

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY, KUMASI

COLLEGE OF AGRICULTURE AND NATURAL RESOURCES

DEPARTMENT OF HORTICULTURE

**THE EFFICACY OF NEEM SEED OIL EXTRACT AND VEGETABLE OIL AS
INSECTICIDES FOR COWPEA–WEEVIL (*CALLOSOBRUCHUS MACULATUS*)
CONTROL**

**A DISSERTATION SUBMITTED TO THE SCHOOL OF GRADUATE STUDIES,
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MPHIL POSTHARVEST TECHNOLOGY DEGREE**

BY

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DECLARATION

I hereby declare that except for references made of other people's publications and copyrighted posts, which have been duly acknowledged, this work submitted as a thesis to the Department of Horticulture for the degree of MPhil Horticulture (Post Harvest) is the results of my own research and has not been presented anywhere for the award of a degree.

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ABSTRACT

Callosobruchus maculatus, a major storage insect pest of cowpea, lays its eggs on the pods or sometimes on the seeds both in the field and in storage. The larva perforates the seeds and completes its life cycle there. It can cause loss, if not controlled in time. Other pests are *Maruna spp* and *Laspeyresis spp*. Storage pests are controlled by the use of recommended

insecticides, vegetable oils, fumigation or heat treatment to the stored seeds. The objective of this project was to examine the effect of neem oil treatment on cowpea weevils as an alternative for preserving cowpea at storage. The experimental design for the study was Completely Randomized Design with three replications. Data collected included; grain damage, insect mortality; weight loss and number of survived insects. From the results, insect mortality was 100% for treated cowpea seed samples compared to the untreated sample; the control. The results indicated that the control treatment recorded high weight loss of 110 g, 240 g and 350 g for storage months of 1, 2, and 3 compared to the neem oil treatment [100:0] recording the lowest of 10 g, 20 g and 30 g for storage months 1, 2, and 3. The control treatment had the significantly highest percentage of seeds with holes of 36.87 %, 69.54 % and 94.67 % for storage months 1, 2, and 3 compared to the neem oil treatment which recorded 11.09 % 11.10 % and 11.16 % for storage months 1, 2, and 3 respectively. Study concluded that neem seed oil should be used to store cowpea grains to reduce weevil infestation.

DEDICATION

I dedicate this work to my Big and elder brother DSP Stephen Abanga for his financial support and the advisory role he played throughout my course of studies. This work would not have come into reality without his support.

This work is also dedicated to my wife, Akanaba Monica and my children Israel and Isabella for their cooperation and patience throughout my work.

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ACRONYMS

IPM	Integrated Pest Management
CSIR	Council for Scientific and Industrial Research
SARI	Savannah Agricultural Research Institute
GSS	Ghana Statistical Service
CRD	Completely Randomized Design

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CHAPTER ONE

1.0 INTRODUCTION

Cowpea (*Vigna unguiculata*) is one of the most important food legumes grown in the tropical savannah zones of Africa and other tropical countries of the world (Kebe and Sembene, 2011; Mabonga, 1983). It is a preferred staple food in many regions of Africa. Cowpea is of major importance to the livelihoods of millions of relatively poor people in less developed countries of the tropics. Its desirability reflects the fact that, rural families variously derive food, animal feed, and cash, together with spill over benefits to their farmlands through its residues. Its leaves, immature pods, fresh seeds and dry grain can be eaten or marketed. It plays a critical subsistence role in the diets of many households in many parts of the world, providing cheap and nutritious food that are deficient in cereals (Singh *et al.*, 1997; Korlety, 2009).

Despite this, its production is considered too risky an investment by many growers, because of the numerous pest problems associated with it (Singh *et al.*, 1997). Insect damage is the major constraint to cowpea grain production in most cowpea producing nations. Insect pests and disease infestation which lead to economic losses affect the production of cowpea. Its yields are known to be low in most parts of the tropics because of heavy insect pest problems. From the field up to the places where it is stored, the cowpea is threatened by various insects. It is infested on the field by a number of pests including the cowpea aphid (Baidoo, *et al.*, 2012). Major insect pests that can cause economic loss are cowpea aphids (*Aphis craccivora*), leafhoppers (*Empoasca spp*), thrips (*Megalurothrips sjostedti*), flower eating beetles (*Mylabris spp.* and *Coryna spp.*), blister beetles (*Hycleu slugens*), green stink bugs (*Nezara viridula*) and cowpea weevil (*Callosobruchus maculatus*) among others (Singh *et al.*, 1997; Tiroesele *et al.*, 2014).

Among these, the cowpea weevil is considered a cosmopolitan field-to-store pest ranked as the principal post-harvest pest of cowpea in the tropics. According to Kebe and Sembene (2011), the main problem that farmers face is the conservation of the cowpea crops due to the fact that about 80 to 100 % of grains can be destroyed by two bruchid species namely *Bruchidius atrolineatus* and *Callosobruchus maculatus*, in a period of 2 to 3 months after harvest and virtually all of the grain may have holes by 6 months.

Cowpea weevils particularly, *Callosobruchus maculatus*, damage cowpea after harvest, attack dried cowpeas and other related stored seeds. The weevil prefers dried cowpeas but will attack other beans and peas in storage (Wahedi *et al.*, 2013).

Insect pest infestation results in substantial quantitative and qualitative losses manifested by seed perforation and reductions in weight, market value and germination ability of seeds. Consumers have a strong aversion to grain that has been damaged by weevils, but it still can be effective as seed, although, germination percentage may have been reduced.

Various techniques and control methods have been developed and more are still being developed in order to reduce serious losses experienced during storage. In Sub-Saharan Africa, farmers use a variety of commercial and traditional methods to control bruchids (Baributsa *et al.*, 2012). Management of cowpea seed storage pests relies heavily on the use of chemical insecticides (Tiroesele *et al.*, 2015). However, most of the small-scale farmers have not adopted these new techniques due to financial and technical reasons. Insecticides also have negative impact on the environment, humans, livestock and non-targeted organisms. Such misuse of insecticides on cowpea, coupled with low yields, has led to an intensive search for pest control options with more acceptable methods of controlling insect pests of cowpea in storage with little or zero input from insecticides, or bio-intensive integrated pest management (IPM).

Research efforts towards ensuring sufficient food production for the increasing human population has led to the realisation that loss of produce is perhaps one of the greatest problems confronting food production in sub-Saharan African countries (Ilesanmi and Gungula, 2013). To reduce postharvest losses, harvesting, handling and storage methods must be matched by a sudden increase in crop production and sustainable production requirement. Among the strategies, there is the use of cowpea varieties which are resistant to weevil's attack (Kebe and Sembene, 2011). However, the use of resistant varieties are mostly not phenomenal as large rural farmers do not have access to them.

Resource-poor farmers in Africa employ a range of traditional methods such as use of ash, sand, dry pepper and botanical extracts. Naturally occurring plant products have been used to protect agricultural products against pests for many years in some parts of the world. Different researchers have reported insecticidal effects of plant products against a broad range of pests. Some of the techniques explored include the use of plant products such as garlic, peppermint and chilies (Tiroesele *et al.*, 2015), leaves and seeds powder from various plants such as mint, tobacco, ginger, moringa, lime, neem among others (Ilesanmi and Gungula, 2013; Longe, 2016). Aromatic plants have both medicinal and aromatic properties and contain a variety of volatile oils which have insecticidal, anti-feedant and repellent effects on insect pests (Tiroesele *et al.*, 2015). Medicinal plants are part of human society and are used to combat diseases, not only those of animals but of plants as well, from the dawn of civilization (Naniwadekar and Jadhav, 2012). This study looked at an alternative of using oil from the seeds of the neem tree to explore a way of controlling insect pest in cowpea at storage.

The neem tree (*Azadirachta indica*), from the Maliaceae family, known as margosa has long been recognized for its properties both against insect and in improving human health. The

neem tree has been known as the wonder tree for centuries as it offers answers to some of the major concerns facing humankind. Its mammalian safety and environmental friendliness reports are highly encouraging (Naniwadekar and Jadhav, 2012). The neem tree is very useful as its bark, roots, sap-water, leaves, flowers and seed, all are valued as medicinal. The tree is now grown in most tropical and sub-tropical areas of the world for shade, for reforestation programs and in plantations for the production of compounds which have toxic, antifeedant and repellent properties against insects. Extracts from the leaves and seeds exhibit antibacterial, antiviral, antifungal and antimalarial properties. Millions have been using neem over thousands of years and no hazards have been documented for normal dosages (Naniwadekar and Jadhav, 2012). The general class of these compounds found in neem is triterpenes, within which, the most effective are the limonoids, which are abundant in Neem oil. Azadirachtin, a limonoid, has been found to be the main ingredient for fighting insects and pests, being up to 90% effective in most instances. Azadirachtin is used as insect repellent, feeding inhibitor, laying deterrent, growth retardant and sterilant among others (Ilesanmi and Gungula, 2013; Wahedi *et al.*, 2013). Meliantriol is another feeding inhibitor, which prevents locusts chewing, and has therefore been in traditional use in some parts of the world for instance, India for crop protection. Neem is therefore one of the few sources of bio-pesticides currently attracting research interest world-wide.

1.1 Problem statement

In spite of the great value of cowpea, the production and storage of this important food crop has faced so many constraints, particularly that of pests. Attack by this pest begins in the field, before harvest and continues in storage, causing substantial damage to stored grains as the pest population rapidly increases (Baributsa *et al.*, 2013; Longe, 2016). The higher the infestation levels before harvest, the greater the damage to the seeds in storage. Cowpea

grain, stored after harvest, is the favourite food of insects called bruchids, weevils and other insect pests. Infestation on stored grains, if not checked, may reach 50% within 3-4 months of storage. After two or three months of storage, a granary of cowpea grain that initially had only a few cowpea bruchids can contain thousands. Since each new adult leaves a hole in the cowpea grain in which it developed, the entire store of grain is riddled with holes after a few months. In this way cowpea weevils can turn a high-value, virtually insect-free store of cowpea grain into smelly and worthless powder within a short span of time.

High losses could be incurred during storage. These damaged beans eventually render the stock unviable for cropping and also, get to the market attracting lower prices (Salifu, 2012). According to Longe (2016), cowpea farmers in Africa have depended, over the years, on the use of synthetic insecticides like phostoxin and primiphos methyl (actellic); which though are very effective for stored products protection, but their use has several drawbacks like increasing costs, inconsistent supplies due to scarcity and hazards to human health and the environment, if carelessly and indiscriminately used. Considering the pressing need to improve the welfare of rural and urban poor farmers in the tropics, issues relating to better food supply and greater opportunities for income generation and farm inputs like viable seeds are of paramount importance.

1.3 Justification

The country cannot realise its planned economic growth and poverty reduction without a significant improvement in the performance of the agricultural sector. Storage of food therefore enhances food security through continuous supply of food for processing and distribution as well as for cultivation.

Inappropriate and expensive storage facilities are limitations to agricultural production.

They contribute to high postharvest losses and low returns for farmers and processors.

Minimising postharvest losses and maintaining high quality of produce are crucial for sustainable and profitable agriculture. The nature of storage structures and the type of storage management practices leave much to be desired.

The contribution of cowpea to food and poverty reduction can be substantial in Ghana if both biological and socioeconomic constraints such as storage and marketing are addressed. While attention has been given to genetics, agronomy and pre-harvest pest control, such economic issues as storage which enhances shelf life, marketing quality and consumer preferences are neglected in cowpea research. Cowpea suffers heavily from insects, both in the field as well as when the grain is stored after harvest. Yield reduction caused by insects can reach as high as 95 percent depending upon the location, year and cultivar (Korletey, 2009).

Botanical insecticides have long been touted as attractive alternatives to synthetic chemical insecticides for pest management because botanicals reputedly pose little threat to the environment or to human health (Isman, 2005). However, there is limited information on the use of the plant products as an alternative control method for controlling weevils in storage. The use of plant products may offer a sustainable, environmentally friendly and safer alternative to synthetic insecticides (Tiroesele *et al.*, 2015).

Pest problems on cowpea persist, at least in part because of a lack of diversity in research interests in the control of pests (Singh *et al.*, 1997). Along with these events, and the new visions for pest management, cowpea production has undergone changes, but these are inadequate to address the more difficult pest problems. The pest problem on cowpea is complex, and requires diversified efforts. Without a major breakthrough in the control of the more recalcitrant postharvest field pests of this crop, bridging the gap between present and potential production of cowpea will be a slow and frustrating process. This study is therefore

to be carried out in order to offer an alternative cowpea preservative technique that is low-cost, accessible, and safe as well as has lasting preservative effect and which is indigenous to farmers, marketers and consumers of cowpea.

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1.3 Objective

The main objective of this study is to examine the effect of neem oil on cowpea weevils as an alternative for preserving cowpea during storage in Zebilla, Bawku West District.

1.3.1 Specific objectives

The specific objectives include

1. To determine the optimum components, concentrations and application rate of neem seed oil extract for cowpea seed weevil (*Callosobruchus maculatus*)
2. To determine the survival-time response of cowpea seed weevil (*Callosobruchus maculatus*) to neem seed oil extract and vegetable oil as an insecticide
3. To determine either one (10 or more of; the appearance, the textural, the flavour, the nutritive, or the safety quality tribute(s) or characteristic(s) of cowpea seed after storage.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Origination and distribution of cowpea

Cowpea (*Vigna unguiculata*) is one of the most ancient human food sources and has probably been used as a crop plant since Neolithic times. A lack of archaeological evidence has resulted in contradicting views supporting Africa, Asia and South America as origin of the crop (DAIS, 2011). The precise location of the centre of origin of species of a crop is rather difficult to determine (Ngalamu *et al.*, 2015). However, the wide geographical distribution of the wild and cultivated species throughout sub-Saharan Africa (SSA), especially West Africa, indicates that they could have been domesticated in the Western part of Africa (DAIS, 2011; Ngalamu *et al.*, 2015). Cowpea originated in Africa, where a large genetic diversity of wild types occurs throughout the continent, southern Africa being the richest. The greatest genetic diversity of cultivated cowpea is found in West Africa, in the savannah region of Burkina Faso, Ghana, Togo, Benin, Niger, Nigeria and Cameroon. It is grown extensively in 16 African countries, with the continent producing two-thirds of the world's total (Egbadzor, Yeboah, Offei, Ofori, and Danquah, 2013). Today, cowpea can be found in almost every continent but predominantly in Africa, India, Asia, South and Central America. It has been introduced in Madagascar and other Indian Ocean islands, where it is sometimes found as an escape from cultivation (Ngalamu *et al.*, 2015).

Cowpea is well adapted to environmental conditions that affect crop production such as drought, high temperatures and other biotic stresses compared with other crops. Notwithstanding, growth and development of many cowpea cultivars are affected by drought and high temperatures, especially during floral development. This is because cowpea cultivars tend to have narrow range of adaptation as cultivars developed for one zone usually are not very productive in other zones (Agyeman *et al.*, 2014). In Ghana, cowpea is produced largely by smallholder resource poor farmers under rain-fed conditions (Rafiu, 2012).

2.2 Cowpea production and its socio-economic importance

Reliable production data for cowpea worldwide are generally difficult to obtain (Singh *et al.*, 1997). This is because most countries do not maintain separate records on cowpea. However, it is estimated that the annual world cowpea crop is grown on 12.5 million ha, and the total grain production is 3 million tons although only a small proportion enters the international trade. West and Central Africa are the leading cowpea-producing region in the world. Thus, the African continent alone produces 64 % of the estimated 3 million tons of cowpea seed produced yearly (DAIS, 2011). An average of 143,000 metric tonnes is produced annually on about 156,000 ha making Ghana the fifth highest producer of cowpea in Africa. Ghana has the fastest growing production of the crop in Africa. Annual rates of growth for cowpea for area, yield and production for the period from 1985-1987 to 2005, 2007 were -0.1%, 39.6%, and 39.8%, respectively. It has been projected that the rate of growth for the period between 2010 and 2020 would be 11.1% for cowpea (ICRISAT, 2011). Annual production in 2011 was estimated at more than 235,000 metric tonnes (FRI, 2016). Average yield was about 1.3 metric tonnes per hectare in 2011. Most cowpeas in Ghana are grown in the savannah areas of the north, but farmers can grow cowpeas anywhere in the country.

Cowpea is the second most important legume in Ghana after groundnut (Egbadzor *et al.*, 2013; FRI, 2016; ICRISAT, 2011). It is eaten regularly in almost every household in Ghana and remains important source of food for the population. The dry grain with about 23-25% protein serves as a cheap source of protein for both rural and urban consumers whereas livestock benefit from the residue left over after the grain is harvested. Rural families that make up the larger part of the population of northern Ghana derive from its production, food, animal feed and cash income. According to Singh *et al.* (1997), cowpea represents the main

food legume in tropical Africa. The demand for cowpeas is increasing in Ghana because of high population growth, mainly in urban areas. In 2010, the average consumption of cowpeas in the country was about five kilos per person per year (FRI, 2016). Malnutrition and infant mortality are expected to drop significantly through increased consumption of cowpea from the current level of 9 kg per capita to 15 kg per capita by more households (ICRISAT, 2011).

According to Ngalamu *et al.* (2015), cowpea is a multifunctional crop which does not only serve as food for man and livestock but also a valuable dependable revenue generating commodity in many parts of the world, is widely grown by nearly all the small holder subsistence farmers. Processed products such as cowpea flour, cowpea cake, cowpea fritters, and cowpea chips are sold in village markets. Households earn a good income by producing two or three crops of cowpeas per year. Ghanaian farmers typically store and sell more than 60% of their cowpeas when prices rise during the off-season. Households generate annual income of about GH¢760-800 through increased production due to two or three cycles production per year of improved cowpea varieties.

Going beyond its importance for food and feed, cowpea can be regarded as a pivot of sustainable farming in regions characterized by systems of farming that make limited use of purchased inputs like inorganic fertilizer. The crop can fix about 240 kg/ha of atmospheric nitrogen and make available about 60-70 kg/ha nitrogen for succeeding crops grown in rotation with it (Agyeman *et al.*, 2014). This reduces the amount of money spent by farmers on inputs such as chemical fertilizers resulting in increased incomes of smallholder farmers and increase productivity of both legume and cereal crops and bring about reduction of hunger and food insecurity in the country.

In summary, Cowpea is an important crop in Ghana as a country due to its contribution to national GDP, farmer incomes, food and nutrition security. Cowpea demand is increasing because of high population growth mainly in the urban areas. Farmers store and sell more than 60% of the produced cowpea when prices go up during the off-season (ICRISAT, 2011). These features together make cowpea a particularly attractive crop for the subsistence farmers of sub-Saharan Africa, where about 70% of the world's cowpea is grown, but where the farmer still faces numerous problems in growing and harvesting the crop (Singh *et al.*, 1997).

2.3 Varieties of cowpeas

The Savannah Agricultural Research Institute identified four varieties of cowpea that are cultivated in Ghana. These include *Bengpla*, *Valenga*, *Apagbaala* and *Marfo-Tuya* (SARI, 2012). The improved cowpea varieties under cultivation in the country were mostly released by the Council for Scientific and Industrial Research (CSIR) institutions.

2.3.1 Bengpla

It is a white seeded variety with black eye, matures in 60 days in the Guinea savannah zone, and may be as early as 52 days in the Sudan savannah zone. It produces good yields in a disease-free environment. The potential yields is 1.5 t/ha. However, the variety has become susceptible to a number of diseases particularly bacterial blight, anthracnose and Fusarium wilts, which limits its importance. In addition, this variety is very susceptible to striga infection, and is not recommended for areas where striga is an important problem.

2.3.2 Vallenga

It is a red-seeded that matures in about 70 days. It was released in 1986 after testing with farmers. It produces stable high yields, with a yield potential of 2.0 t/ha. Although the seed coat pigmentation reduces its market value, it is recommended where red seeded types are

preferred. Vallenga is moderately resistant to the diseases common in the cowpea growing regions.

2.3.3 Apagbaala

This variety has white seed coat with small brown eye. The seeds are small in size. It was released in 2003 for cultivation in the Guinea savannah zone of Ghana. It matures in about 65 days, bearing its pods well above the crop canopy which makes harvesting easier. Under good management and favourable weather conditions, yields as high as 1.8 t/ha can be obtained. It has a small stature and high yields are obtained when grown under high plant densities (200,000 plants/ha) (SARI, 2012).

Because of synchronous pod maturity and long peduncles that carry the pods above the canopy, this variety usually have less damage from the Maruca pod borer. The grains have a short cooking time compared with other varieties. This variety is not recommended for cultivation in the Sudan savannah zone (SARI, 2012).

2.3.4 Marfo-Tuya

This is a 70-day variety and has a white seed coat with brown eye. It was released in 2003 for general cultivation in Northern Ghana. The yield potentials is 2.0 t/ha. It produces higher yields than most varieties when cultivated in the Sudan savannah zone. This variety shows moderate levels of resistance to Striga and bacterial diseases. In addition a number of improved types that have not been released are cultivated (SARI, 2012).

2.3.5 Asontem

The variety Asontem can be effectively grown at all locations in the country. The local white is not suitable for the forest and coastal savannah zones but is good for the interior savannah areas.

2.4 Major Constraints to cowpea production in Ghana

The major constraints to cowpea production in Ghana are biotic and abiotic factors such as insect pests (aphid, flower thrips, maruca, pod sucking bugs, bruchid), diseases (bacterial, fungal and viral), Striga (*S. gesnerioides*) and Alectra which can cause yield loss ranges from 15 to 100% depending on the level and severity of infestation, susceptibility/resistance of the variety, drought and low soil fertility. Lack of inputs such as fertilizers, insecticides and improved seeds, poor cultural practices and lack of appropriate machinery for expanding planted area are other constraints experienced (Danso, 2016; ICRISAT, 2011).

In a study by Egbadzor *et al.* (2013) to ascertain current production constraints faced by farmers and consumers' desired traits of cowpea in Ghana, it was revealed that most limiting factor to cowpea cultivation differ from one district to the other as well as for individual farmers. A number of limitations were identified. They were insect pests attack, diseases, drought, *Cyperus spp.* and harvesting difficulty. Among these, insect pest was the most the most impressing constraint was insect pests attack.

Cowpea yields are known to be low in most parts of the tropics because of heavy insect pest problems. In Africa, cowpea growers do not generally use synthetic insecticides; however, in most parts of Asia, dependence on the use of insecticides is common, often with serious environmental consequences. Such misuse of insecticides on cowpea, coupled with low yields, has led to an intensive search for pest control options that will increase yields with little or no input from insecticides, or biointensive integrated pest management (IPM). The major elements of this strategy include host plant resistance, use of beneficial organisms, agronomic practices, and (where adequate results are not obtained) some insecticide input, preferably from plant-based insecticides (Singh *et al.*, 1997).

2.5 Cowpea field pest

Cowpea is very attractive to insects. Insect pests have remained the most important setback to cowpea production, because each phase attracts a number of insect pests. The main pests during the growing season are pod sucking bugs (*Riptortus spp.*, *Nezara viridula* and *Acantomia sp.*), aphid (*Aphis fabae*, *Aphis craccivora*), blister beetle (*Mylabris spp.*) and pod borer (*Maruca vitrata*) (DAIS, 2011).

2.6 Cowpea storage problems

The principal storage pest of cowpea grain in sub-Saharan Africa is the cowpea weevil (*Callosobruchus maculatus* Walp). In low-resource farms, *C. maculatus* infestations start in the field and continue in storage. In the field, gravid females deposit eggs on the surfaces of pods still hanging on the plant. The females prefer mature green pods, but will oviposit on dry, mature pods as well (Ilesanmi and Gungula, 2010, 2013, 2016; Singh *et al.*, 1997). Cowpea weevils damage cowpea after harvest, attack dried cowpeas and other related stored seeds. The weevils prefer dried cowpeas but will attack other beans and peas in storage (Wahedi *et al.*, 2013). Cowpea grain stored after harvest is the favourite food of insects called bruchids. Adult cowpea bruchids are often known as cowpea weevils. Infestation of cowpea grain starts in the field at a low level. In the granary, the female lays her eggs on cowpeas. The eggs hatch in about a week. Each tiny grub-like larva bores through the bottom of its egg and into the seed. There it feeds, grows and develops, passing through four larval and one pupal stage. This takes about four weeks. As soon as an adult female emerges from the seed, it finds a male and mates. She then begins laying her eggs on other cowpeas. About 30 days later the new generation of adults appears, popping out of their seeds and leaving a little round hole. Each pair of adults then begins its own new generation. Each female is prolific, producing some 100 offspring after only a month (Baributsa *et al.*, 2013). Larvae

hatching from eggs on either seeds or pods use their mouthparts to bore through the bottom of the chorion. They tunnel onwards, penetrating the pod wall or the seed testa.

When this to happen, farmers could easily lose confidence in the value of the resistant lines. Low-resource farmers often sell their cowpea at harvest, when prices are lowest in the year, partly because they anticipate storage losses. Being aware of the storage problem, they are interested in better techniques for preserving their grain after harvest. Since emergence holes represent insects that have developed and left the seed, mated, and laid additional eggs, counting emergence holes to assess damage undoubtedly represents only a part of the problem. The next generation of larvae, more numerous yet, will generally still be developing within the grain. When the damage exceeds one emergence hole per seed, the price is usually discounted (Singh *et al.*, 1997).

2.7 Techniques of cowpea storage and Cowpea weevil control

Insect pests in particular can be devastating to cowpea during storage. There are storage insects that cause damage to the seed; it is therefore important to store seed in a protected place. A serious insect pest during storage is the cowpea weevil *Callosobruchus maculatus*. The rising popularity of organic produce lines has created interest in non-chemical disinfestation treatments as the use of chemicals in controlling these insects is becoming a problem (DAIS, 2011). Management of cowpea seed storage pests mostly rely heavily on the use of chemical insecticides. However, most small scale farmers have not adopted these new techniques due to some financial and technical reasons. Insecticides also have negative impact on the environment, humans and non-target organisms. Therefore, there is a need to develop cheap, safe and easy methods of protecting stored cowpeas against cowpea weevil (Tiroesele *et al.*, 2015).

In order to reduce serious losses experienced during storage, various techniques and control methods have been developed and more are still being developed. Farmers in agricultural communities store their cowpea grains in diverse ways, depending on whether the grains are for consumption during the year or the grains are for seed for planting next season.

Cowpea grains for consumption remain in pods and stored in granaries and storage drums.

For seed, many farmers prefer storing the seed grain either within the dry pod, containers with ashes or triple bags. The dried pods are tied in small bundles and hung over the cooking spot in the house kitchen so that whenever food is being cooked the smoke from the fire would drive away invading insects. There are ashes and hot chillies been reported by farmers to scare and repel away invading storage pests (Ngalamu *et al.*, 2015; Salifu, 2012). The storage life of cowpea depends on its moisture content before storage. The lower the moisture content, the better the quality of seeds in storage.

2.7.1 Use of oils and botanicals

According to Singh *et al.* (1997), an effective way to protect dry beans such as cowpea from bruchids is to mix the grain thoroughly with small amounts of vegetable oil. The oil coats the testa, acting as an ovicide, by plugging the egg micropyle, thus hindering the oxygen supply to the embryo. Other factors may be involved as well; in some cases, the oil may deter oviposition or cause mortality of the adult bruchids. Only small amounts of oil are needed to preserve the grain for months (1-5 mL of oil per kg of threshed grain). A variety of oils can be used, including oil from palm kernel, cottonseed, groundnut, neem, soybean, citrus peels, shea nut among others (Pereira, 1983). Some of the techniques that can be explored include the use of plant products such as garlic, peppermint and chillies. Aromatic plants have both medicinal and aromatic properties and contain a variety of volatile oils which have insecticidal, anti-feedant and repellent effects on insect pests. The chemical repellence hypothesis states that non-host plant odours repel herbivores by disrupting their

ability to locate or feed on the host plant. The plant products used for this study produce odours that are believed to repel weevils, thereby preventing them from attacking cowpea seeds (Tiroesele *et al.*, 2015).

Though effective, oil treatment has some negative attributes. It is essential that the oil be thoroughly and evenly applied to the surfaces of the seeds, so that any eggs already present or subsequently placed on the seeds come into contact with it. Treating much grain thoroughly is tedious. Some oils become rancid with time, or have inherently negative properties; neem oil, for example, stains the hands and has an unpleasant "garlic" odour.

Also, oil treatment is often messy and it is easy to pick up dust and debris while applying it. Thus, while oil treatment is effective, its degree of acceptance appears to be limited. The use of other plant materials for the preservation of cowpea is common on low-resource farms in sub-Saharan Africa. This is often as whole plants or leaves of various mints, aromatic or pungent plant materials that are mixed with the stored cowpea grain.

2.7.2 Metal drums

The procedure involves drying the threshed cowpea grain in the sun, then storing the cowpea grain in sealed metal drums. Metal drums vary in capacity from about 45 kg to 150 kg. It is recommended that the drum be sealed for a minimum of 2 months prior to opening. It is vital that the drum has no openings such as cracked seams that might admit Oxygen and that the cap be tightly sealed (Salifu, 2012; Singh *et al.*, 1997). To ensure that it is air-tight, the cap should be greased before tightening. The filled container is sealed, with peanut or other cooking oil used to lubricate the edges of the closure to ensure an airtight seal. Filled drums are kept in the shade or storehouses so that the cowpea grain does not get too hot due to absorption of solar radiation by the drum. In addition to their relatively low initial cost, these drums can be used repeatedly. One disadvantage of the technique is that a drum filled with cowpea is heavy. However, smaller 60 L drums are available, but they are more expensive

per kg of grain stored than the 200 L drums. Another disadvantage of bulk drum storage is the long interval during which they must remain sealed for the treatment to be effective.

2.7.3 Triple plastic bagging

The technique makes use of widely available, low-cost, clear plastic bags, which will hold about 50 kg of threshed grain. Cowpea grain (40-50 kg) is put in a bag and tightly sealed by tying it shut with strong twine. The first bag is then completely enclosed in a second bag, which is likewise tied shut around the first. The third bag is then similarly used to enclose the first two. Because the bags are transparent, it is easy for the owner of the grain to visually monitor grain storage. A disadvantage of the technique is that the bags are somewhat fragile and can burst with rough handling. They may also be vulnerable to rodents.

2.7.4 Co-storage with ash and other abiotic materials Ash

The method commonly used mostly in Sub-Saharan Africa consists of mixing cowpea grain with ash from the cooking fire in the hope of protecting their grain from bruchids (Salifu, 2012; Singh, Mohan Raj, *et al.*, 1997). The mixture is placed in a mud pot granary, clay jar, or any other vessel, and sometimes tamped down to compress it. Results varied with variations in the mode of application, and to the proportion of ash to cowpea grain used. The most important of these is the ash: grain ratio. Ash storage does not provide complete protection against a build-up of cowpea bruchids unless the ratio of ash to grain is 3 or more parts ash to 4 parts grain (Singh *et al.*, 1997). If immature *C. maculatus* are already present in grain at the time the grain is mixed with ash, they will complete their development within the seed and may even emerge from the seed even if covered with ash. Consequently, farmers sometimes put infested grain-but apparently undamaged-into ash and discover when they remove it later that it now has emergence holes, thus evoking doubts about its usefulness. When properly used, ash storage arrests cowpea bruchid population development within the store, but it does not kill the generation already within the seeds.

For this reason, it is important to mix the grain well with ash soon after threshing. It is proper to mix sieved ash from cooking fires with cowpea grain in equal volumes, then put this mixture in a closed vessel, tamp it down firmly, and cover with a 3 cm layer of loose ash. The mode of action of ash in protecting against cowpea bruchid population build-up is in twofold. First, adults developing from larvae already in the grain at storage with ash are immobilized when emerging from seeds; they die before they find mates. Second, gravid females approaching the store from outside will not burrow into the ash and so will not oviposit on the cowpea grain.

2.8 Chemical control of weevils in cowpea storage

Insecticide use on cowpea has a long history. It is the most widely known form of pest control on this crop. Traditional cowpea growers in Africa do not habitually use insecticides, as reflected in the poor yields they obtain. In many countries in Asia, pest control is mainly insecticide based, and for many commercial growers it is the only way. However, insecticides are the fire-fighting analogue in cowpea pest control, a function for which they remain unrivalled. The landscape of insecticide use has changed over the years from dependence on the highly toxic and/or persistent insecticides (e.g., DDT, endosulfan, monocrotophos) of the 1960s and 1970s to an era of great skepticism and reduced usage in the 1980s, typified by a shift towards less toxic and more environmentally friendly and narrow spectrum, target-specific technology. Currently, economic necessity and sensitivity to environmental destruction have rendered insecticide use socially unacceptable, although somewhat unrealistically so. There is also increased advocacy for monitored rather than calendar-based insecticide application, if insecticides must indeed be used. The insecticides used on cowpea can be grouped into seed dressings, foliar sprays, storage sprays, and dusts (Singh, Mohan Raj, *et al.*, 1997).

Use of insecticides to protect cowpea grain in storage is probably more commonplace and controversial than their use on the field crop, because chemical residues are erroneously feared to persist in the bean after cooking. This is a common misconception. If the right insecticides are used in the appropriate manner, there should be little or no concern about residues in cooked food. The most commonly used insecticides for the protection of cowpea in storage are pirimiphos methyl (Actellic® and the formulation synergized with permethrin, Actellic Super®), aluminium phosphide (Phostoxin®, Gastoxin®, Detia®), Malathion, permethrin (e.g., Coopex®, Kaothrin®), deltamethrin) among others. Those available in dust formulations or as liquid-based sprays usually do not pose the same degree of danger as do fumigants (gastoxin, phostoxin). However, despite the greater risks posed by fumigation, especially if used close to living quarters, fumigants are among the most effective products for disinfesting stored cowpea. Furthermore, the relative ease with which they can be dispensed (as tablet or pellet formulations) has greatly expanded the use of fumigants. In general, most clean and uninfested cowpea sold in the marketplace 3 months or more after the end of the growing season is generally treated with insecticides. Use of insecticides invariably raises questions about resistant pest strains. Fortunately, this has really not been an issue in the case of cowpea, probably because compared to other crops such as cotton; the use of insecticides on cowpea is small. Reducing insecticide use on cowpea as currently advocated will make the development of pest resistance to insecticides less likely. Unfortunately, many research institutions which conducted research on insecticides have either shifted emphasis or completely abandoned work on this subject in favour of topics that attract more funding. As a result, we may never know if, or when, resistance to insecticides ever develops (Singh *et al.*, 1997).

2.9 The neem tree distribution and its potentials

Neem (*Azadirachta indica*), is an evergreen tree originated from Southeast Asia (Dave and Vordzogbe, 2015), particularly India where it provides food and insecticide, and is used for its great number of ethno-medicinal properties (Heuzé *et al.*, 2015). Currently, neem is grown in tropical and sub-tropical regions with semi-arid to humid climates including Ghana (Rafiu, 2012). It was naturally distributed in Thailand, Malaysia and Indonesia and has become one of the most widespread trees in tropical and subtropical areas. It has become invasive in the Caribbean (Puerto Rico, Dominican Republic), sub-Saharan Africa (Kenya, Gambia, Senegal, Guinea Bissau, Ghana, Tanzania), and the Pacific (Australia, Fiji, Marshall Islands). However, it has become invasive in only a fraction of the countries where it is cultivated and utilized as a multipurpose tree.

Neem is a medium-sized tree, reaching 15 to 30 m in height, with a large rounded crown up to 10-20 m in diameter. It is mainly evergreen but sometimes shed its leaves during the dry season. All parts of the plant have been used medicinally for centuries. It is widely used in toothpastes, soaps and lotion currently, as well as being a biological insecticide. Wide arrays of biologically active constituents are produced in *Azadirachta indica* like azadirachtin, nimbidin, nimbidolgedunin. Because neem contains a number of useful chemicals, with multiple uses and adaptability to diverse habitats and climatic conditions, interest in the tree has increased globally. Biological and pharmacological activities attributed to solvent extracts and products such as oil from its seeds are as diverse as antiplasmodial, larvicidal, fungicidal, insecticidal, and anthelmintic. Also, the bark of the neem tree is known to have tannins and phenolic compounds and unveils anti-oxidant precursor (Dave and Vordzogbe, 2015). The seeds are an important source of azadirachtin, a limonoid compound (triterpenoid) present in the seeds, and also to some extent in leaves and other tissues. It acts as an insect repellent, inhibiting them from feeding, thus disrupting their growth,

metamorphosis and reproduction. Extracts or crude parts of the tree are often mixed with stored seeds such as maize, rice and beans, in order to protect them against insects. Neem extracts can protect plants from foliage-eating insects without affecting pollinating insects (Heuzé *et al.*, 2015).

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CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Description of study area

This research was carried in Zebilla of the Bawku West District. Bawku West District is one of the thirteen (13) districts and municipalities in the Upper East Region of Ghana. The district is located in the north-eastern section of the region, with Zebilla as its administrative capital. It is bordered to the north by the Republic of Burkina Faso, to the east by Binduri district, to the west by Talensi district and Nabdam district and to the south by East Mamprusi district. Significantly, two tributaries of the Volta River namely the White Volta and the Red Volta run contiguous to the district's eastern and western boundaries respectively. The population of Bawku West District, according to the 2010 Population and Housing Census, is 94,034 representing 9.0 percent of the region's total population with a total number of 15,169 households (GSS, 2014).

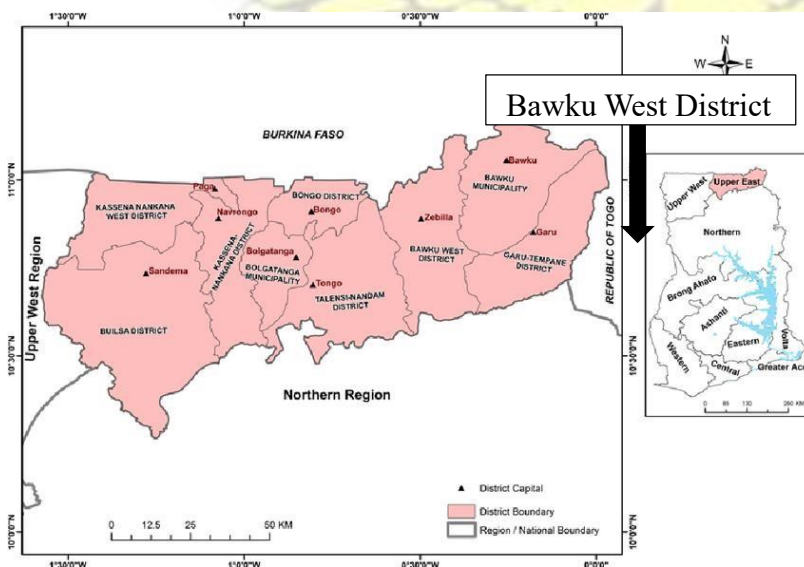


Figure 3.1: Map of Upper East Region showing Bawku West district (arrow inserted)

The district experiences a unimodal rainfall regime lasting 4 to 6 months and a long dry period of 6 to 8 months in a year. The average annual rainfall, temperature and relative

humidity are 956mm, 34°C and 56% respectively, with potential evapotranspiration of more than 2882mm. The district's vegetation is Sudan Savannah consisting of short drought and fire resistant deciduous trees interspersed with open savannah grassland. Grass is very sparse and in most areas the land is bare and severely eroded. In most cases the vegetation is highly degraded by land clearing for farming, fuel wood harvesting, overgrazing, annual bushfires, and harvesting of poles for construction.

The district has agrarian subsistence economy. As high as 93.3 percent of households in the district are engage in agriculture. In the rural localities, 96.5 percent of the total number of households are agricultural households while in the urban localities, 68.4 percent of households are into agriculture. Most agricultural households in the district (98.1%) are involved in crop farming. Chicken and guinea fowl are the dominant animals reared in the district.

3.2 Experimental materials

Mature ripe neem seeds were collected from neem trees readily available throughout the district. The cowpea seeds were purchased from Zebilla Market, Bawku West district. Both infested and non-infested seeds were purchased. Adult cowpea weevils were extracted from infested cowpea seeds obtained from the market. Other materials including vegetable oil, plastic containers with lids, pipette, sieve and a weighing scale were also obtained.

3.3 Extraction of neem seed oil

Oil from neem seeds were extracted using conventional methods such as how shea butter is extracted locally. Majored fallen neem trees were collected from neem trees in Zebilla. The neem seeds gathered were dried in open sunshine for about 8 hours to reduce its moisture content. It was decorticated and fried to further reduce its moisture content and to enable the

easy removal of the endocarp. After frying, it was then milled to paste to facilitate the extraction of the oil. Hot water was added to the milled paste and stirred continuously for about 15 – 30 minutes to separate the oil from the paste. Cold water was later added to the mixed oil and paste to solidify the oil and to make it easy to separate it. The solidified oil was separated and heated to boiling point to remove and remaining water in it and to make it ready for use. Neem kernels contain up to 45% w/w of oil (Naniwadekar and Jadhav, 2012).

3.4 Experimental design

A Completely Randomised Design (CRD) with three replications was used for the experiment. The experiments were setup using non-infested cowpea seeds. The treatment consisted of neem seed oil and vegetable oil mixed in 5 different proportions and a control to obtain a total of six treatments. The amount of oil used for each of the treatments was 25 ml which was added in different proportions. The proportions used and their labels are displayed in Table 3.1.

Table 3.1: Experimental treatments used

Treatment	Proportions	Treatment Label
T1	100	Neem oil
T2	75:25	Neem oil–vegetable oil
T3	50:50	Neem oil–vegetable oil
T4	25:75	Neem oil–vegetable oil
T5	100	Vegetable oil
T6	-	Control (free of both neem seed oil extract and vegetable oil)

3.5 Application of experimental treatments

Experimental set up were done for non-infested seeds. Each treatment consisted of 1kg of cowpea seeds. The weighed seeds were placed in plastic containers and about 25 ml of the treatment oil was pipetted into the weighed seed samples according to the proportions in Table 3.1 except for the control where no oil was applied. The mixtures were vigorously shaken to ensure uniform mixing of the oil and the seeds. About 20 adult weevils were then introduced in each container of cowpea. The containers were covered with a cheese cloth to allow for exchange of gases between the content in the container and the environment. The treatments were then stored at ambient conditions in a storage environment. Plate 3.1 shows the experimental set up of the treatment.



Plate 3.1: A picture showing experimental set up

3.6 Parameters studied

3.6.1 Survival test using neem seed oil

The survival test of the neem seed oil was conducted using 25 ml of the extracted oil. About 20 cowpea weevils were placed in a plastic container and 25ml of the neem seed oil was pipetted into the container. The oil was evenly spread to ensure uniform contact with the

weevils. The duration of exposure of the weevils to the oil and the time of death was then recorded in seconds.

3.6.2 Cumulative Weight Loss of Seeds

The weight loss of the seeds was estimated by measuring the weight loss during the storage period. Weight loss by the grains was measured as the difference between the initial weight of the grains before storage and their final weight for each particular month.

3.6.3 Percentage of Seeds with Holes

The number of seeds with holes were estimated by counting seeds with holes in them. The percentage seeds with holes was calculated using the equation

$$\text{Percentage seeds with holes} = \frac{\text{number of seeds with holes}}{\text{number of whole seeds}} \times 100$$

3.6.4 Insect Mortality

Insect mortality was estimated by counting the number of dead and alive adult weevil in each experimental treatment during the storage period of 3 months. An insect was considered dead when it showed no sign of movement when lightly touched or when it was found lying on its back without kicking (Adu-Acheampong, 1997).

Insect mortality was then calculated using the equation

$$\text{Insect mortality} = \frac{\text{number of dead weevils}}{\text{total number of alive weevils}} \times 100$$

3.6 Data analysis

Data collected were analysed using XLSAT statistical software (Addinsoft, 2016). Analysis of Variance (ANOVA) test was performed. Statistical difference was done using least significant difference test at 5% (P= 0.05). Results were presented in tables and graphs.

CHAPTER FOUR

4.0 RESULTS

4.1 Survival test

Significant differences were observed among the treatments used in the survival test ($p < 0.05$). The results from the analysis (Table 4.1) showed that weevil survival increased as the proportion of neem seed oil was reduced. Neem seed oil (100:0) recorded the shortest time of 10 seconds with Neem-Veg (75:25) recording the second shortest time of 13.3 seconds. However, the control; vegetable oil (100:0) recorded the longest time of 29 seconds.

Table 4.1: Survival test of cowpea weevils as influenced by neem seed oil and vegetable oil

Treatment	Time (seconds)
Neem (100:0)	10.0 ^e
Neem-Veg (25:75)	20.7 ^b
Neem-Veg (50:50)	17.7 ^c
Neem-Veg (75:25)	13.3 ^d
Vegetable (100:0)	29.0 ^a
Pr > F	0.001
Significant	Yes

Means with different letters (a, b, c, d, e) are significant from each other ($p < 0.05$)

4.2 Cumulative weight loss

Significant differences were observed in weight loss among the treatments especially between the Neem oil (100:0) and the control, Neem-Veg (25:75) and Neem-Veg (50:50) in Month 1, 2 and 3 respectively ($p < 0.05$).

Table 4.2 shows the cumulative weight of the cowpea seeds in storage. The results indicate that the control treatment recorded high weight loss of 110g, 240g and 350g

for storage month 1, 2 and 3, followed by the treatment Neem-Veg (25:75) with a weight loss of 30g, 60g and 90g for storage month 1, 2 and 3. Neem oil treatment (100:0) recording the lowest

Table 4.2: Cumulative weight loss (g) cowpea seeds in storage

Treatment	Month 1	Month 2	Month 3
Control	110 ^a	240 ^a	350 ^a
Neem-Veg (25:75)	30 ^b	60 ^b	90 ^b
Neem-Veg (50:50)	27 ^b	53 ^b	80 ^b
Neem-Veg (75:25)	23 ^{bc}	47 ^{bc}	70 ^{bc}
Vegetable (100:0)	20 ^{bc}	40 ^{bc}	60 ^{bc}
Neem (100:0)	10 ^c	20 ^c	30 ^c
Pr > F	0.001	0.001	0.001
Significant	Yes	Yes	Yes

Means with different letters (a, b, c) are significant from each other ($p < 0.05$)

4.3 Percentage of seeds with holes

Statistical significant differences were observed in the percentage of seeds with holes among the treatments for Month 1, 2 and 3 respectively ($p < 0.05$).

Table 4.3 shows the percentage of seeds with hole. The results show that the control treatments had the highest percentage of seeds with holes of 36.87%, 69.54% and 97.67% for storage month 1, 2 and 3. However, the neem oil treatment (100:0) recording the lowest percentage of seeds with holes of 11.09%, 11.10% and 11.16% for storage month 1, 2 and 3 respectively.

Table 4.3: Percentage of seeds with holes

Treatment	Month 1	Month 2	Month 3
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Control	36.87 ^a	69.54 ^a	97.67 ^a
Neem-Veg (25:75)	15.88 ^b	14.65 ^b	14.81 ^b
Neem-Veg (75:25)	14.63 ^{bc}	14.60 ^b	13.14 ^{bc}
Neem-Veg (50:50)	14.21 ^{bc}	14.06 ^{bc}	14.27 ^b
Vegetable (100:0)	12.42 ^{cd}	12.51 ^{bc}	12.60 ^{bc}
Neem (100:0)	11.09 ^d	11.10 ^c	11.16 ^c
Pr > F	0.001	0.001	0.001
Significant	Yes	Yes	Yes

Means with different letters (a, b, c, d) are significant from each other (p<0.05)

4.4 Insect mortality of cowpea weevils

Significant differences were observed in insect mortality between the treated samples and the control experiment (p<0.05). The results for insect mortality of the cowpea weevil is shown in Table 4.4. The results showed that insect mortality was 100% for all treated cowpea seed samples. The insect mortality for the control samples reduced gradually over the storage period with month 1 recording 42.66% mortality, month 2 recording 37.13% and month 3 recording 6.91% insect mortality respectively.

Table 4.4: Insect mortality of cowpea weevils

Treatment label	Month 1	Month 2	Month 3
Control	42.66 ^b	37.13 ^b	6.91 ^b
Neem (100:0)	100.00 ^a	100.00 ^a	100.00 ^a
Neem-Veg (25:75)	100.00 ^a	100.00 ^a	100.00 ^a
Neem-Veg (50:50)	100.00 ^a	100.00 ^a	100.00 ^a
Neem-Veg (75:25)	100.00 ^a	100.00 ^a	100.00 ^a
Vegetable (100:0)	100.00 ^a	100.00 ^a	100.00 ^a

Pr > F	0.001	0.001	0.001
Significant	Yes	Yes	Yes

Means with different letters (a, b) are significant from each other (p<0.05) **CHAPTER FIVE**

5.0 DISCUSSION

5.1 Survival test

The results showed that weevil survival increased as the proportion of neem seed oil was reduced. Neem seed oil (100:0) recorded the shortest time of 10 seconds with Neem-Veg (75:25) recording the second shortest time of 13.3 seconds. However, the control; vegetable oil (100:0) recorded the longest time of 29 seconds. This clearly demonstrates that the neem seed oil contains an important active ingredient (Azadirachtin) which serves as a repellent to the weevils under study. Boeke (2004) demonstrated the insecticidal and repellent properties of *A. indica* seed oils against storage insects. Its compounds, particularly Azadirachtin causes a digestive disturbance, disrupts the metamorphosis of insect larvae by inhibiting molting. This prevents larvae from developing into pupae and they die without producing new generation (Schmutterer, 1995). According to Belanger & Musabyinama (2005) at least 12 mechanisms of action of neem compounds were reported. The results of this study confirms some of these findings. Furthermore, the results from this work, where, the efficacy of the neem increased with increasing concentration is consistent with results of Adu-Acheampong (1997) who found that neem extracts controlled aphids on cocoa in a dose dependent manner. In a study by Ilesanmi and Gungula (2010), it was found that, all the oil types at various rates significantly protect the cowpea samples against weevil attack compared to the control samples throughout the study except for pure moringa at lower rates.

5.2 Cumulative weight loss

The results indicated that the control treatment recorded high weight loss of 110g, 240g and 350g for storage month 1, 2 and 3. Neem oil treatment (100:0) recording the lowest weight loss of 10g, 20g and 30g for storage month 1, 2 and 3 respectively. Rapid weight loss in stored grains is an indication of deterioration since the essential stored grain reserve gets depleted with time. It is likely possible that the grains treated with neem seed oil were able to control the weevils and therefore the mode of operation of the weevils were reduced. The mode of feeding of the weevil and the resulting powdery product is an evidence of product breakdown which is associated with the loss of weight. Similar findings were reported by Katamssadan et al 2015 about the rapid loss of weight in maize and cowpea grains stored using neem seed oil and vegetable oil.

5.3 Percentage of seeds with holes

The results show that the control treatments had the highest percentage of seeds with holes of 36.87%, 69.54% and 97.67% for storage month 1, 2 and 3. However, the neem oil treatment (100:0) recording the lowest percentage of seeds with holes of 11.09%, 11.10% and 11.16% for storage month 1, 2 and 3 respectively. This could be due to the efficacy of the neem seed oil in killing the cowpea weevils during the period of storage. *Azadirachta indica* seed oils could also inhibit progeny production by non-mechanical mechanisms, especially with *S. zeamais*. Female maize weevil lays eggs inside the grain. If, on treated grains oviposition is not deterred by the presence of the oil, then the development of immature stages could be affected chemically. As the oil has the ability to infiltrate the grains, the larvae of *S. zeamais*, which feed inside the grain would ingest some quantity of azadirachtin and other compounds like nimbin and salanin in *A. indica* oil. These compounds have growth regulatory effects on larvae, such as, disruption of molting, growth

inhibition, malformation, which may block the developmental stages of the weevils or cause mortality of immature stages (Isman, 2006). The control treatment recorded the highest holed grains probably due to the absence of repellants which could prevent the insects from feeding and rapidly reproducing. Similar findings were reported by Katamssadan et al 2015 about the extent of damage in maize and cowpea grains stored using neem seed oil and vegetable oil.

5.4 Insect mortality

The results showed that insect mortality was 100% for all treated cowpea seed samples. The insect mortality for the control samples reduced gradually over the storage period with month 1 recording 42.66% mortality, month 2 recording 37.13% and month 3 recording 6.91% insect mortality respectively. This could be due to the high persistent and efficacy of the neem seed oil treatment as used in this study. One of the basic characteristics of an effective grain protectant is its ability to reduce progeny production in treated grains (Khoshnoud *et al.*, 2008). Results of inhibition of progeny production showed that oils extracted from *A. indica* seeds that were subjected to the four drying regimes completely inhibited progeny emergence of *C. maculatus* and *S. zeamais*, showing their enormous ability to control both insects. The *A. indica* oils might have acted physically or chemically on eggs or immature stages, depending on the insect species. Suppression of emergence in *C. maculatus* could be related to physical action of the *A. indica* seed oil. The coating of the seeds by *A. indica* oil may prevent the eggs from adhering onto the seeds. Therefore, it was not possible for the eggs to hatch in the grains and death ensued. Similar explanations were advanced by other researchers, where *A. indica* seed oil completely inhibited the progeny production of *S. oryzae* and *C. maculatus* (Bamaiyi *et al.*, 2007; Kemabonta and Falodu,

2013; Ilesanmi and Gundula, 2013). In addition, *A. indica* oil, like other vegetable oils, penetrates the chorion of bruchid eggs via the micropyle and the oil might occlude the egg funnel, which blocks exchange with the outside, leading to the asphyxiation of the developing insect, then death follows (Copping and Menn, 2000).

Moreover, *Azadirachta indica* seed oils could also inhibit progeny production by nonmechanical mechanisms, especially with *S. zeamais*. Female maize weevil lays eggs inside the grain. If, on treated grains oviposition is not deterred by the presence of the oil, then the development of immature stages could be affected chemically. As the oil has the ability to infiltrate the grains, the larvae of *S. zeamais*, which feed inside the grain would ingest some quantity of azadirachtin and other compounds like nimbin and salanin in *A. indica* oil. These compounds have growth regulatory effects on larvae, such as, disruption of moulting, growth inhibition, malformation, which may block the developmental stages of the weevils or cause mortality of immature stages (Isman, 2006). Udo (2005) stated that, there is a relationship between F1 progeny emergence and adult mortality. His statement is confirmed by the report of Fekalu *et al.*, (2012) who found that *Gossypium hirsutum* and *Brassica carinata* seed oils reduced adult emergence of *S. zeamais*.

CHAPTER SIX

6.1 CONCLUSION

Results from the study has clearly indicated that there is great potential in using neem seed oil as cowpea seed protectants against pest infestation especially *Callosobruchus maculatus*. The study revealed that weevil survival increased as the proportion of neem seed oil was reduced. Neem seed oil (100:0) recorded the shortest time of 10 seconds with Neem-Veg (75:25) recording the second shortest time of 13.3 seconds. However, the control; vegetable oil (100:0) recorded the longest time of 29 seconds. The results revealed that the control treatment recorded significantly highest weight loss of 110g, 240g and 350g for storage month 1, 2 and 3. Neem oil treatment (100:0) recording the lowest weight loss of 10g, 20g and 30g for storage month 1, 2 and 3 respectively.

Moreover, the study revealed that the control treatments had the highest percentage of seeds with holes of 36.87%, 69.54% and 97.67% for storage month 1, 2 and 3. However, the neem oil treatment (100:0) recorded the lowest percentage of seeds with holes of 11.09%, 11.10% and 11.16% for storage month 1, 2 and 3 respectively.

Furthermore, the results showed that insect mortality was 100% for all treated cowpea seed samples. The insect mortality for the control samples reduced gradually over the storage period with month 1 recording 42.66% mortality, month 2 recording 37.13% and month 3 recording 6.91% insect mortality respectively.

6.2 RECOMMENDATIONS

The following recommendations were made from the study;

1. Future research should focus on the effects of other botanicals (garlic and ginger) on the physic-chemical and antioxidants properties of cowpea.
2. Neem seed oil is recommended for the long term storage of cowpea grains in the study area.



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APPENDIX

ANALYSIS OF VARIANCE (ANOVA)

SURVIVAL TEST

Analysis of variance: survival test

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	4	641.7333	160.4333	66.8472	< 0.0001
Error	10	24.0000	2.4000		
Corrected Total	14	665.7333			

Computed against model $Y = \text{Mean}(Y)$

CUMULATIVE WEIGHT LOSS (g)

Analysis of variance: Month 1

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	5	0.0201	0.0040	65.6727	< 0.0001
Error	12	0.0007	0.0001		
Corrected Total	17	0.0208			

Computed against model $Y = \text{Mean}(Y)$

Analysis of variance: Month 2

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	5	0.0989	0.0198	80.8909	< 0.0001
Error	12	0.0029	0.0002		
Corrected Total	17	0.1018			

Computed against model $Y=Mean(Y)$

Analysis of variance: Month 3

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	5	0.2080	0.0416	75.6364	< 0.0001
Error	12	0.0066	0.0006		
Corrected Total	17	0.2146			

Computed against model $Y=Mean(Y)$

PERCENTAGE OF SEEDS WITH HOLES

HOLES

Analysis of variance: Month 1

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	5	1391.2927	278.2585	124.3193	< 0.0001
Error	12	26.8591	2.2383		
Corrected Total	17	1418.1518			

Computed against model $Y=Mean(Y)$

Analysis of variance: Month 2

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	5	7911.1372	1582.2274	483.1987	< 0.0001

Error	12	39.2938	3.2745
Corrected Total	17	7950.4310	

Computed against model $Y=Mean(Y)$

Analysis of variance: Month 3

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	5	17865.6372	3573.1274	1240.3991	< 0.0001
Error	12	34.5675	2.8806		
Corrected Total	17	17900.2047			

Computed against model $Y=Mean(Y)$

INSECT MORTALITY

Analysis of variance: Month 1

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	5	8219.1779	1643.8356	585.0068	< 0.0001
Error	12	33.7193	2.8099		
Corrected Total	17	8252.8972			

Computed against model $Y=Mean(Y)$

Analysis of variance: Month 2

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	5	9880.2395	1976.0479	14697.0003	< 0.0001
Error	12	1.6134	0.1345		
Corrected Total	17	9881.8530			

Computed against model $Y=Mean(Y)$

Analysis of variance: Month 3

Source	DF	Sum of squares	Mean squares	F	Pr > F
Model	5	21663.7592	4332.7518	594418.4333	< 0.0001
Error	12	0.0875	0.0073		
Corrected Total	17	21663.8467			

Computed against model $Y=Mean(Y)$

