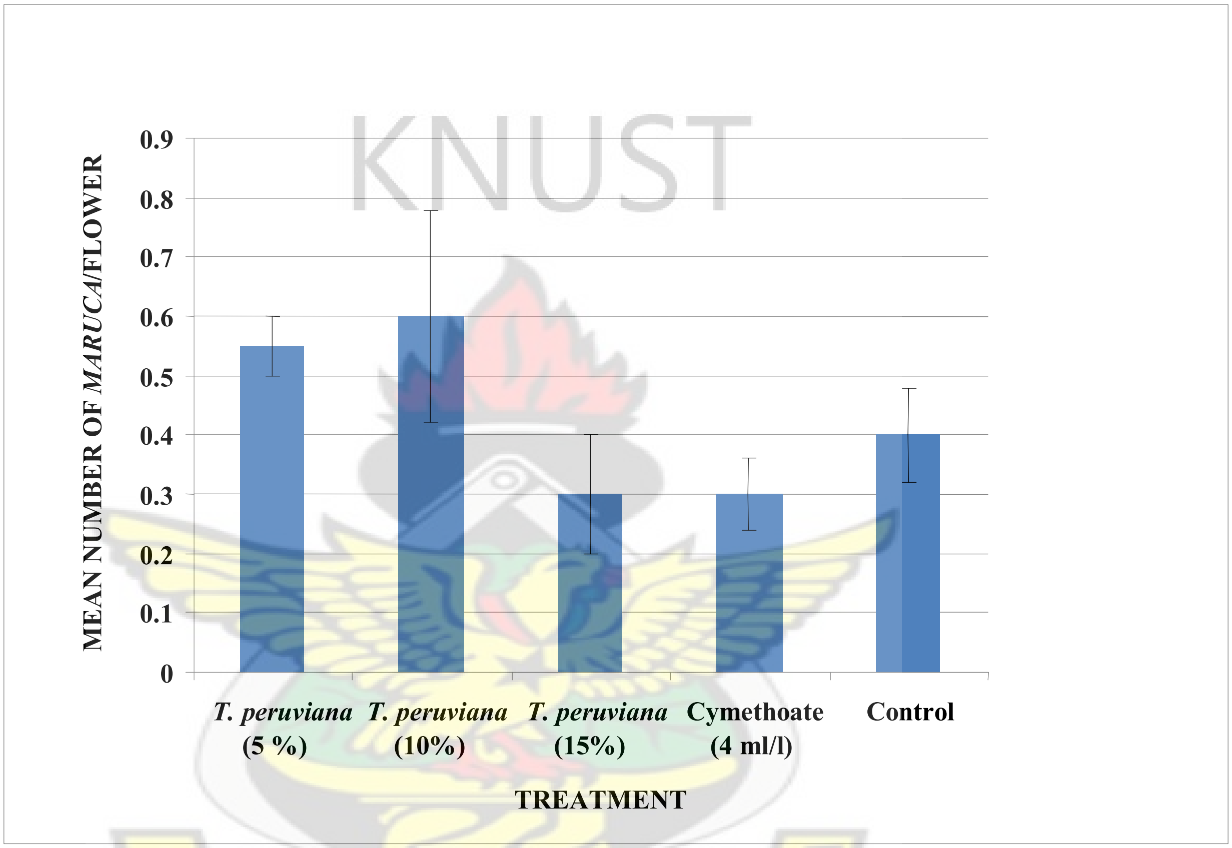


Figure 4.4: Effect of *Thevetia peruviana* root extract and cymethoate on mean number of *Maruca vitrata* larvae per cowpea flower, Kumasi, major season, 2009

Table 4.5: Number of flowers with *Maruca* damage 50 DAP, Kumasi, major season, 2009

Treatment	Percent flowers with *Maruca* damage
T. peruviana (5%)	75±25.19 **a**
T. peruviana (10%)	85±28.55 **a**
T. peruviana (15%)	60±20.15 **a**
Cymethoate	45±15.11 **a**
Control	55±18.47 **a**

All treatment means within the columns followed by the same letter are not significantly different from each other (PÓ0.05) by LSD test.

The pod borer infestation was also assessed by the number of flowers damaged by the larva. From Table 4.5, there was no significant difference between any two of the treatments.

4.2.4. Assessment of Pod Sucking Bugs Infestation

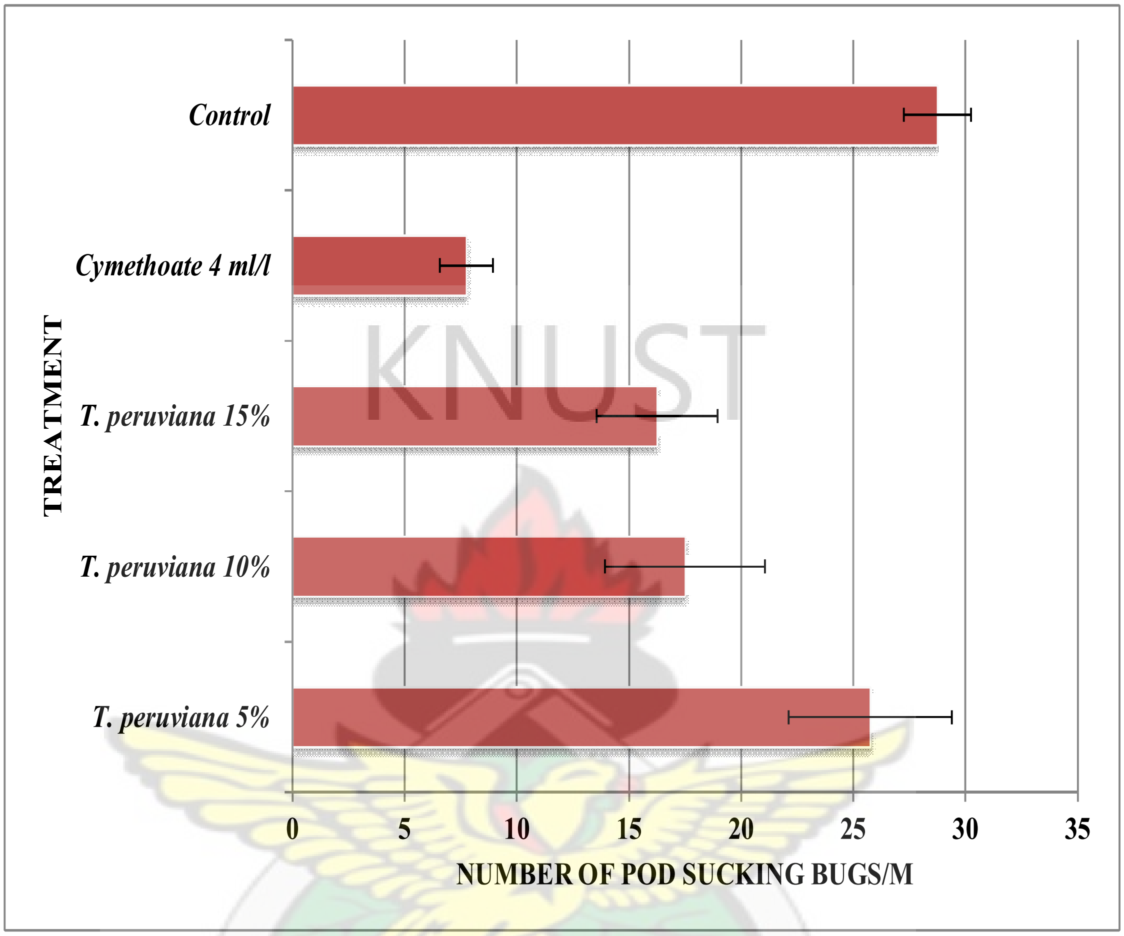


Figure 4.5: Effect of *Thevetia peruviana* root extract and cymethoate on mean number of pod sucking bugs per metre, Kumasi, major season, 2009

All the treatments showed varying effects on the pod sucking bugs with cymethoate showing the highest level of efficacy (Figure 4.5). There were no significant differences between the 5% extract and the control, but the higher extract concentrations (10% and 15%) were not significantly different from each other but both significantly suppressed pod sucking bugs numbers than the control.

4.2.5. Assessment of Yield Parameters

4.2.5.1. Number of flowers per metre at 45 DAP

Figure 4.6 shows the number of flowers that were counted at 45 DAP for the different treatments. The cymethoate treated crop supported significantly largest number of flowers compared with the other treatments.

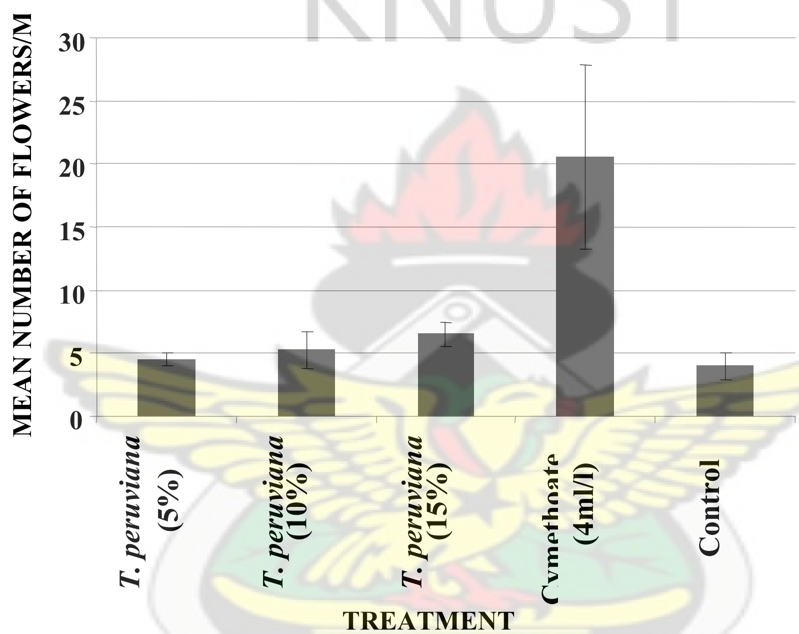


Figure 4.6: Effect of *Thevetia peruviana* root extract and cymethoate on mean number of flowers per metre at 45 DAP, Kumasi, major season, 2009

4.2.5.2. Number of pods per metre at 64 DAP

Table 4.6 shows the mean number of pods that were counted at 64 DAP. The cymethoate-treated plots recorded significantly largest number of pods. Pod load also was significantly greater at 10% and 15% extract treatment than the 5% extract and control treatments.

Table 4.6: Effect of *Thevetia peruviana* root extract and cymethoate on mean number of pods per metre at 64 DAP, Kumasi, major season, 2009

Treatment	**Number of pods/m at 64 DAP**
T. peruviana (5%)	9.5±0.96 **d**
T. peruviana (10%)	16.3±3.01 **c**
T. peruviana (15%)	23.0±3.58 **b**
Cymethoate	92.0±7.88 **a**
Control	9.5±1.32 **d**

All treatment means within the columns followed by the same letter are not significantly different from each other (PÓ0.05) by LSD test.

4.2.6. *C. maculatus* Infestation

The level of *C. maculatus* infestation was assessed in the laboratory. Figure 4.7 shows the mean number of adult bruchids that emerged within 28 days after harvest. The smallest numbers of bruchids emerged from the pods harvested from the cymethoate-treated plots and they were significantly different from the control and all the extract treatments. There was no significant difference between any of the extract treatments and the control.

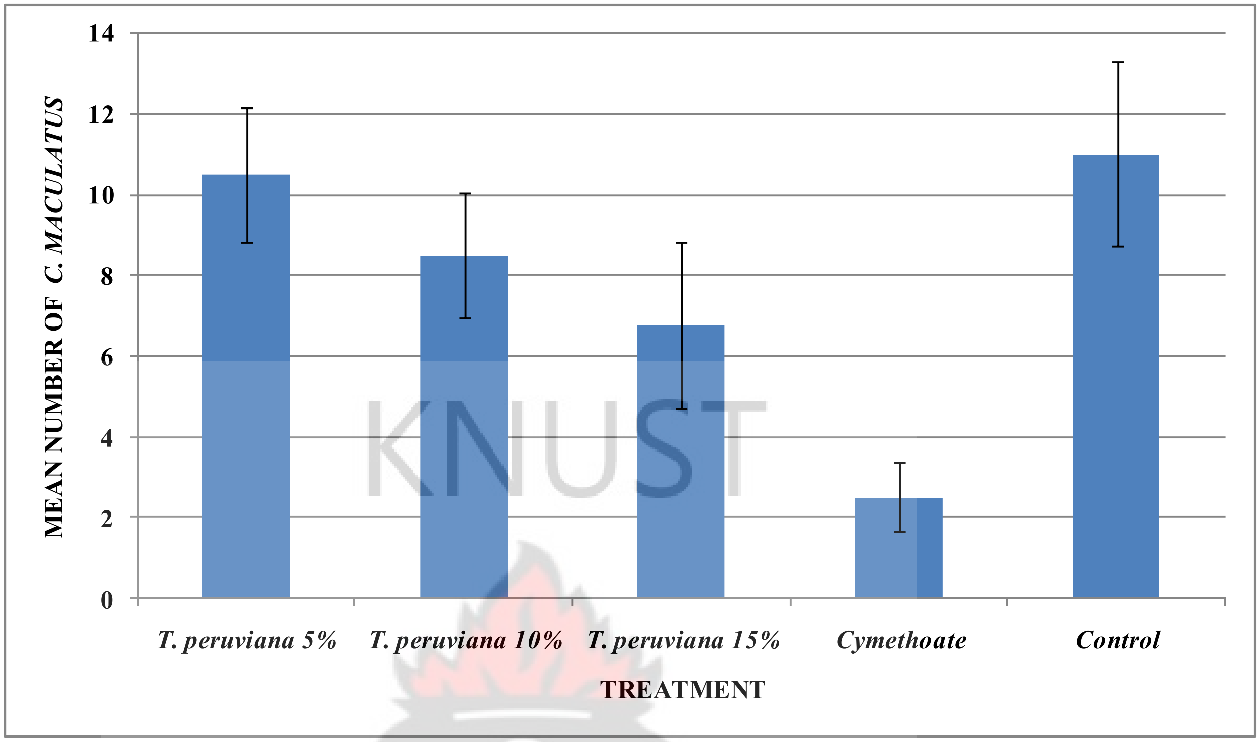


Figure 4.7: Effect of *Thevetia peruviana* root extract and cymethoate on mean number of *Callosobruchus maculatus* adults emerging within 28 days after harvest, Kumasi, major season, 2009

CHAPTER FIVE

DISCUSSION

In this study, the effects of different concentrations of the root extract of *Thevetia peruviana* on five major insect pests of cowpea, *Aphis craccivora*, *Megalurothrips sjostedti*, *Maruca vitrata*, pod sucking bugs and *Callosobruchus maculatus* were studied in two field experiments.

5.1. Preliminary Testing of Ethanolic Root Extract of *T. Peruviana* on Insect Pests of Cowpea

Three concentrations (0.1%, 0.5% and 1%) of the root extract were sprayed on the crop with the intention of controlling the various pests at different stages of the growth of the crop. This spraying method is commonly and frequently used in the field when there is an outbreak of a particular pest and the need for a quick control to reduce the pest burden and keep it below the economic threshold. The results obtained in this experiment indicated that ethanolic root extract was not significantly effective at the concentrations tested.

5.2. Effects of Higher Levels of the extract on the Cowpea Insect Pests

Using higher concentrations (5%, 10% and 15%) of the root extract, different effects were observed on the different insect pests. The effects are compared with the control and the synthetic chemical.

5.2.1. Aphid Infestation Monitoring

Aphids are serious pests of cowpea and they attack the crop at the vegetative stage, particularly the seedling stage. Their control is therefore critical for crop survival. It was evident that on all plots, including the control, aphid populations were very small. These small populations might therefore not be attributed to the effect of the extract. With the maximum aphid score of less than 2.0, most plants had no more than 20 aphids on them. It is therefore likely that other factors such as the heavy rains during the season might have washed the insects off the plants (Karungi *et al*., 1999). However, even though small, the aphid populations were oscillating with spray application, reducing immediately after application but increasing by the time of the next application. It could therefore mean that the root extract was not persistent enough, and therefore may require more frequent applications than synthetic chemicals (Oparaeke, 2007).

5.3.2. Flower Thrips Damage Assessment

There was a high incidence of thrips on plots treated with the root extracts. The effect of the extracts was not significantly different from the control. Scoring at 30 DAP, there was no significant difference between any two of the treatments but subsequent applications resulted in cymethoate performing significantly better than all other treatments. It could therefore be concluded that the root extracts tested could not control thrips at the concentrations tested, even though thrips were successfully controlled with other pesticidal plants such as pepper, neem and pyrethrum at similar concentrations (Rateaver and Gylver, 1993; Anonymous, 2003; Oparaeke, 2006). *T. peruviana* extract was reported to have insecticidal properties on other pests such as termites (Kareru *et al.*, 2010).

5.3.3. Legume Pod Borer (LPB) Assessment

Maruca infestation was generally relatively high in all plots. This could probably be due to the fact that the canopies were denser and the relative humidity (> 85%) was quite high. There was also a comparatively longer vegetative growth phase of the crop because the rains were quite heavy. These provided a conducive environment for *Maruca* to thrive. Denser canopies and its associated humid conditions have been reported to favour *Maruca* multiplication (Karungi *et al.*, 1999), because they provide hiding places for the larvae from predators and insecticide especially contact insecticides. Dense canopies also protect the larvae from desiccation, increasing their chances of survival. Although *Maruca* damage to the flowers was high, relatively few larvae were found in the flowers. Jackai and Daoust (1986) reported that once the larvae enter the flowers they feed and cause damage after which they leave to pupate in the soil.

Of all the plant extracts, only *T. peruviana* root extract at 15% could compare favourably with cymethoate in reducing *Maruca* damage. This could mean that higher extract concentrations could be more effective as was found in a study conducted by Bai and Koshy (2004) who concluded that to be effective on some pests, *T. peruviana* extracts may have to be as high as 40%.

The control plots recorded lower number of *Maruca* per flower than plots treated with *T. peruviana* at 5% and 10%. Insects are known to expend their energy prudently in locating food and oviposition resources. As the control plots had very few flowers it would be more expensive searching the control plots to locate the few flowers hence the smaller damage suffered on the control plots compared with the plots treated with the lower concentrations of the extract.

5.3.4. Assessment of Pod Sucking Bugs Infestation

The presence of pod sucking bugs was determined by counting the bugs found on two rows set aside on each plot. There was delayed pod formation on all the plots treated with extract and very few pods were recorded. The few pods however attracted relatively larger numbers of bugs. This could be because of the ineffectiveness of the extracts to control the bugs as cymethoate, which recorded significantly lower incidence of the bugs. Dimethoate, a component of the cymethoate used in this experiment, is the recommended insecticide for controlling pod sucking bugs in Ghana (Adu-Dapaah *et al*., 2005), and has been recommended for controlling pod sucking bugs elsewhere (Kawuki *et al*., 2005). Although the root extract applied at 10% and 15% could not compare favourably with cymethoate, their effect significantly lowered numbers of pod sucking bugs than the control, indicating that the extracts have the potential of controlling these pests and higher concentrations may be able to compare well with cymethoate (Bai and Koshy, 2004).

5.3.5. Monitoring of *C. maculatus* Infestation

Results obtained indicated that cymethoate could reduce field infestation of *C. maculatus*. Studies have shown that ethanolic root extracts could control adult *C. maculatus* (Mollah and Islam, 2007) but the results obtained in this study did not support this probably because of the concentrations used. Although there have been reports of high efficacies of plant-based insecticides at low concentrations (Scott *et al*., 2003; Dadang and Djoko, 2009), some require comparatively high concentrations before they can demonstrate efficacy depending on the stage of the insect being controlled (Bai and Koshy, 2004).

5.3.6. Yield Assessments

The potential yield of cowpea on the various plots was assessed by counting the number of flowers and pods. There was a positive correlation (r=0.99; P' 0.5) between flowering and pod formation in all treatments. The highest number of flowers and pods was recorded in the cymethoate-treated plots. The ability of cowpea to produce a good yield may be influenced by many factors at the various stages of production (Oparaeke *et al*., 2005). In all instances, pest populations on cowpea plots treated with cymethoate were relatively low so it was not surprising when those plots produced the highest number of pods. The relatively higher incidence of aphids, thrips and *M. vitrata* on the other plots resulted in fewer, less wholesome pods.

There was delayed pod formation on all *T. peruviana* treated plots. This could have been due to the impact of pests during the earlier growth stages of the crop. Thrips infestation in particular is known to delay flower and pod formation as their infestation at the budding stage results in massive bud abscission.

Although none of the *T. peruviana* levels compared well with the synthetic chemical, a further increase in concentration of *T. peruviana* might have been more effective as the extract applied at 15% produced more pods than the lower concentrations.

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusions

This study was carried out to determine the relative efficacy of different concentrations of ethanolic root extract of *Thevetia peruviana* against five major insect pests of cowpea namely *Aphis craccivora, Megalurothrips sjostedti, Maruca vitrata*, pod sucking bugs and *Callosobruchus maculatus*. In view of the results of the study, some conclusions can be drawn.

Cymethoate was consistently superior to all other treatments in substantially reducing the population densities and or damage of all the insect pests. Cymethoate is a recommended cocktail insecticide for controlling all field insect pests of cowpea.

The highest concentration of *Thevetia peruviana* root extract (TP_{15}) exhibited the highest efficacy against all the pests when compared with the lower concentrations (5% and 10%).

Some of the pests were better controlled than others with the same concentration of extract. For instance, *T. peruviana* extract at 15% could control *M. vitrata* better than thrips. Thrips were the most difficult to control as the highest extract concentration of 15% could not cause any appreciable level of control (PÓ0.05).

Concentrations of 10% or more significantly reduced both pod sucking bugs and *C. maculatus* numbers. These concentrations also increased the number of flowers and pods obtained on plots treated with the concentrations.

The results of this study indicated that *T. peruviana* has the potential to control insect pests of cowpea but higher concentrations need to be used and different extraction methods could also be employed.

6.2. Recommendations

6.2.1 Laboratory Bioassays

It is recommended that the study be repeated to validate the results obtained. Laboratory bioassays should be carried out. Although the study was to control field pests, performing bioassays could give an idea of the concentrations that may be effective in the field.

6.2.2. Preparation of Plant Extract

Aside testing extracts from sophisticated equipment, crude ethanolic and aqueous extracts could also be tested to determine if the plant materials could be utilized directly by farmers with their own technology. To ensure the transfer of applicable knowledge to farmers, efficient extracts that are easy to prepare should be tested so that the resource-limited farmer can effectively control the pests. Although more compounds are likely to dissolve better in ethanol, it will be better if aqueous extracts of the material are developed and tested as water is the most readily available solvent for farmersô use. The aqueous extracts could be modified with readily available additives that could improve the adhesion of compounds of the plant materials in the water extract. For instance, aqueous and soap extracts of *Tephrosia* species have been proven to contain five times more rotenoids than aqueous extracts only (SAPP, 2009).

Although the pesticidal properties of *T. peruviana* have been determined on the cowpea pests, the method of extraction cannot be adopted by farmers. The method of soxhlet extraction is too complex and not available for farmersô use. It is cumbersome and time-consuming especially for field experiments that require large volumes of extracts for application. An alternative could be to chop, dry and produce a coarse powder of the plant using the pestle and the mortar. The powder can then be soaked in locally-produced alcohol (akpeteshie) for some hours. The filtrate can then be used. This alternative solvent is cheaper and readily available. The use of the refined commercially-produced ethanol was expensive and would not be able to help achieve the aim of reducing cost of cowpea production and maximizing profit.

6.2.3. Source and Choice of Experimental Plant and/or Plant Part

Before recommending any plant species for farmersô use, analysis should be carried out to determine the composition of such species. The analysis will also ensure the right plant part and stage is harvested for optimum efficacy. Other parts of *T. peruviana* could also be studied on the various pests of cowpea. Studies have shown that *T. peruviana* roots have the highest concentration of compounds (Mollah and Islam, 2007). All other plant parts are also toxic. The leaves, flowers and seeds may contain different compounds which may be more effective in the control of these pests. Although the plant is perennial and evergreen, the use of its roots may not be sustainable. Therefore other plant parts such as the leaves, flowers and seeds could be tested as harvesting these parts may be less destructive and damaging to the plant. Roots dried for different durations and intensities could also be tested to determine which stage is the most potent. The effect of dried roots could also be compared with fresh roots to determine which one is more effective. Drying is known to help reduce the moisture content of the plant. However, some volatile compounds may be lost alongside the water. Drying may also take time which may therefore delay the preparation of the extract and subsequent application.

In this study, the whole roots were used. It is known that the bark of the roots has a higher concentration of the compounds. The bark only could therefore be tested as this may achieve better and more reliable results.

6.2.4. Effects of the Extract on Insect Pests

Higher concentrations of *T. peruviana* could be tested to determine if they could better control the pest. The extracts showed some biological activity so higher concentrations of extracts from this plant may cause higher mortality. The LC_{50} and LC_{90} values should be determined for the extract. It could, therefore, be easier to recommend effective concentrations and doses to farmers for effective pest control. This way, problems associated with over-dosage, contamination of produce and wastage of chemicals may be addressed.

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