LABORATORY EVALUATION OF ETHANOLIC EXTRACTS OF GARLIC (*Allium sativum*) CLOVES AND *Aloe vera* LEAVES AS PROTECTANTS AGAINST THE MAIZE WEEVIL, *Sitophilus zeamais*

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A project report submitted in partial fulfillment of the requirements for the award of a Master of Science (M.Sc) degree in Entomology in the Department of Zoology and Environmental Biology, Faculty of Biological Sciences, University of Nigeria, Nsukka

Supervisor: Prof. (Mrs.) P. O. Ubachukwu

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TOPIC

Laboratory Evaluation of Ethanolic Extracts of Garlic (*Allium sativum*) cloves and *Aloe vera* leaves as Protectants against the Maize weevil, *Sitophilus zeamais*

CERTIFICATION

Miss NWAONUMA, MARYANN U., a postgraduate student of the Department of Zoology, University of Nigeria with registration number, PG/M.sc./12/62250 has satisfactorily completed the requirements for the award of the degree of Master of Science (M.Sc.) Zoology (Entomology) of the Department of Zoology. The work embodied in this project report is original and has not been submitted in part or full for any other diploma or degree of this or any other institution.

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DEDICATION

This work is dedicated to God Almighty for His infinite goodness, kindness and mercy towards me and to my beloved parents ï Late Mr. Benjamin Nwaonuma and Mrs. Martina Nwaonuma.

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It is my pleasure to use this opportunity to show my warm and sincere gratitude to all those who in one way or the other made possible and contributed to my educational pursuit and those who made this research work a reality.

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ABSTRACT

This study evaluated the efficacy of ethanolic extracts of *Allium sativum* (Woodwill) cloves, *Aloe vera* (Miller) leaves and their mixture against *Sitophilus zeamais* (Motschulsky) under laboratory conditions. Experiments were carried out to check the toxicity, adult mortality, oviposition inhibition, repellent and anti-feedant effects of these plant extracts against *S. zeamais*. The experiments were laid out in a completely randomized design of ten treatments replicated three times. The adult weevils were exposed to maize grains treated with three concentrations (200, 250 and 300mg/ml) of *A. sativum* and *A. vera* and equal proportion (50:50) of their mixture while the control was treated with only the solvent. Probit analysis revealed *A. sativum* had lower LC_{50} than *A. vera* and therefore was more toxic than it. All treatments recorded higher significant ($p<0.05$) mortality than the experimental control. Most mortality occurred within 24hr of treatments showing that the extracts had no residual toxicity. Among the extracts used, the mixture recorded highest adult mortality (43.3%) followed by *A. sativum* (35%) and *A. vera* (30%). The plant extracts showed varying levels of repellency to the weevils with the highest repellency (66.7%) recorded by the mixture followed by *A. sativum* (44.4%) and *A. vera* (26.7%). The extracts were able to lower progeny production with the mixture having the highest percentage inhibition rate (61.5%) while the control recorded the lowest (19.1%). Percentage grains weight losses was significantly higher in the untreated check than in the treated grains and are recorded as follows: mixture (3.5 %), *A. sativum* (6.1%), *A. vera* (14.1%) and control (29.3%). The overall results show that the mixture of the two plant extracts performed better in all bioassays than individual plant extracts. The efficacy of the treatments were in the order: mixture > *A. sativum* > *A. vera.* Results also proved that *A. sativum* was a better biopesticide than *A. vera.* The effectiveness of the extracts was dose-dependent with the severity of their effects increasing with increase in concentration (200 < 250< 300mg/ml). The results in the present study shows that the ethanolic extract of *A. sativum* cloves, *Aloe vera* leaves and their mixture are toxic to *S. zeamais* and can suppress progeny development of the weevils by having oviposition inhibition and anti-feedant effects, thus resulting in better protection of maize grains from pest damage.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Insects are a problem in stored grain throughout the world because they reduce the quantity and quality of grain. Grains constitute the most important staple foodstuff for the ever-growing population in the tropics. Grains are small, hard, dry seeds (with or without hull or fruit layers attached) harvested for human food or animal feed (Babcock, 1976). Agronomists also call the plants producing such seeds 'grains' or 'grain crops'. Grains include cereals such as maize, rice, wheat, oats, barley, sorghum, millet etc; legumes or pulses such as soybean, cowpea, pigeon pea, peanut etc and oilseeds such as mustard seeds, canola, sunflower seeds etc. Cereal grains are all members of the grass family and they contain much starch, a carbohydrate that provides dietary energy. Legumes have higher protein than most other plant foods. They also contain starch or oil (Vaughan *et al.,* 1997). Harvested, dry grain has advantages over other staple foods such as the starchy fruits (e.g., plantain, breadfruit) and roots/tubers (e.g., sweet potato, cassava, yam) in being easy to store, handle and transport. In particular, these qualities have allowed mechanical harvesting, transporting by rail or ship, long-term storage in grain silos, large-scale milling or pressing and industrial agriculture in general. Thus, major commodity exchanges deal with soybean, rice, wheat, maize and other grains but not in vegetables, tubers, or many other crops.

Grain storage has often resulted in quantitative and qualitative losses due to physical, chemical, and most importantly biological factors such as pests which may be birds, rodents, fungi, or insects (Oke and Muniru, 2001). The most important among storage pests are insects because apart from their direct damage they create conditions that allow secondary infestation by rot organisms mainly fungi (Lale, 2002).

Conservation of reserved food grain stocks is necessary to ensure a continuous supply at stable prices (Talukder, 2005). Losses are the most serious problem from insect infestation in grain storage, particularly in the developing countries, where poor sanitation and use of inappropriate storage facilities all encourage insect attack. It was estimated that over 20,000 species of field and storage pests destroy approximately one-third of the world's food production, valued annually at over $100 billion, among which the highest losses (43% of potential production) occur in developing Asian and African countries (Jacobson, 1982; Ahmed and Grainge, 1986). Control of these pests relies on the widespread use of various synthetic chemical insecticides and fumigants. However, the application of various synthetic insecticides and fumigants to stored grains over the years has led to a number of problems such as environmental pollution, pesticide residue in food grains, development of insecticide resistance and toxicity to non-target organisms (Yusof and Ho, 1992; Cosimi *et al*., 2009).

The increasing public concern over pesticide safety and possible damage to the environment has resulted in increasing attention being given to natural products for the control of stored grain pests (Rajendran and Sriranjini, 2008). In this context, many plant products have been evaluated for their toxic properties against different stored grain pests, especially in the form of essential oils (Regnault-Roger, 1997; Rajendran and Sriranjini, 2008).

In recent years, many researchers have focused on the search for natural products derived from terrestrial plants as natural insecticides. Terrestrial plants are known to contain a rich source of bioactive metabolites which show anti-feedant, repellent and toxic effects in a wide range of insects (Ukeh *et al*., 2009; Mondal and Khalequzzaman, 2010). Studies have been carried out on some of these plants by different researchers. For example, *Allium cepa* and *Piper guineense* were tested and found to be effective in controlling insect pests of stored grains. Adedire and Ajayi (1996) reported 48.75% adult mortality of *Sitophilus zeamais* treated with *A*. *cepa* after 28 days post treatment and 100% mortality when treated with *P*. *guineense* after 3 days post treatment. Umar (2008) obtained results which showed that at the rate of 2.0g/ 20.0g seeds, the leaf powder of *Jatropha curcas* reduced oviposition by 30% when he worked on the comparative potentials of leaf, bark and wood powders of *J*. *curcas* as protectants of stored cowpea against *Callosobruchus maculatus*. Parugrug and Roxas (2008) worked on the insecticidal action of five plants against maize weevil, *S*. *zeamais* and found that at 24 hours of exposure, ratings of 7.00 (High Repellency) were recorded in powdered *Azadirachta indica* and carbaryl, whereas *Cymbopogon citratus* had repellency rating of 5.80 (Moderate Repellency) at the same hour. Rahman and Talukder (2006) investigated the oviposition inhibition, surface protectant, residual toxicity and direct toxicity effects of plant extracts, powder, ash and oil from nishinda (*Vitex negundo* L.), eucalyptus (*Eucalyptus globules* Labill.), bankalmi (*Ipomoea sepiaria* K.), neem (*Azadirachta indica* L.), safflower (*Carthamus tinctorius* L.), sesame (*Sesamum indicum* L.) and bablah (*Acacia arabica* L.) on *C. maculatus.* The results showed that plant oils were effective in checking insect infestation. The powdered leaves and extracts of nishinda, eucalyptus and bankalmi, at a 3% mixture, provided good protection for black gram seeds by reducing insect oviposition, F_1 adult emergence, and grain infestation rates. Similarly, Sagheer *et al*., (2011), reported the efficacy of acetone extracts of *Amaranthus hybridus* (Cholai), *Calotropis procera* (Ak), *Salsola baryosma* (Khar booti) and *Cuminum cyminum* (Zeera) in the control of the red flour beetle, *Tribolium castaneum.*

1.1.1 Justification of the Study

The use of conventional insecticides in the management of stored grains leads to a lot of problems ranging from toxic residues in food to development of resistance by the pests, hence the search for more natural products that are safer to use, readily available and inexpensive. Interest in plants with insecticidal properties has been on the increase recently around the world today, either singly in integrated pest management or in conjunction with synthetic pesticide and many of these plants have been reported by various authors to be highly effective. It is hoped that these concerted efforts shall eventually bring forth botanicals that can be used as alternative bioinsecticides. *Allium sativum* and *Aloe vera* have been proven effective against various insect pests of stored grain. However, there is a dearth of studies on the comparative evaluation of the insecticidal action of extracts from these plants and their mixture against *Sitophilus zeamais,* hence the need for this research. Understanding the toxicity of compounds to adults and immature stages is very important as it would indicate the appropriate time to apply them for adequate control of the insect pests. The present study would therefore provide the needed information on the toxicity of the extracts from *A. sativum*, *A. vera* and their mixture to adult, larva, and egg of *S. zeamais*.

1.1.2 Objectives of the Study

This study was designed to:

1) identify the potency of *Allium sativum* and *Aloe vera* extracts for the protection of maize grain in storage.
2) determine the minimum effective dosage of *Allium sativum* and *Aloe vera* extracts against maize weevil.
3) determine if the plant extracts have residual toxicity effects against the maize weevil.

4) determine repellence effects of *Allium sativum* and *Aloe vera* extracts against maize weevil.
5) determine oviposition inhibition effects of these plant extracts on maize weevil.
6) evaluate the effects of the plant extracts on progeny production by the maize weevil.
7) compare the effect of the mixture of the two plant extracts to the effect of each individual plant extract.
8) assess the protection potentials of the plant extracts against damage done by maize weevils to stored maize grains.

1.2 Literature Review

1.2.1 Origin of maize production

Maize, *Zea mays*, belongs to the family Gramineae. It is a cereal grass related to wheat, rice, oat and barley; ranking second after wheat and is followed by third-ranking rice in order of world grain production (Parugrug and Roxas, 2008). Maize (*Zea mays*) alternatively called corn originated from the American Continent where it was cultivated by Indians long before the arrival of Columbus. It is the most important crop grown in the U.S and valued at over four billion dollars annually. It is the most domesticated of all field crops and of all the total maize cultivated in U.S, 88% is harvested as grain,7% as fodder, 5% as silage while 90% of the grain is fed directly to livestock, 8% milled, 1 ½% goes to production of alcohol, about ½% is used for breakfast food primarily corn flakes (FAO, 1985). The European discovery of corn came in 1492 after explorer Christopher Columbus arrived in the Caribbean. Columbusô son Ferdinand wrote that his father saw a grain ñthat they call maize and is most tasty boiled, roasted or ground into flourò. Columbus took the seed home and by 1500s, Kasther wrote that corn was growing not just in Spain but in Bulgaria and Turkey, slaves carried corn to Africa. The Portuguese-born Spanish explorer Ferdinand Magellanös men dropped maize seed off in the Philippines and Asiaò This corn boom had begun. According to Harlin a corn farmer in the Finger Lakes region of New York, in United States gave description of some of the wonders of corn as a work of both art and brilliant mathematics. The Portuguese first introduced maize to West Africa in the Sixteenth Century. It is a crop of world importance (Anyanwu *et al*., 1979). However, nearly all the West African maize production is utilized locally in most West-African countries including Nigeria. The world greatest producers of maize are U.S.A., Brazil, the Soviet Union and Mexico.

1.2.2 Varieties of maize

Maize varieties are primarily distinguished by the type of endosperm and the grain colours (Anyanwu *et al*., 1979). Maize can be classified based on the endosperm character as follows:

Dent corn (*Z. mays indentata*): The most widely varied in colour but some are yellow or white in most commercial varieties. Dent type is high in lysine content with essential amino-acid.

Flint corn (*Z. mays indirata*): The endosperm is usually soft and starchy in centre, but enclosed by a corneous out layer. It is widely grown in California.

Floury corn (*Z. mays anylacea*): This has a very thin pericarp and a large amount of white starchy endosperm, which is soft. This type is very susceptible to weevil attack.

Pop corn (*Z. mays truricata*): The kernel is enclosed in a pod of husk. The typical podded ear will never breed true.

Sweet corn (*Z. mays sacchorata*): In this type, instead of all starch granules, we have sugary granules with very little starch granules. This type wrinkles on drying.

In West Africa, various varieties have been introduced in an effort to produce varieties that are resistant to maize rust and adaptable to local conditions. In Nigeria, such varieties include: NSI, Haiti Tsola and various Mexican varieties (Anyanwu *et al*., 1979).

1.2.3 Economic importance of maize

Maize is regarded as versatile and with many uses since it can thrive in diverse climates; hence, it is grown in many countries than any other crop.

Maize is a very important crop in Nigeria. It is utilized mainly for human consumption and form about 50 to 70% of the constituents of livestock feed. Maize is a staple food and is sometimes grown on a garden scale where it cannot be grown as a farm crop (Anyanwu *et al*., 1979). It is an important source of carbohydrate and if eaten in the matured state, it provides useful quantities of vitamins C.

The yellow grain varieties have vitamin A. It is a source of income and maize leaves and stalks contain about 30% of the total nutrients in corn plants; hence it is utilized for pasture. Some maize are cut and fed green to livestock. Maize occupies a central position in having a meaningful, workable and effective food security system in any society. The dietary importance of maize in the diet of Nigerians cannot be overemphasized; maize, millet, sorghum, rice and other cereals account for about 70% of the total caloric intake in most African countries. The dietary importance of cereals and legumes is increased by the fact that they complement one another. Legumes generally have a high content of lysine, which together with tryptophan tend to be limiting (insufficient) in cereals. On the other hand, cereals are relatively rich in methadone that tends to be limiting in legumes (Aworh, 1999). Consequently, maize and beans provide a better nutrition when eaten together than separately. Maize flour is obtained from processed maize after the removal of the testa by mechanical or local method and is used for dough, pap, bread and biscuit making.

Apart from being one of the major sources of food for both human and animals, maize is also processed into various food and industrial products including starches, sweeteners, oil, beverage, industrial alcohol and fuel ethanol (Parugrug and Roxas, 2008). Moreover, thousands of foods and other everyday items such as toothpaste, cosmetics, adhesives, shoe polish, ceramics, explosives, construction materials, metal moulds, paints, paper goods and textiles contain maize components. In addition, maize products are rapidly replacing petroleum in many industrial applications. Polylactide (PLA), a biodegradable polymer made from corn is being used successfully in the manufacture of a wide variety of everyday items such as clothing, packaging, carpeting, recreational equipment and food utensils of renewable resource (Dick, 1988). Maize is also used as the major study plant for many academic disciplines such as genetics, physiology, soil fertility and biochemistry.

1.2.4 Insect pest problems in maize storage

As in field crops, a wide range of insect pests attack stored products with the commonest among them being adult and larval stages of beetles (Coleoptera) and larval stage of moths (Lepidoptera) (Obeng - Ofori *et al*., 1997). All may be a problem by their presence, either alive or dead, in grain that is to be processed for food. Food grain losses due to insect infestation during storage are a serious problem, particularly in the developing countries (Dubey *et al*., 2008). In many tropical countries, maize is a staple crop and *Sitophilus zeamais* (Coleoptera: Curculionidae) is the major pest in storage with percentage weight losses in storage estimated to be 30 to 80% using damaged grains and a conversion factor. Loss of maize grain, caused by *Sitophilus zeamais,* means that resources such as time, labour, land and money spent in growing the crop are wasted (Agoda *et al*., 2011). Other insects pests of maize in storage include larger grain borer *(Prostephanus truncates* Horn); *Sitophilus oryzae*, *Sitophilus granarium*, *Trogoderma granarium*, Angoumois grain moth *(Sitotroga cerealella* Olivier), red flour beetle *(Tribolium* spp*)* and others. However, in most cases maize weevil has the highest prevalence of up to 100% in all the agro-ecological zones (Ofuya and Lale, 2001).

Maize weevil, *Sitophilus zeamais* (Motschulsky), is a pest of stored maize and of cob maize prior to harvest. Infestations initiated in the standing crop may further develop in storage as the grain dries whether stored as cobs or bulk grain. It may also infest other cereals if the moisture content is moderate or high. Apart from the indirect effects, arising from the production of heat by the insects, the major effect of infestation by the maize weevil *Sitophilus zeamais* is the damage to grain by feeding activities of the adults and the development of immature stages within the grain. Maize weevil cause severe damage to maize resulting in losses in weight, seed viability and nutritive quality of the commodity. It not only reduces the grain quality but also produces a considerable amount of grain dust mixed with frass (CABI, 2010).

1.2.5 Life history of maize weevil

Distribution

S. zeamais occurs throughout the warmer, more humid regions of the world, especially where maize is grown (Longstaff 1981). It has also been recorded from Canada, Polynesia, Argentina, Brazil, Burma, Cambodia, Greece, Japan, Morocco, Spain, Syria, Turkey, USA, Yugoslavia (Mason, 2003). *S. zeamais* is widely distributed throughout growing areas of Northern Australia.

Description

A close relative of the rice weevil, the maize weevil has a length of 2.5 mm to 4 mm. This small, brown weevil has four reddish-brown spots on the wing covers (elytra). It has a long, thin snout, and elbowed antennae. *Sitophilus zeamais* appears similar to the rice weevil (*Sitophilus oryzae*), but has more clearly marked spots on the wing covers, and is somewhat larger. It is also a considerably more proficient flier, allowing it to distribute itself more easily. Its main endemic areas are areas that are tropical and temperate, where maize is grown.The larvae of maize weevils are white, fleshy and legless (CABI, 2010).

Host Range

S. zeamais is commonly associated with corn and rice in tropical storage and to a lesser extent in other raw or processed cereals, including wheat, oats, barley, sorghum, rye and buckwheat. The range of moisture contents within which it will breed has been found to be much wider than that of *S. oryzae* and it has been found to attack fruit, such as apples, in storage. *S. zeamais* commonly infests standing crops prior to harvest, particularly maize, where the moisture contents can exceed 20% (Longstaff, 1981).

Reproduction

The adult female Maize weevil lays her eggs within the actual grain kernels of the maize at a rate of 25 per day spread over 100 days. The maize weevil bores into the grain with her long snout, deposits her ovipositor and lays a single egg. It then secretes a mucilaginous plug to enclose the egg as the ovipositor is withdrawn. The plug rapidly hardens, leaving a small raised area above the seed surface, which provides the only external evidence that the kernel is infested.The eggs hatch in approximately 3 days, depending on the humidity and moisture content of the grain (Mason, 2003).

Development

The larvae, which are approximately 4 mm, white and legless, begin to eat the internal contents of the maize while developing, which takes approximately 18 to 23 days. There are four larval instars all of which remain within the grain. Immediately on hatching, the first instar feeds by burrowing through the tissues of the grain. At the end of the fourth instar the larva uses a mixture of frass and larval secretion to close off the end of the burrow, to form a pupal cell. Under normal developmental conditions, weevil larvae allow their frass to accumulate around them inside the grain in which they are feeding. However, if the carbon dioxide level exceeds 5%, the fourth instar larva makes a small hole in the grain and ejects much of the frass. The larva then assumes a prepupal form for a short period before transforming into the pupa (Longstaff, 1981). This process takes approximately 6 days. During these 6 days, the pupa does not eat or move. It then transforms into adult.

Maturation

When the adult has developed, it remains inside the grain for several days before emerging, with the time varying with temperature (Longstaff, 1981). During this time, its cuticle hardens and matures. Adults emerge by cutting a small circular hole in the grain and females move to a surface above the food to release sex pheromone. Males are attracted to this pheromone for mating (Mason, 2003) and the process begins all over again. The entire process takes about 30 to 45 days to complete. The adult maize weevil will also feed on the maize during its lifespan, which is approximately 5 to 8 months long, before dying. Breeding conditions require temperatures between 15 and 34° C and 40% relative humidity (CABI, 2010).

Figure 1: Newly emerged *Sitophilus zeamais* adults on maize seed, showing emergence holes.

Source: CGC (2013)

1.2.6 Conditions favouring insect pest attack

Insect pest populations in stored maize are influenced by availability of food, relative humidity, temperature and moisture content of the grains. Temperatures of 27-31°C are optimal for development of pests of stored maize grains. At temperatures below 14°C and above 42°C development generally does not take place. Most storage pests die at temperatures below 5°C and above 45°C. The optimum relative humidity for most storage maize pests lies at around 70%, the minimum being 25-40% and the maximum 80-100% (Koona and Njoya, 2004). Very few species are able to survive in extremely dry conditions. Storage pests for maize grains are not able to develop quickly or breed successfully in very dry conditions. Their rates of development below 11.5% moisture content are distinctly slower, and at moisture contents below 8% many fail to breed at all. Moisture contents of 12-18% favour rapid increase especially in suitable temperature and relative humidity conditions. Moisture contents of maize in store should be below 13.5%. With insufficient drying, the grain is usually extremely satisfactory for insect breeding. In addition to these factors, poor storage hygiene and the presence of old stock encourage early infestation of the new stock. Also infestations in store result from the pests flying into storage facilities, probably attracted by the odour of the stored grain. In stored maize, heavy infestation of this pest may cause weight losses of as much as 30-40%, although losses are commonly 4-5% (Casey, 1994).

1.2.7 Damage caused by stored maize pests

Maize storage insect pests fall under two main categories:

Primary pests: These are able to feed inside whole, healthy and well-stored maize grains. **Secondary pests:** These can only attack broken grain, moist and thus soft grain; grain damaged by primary pests or processed products like flour.

Sitophilus zeamais is a primary pest and damage is caused by adult feeding and larvae tunneling within the grains (Mason and Obermeyer, 2010).

The pest causes hollowing of whole previously undamaged grains. In severe infestations only the grain hull is left along with powdery white frass (insect waste). The large emergence holes with irregular edges are characteristic. Grains which float in water often indicate larval damage. Direct-feeding damage by insects reduces grain weight, nutritional value, and germination of stored grain. It also brings about increased respiration in the cereal (hot spots), which promotes evolution of heat and moisture and in turn provides favorable living condition for moulds leading to production of aflatoxin. Subsequently, at very high moisture levels, bacterial growth is favoured which ultimately gives rise to depreciation and finally total loss (Dahiya, 1999). Infestations make grains unfit for processing into human and animal food and commercial grain buyers may refuse to accept delivery of insect contaminated grain, or may pay a reduced price.

1.2.8 Pesticides application and their side effects

Since the 1950s, synthetic insecticides have been used extensively in grain facilities to control stored product insect pests. Fumigants such as methyl bromide, phosphine, cyanogens, ethyl formate, or sulfuryl fluoride rapidly kill all life stages of stored product insects in a commodity or in a storage structure. Fumigation is still one of the most effective methods for the prevention of stored product losses from insect pests. But pests develop resistance and stored products were showing a slow upsurge in fumigation resistance (Donahaye, 2000). Resistance to phosphine is so high in Australia and India, it may cause control failures (Rajashekar *et al*., 2006; Leelaja *et al*., 2007,). Methyl bromide has been identified as a major contributor to ozone depletion (WMO, 1994) and has been banned in developed countries, and developing nations have committed to reducing the use by 20% in 2005 and phase out in 2015.

Contact insecticides such as malathion, chlorpyrifos, or deltamethrin are sprayed directly on grain or storage structure for protection from infestation for several months. The incidence of insecticide resistance is a growing problem in stored-product protection. Resistance to one or more insecticides has been reported in at least 500 species of insects and mites (Georghiou, 1990). Champ (1985) reported that resistance to pesticides used to protect grain and other stored food stuffs is widespread and involves all groups of pesticides and most of the important pests. Some of the contact insecticides have become ineffective because of widespread resistance in insect population. Resistance to malathion is widespread in Canada, USA and Australia (Subramanyam and Hagstrum, 1995). Stored product insect pests were found to be resistant against different insecticides including the cyclodienes, chlorpyrifos, cyanophos, carbaryl, cypermethrin, dichlorodiphenyltrichloroethane (DDT), deltamethrin, diazinon, dichlorovos, ethylene bromide, ethyl formate, permethrin, pyrethrins, and propoxur. Although chemical insecticides are effective; their repeated use has led to residual toxicity, environmental pollution and an adverse effect on food besides side effects on humans (Dubey *et al.,* 2007). Their uninterrupted and indiscriminate use not only has led to the development of resistant strains but also accumulation of toxic residues on food grains used for human consumption that has led to the health hazards (Sharma and Meshram, 2006). In view of all these problems, several insecticides have either been banned or restricted in their use.

1.2.9 Botanicals as alternatives to synthetic pesticides in stored grain protection

Botanical pesticides have long been touted as attractive alternatives to synthetic chemical pesticides for pest management because botanicals reputedly pose little threat to the environment or to human health. The increasing serious problems of resistance to pesticides and contamination of the biosphere associated with large-scale use of broad spectrum synthetic pesticides have led to the need for effective biodegradable pesticides with greater selectivity. This awareness has created a worldwide interest in the development of alternative strategies, including the discovery of newer insecticides (Dayan *et al.,* 2009). However, newer insecticides will have to meet entirely different standards. They must be pest specific, non-phytotoxic, non-toxic to mammals, eco-friendly, less prone to pesticide resistance, relatively less expensive, and locally available. This has led to re-examination of the century-old practices of protecting stored products using plant-derivatives, which have been known to resist insect attack (Ewete *et al.,* 1996; Sahayaraj, 2008). Plant derived materials are more readily biodegradable, less likely to contaminate the environment and may be less toxic to mammals. Therefore, today, researchers are seeking new classes of naturally occurring insecticides that might be compatible with newer pest control approaches (Talukder and Howse, 1995). Since ancient times; there have been efforts to protect harvest production against pests. The Egyptian and Indian farmers used to mix their stored products with fire ashes (Abdel-Gawad and Khatab, 1985; Varma and Dubey, 1999). The ancient Romans used false hellebore (*Veratrum album*) as a rodenticide, the Chinese is credited with discovering the insecticidal properties of *Derris* species, whereas pyrethrum was used as an insecticide in Persia and China (Ahmed and Grainge, 1986). In many parts of the world, locally available plants are currently in wide use to protect stored products against damage caused by insect infestation (Khater, 2012). Indian farmers used neem leaves and seed for the control of stored grain pests (Ahmed and Koppel, 1985).

In Northern Cameroon, cowpeas are traditionally mixed with sieved ash after threshing and the mixture put into mud granaries or clay jars (Wolfson *et al.,* 1991). In Eastern Africa, leaves of the wild shrub *Ocimum suave* and the cloves of Eugenia aromatic are traditionally used as stored grain protectants (Powel, 1989). In Rwanda, farmers store edible beans in a traditional closed structure (Imboho) and whole leaves of *Ocimum canum* are usually added to the stored foodstuff to prevent insect damage within these structures (Weaver *et al.,* 1991). Owusu (2001) suggested natural and cheaper methods for the control of stored product pests of cereals, with traditionally useful Ghanaian plant materials. In some south Asian countries, food grains such as rice or wheat are traditionally stored by mixing with 2% turmeric powder (Chatterjee, 1980; Saxena *et al.,* 1988). The use of oils in stored-products pest control is also an ancient practice. Botanical insecticides such as pyrethrum, *Derris,* nicotine, oil of citronella, and other plant extracts have been used for centuries (Singh and Upadhyay, 1993). Turmeric, garlic, *Vitex negundo*, Gliricidia, castor, Aristolochia, ginger, *Agave americana*, custard apple, *Datura*, *Calotropis*, *Ipomoea* and coriander are some of the other widely used botanicals to control and repel crop pests. Talukder (2006) listed 43 plant species as insect repellents, 21 plants as insect feeding deterrents, 47 plants as insect toxicants, 37 plants as grain protectants, 27 plants as insect reproduction inhibitors, and 7 plants as insect growth and development inhibitors. Eighteen species showed insecticidal potential and anti-ovipositional properties against *Sitophilus oryzae* (Devi and Devi, 2011).

1.2.10 Classification and Mode of Action of Pesticidal Plants

Plants contain thousands of constituents and are valuable sources of new and biologically active molecules possessing insecticidal properties. The ultimate aim of recent research in this area has been the development of alternative control strategies to reduce dependency on synthetic pesticides. Tissues of higher plants contain arrays of biochemicals known as secondary metabolites which are defensive in function. They include alkaloids, saponins, steroids, resins, phenolics, tannins, essential oils, various organic acids and other compounds. It is well known that secondary plant metabolites may act as kairomones, allomones, stimulants or deterrents of feeding and oviposition and as anti-feedants, insecticides and insect hormone mimics (Talukder, 2006). On the basis of physiological activities on insects, plant components have been conventionally classified into 5 groups, namely:

- Repellents
- Feeding deterrents/anti-feedants
- Toxicants
- Reproduction inhibitors
- Growth retardants

Repellents

Repellents are desirable chemicals as they offer protection with minimal impact on the environment as they drive away insect pests from the treated materials by stimulating olfactory or other receptors of insects. Repellents are the substances which as stimulants elicit avoiding reactions and are non-poisonous. Repellent substances cause insects to orient their movement away from a food source. Repellents are usually volatile chemicals and express their activity in volatile phase. A strong repellent will be sensed by insects from a few centimetre distance causing them to fly or crawl away (Dwivedi and Shekhawat, 2004). Many researchers have reported the repellent effects of some plants.

Feeding deterrents/Anti-feedants

Feeding deterrents sometimes referred to anti-feedants are defined as chemicals which retard or disrupt insect feeding by rendering the treated materials unattractive or unpalatable. Anti-feedants do not kill insects directly. Insects remain on treated food indefinitely and eventually starve to death without eating (Saxena *et al*., 1988).

Toxicants

Toxicants are specific types of chemicals which directly kill insects. They are also referred to as insecticides. Worldwide reports on the toxicity of different plant derivates showed that many plant products are toxic to stored-product insects (Talukder and Howse, 1994; Obeng-Ofori *et al*., 1997; Tripathi *et al*., 2000).

Reproduction inhibitors

Reproductive inhibitors are chemicals that inhibit or retard reproductive abilities of insects by suppressing fecundity and fertility in them. Many researchers have reported that plant parts, oil or extracts mixed with grain reduced insect oviposition, egg hatchability, post-embryonic development and progeny production (Schmidt *et al*., 1991). Plant derivatives also reduce the survival rates of larvae and pupae and adult emergence (Mukanga *et al*., 2010). Development of eggs and immature stages inside grain kernels are also inhibited by plant derivatives (Tripathi *et al*., 2000).

Growth retardants

Growth retardants are chemicals that retard growth and development in insects, cause mortality of larva, cuticle melanization and high mortality in adults. It has been proven that plant derivatives show deleterious effects on the growth and development of insects and reduced larval, pupal and adult weight significantly, lengthened the larval and pupal periods and reduced pupal recovery and adult eclosion (Sagheer *et al*., 2011).

Pesticidal plants are utilized in two main ways: first, the active compounds are isolated, identified and chemically synthesized. If feasible these compounds or their active analogues are synthesized and marketed by the chemical industry. For example, nicotine, pyrethrins, azadirachtin and rotenoids have been isolated, characterized and developed as commercial insecticides. The second approach is suitable for farmers in developing countries and for organic farming. Plant tissues or crude products of the plant, such as aqueous or organic solvents extracts, are used directly. These practices are labour intensive, but are often economically and ecologically sound and do not require sophisticated technology (Yang and Tang, 1988).

1.2.11 Importance of Pesticidal Plants in IPM

The control of insects includes everything that makes life hard for insects and tends to kill them. Integrated Pest Management (IPM) involves the careful consideration of all available pest control techniques and subsequent integration of appropriate measures that discourage the development of pest populations and keep pesticides and other interventions to levels that are economically justified and reduce or minimize risks to human health and the environment. IPM emphasizes the growth of a healthy crop with the least possible disruption to agro-ecosystems and encourages natural pest control mechanisms. The use of pesticidal plants in stored-product insect pest management is one of the strategies of Integrated Pest Management since these plants are eco-friendly and pose no hazard to non-target organisms. The chemical control is a very quick, effective and popular method of pest control, but these create acute and chronic poisoning in man and other non-target animals. Therefore, it is used as a last resort in IPM when other control methods fail or when careful monitoring indicates they are needed according to preestablished guidelines. When required, the least hazardous and the lowest effective amount of pesticides are used. It is recommended that farmers switch to more natural means of stored grain insect pest control through the use of pesticidal plants and other natural methods and rely less on chemical control.

1.2.12 Description of research plants

Allium sativum

Allium sativum (garlic) is from the Alliaceae family like its close relatives chives, leek and onions. The edible herb most commonly associated with the name is the bulb of garlic cloves that is found underground, below the leafy, scallion-like growth. Garlic is a bulbous perennial plant growing up to 1 metre in height. It grows from an underground bulb which sends shoots up in the air. A garlic bulb, composed of 4-60 cloves, can be 1.5 to 3 inches in diameter (4 to 7.5 cm) and grow to a height of 10 inches to 5 feet (10 cm to 1.5 m). The flowers are white with a rose or green cast. The bulbs themselves are creamy white and may have a purplish hue, as may the paper-like covering that surrounds the bulb and encloses each clove. With a history of human use of over 7,000 years, garlic is native to central Asia but it is now grown all over the world, preferring full sun and well drained soil (Fields *et al*., 2001).

Garlic has long been a staple crop in the Mediterranean region, as well as a frequent seasoning in Asia, Africa, and Europe. Previous works on the phytochemistry of garlic suggests that it is rich in biochemical constituents like alkaloids, glycosides, saponins, steroids, tannins, terpenoids, reducing sugars and acidic compounds, mainly sulphuric compounds. Hence, it has been proven by many researches that garlic has anti-feedant, bacterial, fungicidal, insecticidal, nematicidal and repellent properties (Loth *et al*., 2007). Garlic is effective against a wide range of insects at different stages in their life cycle (egg, larvae, adult). This includes ants, aphids, army worms, caterpillars, Colorado beetle, diamondback moth, pulse beetle, whitefly, wireworm, false codling moth, imported cabbage worm, khapra beetle, mice, mites, moles, Mexican bean beetle, peach borers and termites as well as fungi and bacteria.

Figure 2: Garlic bulbs and individual cloves, one peeled.

Aloe vera

Aloe vera (synonym: *Aloe barbadensis* Miller) is a perennial succulent plant belonging to the Liliaceae family (subfamily of the Asphodelaceae) (Eggli, 2001). From 400 *Aloe* species, *Aloe vera* is most widely accepted and used for various medical and cosmetic purposes (Grindlay and Reynolds, 1986; Khaing, 2011). It is a shrubby or arborescent, xerophytic, pea-green colour plant. It grows mainly in the dry regions of Africa, Asia, Europe and America.

The plant is made of triangular fleshy leaves joined at the stem in a rosette pattern. Each leaf consists of three parts: an inner clear pulp (gel), a middle layer of latex which is the bitter yellow sap and an outer thick layer called rind which has protective function. The margin of the leaf is serrated and has small white teeth. *Aloe vera* leaves contain phytochemicals under study for possible bioactivity, such as acetylated mannans, polymannans, anthraquinone C-glycosides, anthrones, anthraquinones such as emodin, and various lectins (Boudreau and Beland, 2006). Some of these compounds are used to manufacture insecticides (Yang *et al*., 2003).

Figure 3: *Aloe vera* plant with flower detail inset

CHAPTER TWO

MATERIALS AND METHODS

2.1 Collection and Preparation of Plant Materials

The cloves of garlic, *Allium sativum* and the leaves of *Aloe vera* were purchased from Ogige Market, Nsukka, Enugu State. The garlic cloves were peeled, chopped into small pieces and air-dried in the laboratory until dry. The fresh *Aloe vera* whole leaves were washed with distilled water, cut into smaller sizes and dried in the oven at 50°C for 48hours (Oparaeke and Kuhiep, 2006). The plant materials were ground separately to powder with a laboratory milling machine. The powder was then passed through sieve of 0.25 mm mesh size to obtain uniform particle size after which they were stored in plastic air-tight containers in refrigerator until when needed.

2.2 Preparation of Plant Extracts

The plant extracts were prepared using the method of Yankanchi and Gadache (2010). The powdered plant materials were separately subjected to ethanol extraction with Soxhlet apparatus for 6hours. The extraction of the plant materials was done in the Department of Biochemistry, University of Nigeria, Nsukka. Boiling flasks of 250 ml capacity were washed with detergent and were properly rinsed with clean water. They were dried in the oven at 105 to 110°C for about 30 min. 20 g of the powdered materials were weighed into a labeled thimble. The boiling flask was filled with 250 ml of ethanol and the extraction thimble was plugged tightly with cotton wool. The soxhlet apparatus was then assembled to allow for reflux for about 6 h. After 6 h, the thimble containing the sample was removed with care and the ethanol on the top was drained into a container. The extract was then concentrated using water bath, which removed the ethanol component leaving behind semi-solid residue. The residue obtained was weighed and stored in air-tight containers and maintained in a refrigerator (4^{o}C) until further use.

Plate 1: Preparation of plant extracts using Soxhlet apparatus

2.3 Collection and Preparation of Maize grains

Maize that was used in this study was obtained from Crop Science Department Farm Shop. The maize was air-dried for 3 days and placed in a deep freezer at a temperature below $0^{o}C$ for two weeks to kill any storage insect pests. After disinfestations, the maize grains were air-dried to prevent mouldiness. The grains were then packaged in polythene bags and kept pending use.

2.4 Culturing of *Sitophilus zeamais*

Insect culture was done using the method of Denloye (2010). The insect culture was done at the Entomology Laboratory, Department of Zoology and Environmental Biology, University of Nigeria, Nsukka. *Sitophilus zeamais* that was used to establish the culture was obtained from naturally infested maize grains from Ogige market, Nsukka, Enugu State. Disinfested grains were measured into two clean transparent jars. Each jar contained 200 g of maize into which 100 adult *S. zeamais* (50 males, 50 females) were introduced. The jars were capped with muslin cloth held in place with rubber band to allow ventilation and to prevent possible escape of insects or entry of mites and other insects. The insects were allowed to mate and oviposit for seven days after which they were sieved out with a sieve of 2 mm mesh size.The jars were monitored until adult weevils started emerging. The emerging adult insects were collected and kept in separate jars according to their age. Adults that emerged on the same day were considered of the same age. These F_1 progenies were used for all experiments.

2.5 Experimental Design

2.5.1 Toxicity test

The toxicity test was carried out using the method described by Sagheer *et al.* (2011). Filter papers were treated with different concentrations of the plant extracts ranging between 25mg/ml and 450mg/ml and allowed to dry. These filter papers were placed at the bottom of the disposable plastic cups, 5g of maize was placed on them and 30 adult insects (2-3 day old) were introduced into the cups. The cups were covered with muslin cloth and held in place with rubber bands. The experiment was done in three replicates. For the control, only the solvent (Tween 80) was applied to the filter papers. The set up was examined daily for 3 days and number of dead insects (those that did not respond to pen probes) was recorded every 24 h. Probit analysis was used to determine the concentration of the plant extracts that killed 50% of the test population (LC_{50}) and subsequent experiments were carried out using concentrations below it.

2.5.2 Adult mortality test

The adult mortality test was carried out using the method of Yankanchi and Gadache (2010). Three concentrations (200, 250 and 300mg/ml) of each plant extract were prepared. Each concentration of plant extracts was mixed for about five minutes with maize grains separately (1 ml/50 g maize) and air dried for 10 minutes. Twenty-four hour old adult male *Sitophilus zeamais* (20 in number) were released into bottles containing plant extracts-treated maize and covered with muslin cloth. The muslin cloth was held in place with rubber bands. Another experiment was set up exactly as above but this time mixtures (50:50) of the plant extracts were used. Three replicates were maintained for each concentration of the individual plant extract and for the mixtures. Same quantity of solvent-treated grains served as control. Insect mortality count was taken on 1st, 2nd, 3rd, 4th, 5th, 10th, 15th and 20th day after treatment by sieving out the contents of each bottle into a tray and carefully counting the number of dead insects. The maize grains and surviving insects were returned to their original jars after each exercise and Percentage adult mortality was determined using the formula:

$$\% \text{ mortality} = \frac{\text{No. of dead insects}}{\text{Total no. of insects}} \times 100$$

2.5.3 Repellency test

The method of Talukder and Howse (1995) was adopted in carrying out the repellency test. Three concentrations (200, 250 and 300mg/ml) of the plant extracts were prepared. Whatman filter papers were cut into two equal halves, one half of each filter paper was treated with various concentrations of extracts as uniform as possible by using micro pipette. The other halves of the filter paper were treated with only the solvent as control. The plant extracts-treated and solvent-treated filter paper halves were air-dried to evaporate the solvent completely. Plant extracts-treated and solvent-treated filter paper halves were then attached lengthwise, edge-to-edge with adhesive tape and placed at the bottom of glass petri dishes. Ten adult weevils were released at the centre of the petri dishes and the petri dishes were covered and kept in dark. Three replicates were set for each concentration of plant extracts and for the mixtures. Number of insects present on both treated and untreated halves was recorded after 1 hour and up to 6 hours in mild light.

Percentage repellency was computed as follows:

$$PR = \frac{N_c - N_t}{N_c + N_t} \text{ X } 100\%$$

where: PR = percentage repellency

Nc = insect number present on control strip

Nt = insect number present on treated strip

Negative PR values were treated as zero

2.5.4 Oviposition inhibition test

Laboratory tests for oviposition inhibition effects were conducted according to the method of Talukder and Howse (1994). Whatman filter paper discs were soaked in different concentrations (200, 250 and 300mg/ml) of each plant extract and also in the mixtures and air-dried for an hour. The control filter papers were soaked with the solvent only. The treated and control filter paper discs were placed singly at the bottom of Petri dishes (90 mm diameter) and 30g of maize grains was placed on the papers. Five pairs (5 females and 5 males) of a-day old adult *Sitophilus zeamais* were released into each Petri dish and covered for the next 7 days allowing them to lay eggs. Three replicates were maintained for each concentration of plant extract and for the mixtures.

The adults were then removed and the following data was recorded:

- Number of eggs per 50 seeds in each Petri dish
- Number of F_1 adults emerged in each Petri dish from day 27 ï 42

42 days after the setup the following was calculated:

Percentage of eggs hatching = $\frac{\text{[illegible]}}{\text{[illegible]}} \times 100$

Percentage Inhibition rates, IR% = $\frac{\text{[illegible]}}{\text{[illegible]}} \times 100$

where: Cn = Number of insects in control dish

Tn = Number of insects in treated dish

2.5.5 Damage assessment test

The method of Ileke and Oni (2011) was adopted in carrying out this test. Fifty pairs (25 males and 25 females) of a-day old adult *Sitophilus zeamais* were introduced into plastic containers containing maize grains treated with three concentrations (200, 250 and 300mg/ml) of each plant extract (2ml/100g) and also the mixtures and covered with muslin cloth held in place with rubber band. The set up was left to stand for 42 days. In the control, the maize grains were treated only with the solvent. At the end of the 42-day observation period, the weevils were sieved out and the maize grains in each container weighed. The extent of weevil damage was assessed in terms of weight loss of the maize grains using the formula:

$$\%\text{Weight loss} = \frac{\text{[illegible]}}{\text{[illegible]}} \text{ X } 100$$

2.6 Statistical Analysis

Data was analyzed using Statistical Package for Social Sciences (SPSS version 17.0). Results of repellency test were transformed to percentages. One-way Analysis of Variance (ANOVA) followed by New Duncanôs Multiple Range Test (NDMRT) were used to compare percentage repellency, adult mortality, oviposition and damage assessment test of the treated and control groups. Lethal concentration (LC_{50}) of test plants was calculated using probit analysis. All analyses were done at the 5% level of significance.

Plate 2: Experimental set up for the bioassays

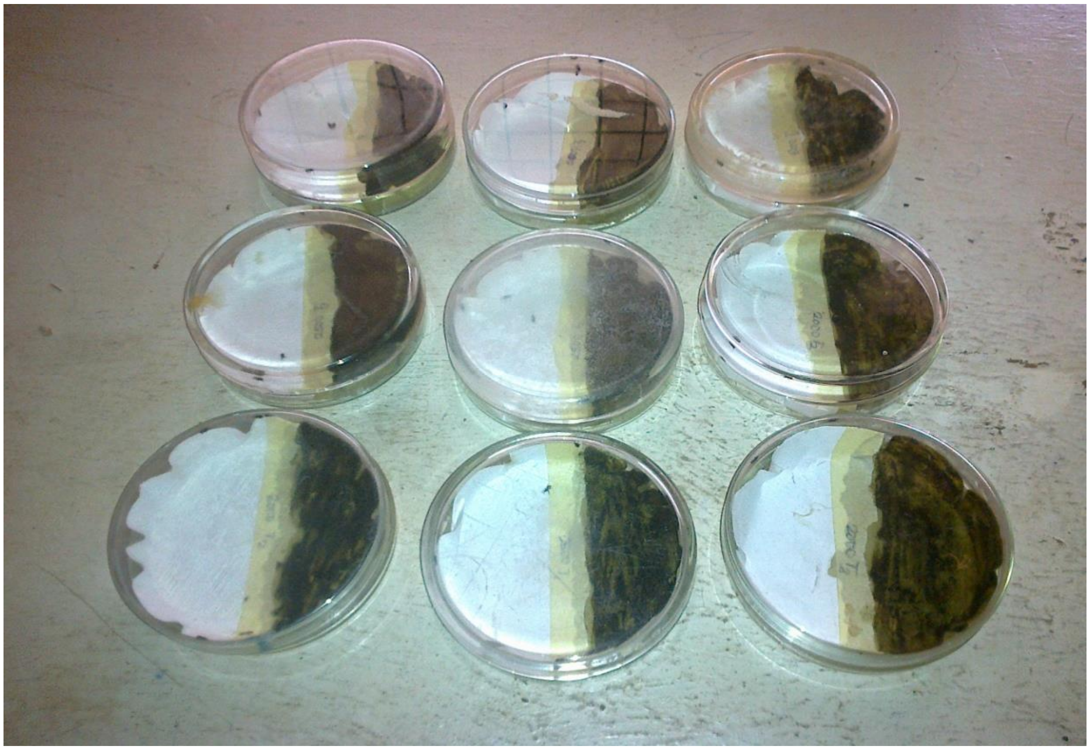

Plate 3: Experimental set up for repellency test

CHAPTER THREE

RESULTS

3.1 Acute Toxicity Results of Ethanolic Extracts of *Allium sativum* and *Aloe vera*

Probit analysis revealed that at 24h of exposure of maize weevil to test extracts, the concentration of garlic extract that killed different percentage number of weevils was lower when compared to that of *A. vera* extract. Comparing lethal concentrations of the two extracts at 48h, *Aloe* extract was more efficient at lower concentrations while at 72h, lower lethal concentrations were recorded for garlic extract.

Table 1: Lethal concentrations of garlic and *A. vera* extracts at different durations of treatment

Lethal concentrations (mg/ml)	24h		48h		72h	
	Garlic extract	*Aloe vera* extract	Garlic extract	*Aloe vera* extract	Garlic extract	*Aloe vera* extract
LC_{25}	228.07	270.67	4923.17	954.96	1426.08	2576.09
LC_{50}	415.12	454.32	23487.29	2278.54	3700.27	7991.83
LC_{75}	755.54	762.57	112052.69	5434.00	9601.12	24793.01
LC_{90}	1295.22	1215.39	457271.14	11883.73	22647.19	68684.65

3.2 Adult Mortality Effects of Ethanolic Extracts of *Allium sativum*, *Aloe vera* and their Mixture

The mean weevil mortality of the mixture of garlic and *Aloe* performed significantly better ($P<0.05$) than just garlic or *Aloe* at 24h for 200 and 250mg but there was no significant difference ($P>0.05$) in the mortality of weevils at 300mg for the different extracts used. Comparing individual test extract at different concentrations, it was observed that except for the mixture, 300mg of extract caused significantly higher ($P<0.05$) mortality than 200 and 250mg at 24 h. At 48 and 72h, there was no marked difference in the mortality of weevils for individual extract (Table 2).

Significantly higher ($P<0.05$) percentage mortality was recorded at all concentrations in the mixture of the two extracts than when they were used singly. Comparing the percentage mortality of the two extracts singly, the garlic extract had significantly higher ($P<0.05$) performance than *Aloe* (Table 2). No mortality was recorded in the untreated check.

Table 2: Mortality of maize weevils exposed to sublethal concentrations of extracts

	Garlic	*Aloe*	Mixture	Control (solvent-treated)
24h				
200	4.67 ± 0.33^{b3}	4.33 ± 0.33^{b3}	5.67 ± 0.33^{a2}	0.00 ± 0.00
250	6.33 ± 0.33^{b2}	6.00 ± 0.00^{b2}	7.33 ± 0.33^{a1}	
300	7.67 ± 0.33^{a1}	7.67 ± 0.33^{a1}	7.67 ± 0.33^{a1}	
48h				
200	0.00 ± 0.00^{a1}	0.00 ± 0.00^{a1}	0.67 ± 0.33^{a2}	0.00 ± 0.00
250	0.67 ± 0.33^{ab1}	0.00 ± 0.00^{b1}	1.33 ± 0.33^{a12}	
300	1.33 ± 0.33^{a1}	0.00 ± 0.00^{b1}	1.67 ± 0.33^{a1}	
72h				
200	0.00 ± 0.00^{a1}	0.00 ± 0.00^{a1}	0.33 ± 0.33^{a1}	0.00 ± 0.00
250	0.00 ± 0.00^{a1}	0.00 ± 0.00^{a1}	0.33 ± 0.33^{a1}	
300	0.33 ± 0.00^{a1}	0.00 ± 0.00^{a1}	1.00 ± 0.00^{a1}	
Percentage mortality				
200	23.33 ± 2.89^{b3}	21.67 ± 1.67^{b3}	33.33 ± 1.67^{a3}	0.00 ± 0.00
250	35.00 ± 0.00^{b2}	30.00 ± 0.00^{c2}	45.00 ± 0.00^{a2}	

300	46.67 ± 1.67^{b1}	38.33 ± 1.67^{c1}	51.67 ± 1.67^{a1}

Mean values with alphabets as superscript are used to compare extracts at different concentrations (rows). Mean values with figures as superscripts are used to compare concentrations for the different extracts (columns)

3.3 Repellency Effects of Ethanolic Extracts of *Allium sativum*, *Aloe vera* and their Mixture

Fig. 1 compares the percentage repellency of extracts at different concentrations. Consistently, at all concentrations, all extract tested were able to significantly repel more ($P<0.05$) weevils when 300mg is used compared to other concentrations. Except for 200mg/ml, the percentage repellency of *Aloe* extract was significantly lower ($P<0.05$) than that of garlic.

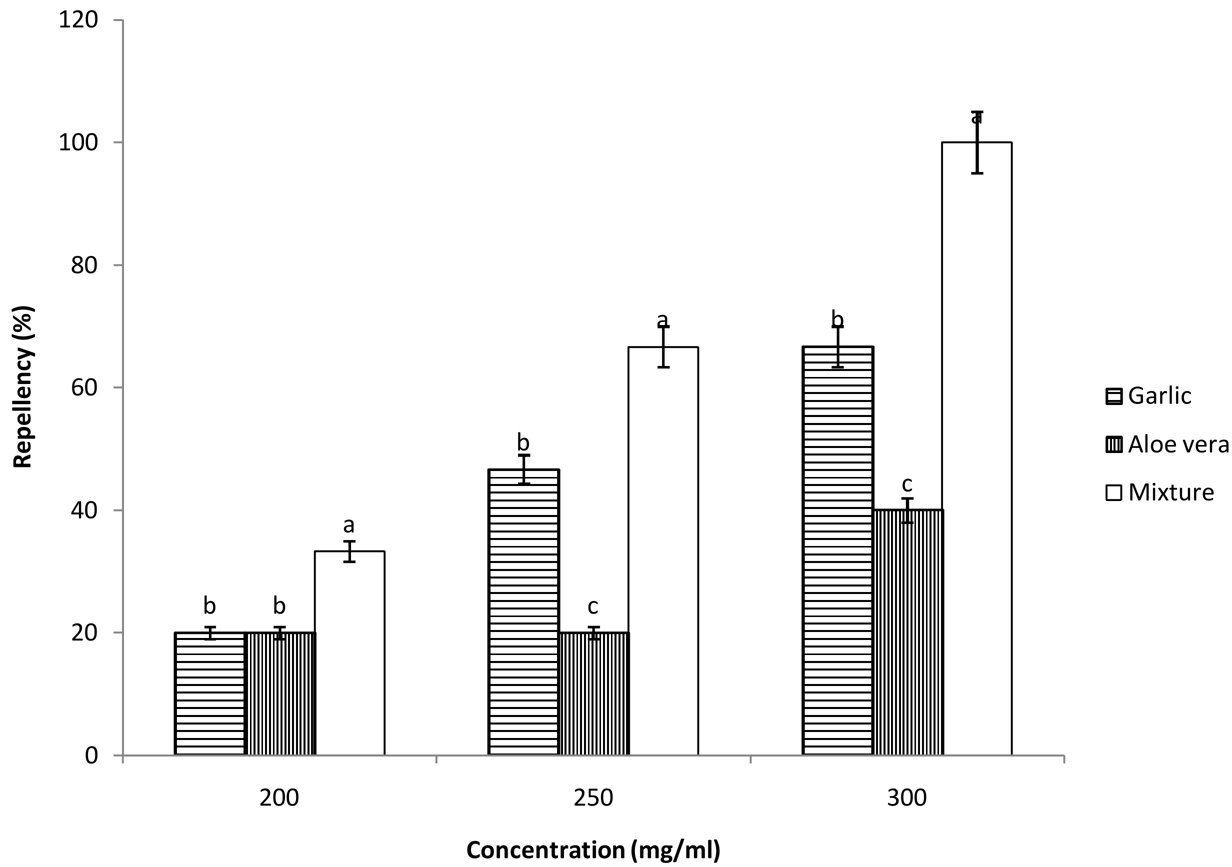

Fig 4: Comparison of percentage repellency of extracts at different concentrations
Mean values with different alphabet are significantly different (P<0.05)

For garlic and the mixture extracts, percentage repellency was significantly different ($P<0.05$) in the following order: 300mg/ml>250mg/ml>200mg/ml. Using *Aloe* extract, 300mg/ml had significantly higher repellency ($P<0.05$) than the other two concentrations while between 200 and 250mg/ml concentrations of *Aloe*, there was no significant difference ($P>0.05$) in percentage repellency (Fig. 2).

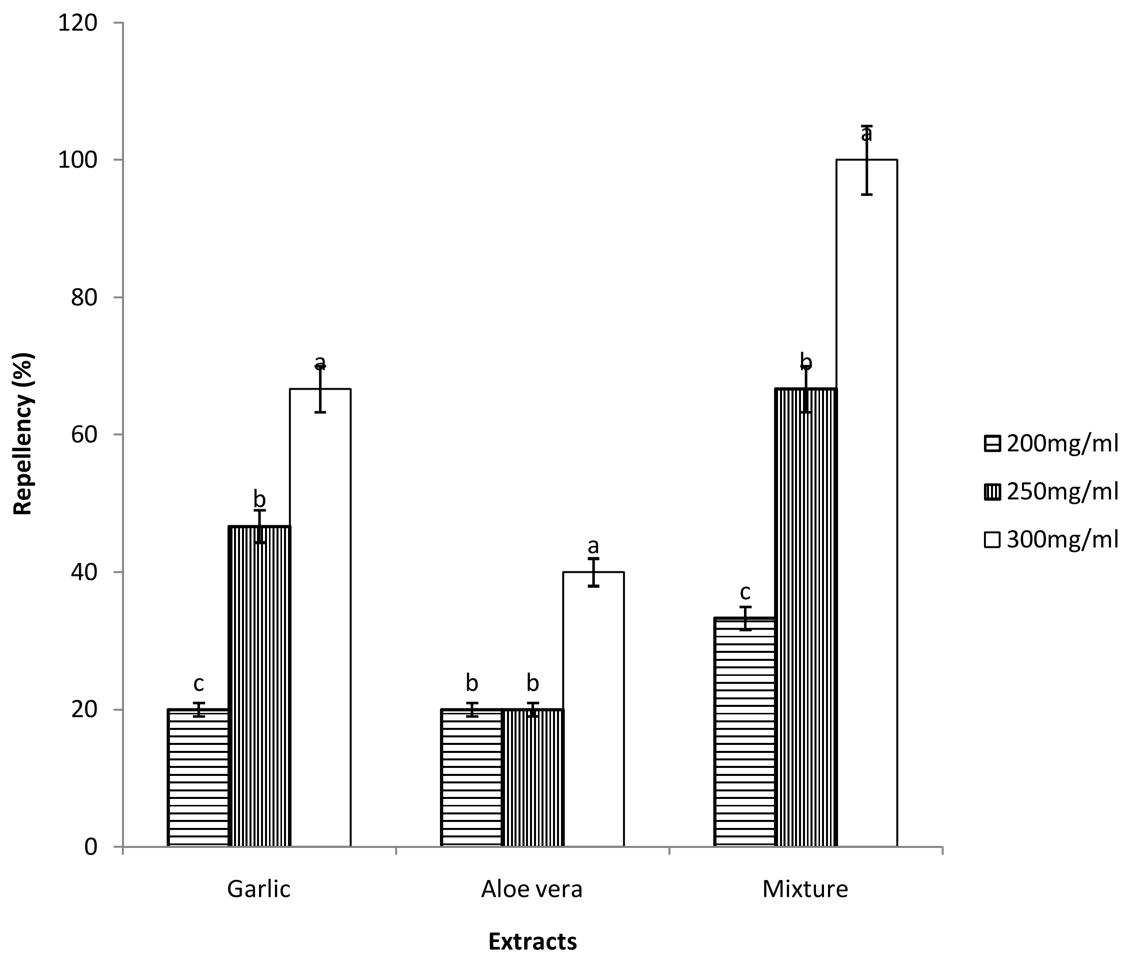

Fig. 5: Comparison of percentage repellency at different concentrations for individual extracts
Mean values with different alphabet are significantly different (P<0.05).

3.4 Oviposition Inhibition Effects of Ethanolic Extracts of garlic, *Aloe vera* and their Mixture

Comparing extracts at different concentrations, number of eggs and F1 adults hatched was significantly reduced ($P<0.05$) in the mixture followed by garlic and *Aloe* extracts while it was higher in the untreated check. For individual extract type, the number of eggs and FI adults hatched differed significantly ($P<0.05$) in different concentrations in the following order: 300mg/ml>250mg/ml>200mg/ml.

At 200mg/ml, there was no significant difference ($P>0.05$) in the percentage eggs hatching in garlic and *Aloe* extracts while in the mixture extract, the number of eggs hatching was significantly lower ($P<0.05$). When 250mg/ml extract was applied, the percentage number of eggs hatching was significantly higher in *Aloe* extract compared to other extracts and at 300mg/ml, percentage eggs hatching was significantly reduced ($P<0.05$) in garlic extract compared to those of other extracts. For specific extract performance at different concentrations, the percentage number of eggs hatching was significantly different at the different concentrations for garlic and *Aloe* extracts, with 300mg/ml having the least percentage number of eggs hatched. However, in the mixture, there was no significant difference ($P>0.05$) in the number of eggs hatched at different concentrations.

The mixture extract and 300mg/ml concentration had significantly higher ($P<0.05$) percentage inhibition than other extract and concentrations, respectively (Table 3). The lowest percentage inhibition was recorded in the untreated check.

Table 3: Effect of extracts on oviposition of maize weevils

	Garlic extract	*Aloe* extract	Mixture	Control (solvent-treated)
Number of eggs				
200mg/ml	30.33 ± 0.33^{b1}	37.00 ± 0.33^{a1}	25.67 ± 0.33^{c1}	45.27 ± 0.84^{d}
250mg/ml	26.67 ± 0.33^{b2}	34.33 ± 0.33^{a2}	20.67 ± 0.33^{c2}	
300mg/ml	21.67 ± 0.33^{b3}	32.00 ± 0.58^{a3}	17 ± 0.00^{c3}	
F1 adults				
200mg/ml	27.67 ± 0.33^{b1}	34.67 ± 0.33^{a1}	21.33 ± 0.33^{c1}	43.09 ± 0.32^{d}
250mg/ml	21.67 ± 0.33^{b2}	34.33 ± 0.33^{a2}	16.67 ± 0.33^{c2}	
300mg/ml	17.00 ± 0.58^{b3}	28.67 ± 0.33^{a3}	14.00 ± 0.00^{c3}	
Percentage eggs hatching				
200mg/ml	91.20 ± 1.05^{a1}	92.07 ± 1.47^{a1}	83.13 ± 1.88^{b1}	95.35 ± 1.45^{d}
250mg/ml	81.30 ± 1.97^{b2}	89.33 ± 0.94^{a2}	80.67 ± 0.33^{c1}	
300mg/ml	78.43 ± 1.71^{c3}	89.60 ± 0.85^{a3}	82.40 ± 0.00^{b1}	
Percentage inhibition				
200mg/ml	38.53 ± 0.73^{c3}	22.93 ± 0.73^{b3}	52.67 ± 0.73^{a3}	19.09 ± 0.00^{d}
250mg/ml	51.83 ± 0.73^{c2}	31.83 ± 0.73^{b2}	62.93 ± 0.73^{a2}	

300mg/ml	62.20 ± 0.27^{c1}	36.33 ± 0.73^{b1}	68.90 ± 0.00^{a1}

Mean values with alphabets as superscript are used to compare extracts at different concentrations (rows). Mean values with figures as superscripts are used to compare concentrations for the different extracts (columns)

3.5 Damage Assessment Test Results

There was significant difference in the final weight of maize grains after treatment, with more weight lost in the untreated check followed by grains treated with *Aloe* extract. Considering concentration, the weight of grains was significantly low in 200mg/ml for all extracts. Similarly, there was significant higher weight loss for grains treated with *Aloe* extract and 200mg/ml concentration when compared to those of other extracts and concentrations (Table 4).

Table 4: Effect of extracts on weight of maize grains after treatment

	Garlic extract	*Aloe* extract	Mixture	Control (solvent-treated)
Final weight				
200mg/ml	92.15±0.55^{b3}	82.92±0.38^{c3}	94.49±0.20^{a3}	70.64±0.34^{d}
250mg/ml	94.17±0.31^{b2}	85.71±0.26^{c2}	96.90±0.08^{a2}	
300mg/ml	95.41±0.24^{b1}	89.20±0.52^{c1}	98.19±0.12^{a1}	
Percentage weight loss				
200mg/ml	7.87±0.57^{b1}	17.10±0.36^{a1}	5.50±0.21^{c1}	29.31±0.71^{d}
250mg/ml	5.80±0.82^{b2}	14.30±0.25^{a2}	3.13±0.19^{c2}	
300mg/ml	4.60±0.23^{b3}	10.80±0.52^{a3}	1.80±0.12^{c3}	

Mean values with alphabets as superscript are used to compare extracts at different concentrations (rows). Mean values with figures as superscripts are used to compare concentrations for the different extracts (columns).

CHAPTER FOUR

DISCUSSION

The constraints of synthetic pesticides have led to increased interest in the application of botanical pesticides for crop production. Pesticidal plants are considered to be non-pollutant, less toxic and easily biodegradable. The active components in pesticidal plants are non-persistent with many being UV labile and others broken down through oxidation or by microorganisms thus presenting lower risks to consumers (Pugazhvendan *et al*., 2012). In recent years the use of plant derived insecticides is gaining importance and many plants have been screened for their insecticidal properties by many researchers.

In the present study, garlic, *Allium sativum* (Alliaceae) and *Aloe vera* (Liliaceae) were screened for their insecticidal action against *Sitophilus zeamais.* Members of the genus *Allium* and *Aloe* have been known to demonstrate repellent and insecticidal properties against economically important insect pest species and a few workers have reported their potency against other insects. Ekeh *et al*. (2013) investigated the effectiveness of some plant powders, *Allium sativum* being one of them, for the protection of some legumes and found that powder of *Allium sativum* is effective in killing adult *Callosobruchus maculatus.* Omotoso and Oso (2005) demonstrated that *Aloe vera* leaf powder and root ash have insect productivity reduction capacity on the beetle, *Tribolium castaneum.* Dauda *et al*. (2012) worked on insecticidal activity of garlic oil on *Callosobruchus maculatus* in post- harvest cowpea and found that grain treatment with garlic oil before oviposition drastically suppressed oviposition, egg-hatch and development by the cowpea bruchid and consequently the emergence of few to zero adults. Both pre- and post- oviposition grain treatments were effective in the management of the beetle. Garlic emulsion (Sirinol) has been demonstrated to have repellency effects on adults of cigarette beetles, *Lasioderma serricone* and red flour beetles, *Tribolium castaneum*. The insects were exposed to 0, 0.5, 1.5, and 10% concentration and the maximum percentage repellency occurred at 10% concentration. Results also showed that *T. castaneum* was more susceptible to Sirinol than *L. serricone*. (Jahromi *et al.,* 2012). Denloye (2010) investigated the bioactivity of powders, extracts, and essential oils from garlic and spring onions against adults, eggs, and larvae of *Callosobruchus maculatus* and reported that at 48 h median toxicity (LC_{50}), test plant powders and extracts from garlic were more toxic to *C. maculatus* adults than those from spring onions. Sarwar *et al.,*

(2012) evaluated the potential of *Nicotiana tobacum*, *Citrullus colocythis* and *Aloe vera* for the management of the cowpea bruchid, *Callosobruchus analis* population in gram seed (*Cicer arietinum*), during storage and reported that seeds treatment with *A. vera* followed by *N. tabacum* reduced maximum pest damage over *C. colocythis,* which proved least effective to control *C. analis* population. Fekadu *et al*. (2012) conducted a study to evaluate selected locally available botanical powders and cooking oils for their effectiveness as grain protectants against maize weevils. Among the botanicals used, *Chenopodium ambrosoides*, *Tagetus erecta*, *Azadirachta indica* leaf and bark powder registered higher adult mortality (70%) followed by *A. indica* kernel (65%), *C. citratus*, *Maesa lanceolata* and *Echinops kebericho* (55%) and *Allium sativum* (50%). Highest *S. zeamais* adult mortality of 95% and 100% was recorded from *Brassica carinata* oil and *Gossypium hirsutum* oil.

The results in the present study have shown that the ethanolic extract of garlic cloves and *Aloe vera* leaves and their mixture are toxic to *S. zeamais* and can suppress progeny development of the weevils by having oviposition inhibition and anti-feedant effects, thus resulting in better protection of maize grains from pest damage. This reinforces earlier observations that members of the genus *Allium* and *Aloe* are potent against a variety of insects.

The result of the probit analysis shows that garlic extract has lower LC_{50} value at 24h and therefore was more toxic than *A. vera* extract. Consequently, garlic extract caused higher mortality (35%) than *A. vera* extract (30%) in increasing concentration but their mixture performed better (43.3%). This finding is in conformity with the work of Fekadu *et al.,* 2012 who reported that *A. sativum* powder applied at the rate of 0.5g/250g of maize caused 50% mortality of *Sitophilus zeamais.* It also agrees with the work of Danjuma *et al*., (2009) who reported that *A. sativum* powder caused 96.67% mortality of *Sitophilus zeamais* applied at the rate of 2.0g/50g of maize grains. The disparities in the percentage mortalities maybe due to differences in the application rates.

The higher toxicity shown by *A. sativum* may be because the active principles responsible for the activity of the test extracts were present in higher quantities in the *A. sativum* than *A. vera*. Also *A. sativum* may have been very potent because of the strong choky odour it produces which may have exerted a toxic effect by disrupting normal respiratory activity of the weevils, thereby resulting in asphyxiation and subsequent death (Adedire and Ajayi, 1996). In addition, *A. sativum* may contain other compounds not contained in *A. vera*.

In the present study, both plant extracts achieved very low mortality of the weevils with increasing time of exposure which proves that the extracts have little or no residual toxicity against the weevils. This observation disagrees with the work of Oparaeke and Kuhiep, (2006) who reported that the powdered leaves of *A. vera* was more persistent with longer time of exposure and thus achieved high mortality of *S. zeamais.* Also this observation is contrary to the report of Arannilewa *et al*., 2006 who observed that the effectiveness of the petroleum ether extract of *A. sativum* increased with time. This could be due to differences in the form in which the botanicals were used, application rates of the botanicals and the solvent used for the extraction.

The results of the repellency test proved that both plant extracts repelled the weevils moderately with the mixture of the two extracts achieving highest repellency followed by *A. sativum* extract and then *A. vera* extract. The *A. sativum* extract was able to achieve higher repellency than *A. vera* extract also evidently because of its choky odour caused by the volatile sulphate substance allicin (diallyl thio sulphinate) synthesized in garlic when the tissues are damaged and the substrate alliin (S-allyl-L-cysteine sulphoxide) mixes with the enzyme alliin-lyase (Malkhan *et al*., 2012).

The results of this study also proves that the plant extracts and their mixture have potential as oviposition inhibitors which was evident in the reduced number of eggs and F1 adults hatched in the treated groups. This could be due to adult insect mortality, ovicidal effects and certain nutrition inhibition effects of the plant extracts (Borisade, 2009). Also the act of weakening of adults by plant extracts may make them lay fewer eggs than normal leading to less hatchability to larvae and final metamorphosis to adults.

The results of this study also prove that the plant extracts and their mixture were able to protect the maize grains against direct-feeding damage and weight loss. This was evident in lower percentage weight loss recorded in the treated groups. This could be due to mortality and anti-feedant effects of the extracts. The anti-feedant properties of the plant extracts might have resulted from their smell and bitter taste that rendered the maize grains unpalatable for the weevils (Mukanga *et al*., 2010).

In all bioassays, the effectiveness of the plant extracts was dose-dependent with the severity of their effects increasing with increase in concentration. Different botanicals effectiveness at higher dosage to various storage pests have been reported by several authors (Talukder and Howse, 1994; Tripathi *et al*., 2000; Yankachi and Gadache, 2010; Ileke and Oni, 2011; Ekeh *et al*., 2013). The mixture of *A. sativum* and *A. vera* extracts performed better in all bioassays than the individual extracts and this could be attributed to the effect of the combined potency of the two extracts.

The exact mechanism of activity of these plant extracts against *Sitophilus zeamais* is not very clear but their insecticidal actions may be attributed to fumigation effects, repellency effects, stomach poisoning effects; in which case the insects feed on the admixed seeds and contact poisoning effects which results in physical abrasion of the insect cuticle with consequent loss of body fluid or blockade of the spiracle (Ogunwolu *et al*., 1998).

4.1 Conclusions

The indiscriminate use of synthetic insecticides for storage protection may leave toxic residues in food and result to health risk to consumers of the treated products. It can also lead to development of resistance by insects. Unlike conventional insecticides which are based on a single active ingredient, plant-derived insecticides consist of a mixture of biologically active compounds and hence insects are not exposed to the same selection pressure as with conventional insecticides and develop resistance slowly. They can be broad-spectrum in activity, safe to use, unique in the mode of action and easy to process and apply. They also tend to be less toxic to higher animals and the environment and can be produced by farmers and small-scale industries.

In the present study, the ethanolic extracts of *Allium sativum*, *Aloe vera* and their mixture displayed some level of insecticidal activity against the maize weevil, *Sitophilus zeamais* and this strongly suggest the possibility of using them as toxicants, repellents, reproduction inhibition agents, feeding deterrents and food poisoning agents in integrated pest management strategies of *Sitophilus zeamais*. Therefore, these results would add to the database of plant products available for use as biopesticides.

4.2 Recommendations

The search for more selective and biodegradable insecticides is a promising field within stored-product pest management strategies. Researches to improve the effectiveness of botanical derivatives as insecticides should be encouraged as this will benefit agricultural sectors of developing countries because these substances are not only of low cost, but also have less environmental impact in terms of insecticidal hazard.

The active principles in *Allium sativum* and *Aloe vera* need to be isolated, identified, characterized and synthesized for further tests as such compounds may be more potent in their pure form than is the case with the crude form which would enable maximum exploitation of their potential as grain protectants. It would be advantageous to standardize methods of extraction and testing so that the search for new biologically active plant products could be more systematic and interpretation of results would be facilitated. In addition, there is the need to investigate the shelf life of the extracts and to find out if repeated application is needed after a given period. Furthermore, more research should be carried out using mixture of different plant extracts to discover the plants that will offer better protection when used together than when used singly so as to increase advantages provided by the use of pesticidal plants in stored grain protection.

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