EVALUATION OF SOME PLANT PRODUCTS FOR THE CONTROL OF THE BRUCHID *Callosobruchus subinnotatus* PIC. ON BAMBARA GROUNDNUT (*Vigna subterranea* (L.) Verdcourt

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DECLARATION

I declare that the work in the project entitled Evaluation of Some Plant Products for the Control of the Bruchid, *Callosobruchus subinnotatus* Pic. on Bambara Groundnut (*Vigna subterranea* (L.) Verdcourt has been carried out by me in the Department of Crop Protection under the supervision of Professors M. C. Dike, I. Onu and A. M. Oparaeke. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis has been submitted for any degree or diploma at any University.

______________________	____________________	________________
Jamila Abdullahi	Signature	Date

CERTIFICATION

This dissertation titled LABORATORY EVALUATION OF SOME PLANT PRODUCTS FOR THE CONTROL OF THE BRUCHID, *Callosobruchus subinnotatus* PIC. ON BAMBARA GROUNDNUTS *Vigna subterranea* (L.) Verdcourt by Abdullahi, Jamila meets the regulations governing the award of the degree of Doctor of Philosophy in Crop Protection of the Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

With gratitude to Allah (SWT) for everything, I dedicate this work with love to my late father Alhaji Abdullahi Ibrahim who more than anybody wanted to see me do this. This is for you and our dearest mother Hajiya Adama Abdullahi who ensured I did it.

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ABSTRACT

Laboratory Evaluations of some plant Products for the Control of adult *Callosobruchus subinnotatus* Pic. on Bambara groundnut (*Vigna subterranea* (L.)) Verdc. was undertaken in 2008 and 2009. The objectives of the research were to investigate the effect of the plant products *Artemisia annua* crude aqueous extract, *Aloe vera* gel, *Gmelina arborea* drupe juice and oils from seeds of *Moringa oleifera* and *Mangifera indica* on the mortality of *C. subinnotatus* at three levels of applications of 2 ml/kg, 4 ml/kg and 8 ml/kg. The treatments were compared with Actellic 2% dust at 10 g/kg, *Azadirachta indica* seed oil at 2 ml/kg as standards and an untreated control. The mode of activity of the plant products, the effect of the treatments on bambara groundnut seed viability, weight, moisture content and progeny emergence were studied under prevailing laboratory conditions. The design of the experiment was completely randomized design repeated three times. Data was analyzed using statistical softwares Microsoft Excel 2003, SPSS 13.0 and SAS 9.0. Results showed treatments with *M. oleifera* at all three levels of application caused significant mortality of the bruchid from 24 hours post application and compared favourably to the standards at 5% level of significance (Student Newman Keul's (SNK)) during both years of the trial. Treatments with *G. arborea* at application rate of 4 ml/kg caused comparable mortality to the standards by the 24th hour in 2008 and 48 hours in 2009. The results also showed the oil of *M. oleifera* had contact, repellent and some fumigant toxicity and *G. arborea* had positive repellent toxicity against the bruchid. The treatment *A. vera* at all three rates of application showed poor performance in causing the mortality of the bruchid up to 72 hours post application as compared to the standards and the treatments were not significantly different from the control in 2008. The gel showed positive contact toxicity 72 hours post treatment that compared favourably to the standards (SNK P= 0.05). Treatments with *A. annua*

and *M. indica* showed positive fumigant toxicity. However, treatments with *A. annua* at the rate of 2 ml/kg did not adequately protect the seeds from weight loss and F1 progeny emergence. All the treatments had an effect on the viability of treated seeds causing poor germination. The edible oil *M. oleifera* can be used as a protectant of bambara groundnut seeds against *C. subinnotatus* at application rates of 2 ml/kg, however, like many plant oils it should be used for treatments of seeds meant for planting with caution as its physical and chemical property may impair germination. All the plant products showed some biological activity against the bruchids, they can therefore be used for the protection of bambara groundnuts at their effective levels taking advantage of their modes of activity. They can also be incorporated into an effective Integrated Pest Management Programme and future efforts should be geared towards the isolation and standardization of their active ingredients with a view to manufacturing plant based pesticides for use in grain storage by farmers and grain merchants

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CHAPTER ONE

1.0 INTRODUCTION

Agriculture plays an important role in ensuring adequate food to the masses for a healthy and productive life. Globally, enough food is produced for all, but more than 780 million people are chronically undernourished (FAO, 1992; 1996; Ogunbanjo, 1997: University of Sidney, 2004). It has been reported that over 800 million people representing about one sixth of the world's population are severely undernourished and two billion people suffer nutrient deficiencies (www.agric.usyd, 2004; http://www.pc, 2007). Poverty is the main cause of hunger and malnutrition is aggravated by rapid population growth, policy inadequacies or weak administrative capabilities and unhealthy food storage and processing techniques. In sub Saharan Africa, some 215 million people or about 43% of the population lack adequate amounts of food that are rich in the nutrients needed for a healthy and productive life (FAO, 1996 and Sanni, 1999). Deficiencies of iron, vitamin A and iodine are also widespread; about 300 million people are affected yearly, and a much greater number are at risk of these deficiencies (FAO, 1996).

Malnutrition increases people's vulnerability to infections, causing numerous deaths. Even where the food is available in the developed and rich countries, eating the wrong kind of foods can lead to debilitating conditions and diseases some of which are potentially fatal. Illnesses like heart disease, obesity and diabetes can result from poor diet. Type-two diabetes, which is the non-insulin dependent diabetes or adult onset diabetes is caused by poor nutrition and among other things has been found to affect more blacks (>70%) than whites in the United States of America (Beverly, 2007). More people are found to die from complications of diabetes than from Acquired Immune Deficiency Syndrome (AIDS) and breast cancer combined (Ebony, 2002; 2005).

In terms of dietary balance, grain legumes or pulses contain more proteins than cereals and about ten times as much protein as most roots and tubers (FAO, 1968). Food legumes have a major role to play in the fight against malnutrition. It is therefore necessary that their level of consumption, which is already too low in many developing countries, should be increased (Borget, 1992). Legumes serve as a source of protein to a large proportion of the population in the developing countries of the world by being the least expensive and easily stored and transported non-processed protein source for rural and urban dwellers (Rachie and Silvestre, 1997). The high carbohydrate (65%) and relatively high protein (18%) content of bambara groundnut, *Vigna subterranea* F. make it a complete food (Doku, 1995). The pulse also has important nutritional value as extenders of animal proteins that are scarce and costly. Foods like bambara groundnut can be used in the case of debilitating illnesses like diabetes and obesity because of their special nutritional value. These traditional crops have numerous advantages, especially in terms of household food security as they are particularly important in the cropping strategies and consumption patterns of poor people. They provide a varied diet often rich in protein, carbohydrate, dietary fibre, minerals, vitamins A and B complex, iron and calcium. The gross energy value of the seed is greater than that of other common pulses such as cowpea, lentils and pigeon pea (FAO, 1982). The crop may also have a future as a nutraceuticals (a term combining nutrition and pharmaceuticals), being a functional food. Nutraceuticals are foods rich in nutrients that can prevent disease, cure illness, build muscles and reduce weight (Wolfenbarger, 2000).

Bambara groundnut, *V. subterranea* are regarded as one of the underutilized and neglected food crops (Heller *et al.*, 1997). They are also secondary legumes and thus constitute an important crop in certain regions. Even though they are not as widely grown as other food crops, they can enhance nutritional status, increase household food security, increase crop productivity by reducing buildup of pests and diseases, conserve soil, increase soil fertility and aid in increasing household and national income by trading in the crop.

The crop has advantage over more favoured species in terms of nutritional value and tolerance to adverse environmental conditions. As one of the most drought- tolerant legumes, it deserves more attention than it has received from the scientific society. It is commonly referred to as the poor man's food [Mungate, 1995]. Most human food requirement is provided by fewer than 20 crop species. There remain wide repositories of many hundreds of underutilized species that have been grown locally for centuries and which contribute to the food security of the world's poorest people. Many of these crops are grown in hostile, tropical environments by small-scale farmers without access to irrigation or fertilizers and with little guidance on improved practices and feasible alternatives among others i.e. herbicides, insecticides e.t.c. (FAO, 2005).

While in the field, bambara groundnuts whose pods are underground, are thought to be free from attack by most pests that limit the yield of legumes whose pods are exposed since they bury their pods in the ground. However, pests like rodents, cutworms and termites (Dike, 1997) are found to attack the underground part. Some disease pathogens have also been found associated with the plants in the field during pod formation after heavy rains (Linnemann and Azam-Ali, 1993b).

In storage, the insect pests *Callosobruchus subinnotatus* Pic. and *Callosobruchus maculatus* F. have been found to attack bambara groundnuts (Ngugi, 1997; Cudderford, 1997; Lale, 2002). Together the bruchid species cause up to 20 % w/w loss of bambara groundnut after five months storage (Ofuya and Lale, 2001). Of the two *C. subinnotatus* is almost restricted to bambara groundnuts. Many methods are used for the control of insect pests in storage, among which is the use of natural products. Plants are nature's chemical factories that possess insecticidal property that are readily available and accessible including the fact that they cost little or nothing. Also, they do not often require technical know-how for their application

The use of synthetic pesticides is regarded as the panacea to agricultural development in order to cope with the teeming human population. Insect damage in stored grains and pulses may amount to between 10 to 40% in countries like Nigeria where modern storage technologies have not been developed or introduced (Shaaya *et al.,* 1997; Olumeko, 1999; Adejumo and Raji, 2007). Nigeria loses about 2.4 billion tones of food yearly due to poor harvest and storage facilities (Olumeko, 1999). This has resulted in considerable waste in agricultural output and therefore loss in the economy.

Currently, measures used to control insect pest infestation in grains and dry foods rely heavily on the use of synthetic gaseous and liquid insecticides, which pose possible health hazards to warm-blooded animals. Their use is further limited by the economic realities in Nigeria as well as the lack of technical know-how by farmers, traders and consumers in handling these poisonous liquid and gases. Continuous heavy usage of some insecticides has created serious problems, the most obvious being direct toxicity to non target organisms and humans. Certain chemicals may also be concentrated in food chains and some organisms develop resistance to these insecticides. At present, most, if not all of the pesticides used in Nigeria are imported. Where it is produced or manufactured locally, the chances are high that the raw materials are imported. This has necessitated the need for the searching and using of alternative methods of pest control in all stages of agricultural production including storage. Considerable research is needed in addition to that which has been undertaken to provide alternative botanical methods of pest control. Use of natural plant products for the control of insect pests is not a new practice. These plants are available in nature. They confer little or no damage to the environment and have little or no residual toxicity. They can safely be used for the protection of seeds and even food grains by peasant farmers at farm levels and they often do not require technical know-how for their application. Their active principles can safely be used locally as raw materials for the large scale production of pesticides to reduce the further depletion of already lean financial resources.

1.1 Justification of the Study

Insect damage in stored grains and pulses may amount to 10-40 % in countries where modern storage technologies have not been introduced or adapted (Shaaya *et al.*, 1991). Currently, the measures to control insect pests in storage rely heavily on the use of different formulations of potentially hazardous synthetic insecticides. These pose health hazards to humans, other living plants and animals and the environment. These pesticides are toxic, too expensive and difficult to obtain by the farmer and coupled with the problems of development of multiple resistances by insect pest species and high cost of production of insecticides call for the use and application of less hazardous substances and safe alternatives that are readily available in nature, affordable by the common farmer and commodity traders, are simple, convenient to use and environmentally friendly.

Bambara groundnut is an important grain legume in Nigeria, being susceptible to the bruchid, *C. subinnotatus* especially in places like northern Nigeria where it is identified to be native; there is the need to evolve different means for the protection of its seeds from damage by the bruchid. Synthetic pesticides that have been used for the protection of stored grains and pulses have rather proven not to be the best and safe option for all users. This therefore calls for the use of all measures available in nature including natural plant products. Biopesticides are readily available, safe and easy to use. Many spices and herbs and their extracts are known to possess insecticidal properties. This method of insect control is convenient and inexpensive for the protection of agricultural produce in particular stored seeds in households and on small farms (Shaaya *et al.,* 1997). Many plant products have been tested for their insecticidal potential against storage insect pests in Nigeria. However, very few have been tested against *C. subinnotatus* an important field to store pest of bambara groundnut in northern Nigeria. The present study was undertaken to investigate the insecticidal potential of some plant products on the bruchid. The mode of activity of the plant products was undertaken to investigate the specific ways these plant products exert their insecticidal activity. The effect of the plant products in stopping or reducing the activity of the bruchid on bambara groundnut was undertaken.

1.2 Objectives of the Study

The present study had the following as its objectives

i. To determine the effect of some plant products on the mortality of *C. subinnotatus.*
ii. To study some behaviour of the bruchid *C. subinnotatus*
iii. To determine the mode of activity of the plant based insecticides
iv. To determine the effect of insect activity on the germinability of treated bambara groundnut seeds.
v. To determine the effect of the plant products on the progeny emergence, weight and moisture content of bambara groundnut.
vi. To determine the effective concentration of plant products on mortality of *C. subinnotatus*

CHAPTER TWO

LITERATURE REVIEW

2.1 Bambara Groundnut

There are many different types of bambara groundnut, which are classified based on growth habit. These include open or spreading types, compact or bunched types and intermediate or semi-bunched types. The plant belongs to the genus *Vigna* and tribe phaseoleae, in the family Fabaceae (formerly leguminoseae) and sub-family Papilionoidea (Hepper, 1970). The genus *Vigna* contains several species that are of considerable economic importance in many developing countries (Fery, 2002). The genus contains about 80 species and occurs throughout the tropics (Brink *et al*., 2006). Two botanical varieties are distinguishable, *Vigna subterranea* var. *spontanea*, which include wild forms of bambara groundnut that are restricted to Cameroon and *Vigna subterranea* var. *subterranea*, which are the cultivated varieties (Hepper, 1970) found in many parts of the tropics, particularly sub-Saharan Africa. There are considerable morphological differences between the wild and the domesticated varieties.

The crop was first mentioned in the 17th century (Marcgrav de Liebstad, 1684) where it was referred to as Mandubi d'Angola. In 1763, Linnaeus described it in species plantarum and named it *Glycine subterranea* in accordance with his system of nomenclature. Du Petit-Thours in 1806 found the crop in Madagascar under the vernacular name "*Voanjo"* subsequently written *Voandzou* in French. He then proposed the name *Voandzeia subterranea* (L.) Thours, which was widely used by subsequent researchers for over a century. Detailed botanical studies by Marechal *et al*. (1978) found great similarities between it and species of the genus *Vigna.* This confirmed studies by Verdcourt who in 1980 proposed the present name of *Vigna subterranea* (L.)Verdc. (Linneman and Azam-Ali, 1993a).

Bambara groundnut, *V. subterranea* (L.) Verdc. has been known variously before Verdcourt (1980) gave it its present name. Synonyms by which it is known are *Voandzeia subterranea* L.Dc (1825) Thours and *Glycine subterranea* L. (1763), *Vigna possoni* Chev. The crop is also known variously in English and the vernacular as bambaranut, bambarranut, African ground bean, *Njugo* bean, Congo goober, *Baffin* pea, bambar[r]a groundnut *Kaffir* pea, Madagascar groundnut, *juga* nut and earthnut. In French, it is known variously as *haricot, pistache, voandzou, pois de souterrain* and *pois de terre* (Cudderford, 1997. In Sudan, it is called *ful abugawi* and *gertere*. In Kenya, it is known as *njuga mawe.* In Malawi, it is called variously as *isugu, njama, nzama, sugama* and *niumu* by the different ethnic groups. In Zimbabwe, it is called variously as *nzama, nyimo, indlubu* and *tinjuwu.* In Zambia, the nut is called *nzama, nzuwu, zindulube, zinzuwu* and *ntoyo*. In Uganda, it is called *mpande* and *isuk* (Cudderford, 1997). Begemann (1988) listed 43 common names used for bambara groundnut, while Duke *et al*. (1977) indicated that 45 known common names are in use. The previous genus name, *Voandzeia* now incorporated into the genus *Vigna* for bambara groundnut is thought to have come from the Malagasy name "Voanjo" where "voa" stands for seed and "anjo" stands for that which satisfies well. In Nigeria bambara groundnut is known in Hausa as *gujiya* and *kwaruru*, while in Ibo it is known as *okpa* and in Yoruba as *epiroro*.

There are many different types of bambara groundnut, which are classified based on growth habits of open or spreading types, compact or bunched types and intermediate or semi-bunched types. The common English name appears to be derived from a tribe of agriculturalists, the Bambara who nowadays live mainly in Mali (Williams, 1993). Bambara groundnut is generally very drought-tolerant, and does well in soils that may be too poor for groundnut. Bambara groundnut is probably the most drought–resistant of the grain legumes and may be found growing successfully where annual rainfall is below 500 mm and optimum between 900–1000 mm per year (Ocran *et al*., 1998). The crop is known variously by different synonyms such as Madagascar nut and earthnut. It is a small annual herb of the family leguminoseae and sub family papilliodeae. It is an erect plant, with long stalk and trifoliate leaves. It matures in 90-150 days forming its fruit underground. Its agricultural requirement is similar to that of peanut but it grows in soils even poor for groundnuts. It is grown widely in Africa especially in semi-arid Africa to sub-humid fringes of the Sahara. The crop is a legume species of African origin (Borget, 1992) and is widespread south of the Sahara (Ocran *et al*., 1998). It is also grown in the Caribbean, India and the Indian Ocean, Comorro Islands, Madagascar and Mauritius. It is however thought to be native only to Nigeria, Cameroon and the Central African Republic. The major producers are Nigeria, Niger and Ghana where it is third in importance only to cowpea and peanuts (Howell, 1993).

2.1.1 Description of Bambara Groundnut

Bambara groundnut, *Vigna subterranea* is a leguminous crop belonging to the family Fabaceae formerly Leguminoseae/Papilionaceae, sub- family Papilionoidea and tribe Phaseoleae (Hepper, 1970; Fery, 2002). The plant is an annual bunched herb with short creeping or trailing much branched stems, rooting at nodes with short internodes. The lengths of the internodes vary, resulting in bunched, intermediate and spreading types (Doku, 1969). The cultivated varieties usually have a bunched growth habit. The plant shows a wide range of growth habits. The leaves are pinnately trifoliate with long stiff and grooved petioles that are erect and thickened. The base may be pink, purple or bluish green in colour according to types. The terminal leaflet is subtended by two stipels, while the lateral leaflets have one each. The terminal leaflets are larger than the lateral ones [Hepper, 1970]. There is also an erect form. The leaves are compound with three leaflets, usually held upright on long stalks thickened at the base. Leaflets are elliptic, smooth, dark green above, paler below with margins entire. Local cultivars vary in leaf size and shape (FAO, 1982).

Leaflets are oblong or lanceolate 2.7-7.5 cm long, and 0.8-3.0 cm wide (Hepper, 1970). One to three papilionaceous flowers are borne on a raceme on long hairy peduncles, which arise from the nodes on stems. Flowers are bisexual, papilionaceous, shortly pedicelled; calyx with tube c. 1 mm long and five lobes c. 1 mm long; corolla whitish-yellow, standard obovate, 4-7 mm long, wings and keel slightly shorter; with filaments fused for more than half their length and one free; ovary superior, one celled, style bent (Brink *et al*., 2006). The petals are yellow to reddish pink 4.0-7.0 mm long (FAO, 1982). Other colours found are yellowish-white and light pink petals, sometimes with reddish brown striations. Self pollination is a common rule, although cross-pollination by ants has been reported in Ghana for spreading types (Doku and Karikari, 1971). After flowering, the flower stalks bend down to the soil and penetrate into the surface, where the fruits develop. The bud tips create a tunnel through which the fertilized flower, attached just behind the tip, is drawn into the soil. Reproductive development is not completely inhibited by light. The pod grows first and reaches maturity 30 days after fertilization. The seeds develop in the following ten days. Root system consists of a tap root and lateral roots lower down, with rounded and sometimes lobed nodules. Mean temperature during the seasons influences the time taken to reach physiological maturity. Bunched types tend to mature earlier than spreading types. Fruit development has been reported to be influenced by photoperiod

(Linnemann and Azam-Ali, 1993a). Long photoperiods delay or even prevent fruit set in some cultivars. Pods develop and mature over a long period because of the indeterminate habit of bambara groundnut. The first pods normally develop from early flowers, close to the axis of the plant. During the growing season the branches develop and pods continue to be produced. However, small farmers often solve the problem of uneven maturity by harvesting the pods as they mature (Kay, 1979). Typical fruits are rounded, 2 - 3.7 cm in diameter depending on the number of seeds they contain (Goli, 1997). Some varieties produce two to three seeds. The mature pods are indehiscent and often wrinkled when mature. The fruits usually contain one or more seeds, which are round (typically 1.5 cm in diameter); the seeds often have a seed patterned seed coat (sometimes with an eye around the hilum) (Goli, 1997).

Seeds are round smooth and very hard when dried. Seed colour varies and may be white, creamy, red, purple, brown, black or mottled in any of these shades (Cobley, 1976) (Plate I). Various testa patterns are found including mottled, blotched and striped, as well as the predominantly uniform coloured seeds. Bambara groundnut is similar to peanut in both culture and gross morphology. The underground fruit is significantly different in etiology from peanut (Purseglove, 1968). The root contains many Nitrogen fixing nodules (Stephens, 2003).

From the cytological point of view bambara groundnut is known to have a chromosome number $2n = 22$ like many other legumes (Brink *et al.*, 2006). Reports on critical genetics and breeding of the plant are very limited. Through many years of successive cultivations, farmers have selected desirable traits such as high yields and bunched habits (Smart, 1985).

Plate I: Bambara Groundnut Seeds Showing Variability of Colours, Shapes and Designs

2.1.2 Genetic Resources of Bambara Groundnut

Bambara groundnut germplasm is abundant in sub-Saharan Africa, as the crop is grown in every tropical region of the continent. So far, wild relatives of cultivated bambara groundnut have only been found in north eastern Nigeria and northern Cameroon. It is believed that the crop originated from this part of the continent. There are no scientifically improved varieties of bambara groundnut, and marginal and subsistence farmers grow locally adapted landraces (Shravani *et al*., 2004). Despite its potential as a crop, no coordinated plant breeding or improvement programmes have been established for the crop. For many centuries, farmers have grown locally-adapted landraces consisting of a mixture of genotypes and often resulting in low and unpredictable yields. Nigeria is believed to be one of the centres of origin of this crop, and possesses extensive bambara groundnut genetic resources (Tanimu and Aliyu, 1995). This can be observed from the wide range of differences in seed coat colour, seed sizes, pigmentation around the eye, pod shape, growth habit and other characters.

Molecular mapping has proven to be a powerful tool for gene localization, gene isolation, marker-assisted selection and evolutionary studies. Knowledge of the organisation of genes controlling economically or agronomically important traits can make a considerable contribution to plant improvement programmes. No gene or DNA sequence information is available for bambara groundnut. Isozyme patterns, as observed in other species of the genus *Vigna*, such as *V. unguiculata* (cowpea) (Panella and Gepts, 1992; Pasquet, 1993; Vaillancourt *et al*., 1993), were found to be very similar to those observed in bambara groundnut (Pasquet, 1993). This suggests a genetic synteny (occurrence of multiple genes on a single chromosome) between cowpea and bambara groundnut.

2.1.3 Races of Bambara groundnut

Different races of bambara groundnut exist. Seven races have been collected from Botswana, others were also collected from Zimbabwe and one from Tanzania (Doku and Karikari, 1971). Studies have shown that wild accessions of bambara groundnut have useful genetic variability for maturity period, yield and seed characteristics. Variability in landraces in Botswana, Zimbabwe and Tanzania showed that there are early maturing (120 days), medium duration landraces (120 - 128 days), and late season maturing in 133 - 165 days. Early maturing landraces have been found to be high yielding because they emerged rapidly, flowered within 40 days and had enough time for their pods to mature before the advent of unfavourable weather due to less moisture and decreased temperature (Gibbon and Pain, 1985)..

2.1.4 Origin of Bambara Groundnut

Bambara groundnut is considered to be an indigenous crop of Africa. The exact center of origin is not very clear; however, wild varieties of the crop have been found in Jos Plateau and Yola in northern Nigeria; in Garoua (Cameroon); and possibly in the Central African Republic (Hepper, 1970; Cobley, 1976). Wild forms of the plant have also been found in the vicinity of Bakel (Senegal) (Guillemin *et al*., 1890). Once thought to be an Arabic importation from Madagascar, no wild forms where found there [Godwin, 1939]. The centre of origin of bambara groundnut is also thought to be probably north – eastern Nigeria and northern Cameroon. It is found in the wild from central Nigeria eastwards to southern Sudan, and is now cultivated throughout tropical Africa and to a lesser extent in tropical parts of America, Asia and Australia (Brink *et al*., 2006). Bambara groundnut was domesticated in the semi-arid zone of West Africa, probably around the headwaters of the Niger River, from where it spread in ancient times to

Central Africa, and more recently to the Malagasy Republic, Asia and South America (Tweneboah, 2000).

The plant is wild in isolated locations in the savannah zone of West Africa and may have been domesticated around the head waters of the Niger River (Cobley, 1976). The name bambara appears to have come from the district of Bambara near Timbuktu in present day Republic of Mali. Guillemin *et al*. (1832) as well as Holm and Marloth (1940) are of the opinion that the crop originated from this place. However, no spontaneous or wild forms have been found in this area. The name Bambara is used to describe an African tribe that inhabits the valley of the upper Niger River in Mali. Guillemin *et al.* (1832) reported probable wild forms from Senegal near Mali. Bambara groundnut was however, found by Dalziel in its genuinely wild form in 1901, in Yola in northern Nigeria. He reported that Ledermann also found the wild plants in Garoua, northern Cameroon. The distribution of wild Bambara groundnut is now known to extend from Jos Plateau and Yola in northern Nigeria, to Garoua in Cameroon. Begemann (1988) carried out detailed analyses on the seed- pattern diversity and found that samples collected within 200 km from the putative centre of origin, between Yola and Garoua, consistently showed greater seed pattern diversity. Investigators interested in the origin of bambara groundnut (Dalziel, 1939; Jacques-Felix, 1946; Rassel, 1960; Hepper, 1963; Begemann *et al*., 1988) all agreed that the crop originated from the African continent.

Karikari (1971) believed that the plant appears to have evolved in the savannah probably in West Africa, as its compact habit seems to be an adaptation to growing in hot, windy environments. Its use as a pulse in West Africa was recorded by Arabic travellers in the 14th century. Its importance declined after the introduction of groundnut from the new world tropics.

2.1.5 Geographical Distribution of Bambara Groundnut

Bambara groundnut is regarded as an indigenous grain legume in the drier parts of sub-Saharan Africa. As such it is cultivated widely in West Africa (Rachie and Roberts, 1974). It is found widely in Africa especially in the semi-arid to sub-humid southern fringes of the Sahara, in eastern Africa from Sudan to Rhodesia, and the Malagasy Republic. Major producers are Nigeria, Niger and Ghana. In the African continent the crop is the third most importance grain legume after cowpea and peanut (Howell, 1993). In Nigeria however, it ranks fourth after groundnut, cowpea and soybeans (Tanimu, 2005). Some bambara groundnut is also found in Guinea, Senegal and Sudan.

In Africa, it is found as widely distributed as in Angola, Benin, Cameroon, Central African Republic, Chad, Ethiopia, Guinea Bissau, Namibia, Ivory Coast, Kenya, Mali, Mozambique, Senegal, Sudan, Swaziland, Tanzania, Togo, Uganda, Zaire and Zambia. In the Caribbean, the plant is cultivated in the Dominican Republic, while in the Indian Ocean it is found in the Comorro Islands, Mauritius and Madagascar. The plant was introduced into Argentina from Australia in 1951 (Escalante, 1956).

Bambara groundnut is found distributed in Asia in places like India, Sri- Lanka, Indonesia, The Philippines and Malaysia. In the Americas, it is found in Iowa (USA) and tropical Central America. It is also found in Northern Australia, New Zealand, Surinam, New Caledonia and Brazil (Rassel, 1960; Duke *et al*., 1977). It was carried to America by slaves (Stephens, 2003). Presently, its degree of cultivation outside Africa is negligible.

Europeans are believed to spread bambara groundnuts to South America in the 17th century and south east Asia in the 18th century. However, it has not become important outside central Africa (Purseglove, 1968).

In Nigeria, bambara groundnut is widely cultivated by small farmers. Its cultivation gains importance from north to south. The most important areas of cultivation are located in the savanna and include west of Makurdi from Otukpo and Ayangba in Kogi State, and to a lesser extent in Borno, Yobe, Kaduna, Katsina, Sokoto, Zamfara, Kebbi, Niger, Adamawa and Taraba States (Linneman, 1988). The most important producing areas are Kano, parts of Anambra, Plateau, Sokoto and Benue States.

Bambara groundnut importance has been replaced in many countries by peanuts *Arachis hypogaea* L. Nevertheless, bambara groundnut cultivation still has wide distribution from Senegal to the Ethiopian lowlands, as well as in South Africa and Madagascar (Jacques-Felix, 1950). In the 17th century, it was introduced into South America, as Linneaus (1763) described it as a plant from Surinam. Later it was carried to the Philippines and Indonesia (Brouk, 1975).

2.1.6 Bambara groundnut Production

Cultivation of bambara groundnut on a large scale and pure stands is not very common. The crop is mostly grown by women, intercropped with major commodities such as maize, sorghum, millet, yam, cassava, peanut and cowpea. Grown in rotation, bambara groundnut improves the nitrogen status of the soil (Mukurumbira, 1985). Reliable production figures for the crop are difficult to obtain, because the crop is mainly grown for home consumption and sale at local markets.

The crop is planted extensively in Western Africa where about 333,000 million tonnes of dry seed were produced on 400,000 ha annually (Rachie and Roberts, 1974). The major producing areas are Nigeria (100,000 tonnes/annum), Niger (30,000 tonnes/annum) and Ghana (20,000 tonnes/annum) (Rachie and Roberts, 1974). Production outside Africa is negligible. Its cultivation seems to have preceded that of the common groundnuts (*Arachis hypogaea* L.), of American origin (Goli, 1997). World annual production of bambara groundnut was estimated at 330,000 metric tonnes in 1982, with about half being produced in the Savannah regions of West Africa. Available estimates in Botswana revealed that the crop is grown on 1500 hectares of land from which a total yield of 40 metric tonnes (i.e. 26.6 kg/ha) of seed was obtained annually (Karikari, 2000). Most production is carried out at subsistent level or small-scale farms which make mechanization impossible or large scale production using mechanization difficult. The crop is grown widely in eastern and southern Africa and in Madagascar. The main exporting countries are Burkina Faso, Chad, Mali, Nigeria and Senegal; they supply markets in Benin, Ghana, Nigeria and Togo.

Bambara groundnut yields are low because the production environments are characterized by various biotic and abiotic stresses. However, even under optimal conditions the yields are variable and unpredictable and this is partly due to variability in growth and development of individual plants in landraces (Squire *et al.,* 1997). Nigeria is a major producer of the crop (Rachie and Silvestre, 1997). Seed yields in Africa average 650-850 kg/ha (Stanton *et al*., 1966). Maximum yields of dry seed are about 2,600 kg/ha and average about 750 kg/ha (Rachie, 1979). In an experiment conducted in Samaru, Nigeria, mean yield of bambara groundnut in a field protected from pests was 663 kg/ha in 1993 and 493 kg/ha in 1994 (Dike, 1997), while the yield in the unprotected control plots was 194 kg/ha in 1993 and 282 kg/ha in 1994, representing losses of 71% and 43%, respectively. The experiment recorded mean pod yields from the protected plots as 1099 kg/ha in 1993 and 878 kg/ha in 1994. The mean pod yield from the unprotected plots was 361 kg/ha and 540 kg/ha or losses of 67% and 38% in 1993 and 1994, respectively. The mean pod and seed yields from plots protected with the insecticide were significantly greater than equivalent yields from the unprotected plots. It has been shown that farm yields are in the region of 500 kg/ha, yet under research conditions, the figure can be raised to 4000 kg/ha (Dike, 1997).

2.1.7 Food uses of Bambara Groundnut

Majority of farmers cultivate the crop at a subsistence level for home consumption and may sell part of the excess for money to take care of their financial needs. Most of the bambara groundnut produced in Nigeria are consumed locally as no industrial use of the crop has been reported. Bambara groundnut is grown primarily for its seeds, which are used for many types of foods. When roasted or boiled, even the mature seeds are sweet and pleasant tasting. The seeds are often roasted and ground into nutritious flour. Seeds contain 14-24% protein and about 60% carbohydrate. The protein is reported to be higher in the essential amino acid methionine than other grain legumes. Bambara groundnuts contain 6-12% oil, which is less than half the amount found in peanuts, making them not useful as an oilseed crop (Stephens, 2003).

The edible part of the bambara groundnut plant are the seeds, young pods and leaves .When young the seeds are eaten raw or cooked, but when mature, they become too hard to be eaten raw (Swamithan and Kochhar, 1989). Ideally, the seeds should be eaten after cooking as the raw seeds contain a trypsin inhibitor which reduces the body’s ability to digest the protein. Roasted seeds are eaten as a snack after they are roasted in wood ash, soaked in salt water and dried, like groundnuts or as a relish accompanying the main starchy food. Occasionally, they are used as coffee substitute. They are also ground to powder, which is used in many dishes. Fresh ripe seeds are first parboiled to soften the tough seed coat, which is then removed and the remaining kernel thoroughly boiled. Seeds can also be pounded and made into a stiff porridge, which is often kept for a long period (Holm and Marloth, 1940).

In Ghana, seeds are soaked for 24 hours before cooking and then boiled to become soft before eaten. They are often made into a type of porridge with beans floating in it (‘*oboboe’*). They may be eaten hot or cold, together with red pepper, salt and often sugar. In Cameroon, they are eaten raw or after cooking, are ground and added to soups and stews. In Tanzania, the boiled seeds are crushed, groundnut paste is added and the mixture is eaten with *“ugali”*. In Madagascar, they are often added to meat stews with rice or eaten with green leafy vegetables. Dried seeds boiled or split, are mixed with maize or plantains and then boiled. Sometimes seeds are soaked in water and ground into a paste, which is used to prepare fried or steamed dishes. Vegetable milk and fermented products can be made from the seeds.

Bambara groundnut flour has a chestnut flavour when boiled (Swamithan and Kochhar, 1989). In Southern Africa, the flour is mixed with butter or oil and eaten with meat, or it is made into balls or cakes. It is sometimes boiled with maize or millet flour to form stiff dough, which when salted and made into balls, will keep for several days. When roasted or boiled, the major seeds are considered to be sweet and pleasant tasting (Stephens, 2003). Immature green pods and/or young leaves of bambara groundnut are boiled, dried, and stored for future use. These dried leaves are known as ‘mfutso’ in Malawi. The green seeds are eaten after soaking and boiling and are easier to cook than ripe seeds, which are often roasted and ground to flour (Purseglove, 1968; Joplin, 1975).

In Nigeria, it is boiled in pods and eaten or roasted and taken as a snack. In Niger State, dried and preserved nuts are soaked and cleaned then ground to a paste with pepper, onions and other condiments and deep fried in vegetable oil. In other cases, to same combination oil is added and batches are wrapped in leaves or polythene bags and steamed (Obizoba, 1983). In some parts of Nigeria, bambara groundnut and pigeon peas are consumed after appropriate washing, dehulling and boiling or roasting or steaming, they may be eaten alone or mixed with sauce, condiments, spices and other foods (Igbedioh *et al.*, 1994). Another favourite Nigerian dish is "*okpa",* which is a doughy paste that is wrapped in banana leaves and boiled. Canning of bambara groundnut was a successful manufacturing industry in Ghana. The beans used to be canned in gravy at GIHOC cannery in Nsawam. The product was thus available throughout the year, and over 40 000 cans of various sizes were produced annually (Doku and Karikari, 1971; Begemann, 1988). A trial of bambara groundnut milk was carried out, which compared its flavour and composition with that of cowpea, pigeon pea and soybean (Brough *et al*., 1993). Bambara groundnut was ranked first, and while all milks were found to be acceptable, the lighter colour of the bambara groundnut milk was preferred. In western Kenya, seeds are cooked with maize (occasionally after overnight soaking), or on their own, then mashed, fried and made into stew. It is reported by some that bambara groundnut requires careful preparation, as it has a rather bitter taste. Different areas have various methods for the preparation of the crop either as a food supplement, main meal or a relish. Bread made from bambara groundnut flour has been reported in Zambia (Linnemann, 1990).

2.1.8 Other Uses of Bambara Groundnut

The seeds of bambara groundnut are fed to pigs and poultry and the leafy shoots are used as fodder. In Senegal, leaf preparations are applied to abscesses and infected wounds; leaf sap is applied to the eyes to treat epilepsy and the roots are sometimes taken as an aphrodisiac. Pounded seeds mixed with water are administered to treat cataract. The Igbos in Nigeria, use the plant to treat venereal diseases. In some areas, it is used in traditional ceremonies such as funeral rites and for gift exchanges. In Botswana, the seeds of the black landrace are used for traditional medicine. Bambara groundnut has long been used as animal feed and the seeds have been successfully used to feed chicks (Oluyemi *et al.*, 1976). The haulm was found to be palatable (Doku and Karikari, 1971) and the leaves are rich in nitrogen and phosphorus and therefore suitable for animal grazing (Rassel, 1960).

2.1.9 Economic Importance of Bambara groundnut

The plant has the inherent ability to fix atmospheric nitrogen, which increases the productivity of the soil. This is an important requirement especially in African soils that have natural lower productivity compared to European soils (Mungate, 1995). Bambara groundnut is highly nutritious and the seeds contain 6 - 9% fat, 17 - 25% protein and 55 - 70% carbohydrate thus serving as a source of rich diet for humans and livestock (Oliveira, 1976; Oluyemi *et al.*, 1976). The mature seed has more methionine (an essential amino acid) than found in other legumes. The importance of the crop as a source of protein cannot be overemphasized. This is because animal proteins have continued to be in short supply in diets consumed by a large segment of the population in developing countries. In many of these countries like Nigeria, traditional sources of animal proteins obtained from game or wild animals as well as domestic production of animal products are prohibitively expensive. Legumes, like bambara groundnuts, are some of the low priced sources of protein-rich foods that have been important in alleviating protein malnutrition. Bambara groundnut seeds make for a complete food as it contains sufficient quantities of protein, carbohydrate and fat. The seeds of bambara groundnuts are valued for both their nutritional and economic importance.

Three Anthocyanins have been isolated from bambara groundnut. These are delphinidine 3-O-β-glucoside, petudine 3-O-β-glucoside, and malvidin 3-O-β-glucoside. These three are among five anthocyanin pigments isolated in Burkina Faso (Pale *et al*., 1997). Anthocyanins give certain fruits like tart cherries their deep-red colour and are said to have anti-inflammatory properties similar to those in aspirin (Readers Digest, 2006). Besides chlorophyll, anthocyanins are probably the most important group of visible plant pigments. Anthocyanins, a flavonoid category, were found in one study to have the strongest antioxidizing power of 150 flavonoids (Approximately 4,000 different flavonoids have been identified) (Sterling, 2001). Studies show anthocyanins' positive influences on a variety of health conditions. One reason is their anti-inflammatory properties, which affect collagen and the nervous system. Their ability to protect both large and small blood vessels from oxidative damage derives from a range of effects, including mitigating micro vessel damage from high blood-sugar levels that cause complications in diabetics. By the same token, diabetic retinopathy, which damages eyesight, is caused by leaking, damaged capillaries. Anthocyanins are also found to be beneficial in nervous system (Joseph, 1999), eyesight (Nakaishi, 2000) large and small blood vessels (Adriambeloso, 1998; Pawlowicz, 2000) and diabetes (Adriambeloso, 1998).

The present status of bambara groundnut is that of a neglected and underutilized African legume. The crop is one of a number of species that have been neglected in a varying degree by researchers, or have been underutilized economically. The crop has advantages over more favoured species in terms of nutritional value and tolerance of adverse environmental conditions. The crop also has a number of production advantages in that it can yield on poor soils with little rainfall as well as produce substantial yield under better conditions. It is nutritionally superior to other legumes and is the preferred food of many local people

(Linneman, 1990; Brough *et al.,* 1993). The seeds are valued both for their nutritional and economic importance. The seeds command high market price with demand far outweighing supply in many areas (Courdert, 1982). Unconfirmed observations indicate that the crop can suppress *Striga* species, a parasitic plant found in sandy soils. However, despite these important attributes, the ecological and genetic potential of bambara groundnut have not been fully realized nor its full economic significance determined. The crop is still cultivated from landraces rather than varieties bred specially for particular agro-ecological conditions or production systems. Bambara groundnut is reported to be used as possible home-prepared and commercially processed weaning formulations, which are expected to meet the protein-energy needs of small children at the lowest cost.

2.1.10 Bambara Groundnut Cultivation

Bambara groundnut grows best in climates suitable for peanuts. It needs bright sunshine, high temperatures, at least four months free of frost, and frequent rains to grow best. The plant, however, is highly adaptable and tolerates harsh conditions better than most crops. It yields under conditions too dry for peanuts, such as in the Bambara district near Timbuktu on the Sahara Desert's southern fringe. It also is known to grow in rain forest areas, and the cool moist highlands of Zimbabwe. Farmers do not normally apply chemical fertilizers to bambara groundnut fields. The nitrogen requirement is met by natural nitrogen fixation, as indicated by several nodulation studies (Doku, 1969a; Samasegaram *et al*., 1993).

Reports from various parts of Africa show large variations in seeding rates (Linnemann, 1992). Studies in the highly leached soils of Bukoba in North-western Tanzania [Dunbar, 1969] indicated that farmers sow bambara nuts at an average spacing of 30 cm x 30 cm. In West Africa, Ameyaw and Doku (1993) recommended a spacing of 60 cm x 30 cm. Duke *et al*. (1977), similarly reported seed rate variations from 25-75 kg ha^{-1}, with inter-row spacing varying from 60 cm x 30 cm and 10-50 cm, respectively. Bambara groundnuts' reaction to population density also varies with location and cropping systems (Ameyaw and Doku, 1993).

Bambara groundnut genotypes differ in their response to photoperiod (Linnemann, 1990). This has implications for breeding locally adapted varieties. In a photoperiod sensitive genotype from Ankpa in Kogi State of Nigeria, for example, the onset of flowering, progress of flowering, onset of podding and progress in pods growth in plants under photoperiods of 14 hours or longer were all retarded compared with plants under photoperiod of 13 hours or less (Linnemann, 1995). Podding was affected more than flowering. Some plants flowered but failed to produce pods under photoperiod of 14 and 16 hours. These plants had many undeveloped ovaries, some of which developed into full grown pods after transference to 12 hour photoperiod regime. Hence the delay or absence of photoperiods of 14 hours or longer was attributed to growth of fertilized ovaries being checked. Transference from a 14 hour to a 12 hour photoperiod for 16 days or less was sufficient to induce podding in this genotype from Ankpa (Linnemann, 1995). Pods were only produced by plants which were not returned from the 12 to the 14 hour photoperiod (Linnemann and Azam-Ali, 1993a; Linnemann, 1995).

2.1.11 Nutritional value of Bambara groundnuts

Bambara groundnut is a legume and like all legumes it contains and supplies the protein needs of a large segment of human population, as well as serves as animal feed and soil conditioner. The legumes are the low -priced sources of protein-rich foods that have been important in alleviating protein malnutrition. Despite the large number of existing grain legumes in Nigeria, their consumption as staple foods has centered mainly on cowpea and groundnut. Livestock especially goats, are very fond of the haulm, which they are allowed to graze on at the end of the season, after the pods have been harvested. The seeds of the mature black landrace are used in traditional medicine.

Bambara groundnut is a rich source of protein (16-25%) (Fery, 2002) and contain about 60% carbohydrates (Stephens, 2003). Protein is essential for growth and development of living organisms and it constitutes 80-90% of all organic substances in animal body. In some parts of the world, they have important nutritional value as extenders of animal proteins. Unlike peanuts, it contains very little oil (6-12%) (Stephens, 2003). Bambara groundnuts have more methionine (essential amino acids) than found on other legumes (Stephens, 2003). Essential amino acids include lysine, leucine, isoleucine, methionine, phenylalanine, threonine, tryptophan and valine. Bambara groundnut assessed by chemical methods gave a total lysine content of 6.5 g/16 g N in the available form using 1,fluoro,2,4-dinitrobenzene method (FDNB) (Adewusi *et al.*, 1991). The seeds are shown to contain reducing sugars. Bambara groundnut is the only legume whose seeds are referred to and used as complete food because they contain protein, carbohydrate and fat in sufficient proportions to provide a nutritious food (Poulter and Caygill, 1980; Goli, 1997). The ripe seeds contain on average 10% water, 15 – 20 % protein, 4 – 9 % fat, 50 – 65 % carbohydrate and 3 - 5% fibre (Baudoin and Mergeai, 2001).

These analysis show that the constituents are present in suitable proportions for feeding purposes, and that the seeds would be valuable food if used in addition to a well proportioned diet of the usual type (Doku, 1995). Raw immature bambara groundnut seeds are found to contain per 100 g edible portion: water 57.3 g, energy 636 kJ (152 kcal), protein 7.8 g, fat 6.2 g, carbohydrate 30.0 g, Fe 1.2 mg. The composition of mature dry seeds per 100 g edible portion is: water 10.3, energy 1537 kJ (367 kcal), protein 18.8 g, fat 6.2 g, carbohydrate 61.3 g, fibre 4.8 g, ash 3.4 g, Ca 62 mg, P 276 mg, Fe 12.2 mg, β-carotene 10 μg, thiamin 0.47 mg, riboflavin 0.14 mg, niacin 1.8 mg and ascorbic acid traces. The content of essential amino acids per 100 g food is: tryptophan 192 mg, lysine 1141 mg methionine 312 mg, phenylalanine 991 mg, threonine 617 mg, valine 937 mg, leucine 1385 mg and isoleucine 776 mg. As in other pulses, the sulphur- containing amino acids, cystine and methionine are limiting. The main fatty acids in the seed oil are palmitic acid, 18-24%, stearic acid, 5-12%, oleic acid, 18-24%, linoleic acid, 2-3% and behetnic acid, 3-7%. A content of 21% linolenic acid and no oleic acid, however, has also been recorded. The ratio of saturated and unsaturated fatty acids is approximately 1:2. The oil content is too low for the seed to be used as a source of oil (Agrodok, 2004).

Two varieties each, of bambara groundnut, together with kidney bean (*Phaseolus vulgaris* L.), Lima bean (*Phaseolus lanatus* L.), Pigeon pea (*Cajanus cajan* (L.) Millsp.) and one variety of Jack bean (*Canavaliea ensiformis* (L.) Dc.) Werw. were investigated in Nigeria (Adewusi *et al.*, 1991) for their proximate composition, mineral and amino acid contents, in the raw, cooked and autoclaved forms. The results indicated that the chemical compositions of the crude protein ranged from 20.6% in one variety of bambara groundnut to 27.7% in Lima beans. This remarkable high level of proteins underscores their importance as sources of this vital nutrient. Ether extracts of bambara groundnuts gave a value of 6.6%, which is low thus limiting the contribution of the seed as source of edible oil. Crude fiber of bambara groundnut in all cases was lower than that of other seeds. Total ash and N-free extracts compared favourably with the other test legumes. Macro and micro nutrients like calcium, phosphorus, magnesium, manganese, zinc etc were found in small quantities.

The seeds contain tannins, mainly in the seed coat. In comparative studies in Botswana and Ghana, tannin levels were found to be lowest in cream-coloured seeds, intermediate in red seeds and highest in black seeds. Ripe seeds are often preferred for red and black seeds, because they are less bitter (‘sweeter’) and take less time to cook. Large seeds are preferred over smaller ones, e.g. for use as snack: smaller seeds are ground into flour for use in various recipes. Dried leaves for fodder contain crude protein 15.9%, crude fibre 31.7%, ash 7.5% and fat 1.8 % (Doku, 1995).

2.1.12 Anti-nutrients of Bambara Groundnut

Bambara groundnuts like other legumes contain certain anti-nutrients. These anti-nutrients hinder the efficient utilization, absorption or digestion of nutrients and thus reduce their bioavailability and nutritional quality. One of the anti nutrients phytic acid (myo-inositol-1,2,3,4,5,6,-hexakinase hydrogen phosphate) forms complexes with protein (protein phytate complex and chelates essential dietary minerals such as zinc, magnesium, calcium and iron thus decreasing their utilization. Trypsin inhibitors have been found in bambara groundnuts. This plays a role as an anti nutritional factor associated with growth retardation, thus the seeds are not recommended as feed. However, the body is able to compensate trypsin activity by producing more enzymes (Swamithan and Kochhar, 1989). Cooking and other forms of processing (e.g. soaking, milling, hulling, germination, fermentation) reduced the concentration of anti nutritional factor. Some processing and cooking done before consumption has been found to reduce the phytic acid level. Sprouting and boiling of bambara groundnut has been demonstrated to reduce the phytic acid level by 56% in bambara groundnuts (Igbedioh *et al*., 1994). Sprouting also increased the protein level and moisture level significantly ($p<0.05$), while roasting tended to reduce other constituents except carbohydrate. Soaking was also found to reduce the phytic acid level through the leaching out of the phytate ions into the soaked water.

2.1.13 Breeding for Resistance of Bambara groundnut

Bambara groundnuts like other grains suffer loss and enormous damage from insect pests and pathogens during storage. As a result seed varieties have been screened and bred for resistance. Out of 31 varieties of bambara groundnut screened, six were found to be resistant to *C. maculatus* and *C. subinnotatus* (Ahmed and Yusuf, 2007). This is an inexpensive technology for the farmer and is an effective means of controlling insect pests on stored grains and poses little or no environmental hazards.

Results from screening 21 varieties cultivated in West Africa for resistance against *C. subinnotatus* revealed that three accessions (TVSu 1038, TVSu346 and TVSu 688) were moderately resistant (Mbata, 1993). Mbata (1992a) also reported three bambara groundnut accessions (TVSu 119, TVSu 9 and TVSu 11) that showed high resistance against *C. maculatus* out of ten varieties screened.

2.1.14 Insect Pests and Diseases of Bambara Groundnut

Bambara groundnut has a reputation for resisting pests. Dike (1997) observed up to 16 insect pests on bambara groundnut in Samaru, Nigeria. He recorded six sucking bugs, *Agonoscelis versicolor* Fab., *Clavigralla tomentosicollis* Stal., *Mirperus jaculus* Thunby, *Locris rubens* Erich, and *Poophilus spp.* as prevalent pests during the reproductive stage of bambara groundnut.

Bambara groundnut is susceptible to infestation by field-to-store pest bruchids. It is often infested simultaneously by two species of bruchidae, *C. maculatus* and *C. subinnotatus* in West Africa (Haines, 1991; Mbata, 1992b). Of the two bruchids, *C. maculatus* is more destructive on account of its shorter life cycle and higher intrinsic rate of increase (Lale and Vidal, 2001). Whilst *C. maculatus* attacks a wide range of grain legumes, *C. subinnotatus* is restricted to bambara groundnuts (Haines, 1991). Where seeds are left very long in the soil, they were found to be attacked by termites in Kenya (Ngugi, 1997).

In Nigeria, the crop is grown during the rainy season, a period of high temperatures and humidity; they are highly susceptible to fungal diseases. Fungal diseases like rust and leaf blight caused by *Puccinia* sp. and *Colletotrichum* sp., respectively have been reported in northern Nigeria associated with bambara groundnuts. Isolated cases of rosette have also been reported. Insect pests such as aphids, scale insects and mealybugs have been found transmitting viral diseases. In humid environments however, fungal diseases, such as *Cercospora* leaf spot, *Fusarium* wilt and *Sclerotium* rot are common (Begemann, 1986; 1988). In such circumstances spraying with the fungicide benlate [1kg/ha] has proved beneficial.

Viral diseases are widespread in most environments, especially in areas where other grain legumes such as cowpea are grown. Common diseases are cowpea mottle virus (CMeV) and cowpea aphid-borne mosaic virus (AbMV) (Ng *et al*. 1985). A combination of an unusually heavy virus attack and *Cercospora* leaf spot in one accession (TVSU218) resulted in zero yields in Kaboinse, Burkina Faso (Goli *et al*., 1991).

Simultaneous maturity of all fruits on one plant is a desirable trait in food production systems where harvesting is a single event. Fruits that mature long before the harvest date are more likely to be attacked by pests and diseases and/or to germinate prematurely resulting in large yield losses. In the humid environment of Ibadan, Nigeria, bambara groundnut was found to have 1.5% rotten pods at 115 days after sowing and 37.3%, 35 days later (Goli and Ng, 1988).

2.2 *Callosobruchus subinnotatus* Pic.

2.2.1 Description of *C. subinnotatus*

The bruchid belongs to the order Coleoptera, which comprise the largest order in the animal kingdom (Hinton and Corbet, 1949) and family bruchidae, tribe bruchini. Among fauna associated with stored products, Coleoptera are the most numerous with varied orders (Tropical Products Research Institute, 1972). The bambara groundnut bruchids species *C. subinnotatus* was originally described as a variety of *C. maculatus* [under a synonym] but was later recognized as a distinct species by Southgate (1958). The body colour is uniformly brown to black with pale setae, which is dull whitish and golden in male and whitish but with a dark setae forming vague lateral patches on the elytra in the females. The body is lighter than *C. maculatus* and *C. chinensis.* The body measurement is 4.5 - 5.5 mm long (Ofuya, 2001). The insect is about twice as large as *C. maculatus* (2 - 4 mm long). The inner tooth of the hind femur is acutely triangular and is slightly longer than the outer tooth.

As with *C. maculatus* there is sexual dimorphism with the female being larger than the males. Females of *Callosobruchus* species are known to produce pheromones that attract males. The males were found to be able to follow odour trails to the source in windless conditions of storage environment (Mbata and Ramaswamy, 2000). The highest percentage of males reached the source when the components of the pheromones (3-methyl-2-heptonoic acid (E32A) and (Z)-3-methyl-2-heptonoic acid (Z32A), were formulated in a 50:50 or 25:25 ratio. Males of *C. maculatus* responded to sex pheromones of *C. subinnotatus*, but males of *C. subinnotatus* did not respond to that of *C. maculatus*. The two pheromone components of *C. subinnotatus* are also constituents of *C. maculatus* sex pheromones. These two components may be potentially useful in monitoring the population of both species in stored legumes.

Rearing of *C. subinnotatus* under crowded condition at 30'C and 80% R. H. produced active males and females (Mbata *et al*., 1999). The active individuals emerged later than normal individuals and were lighter in weight (Mbata *et al*., 1999). The adult female lives an average of 12.2 days, while the male has an average lifespan of 22.2 days. An opening, 25- 30 µm in diameter, occurs distally on the medial surface of fore coxae of both males and females *C. maculatus and C. subinnotatus* (Ramaswamy and Monroe, 1997; Shu *et al*., 1999). Eggs are white and measure about 1 mm, larvae are white and C- shaped and measure 5 mm, the head capsule is brown.

2.2.2 Geographical Distribution of *C. subinnotatus*

C. subinnotatus is restricted almost entirely to West Africa, even though its host *Vigna subterranea* is grown throughout the arid zones of Africa and parts of Asia. It became established in Puerto Rico and in the small area of South America, but it is not known whether these populations have persisted. They are widely distributed in Nigeria (Amatobi, 2007). The crop is now found throughout tropical Africa as well as in Madagascar, Mauritius, Sri Lanka, Indonesia, the Philippines, Malaysia, the United States of America, New Caledonia, Northern Australia, tropical Central America, Suriname and Brazil (Tanimu, 2005).

2.2.3 Economic Importance of *C. subinnotatus*

The insect is a primary field-to-store pest restricted to attacking bambara groundnut only in sub Saharan West Africa (Dobie *et al.,* 1984; Appleby and Credland, 2001). It may also be found attacking cowpea seeds (Mbata, 1993) where were found in research environment to cause damage and loss to some cowpea varieties comparable to losses caused to the cowpea varieties by *C. maculatus.* Feeding activity by *C. subinnotatus* causes reduction in seed viability and increase in free fatty acids and peroxides which are indices for biochemical deterioration (Mbata, 1993).

2.2.4 Life cycle of *C. subinnotatus*

Infestation by *C. subinnotatus* often commences in the field as soon as bambara groundnut pods are harvested and left to dry. The life cycle of the bruchid has been reported (Dobie *et al*., 1984; Mbata, 1990; 1991). The developmental period under unfavourable conditions (30-35^{o}C and 60-90% r.h.) range from 25-28 days, but this period may be prolonged under variable conditions of temperature and humidity. Under ambient conditions ranging from 22-31^{o}C at an average humidity of 70% r.h. has been reported to be 34-42 days (Dobie *et al.* 1984; Mbata, 1990; 1991). Eggs are deposited on the pods while it is left in the field to dry or in the store. Females lay about 70 eggs almost uniformly distributed on the seeds (Mbata, 1992b). the antennae and labial and maxillary palp are involved in obtaining egg obtaining uniform egg distribution on seeds. Eggs hatch in 5-8 days, larval period lasts 8-16 days and pupation is within the seed in a chamber. Pupal period is about five days with pre-pupal period of two days. Developmental period from egg to adult ranges from 34-42 days (Amatobi, 2007).

2.2.5 Morphs of *C. subinnotatus*

A study of *C. subinnotatus*, the primary pest of bambara groundnut in West Africa found that there are two distinct morphs of this insect: one produces many eggs in the presence of seeds and another, which needs more complex external cues for reproduction to commence. If environmental conditions could be modified to ensure or even increase the production of the latter morph then pest populations would significantly decline (Credland, 2003). A series of laboratory studies was performed to investigate the bionomics of and differences between two apparently different morphs of adult of each sex of this species, here termed 'active' and 'normal' (Appleby and Credland, 2001). Major differences in their morphology, physiology and behaviour were identified and are described in detail for the first time. They provide clear evidence of the existence of an adult polymorphism among populations of this species, which is comparable in certain respects to that previously described for *C. maculatus* and *C. chinensis*. Adults can be separated into the correct morph based on characteristic differences in elytral and pygidial colour and pattern. 'Normal' adults are characterized by having high fecundity, short adult life and are relatively sedentary while 'active' adults exhibit reproductive diapause (suspension of reproductive activity), are long lived, and show (at least in females) increased dispersal tendencies. These characteristics suggest adaptation of the 'active' and 'normal' morphs respectively to the different environments of field and seed stores, and the significance of the polymorphism in the life history of *C. subinnotatus* is discussed in this context. The design of any effective control regime for this bruchid needs to take account of and could potentially be based upon the existence of polymorphism in the bruchid (Mbata, 1993). Almost entire life is spent in the seed. Adult is free-living and is the dispersal stage. It is capable of repeated generations in the store.

2.2.6 Natural Enemies of *C. subinnotatus*

Many natural enemies attack bruchids (Huis, 1991). Natural enemies of the bruchid include predators like spiders, lizards and geckos. *Dinarmus basalis* Rondani parasitizes the bruchid. *D. laticeps* was first described from specimens parasitizing *C. maculatus* (Waterson, 1921) and has been recorded many times on the *C. subinnotatus* and on many Bruchidae. The parasitoid, *Eupelmus vuilleti* Crawf. has been found parasitizing the related bruchid species, *C. maculatus* and *C. atrolineatus* (Huis *et al.,* 1990; Monge and Huignard, 1990; Alebeek, 1996).

2.2.7 Control of *C. subinnotatus*

Stored commodities in the storage environment have been shown to be subject to degradation by various pests which can cause severe quantitative and qualitative losses. The methods to be adopted for reducing post harvest losses due to pests will vary with the pest to be controlled, type of storage structure or container, quantity of commodity and storage duration (Ofuya and Lale, 2001). Many methods are used for control of bruchids in storage and include:

2.2.7.1 Physical Control Methods of *C. subinnotatus*

The susceptibility of *C. maculatus* and *C. subinnotatus* eggs, first and late instar larvae as well as adults to the lethal effect of solar heat of a temperature of 50^{o} C simulated in an incubator was evaluated (Lale and Vidal, 2001). Development of adult progeny was completely suppressed in both species in bambara groundnut seeds bearing one day-old eggs or habouring first instar larvae exposed for four or six hours to this temperature. This level of heat caused 100% mortality of these developmental stages. In *C. maculatus,* 100% of mortality occurred in first instar larvae in two hours, while in *C. subinnotatus* 100% mortality occurred in eggs and first instar larvae exposed for the same duration. On average, *C. maculatus* adults were more susceptible to the lethal effect of this level of heat than *C. subinnotatus.* Based on this result, solar heat could be used as an inexpensive method for the control of these species in developing countries. The use of low temperatures such as freezing temperatures of commodity, have proven effective in the control of storage insect pests.

2.2.7.2 Non-chemical Control of *C. subinnotatus*

Store sanitation is one of the most important methods of control of storage insect pests. Plant materials and oils have been employed for non-chemical control. Other methods include admixture of inert materials, plant oils, extracts and powders. It has been shown that storing grains like cowpea seeds by admixture with dry sand reduces insect damage because the sand reduces inter-granular spaces and prevents free movement of adults for oviposition (Akpaetok, 1974). Ash has also been used as desiccants in storage against bruchids. It acts by causing the desiccation of the eggs, larvae as well as adults; mortalities result from abrasion of the insect cuticle causing dehydration (De Lima, 1987). Ash particles could also clog spiracles and trachea causing suffocation (Wolfson *et al.*, 1991). Creating an airtight environment in storage is another effective method of storage insect control. Airtight situation stops fresh infestation from outside while the CO_2 produced by the grains and insect respiration causes oxygen to be depleted and brings metabolic activity to a stand still. Insects die not from CO_2 but from lack of oxygen.

2.2.7.3 Chemical Control of *C. subinnotatus*

Fumigants like DDVP and Aluminium Phosphide have been used in the control of *C. subinnotatus* (Ofuya and Lale, 2001). Pyrethroids are also effective for the control of the bruchids. The use of synthetic pesticides remains the major weapon in the ‘pest war’ (Hill, 1983). This is true today as it has been in the 1980’s. This is because the method is quick in action, and for majority of pest outbreaks, chemical control still remains the method by which the surest and most predictable results can be obtained. Many conventional insecticides have been demonstrated to be effective against storage bruchids either as dusts or fumigants (Rahman, 1990; Singh and Singh, 1990; Daglish, *et al.,* 1993). Insecticidal dusts of malathion, chloripyrifos-methyl, fenitrothion, methacrifos or pirimiphos-methyl (organo-phosphorus compounds), cabaryl (carbamate) or permethrin and deltamethrin (synthetic pyrethroids) can protect shelled pulses in storage (Ofuya, 2001).

There are many chemicals in the market that kill insects, but only a few can be used to control storage pest insects because of strict regulations on the use of pesticides on or near foodstuff. Insecticides that take a long time to degrade and as such leave residues in the produce are unsuitable for use on stored produce (Agrodok, 2004). Not one single insecticide is safe. Some insecticides can in addition to killing insects poison grains in such a way that can affect their viability as seeds (Hill, 1983).

2.2.7.3.1 Pirimiphos methyl

Pirimiphos-methyl has a low toxicity for humans and warm-blooded animals (Agrodok, 2004). It remains stable, even on relatively wet grain. It is persistent for several months which reduces the risk of re-infestation by second generation insects or new ones from the outside. The chemical is active against beetles, moths and mites and performs well against insects resistant to Malathion. Pirimiphos methyl is a phosphothionate insecticide which is an inhibitor of acetyl choline esterase. Pirimiphos-methyl is available in different formulations: dusts wettable powder, emulsiafiable concentrates and fumigants. Trade names include Actellic, Actellifog, Silosan and Blex (Agrodok, 2004). It is a fast acting organophosphorus insecticide with contact, respiratory and fumigant activity. It is considered a safe insecticide with low residues which can be used for postharvest clean-up in stores.

2.2.7.4 Use of Natural Products for the Control of Insect Pests

From the beginning of civilization, man has experimented with higher plants in an attempt to find remedies against various illnesses and diseases. Plant materials with insecticidal properties provide small-scale farmers with locally available, biodegradable and inexpensive method of pest control for storage. Through trial and error, early mankind has found medicinal properties in seeds, barks and roots of certain plants. Until the early 20th century, the main source of medicines consisted of drug preparations obtained primarily of terrestrial plant and vegetable sources (Madari and Jacobs, 2004). Intensive crop cultivation demands the use of strong chemical insecticides for both field and storage pest control. These are not only expensive but also hazardous for all living things. Use of bio-control agents may be an alternate solution that can reduce pest and disease incidence. Natural products, as crude plant extracts, have been used for thousands of years, and many of these ancient formulations have been recorded in ancient literature (De Pascual *et al.*, 1981). Several higher plants and their constituents have proven successful in controlling plant diseases and insect pests. These have turned out to be non-toxic and harmless (Ramezani, 2006). Essential oils of plant products have been used in medicine and dentistry because of their bactericidal or anti-parasitic properties. They are also used as insecticides, in the making of perfumes, soap and toiletries. They supply flavouring agents for food, alcoholic beverages and soft drinks. Minor applications are as a clearing agent in histological work and as a deodorant (Kio, 1998). Plant oils are used for storage insect control. The oils act as physical barriers to respiration of insect eggs and young larvae (Credland, 1992; Daniel and Smith, 1994), toxicity and repellence by constituents (Don Pedro, 1989; 1990)

Natural methods of plant protection have assumed new importance in an age when a host of commercial products is available, which seems to offer an easy answer to the problems of fighting pests and diseases. However, these have neither solved the purely agricultural problems of the small farmer, nor have they improved his financial situation. On the contrary, they have resulted in a series of consequences, which politically, economically, ecologically and socially are self defeating (Stoll, 1988). The natural plant products are relatively broad spectrum, bio-efficacious, economical, environmentally safe and biodegradable (Ramezani, 2006). Global pesticide use increased rapidly after the Second World War as technological advances led to dramatic expansion in agricultural production (Murray and Taylor, 2001). The new production techniques and the rise of modern chemical manufacturing sector were heralded as part of a 'miracle technology' promising an end to world hunger and disease. Prior to the economic realities in Nigeria, the use of synthetic pesticides was viewed as the panacea to food and health problems. Pesticides where widely used even in storage where produce is used for food preparations. Following the prohibitive cost of these chemicals, and also due to the public cries and complaints aired by environmentalists and other concerned persons in respect to the negative effects of synthetic pesticide usage, the trend has shifted to the search for alternative methods that are effective, safe and inexpensive. Insect damage in stored grains and pulses may amount to 10-40% in countries where modern storage technologies have not been introduced (Shaaya *et al*., 1997). Many farmers are unaware of the use of insecticidal plants despite their use by farmers in other areas and regions. In fact, the majority of farmers do nothing to protect their grain during storage largely because resource-poor farmers find conventional synthetic pesticides too expensive and difficult to obtain. Furthermore, inappropriate use of synthetic chemicals may lead to human and environmental health risks and promote insecticide resistance. Plant materials with insecticidal properties provide small-scale farmers with a locally available, biodegradable and inexpensive method of pest control for storage. Botanicals, such as neem and hot chilli pepper, have been used for generations throughout Africa, Asia and the Americas. Botanicals could, therefore, have advantages over synthetics as a cost-effective and environmentally sustainable alternative for protecting stored food against insect attack. However, information and scientific support on botanicals is generally inadequate, and it is often difficult to recommend particular plant materials as a replacement for chemical insecticides as the efficacy levels of botanicals can vary among storage pests, application methods, and stored products. This led to the research into natural plant products. Many spices and herbs and their extracts are known to possess insecticidal activities, which may frequently present in the essential oil fraction (Brattesten, 1983; Schmidt *et al.*, 1991; Shaaya *et al.*, 1991). Most of the essential oils are monoterpenoids, which are secondary plant chemicals and considered to be of little metabolic importance. The use of edible oils as contact insecticides to protect grains, especially legumes, against storage insects is traditional practice in many countries in Asia and Africa. This method is convenient and inexpensive for the protection of stored seeds in households and on small farms. Many different edible oils have been studied as stored grains protectants against insects (Verma and Pandey, 1978; Oca *et al.*, 1978; Pandey *et al.*, 1981; Messina and Renwick, 1983; Ivbijaro, 1984; Pierrard, 1986; Ahmed *et al.*, 1988; Don Pedro, 1989; Pacheco *et al.*, 1995). Plants with proven insecticidal properties include sweet clover (coumarins), neem (Azadirachtin), *Solanum* (some alkaloids), *Artemisia* (absinthin). Other plants are found to contain insecticidal phytols and diterpines (Shorey and Mckelvey, 1977).

2.3 The Mango Plant and Chemical Composition of Mango Butter

The genus *Mangifera* is one of 73 genera belonging to the family Anacardiaceae in the order Sapindales. Mangoes are grown in tropical and sub-tropical regions around the world (Ploetz *et al*., 1994). Because of their delicious taste, nutritional value, attractive fragrance and beautiful colour, mangoes are now important fruits in the world market.

Mango butter which is extracted from the decorticated mango seed has similar composition as Shea and cocoa butter. It is used for soap making and is attributed with properties for skin beautifying and healing. It is a fatty acid cold pressed from mango seeds with property and chemical composition similar to cocoa and shea butter. Once extracted it has an optimal shelf-life of two years. To prolong shelf-life it should be stored in a dark cool location. When analysed, its chemical composition was found to contain free fatty acids 0.154%, oleic acid 46%, Palmitic acid 6.6%, stearic acid 42%, total monounsaturated oils 40-50, total polyunsaturated 2-4. In Nigeria, the chemical composition of mango was reported to be as follows: 44.4% m.c., 6% protein, 12.8% fat, 32.8% carbohydrate, 2.0% crude fibre, 2% ash, 0.39% tannin, 46.3% stearic acid and 40.0% oleic acid. Other constituents include palmitic, linolenic, arachidonic and beheric in small quantities (<6.6%). Inorganic ions are: P (140mg/100g), mg (100mg/100g), Ca (49mg/100g), while Fe, Na, Mn, and Zn are in small quantities (<11.0mg/100g) (Anonymous, 2008b).

2.3.1 Medicinal and insecticidal properties of *M. indica* Butter

Mango butter is used for many purposes on human skin such as eczema and psoriasis as well as a natural pesticide for farming (Ploetz *et al*., 1994).

2.4 *Aloe vera* (*barbadensis*) (L.) Burm. f. and Chemical Composition of *A. vera* Gel

Aloe barbadensis Mill. (*Aloe vera* L.) belongs to the family Liliaceae. It is classified as a succulent. The plant is known to herbalists and medical folklorists for centuries as the 'medical plant' or 'the potted physician'. Aloe vera's use can be traced back 6,000 years to early Egyptian civilization, where the plant was depicted on stone carvings. Known as the "plant of immortality" aloe was presented as a burial gift to deceased Pharaohs (Anderson undated; Encyclopedia Americana, 1989). About 200 species of *Aloes* have been identified.

Aloe contains a mixture of crystalline glycosides known as aloin (18-25%), resin (16-63%), emodin and volatile oils. It also possesses the anthraquinone glycosides like barbaloin [aloe-emodin anthrone C-10 glycosides]:chrysophanic acid, B-barbaloin, and iso-barbaloin (Ali, 2002). In addition, flavanoids, oxanthraquinones, coumarines, amino acids, monosaccharides, polysaccharides, oils, sterols, triterpenes, vitamin C and group vitamins, citric L-malic and formic acids are present in all aloes. Presence of minerals B, P, Al, Mg, Cu, N, Ca, K, Si, Fe, Pb, Cr, Ba, Ag, Zn, and Sr have been confirmed (Ali, 2002).

2.4.1 Medicinal and Insecticidal Properties of *A. vera*

The plant is known to the ancient Greeks, the juice is used medicinally as a purgative and for treating many medical conditions. It also serves as a dye or pigment and as an insecticide.

Several species are grown for aloe juice (Encl. Americana, 1989). It is also used in treating shingles.

2.5 *Gmelina arborea* Roxb. and Chemical Composition of *G. arborea* Fruits

Gmelina also known as white teak is one of the best timbers of the tropics used for light construction and other uses. The plant is a deciduous tree 12 - 30 m high and 60 - 100 cm in diameter.. Drupes are ovate or pyriform, 2 - 2.5 cm long, smooth, becoming orange-yellow, pulpy, with large egg-shaped stone, having 1 - 4 cells. Seeds are 1 - 4 (Little, 1983).

Gmelina arborea is reported to be tolerant to disease, drought, fire, heat, laterite, light frosts, and slope. Although casting a dense shade itself, it is intolerant to shade as a seedling. [2n =36, 38]. The fruits of *G. arborea* have been found to consist of butyric and tartaric acids, alkaloids, sugars, resins and astringents as well as resinous and saccharine matter, the latter two are also found in the roots, which contain traces of benzoic acid.

2.5.1 Insecticidal Properties of *G. arborea*

Grave yard tests indicate that untreated timber of *G. arborea* may last 15 years in contact with the soil without deterioration (Little, 1983). This may be an indication of the plant having some pesticidal property that confers protection against soil pests.

2.6 *Moringa oleifera* Lam. and Chemical Composition of *M. oleifera* seeds

Moringa oleifera is a fast growing aesthetically pleasing small tree adapted to arid, sandy conditions. It is described as a multi purpose tree. It has wide adaptability and ease of establishment. Virtually every part of the plant is useful in some way. The plant *M. oleifera* is the best known of 14 species of the family Moringaceae. It adapted to a wide variety of soil types. *Moringa* is the only genus in the family Moringaceae. This genus comprises 13 species, all of which are trees that grow in tropical and sub-tropical climates (Annonymous, 2008a). The species is characterized by its long, drumstick shaped pods that contain its seeds. Within the first year of growth it has been shown to grow up to 4 m and can bear fruit within the same year. It is well known for its multipurpose attributes, wide adaptability and ease of establishment. Its chemical description is glucosinolate 4-α-L-rhamnosyloxy benzyl isothiocyanate. The root bark [which is poisonous] yields two alkaloids: moringine and moringinine.

2.6.1 Uses of *M. oleifera* in Pest Control

Moringa oleifera contains coagulant lectins whose insecticidal property caused larval weight disturbances (low larval weight) as well as increase in pupal mortality in the flour moth *Anagasta kuhniella* (de Oliviera *et al.,* 2011). The lectin also caused nutritional disturbances which may have caused the low larval weight. In another study to test the effect of the oil on *Aedes aegyptii* in Pakistan, results indicated significant effect of the treatments and the effectiveness increased with extended exposure interval and enhanced doses (Ashfaq *et al*., 2012).

2.7 *Artemisia annua* (*Absinthium*) Mill. and Chemical Composition of *A. annua*

Artemisia is a common type of wormwood that is native to temperate Asia, but naturalized throughout the world. It is large diverse genus of plants with between 200 - 400 species belonging to the daisy family asteraceae. It comprises hardy herbs and shrubs known for their volatile oils (Anonymous, 2008b). They grow in the temperate climates of the Northern and southern Hemispheres, usually in dry or semi-dry habitats. The fern-like leaves of many species are covered with white hairs. Most species have strong aromas and bitter tastes from terpenoids and sesquiterpene lactones, which exist as an adaptation to discourage herbivory. The small flowers are wind pollinated. The plant contains the anti-malarial agent artemisimin a sesquiterpene lactone. In addition four flavanoids were identified: chryssoplenetin, castinin, eupatin, artemetin (Shorey and Mckelvey, 1977). Highest contents of the principles are found at the full blooming stage.

2.7.1 Insecticidal property of *A. annua*

Absinthin as found in Artemisia is found to act as an anti-feedant to insects (Shorey and Mckelvey, 1977). It is known to be used in the house for repelling fleas.

2.8 Neem *Azadirachta indica* A. Juss. and Chemical Composition of Neem seed

The Neem plant, (*Azadirachta indica*, syn. *Melia azadirachta* L., *Antelaea azadirachta* (L.) Adelb.) is a tree in the mahogany family Meliaceae. It is one of two species in the genus *Azadirachta* and is native to India and Burma, growing in tropical and semi-tropical regions. Neem is a fast growing tree that can reach a height of 15 - 20 m, rarely to 35 - 40 m. It is evergreen but under severe drought it may shed most or nearly all of its leaves. The branches are wide spread. The fairly dense crown is roundish or oval and may reach the diameter of 15 - 20 m in old, free-standing specimens. The neem tree is noted for its drought resistance. Normally, it thrives in areas with sub-arid to sub-humid conditions, with an annual rainfall between 400 and 1200 mm. It can grow in regions with an annual rainfall below 400 mm, but in such cases it depends largely on the ground water levels (Anonymous, 2007). Neem can grow in many different types of soil, but it thrives best on well drained deep and sandy soils (pH 6.2-7.0). It is a typical tropical/subtropical tree and exists at annual mean temperatures between 21-32 °C. It can tolerate high to very high temperatures. It does not tolerate temperature below 4 °C (leaf shedding and death may ensue).

Azadirachtin is a chemical compound belonging to the limonoids. It is a secondary metabolite present in the neem tree seeds. The molecular formula is $C_{35}H_{44}O_{16}$ (fig.3). Azadirachtin is a highly oxidized tetranortriterpenoid which boasts a plethora of oxygen functionality, comprising enol ether, acetal, hemiacetal, and tetra-substituted oxirane as well as a variety of carboxylic esters. It is classified among the plant secondary metabolites (Anonymous, 2007).

Additionally, both secondary and tertiary hydroxyl groups and tetrahydrofuran ether are present. Inspection of the molecular structure reveals 16 stereogenic centers, seven of which are tetra-substituted. These characteristics explain the great difficulty encountered when trying to produce it by a synthetic approach. It was initially found to be active as a feeding inhibitor towards the desert locust (*Schistocerca gregaria* (Forskal)) (Anonymous, 2007). It is now known to affect over 200 species of insect, by acting mainly as an anti-feedant and growth disruptor. It fulfils many of the criteria needed for a natural insecticide if it is to replace synthetic compounds. Azadirachtin is biodegradable and shows very low toxicity to mammals, thus being environmentally sound. This compound is found in the seeds of the neem tree (0.2 to 0.8 percent by weight). Many more compounds, related to Azadirachtin, are present in the seeds as well as in the leaves and the bark of the Neem tree, which also show strong biological activities (Ali, 2002). Azadirachtin is the only correct name for this compound e.g. Azadirachtin A is incorrect.

2.8.1 Uses of neem in Pest and Disease Control

Neem is deemed very effective in the treatment of scabies although only preliminary scientific proof exists which still has to be corroborated, and is recommended for those who are sensitive to permethrin, a known insecticide which might be irritant. Also, the scabies mite has yet to become resistant to neem, so in persistent cases neem has been shown to be very effective. There is also anecdotal evidence of its effectiveness in treating infestations of head lice in humans. It is also very good for treating worms (soak the branches and leaves in lukewarm water and then drink it). Neem oil is described as a successful insecticide (Agrodok, 2004). Among its many benefits, the one that is most unusual and immediately practical is the control of farm and household pests. Some entomologists now conclude that neem has such remarkable powers for controlling insects that it will usher in a new era in safe, natural pesticides. Azadirachtin is a feeding deterrent in insects (Ali, 2002).

2.9 Use of Plant Extracts and Oils for Storage of Grains

Many vegetable oils have been used as protective additives in storage. An advantage is they are easy to apply. Many oils have been used with success including oils of peanuts, coconut, safflower, neem, mustard, castor bean, cotton seeds, soybeans and maize (Agrodok, 2004). Several workers have proven that coating legume seeds with oils extracted from plants is effective in bruchid damage control (Singh *et al.,* 1978; Messina and Renwick, 1983; Don Pedro, 1989). Although very promising, however, high levels are needed for effective pest control (typically 5 or 10 ml/kg of seed). Plant oils are toxic to young larvae, adults and eggs of bruchids. The mode of action is partially attributed to interference with normal respiration resulting in suffocation (Ofuya and Lale, 2001).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Plant Products

The following plant species were collected and processed. Ripe orange coloured drupes of *Gmelina arborea* Roxb., *Azadirachta indica* A. Juss.(“Dalbejiya’ or “Dogonyaro’(Hausa)) seed, whole fully mature leaves of *Aloe barbadensis* [L] Burm. f. and seeds of *Mangifera indica* (‘mangwaro (Hausa))’. These were collected locally around the premises of the Ahmadu Bello University, Zaria, Nigeria. Seeds of *Moringa oleifera* (‘zogale (Hausa)) and dried shrubs (leaves, stems and flowers) of *Artemisia annua* Mill.(‘Tazargade’ (Hausa)) were bought from local markets in Zaria. The plants were chosen for the present study because of either their insecticidal property against some insect species (*M. oleifera, A. vera* and *A. annua)* or to investigate the possibility of their use as insecticides for the control of the bruchid *C. subinnotatus.* Oils from seeds *A. indica, G. arborea, M. oleifera and M. indica* L. were extracted and used while the gel of *A. vera* was extracted manually after washing in distilled water and crude extract of *A. annua* was extracted and used for the experiments.

3.1.1 Juice Extraction Method of *G. arborea*

Ripe orange coloured drupes of *G. arborea* were collected and gently rolled on table top to soften it. A sterile syringe was inserted and the juice extracted. Extracted juice was stored in glass specimen bottles and kept in a laboratory refrigerator for when needed.

3.1.2 Method for Gel Extraction of *A. vera*

Ripe mature succulent leaves of *A. vera* was cut at the base from the main plant washed in clean water to remove the bitter yellow green sap. The outer shiny green leaf was removed with a knife and the gel gently rinsed in distilled water and place in a clean Petri dish. The gel was crushes using a clean fork to obtain approximately uniform constituency for use in the study.

3.1.3 Oil Extraction Method

Oil from seeds of *M. oleifera, M. indica* and *A. indica* seeds were extracted using Soxhlet extraction method (Jensen, 2007). The seeds were air dried on a table under laboratory conditions to avoid solar leaching (Nuhu. Pers. Comm.) for seven days. The dried seeds were separately ground to powder after decorticating and 200g batches were weighed into the extraction thimble of a Soxhlet extractor. The oils were extracted using Petroleum Ether (Boiling Point $60^{o}C$) as extraction Solvent. The solvent was removed and resultant oil purified by simple distillation method under reduced pressure.

3.1.4 Method for Aqueous Extraction of *A. annua*

Market bought plant *A. annua* consisted of dried twigs, stems, leaves and flower buds. This was air dried in a laboratory for one week it was then pounded in a mortar to obtain a fine powder. Five hundred grammes of the powder was weighed and placed in a five litre plastic bucket with a cover. Four litres of water was added and allowed to stay for twenty hours. The extract was sieved with a muslin cloth and this was stored in a refrigerator when not in use

3.2 Sources of Bambara Groundnut

Local Bambara groundnut variety used for the experiment was obtained from local markets in Sabon Gari (kasuwan mata*)* and Giwa (Thursday market) local government areas of Kaduna State, Nigeria. The nuts were each time from the last season's stock and care was taken to ensure that there was no insect activity before purchase. The bambara groundnuts were manually picked to remove broken seeds, seeds with holes and other contaminants and then sieved using a 3 mm mesh sieve to remove insects and remaining contaminants before fumigating using aluminium phosphide (Phostoxin) tablets. One tablet was placed in a donation envelop and placed in a bag of 20 kg Bambara groundnut. This was placed in a polythene bag to create an airtight environment necessary for fumigation. The treatment was carried out for 72 hours after which the envelope containing the fumigant was removed and discarded. Treated Bambara groundnut seeds were spread on a plastic mat on a flat table, covered with wire mesh (2.0 mm mesh size) and allowed to stay for 24 hours in order for the effect of the fumigant to dissipate. Treated bambara groundnut used for the research work was stored in airtight polythene bag to stop re-infestation of the grains.

3.3 Insect Culture

Adult *C. subinnotatus* were obtained from a batch of naturally infested bambara groundnut from local markets in Dawanau and Dabatta (Kano State). These were placed in six Kilner jars containing 500 g bambara groundnut seeds and allowed to oviposit for 15 days before dead and live parent stocks were completely sieved out to await the emergence of F1 generation used for the experiment. The bruchid was identified at the Insect Museum of the Department of

Crop Protection Ahmadu Bello University, Zaria. For the purpose of this experiment, a mass laboratory culture of *C. subinnotatus* was maintained at the different stages of the study.

3.4 Disinfestation of Bambara Groundnut

All bambara groundnuts used for the present experiment were clean, well-formed seeds (Plate II) that were pre-fumigated using Aluminium phosphide tablets at the rate of 0.2 g/kg for 72 hours under airtight conditions to exclude any possible infestation that may affect the treatment. After 72 hours, the fumigated bambara groundnut was spread on a clean table covered with baft cloth to remove any residual effect of the fumigant and to exclude reinfestation. This was done for 48 hours.

3.5 Behaviour, Movement and Size of *C. subinnotatus*

The general behaviour of the bruchid was studied. Observations made included movement, oviposition, and mating habits. This was aimed at monitoring and documenting some characteristics that may be crucial in creating effective management and control methods that may be useful in bringing down the bruchids population. The behaviour and movement observations were carried out visually using hand lens. Twenty adults (ten each of the sexes) were allowed to roam freely on a table and covered with a transparent plexiglass for easy visibility. These were observed for two hours. This was repeated five times. The size of the bruchid was observed under the microscope at magnification of 4 x eye piece. One hundred adult bruchids were measured from the tip of the mouthparts to the caudal end to determine the average length. Readings were taken in microns using a calibrated slide mounted on the microscope. This was converted to millimeters by multiplying by 12.5. The mean number of holes per seed was estimated by randomly selecting 100 seeds from the untreated control and the number of holes per seed counted. This was aimed at estimating the level of damage if effective control measures are not undertaken.

3.6 Effect of Plant Product Treatments on the Mortality and Progeny Emergence of *C. subinnotatus*

For the purpose of this experiment, bambara groundnuts used were clean, well-formed seeds because females of most species of storage pests prefer to oviposit on larger, higher quality hosts (Schmidt and Smith, 1985; Takagi, 1986; Mitchell, 1990; Hardy *et al*., 1992; Fox and Mousseau, 1995). The variety was the cream coloured variety known locally as *gujiya* (Plate II). This is the commonest type of bambara groundnuts found in the Zaria area. Two hundred and fifty grammes each of bambara groundnut was placed in plastic containers and the plant products *G. arborea* drupe juice, crude aqueous extract of leaves of *A. annua, A. vera* and oil of *M. oleifera* and *M. indica* butter were applied at the rates of 2.0, 4.0 and 8.0 ml/kg (i.e. 0.5, 1.0 and 2.0 ml/250g bambara groundnuts). Actellic 2.0% dust (Pirimiphos-methyl) was applied as a check at the rate of 10 g/kg. Neem oil was used as a standard at the rate of 2.0 ml/kg (Agrodok, 2007). There is an untreated control. Five pairs of day old adults (0 - 24 hr old) *C. subinnotatus* were placed in each treatment. The design was Completely Randomized Design and each treatment replicated three times (Plate III) these were placed on a flat table where they received approximately equal light and aeration. All treatments were labeled accordingly.

Mortality readings for the bruchids were taken at 24, 48 and 72 hours after setting the experiment. Thereafter readings were taken at weekly intervals for three weeks. The aim of this was to investigate the duration between application of treatment and mortality of the experimental bruchids. Dead bruchids were counted after sieving out the insects. The live adults were returned to their respective Kilner jars. After the third week, the adults were sieved out and observed for another two weeks to await progeny emergence.

3.6.1 Data Analysis for mortality of Adult *C. subinnotatus*

Data obtained for adult mortality was analyzed using the one-way analysis of variance and differences between means were compared at the 5% level of probability with Student's Newman's Keuls (SNK). All experiments were carried out under prevailing room conditions.

3.7 Determination of Effective Concentration of Plant Product Treatments on Mortality of *C. subinnotatus*

The effective concentration of the plant products of the three levels under investigation in causing the mortality of the adult bruchids was determined. This was done by studying and recording the lowest concentraton that caused mortality within the shortest period of time as against doses that caused later or no mortality within the duration of the mortality study.

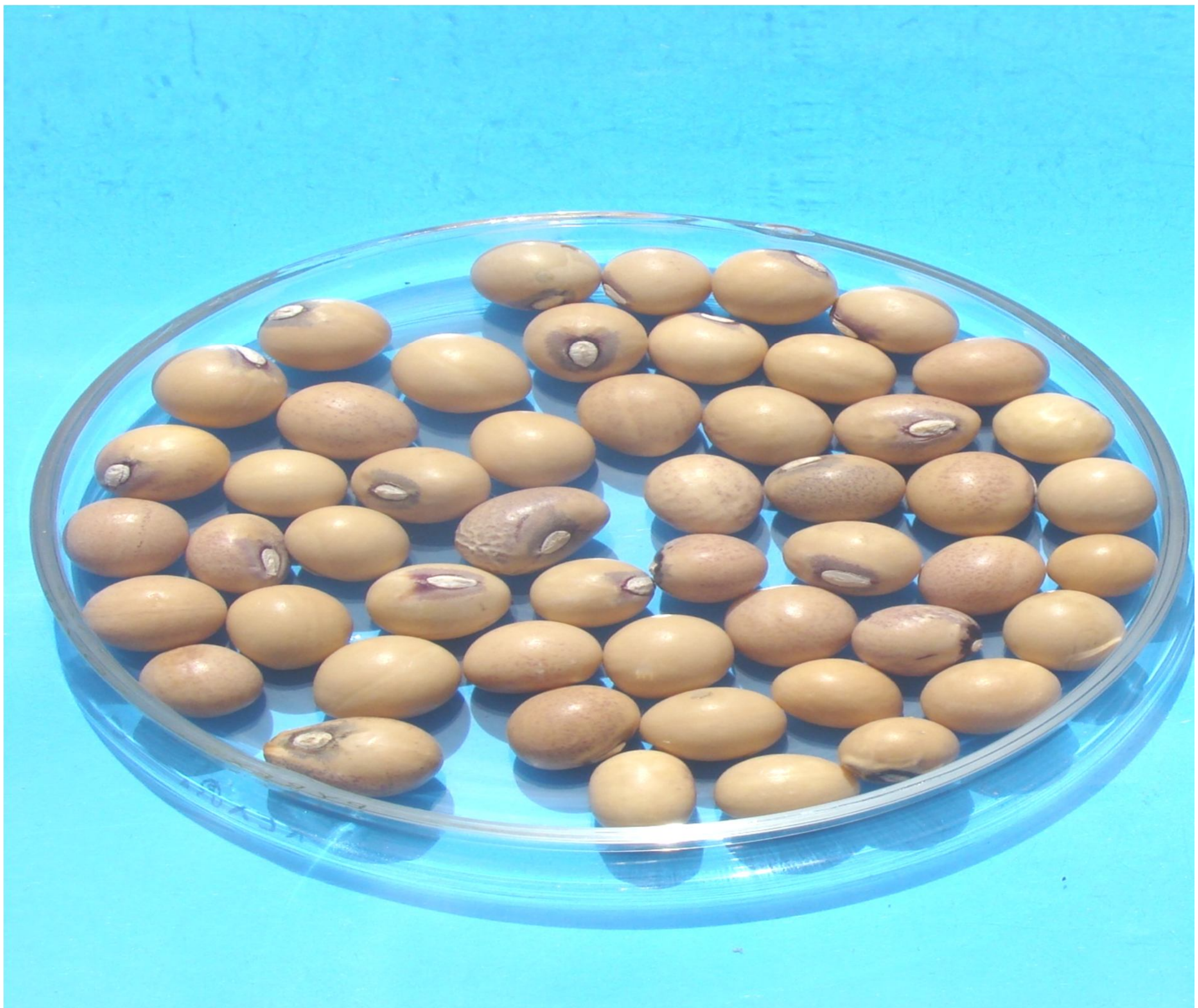

Plate II: Clean Cream Coloured Bambara Groundnut Variety used in the Study

Plate III: Experimental Set-up Completely Randomized Design

3.8 Determination of the Bioactivity of Test Materials on *C. subinnotatus*

3.8.1 Contact Toxicity

Test plant product materials were topically applied (i.e. applied directly to the surface) dorsally to the thorax of ten adult *C. subinnotatus* of both sexes using a micro pipette at the rate of 0.5µl. The insects were then transferred to 50 g of the culture media and observed daily for a week [Liu and Ho, 1999]. Distilled water was used as control.

3.8.1.1 Data Analysis for Contact Toxicity

Mortality count was taken daily for one week, data was analyzed using the one- way analysis of variance and differences between means was separated using SNK at the 5 % level of probability.

3.8.2 Fumigant toxicity

Filter papers were impregnated with 25 µl of each test material and placed on the underside of the cover of a clean glass specimen bottle (capacity 55 ml) containing ten day old test insects (five pairs) and screwed tightly. Distilled water was used as control on the underside of the specimen bottle covers. These were incubated for 72 hours and mortality readings taken. After 72 hours, the insects were then transferred to clean specimen bottles containing some culture media (50 g) and observed for 96 hours (Liu and Ho, 1999). This is so that the insects can get ventilation and mortality readings expected to be as a result of toxicity of test materials the insects were exposed to as a fumigant.

3.8.2.1 Data Analysis For Fumigant Toxicity

Mortality count was taken, data was analysed using the one- way analysis of variance (Single factor ANOVA) and differences between means was separated using SNK at the 5 % level of probability.

3.8.3 Repellent Toxicity

Half filter paper discs (Whatman size one filter paper) were prepared by cutting the filter paper to two equal parts with a pair of scissors and 750 µl of each of the test treatment material was applied to one half of the filter paper and distilled water as control was added to the other half disc. Each treated half was attached lengthwise, edge to edge to a control half disc with a thin strip adhesive tape and placed in a Petri dish. Each treatment was replicated five times. The orientation of the seam was changed in the replicates to avoid the effect of any external directional stimulus affecting the distribution of the insects (Sighamony *et al*., 1984; Liu and Ho, 1999). Twenty adult day old bruchids were released in the middle of each filter paper circle and transparent netted cloth was placed and held with a rubber band on top for ventilation and visibility. The number of insects that settled on each half of the filter paper were counted after one hour and then at hourly intervals for five hours.

3.8.4.1. Data Analysis for Repellent Toxicity

The average counts were converted to percentage repellency (PR) using the formula (Liu and Ho, 1999).

$$PR = 2(c - 50)$$

$$\% R = \frac{Nc - Nt}{Nc + Nt} \times 100$$

Where:

Nc is number of insects on the untreated (control) half of the disc

Nt is the number of insects on the treated half of the disc

c is the percentage of insects on the untreated half of the disc.

Positive values expressed repellency and negative values attractancy. The data (PR) was analyzed using ANOVA and DNMRT after transforming them into arcsine percentage values. Arcsine percentage was obtained by dividing the percentage value by 100, taking the square root and then finding the inverse Sin value ($\sin^{-1}$).

3.9 Moisture Content and Weight Loss Determination of Treated Bambara Groundnut

The moisture content of bambara groundnut used in the experiment were determined at the beginning and at the end of each experiment using Microwave oven method and oven dry method (Pixton, 1967). Using microwave oven to determine the moisture content is advantageous because it dries the food material in a minimum amount of time and can provide accurate result (Edward *et al.,* 2002). Weighed samples were placed in a microwave oven for two minutes at a time until uniform weight was obtained three consecutive times and the dried mass was weighed. The microwave used was a domestic microwave oven model Thermocool at a high setting. Nevertheless, care was taken to standardize the drying procedure and to ensure that the microwave energy is applied evenly across the sample this was done by ensuring that the microwave tray was property fitted so that it rotated throughout the drying process.

Oven-dry method was determined by grinding the bambara groundnuts to coarse powder, weighed and placed in aluminium tins of known weight. The tins and samples were placed in an oven running at 120^{o}C for three hours. After removal from the oven, the tins were immediately covered to avoid absorption of moisture and allowed to cool for two hours before weighing. Percentage moisture content (M.C.) was calculated as:

$$\text{M. C.} = \frac{X - Y}{X} \times 100$$

Where:

X =original weight of sample

Y = final weight of sample

X – Y = weight of water in sample

At the end of the experiment, by the fifth week, each experimental unit was weighed to assess the effect of feeding activity and test products on weight of Bambara groundnuts. The data obtained was analysed using single factor analysis of variance and differences between means separated at the 5% level of probability using SNK.

3.10 Effect of Treatments and Insect Activity on Percentage Germination of Bambara Groundnut

After the termination of mortality studies in which Bambara groundnut treated with various concentration levels of botanical preparations were exposed to *C. subinnotatus*, ten seeds were randomly selected from each treatment and separately placed on moist filter papers and observed daily for one week to determine the effect of treatment and insect activity on germination of Bambara groundnut seeds. Additional moisture was added to keep the filter papers wet on a daily basis. This was necessary as Bambara groundnut possesses a hard testa and required adequate moisture in order to germinate. Three replicates were used for each treatment. Germinated seeds were counted and recorded. Percentage germination was calculated and data analyzed using single factor analysis of variance and means separated using SNK at the 5% level of probability.

CHAPTER FOUR

4.0 RESULTS

Observed Behaviour and characteristics of *C. subinnotatus*

The bruchid were active fliers and many flew away as soon as culture containers were opened. Day olds as well as older insects flew readily. The sexes could be differentiated by their colours where the male was almost uniformly brown; the female was dark with black markings on the elytra (Plate IV). When the food stock was depleted, the insects emerging were small in size. During mating, the male made several attempts to mount the female by clasping her abdomen with its fore legs. The female frequently moved away until the male could get a successful grip on the abdomen. At full maturity, and with adequate food supply, the male and female appeared to be almost the same size with the female being slightly longer (mean length of 100 males and 100 females was 3.9 mm and 4.5 mm, respectively).

Completely hollowed out seeds were found to contain large numbers of dead beetles many of which had their mouthparts pointing outwards. In warm temperatures (28^{o}C and above) the adults laid their eggs on all available surfaces in the absence of a suitable medium. These eggs never hatched. Heavily infested seeds had eggs and emergence holes on the seeds from the emergence of the adult beetles and appeared unattractive compared to the clean seeds. These seeds become heavily infested with fungi which caused discoloration of the seeds (Plate V). Infested seeds also showed evidence of qualitative and quantitative deterioration (Plate VI). Number of holes per seed depending on the size of the seed ranged from one to 19 with mean number of holes being 5.9 per seed. Eggs were laid glued to the surface of the seeds (Plate VII) from where they hatched and bored into the seeds to continue development.

Plate IV Adult male (A) and Female (B) *C. subinnotatus*

Plate V: Eggs of *C. subinnotatus* on Bambara Groundnut Seeds

Plate VI: Clean (left) and Damaged (right) Bambara Groundnut with Characteristic Big Round Emergence Holes Produced by *C. subinnotatatus*

Plate VII: Infested Bambara Groundnut Showing *C. subinnotatus* with Eggs and Big Round Emergence Holes

4.4 First Year Mortality Studies

When analysed using single factor (One-way) ANOVA and means separated using SNK at the 5% level of probability in 2008, the effect of treatments on the mortality of *C. subinnotatus,* from 24 hours after treatment showed that the standards Actellic and neem caused significant mortality of the bruchid that was statistically not different from each other ($P>0.05$). The oil treatment of *M. oleifera* at all the three levels under investigation (2, 4, and 8 ml/kg) and the drupe juice extract of *G. arborea* (4 ml/kg) caused mortality of the bruchid that compared favourably to the standard treatments with neem and Actellic ($P> 0.05$) (Table 1) and was statistically higher than the other treatments ($P<0.05$). The other treatments and the untreated control did not compare favourably to the standards ($P<0.05$) and were not significantly different from each other ($P>0.05$). Forty eight hours after treatment, all three levels of application of *M. oleifera* as well *G. arborea* at the application rate of 4 ml/kg compared favourably to the two standards, actellic 2% dust and neem seed oil (Table 2). They showed superior performance over treatments with *M. indica* at application rates of 2 and 4 ml/kg by being statistically higher than these treatments ($P< 0.05$). Two treatment levels of *M. indica* (2- and 4 ml/kg) were statistically the same and compared favourably to the treatment with *A. annua* at the application rate of 8 ml/kg (Table 2). Treatment with *A. annua* at the rate of 8 ml/kg compared favourable to the other treatments but was statistically different from the untreated control and treatments with *A. vera* (2 mg/kg) by being superior to them at the 5% level of significance.

Seventy two hours after introducing adult *C. subinnotatus* on bambara groundnuts treated with the plant products, those treated with *M. oleifera* (2, 4 and 8 ml/kg), *G. arborea* (2 and 4 ml/kg), *A. annua* (4 and 8 ml/kg) caused mortality of the bruchid that was comparable to the standard treatments ($P>0.05$) (Table 3). One week after treatment, *M. indica* butter at 2- and 4 ml/kg compared favourably with the standards (neem and Actellic) as well as *M. oleifera* at all levels and *G. arborea* at 4 ml/kg by not being statistically significantly different (P>0.05) (Table 4). By the first week of investigation into the effect of the plant products on the mortality of the bruchid, all the treatments except *G. arborea* (8 ml/kg) were not significantly different from each other when compared using SNK at the 5% level of probability (Table 4). Two weeks post application of plant materials, all three levels of treatments with *M. oleifera* and *A. annua* as well as treatments with *M. indica* (2 and 4 ml/kg), *G. arborea* (2 and 4 ml/kg) and *A. vera* (8 mg/kg) caused mortality of the bruchid that was statistically comparable to the standards. Treatments with *A. vera* (2 and 4 mg/kg), *M. indica* (8 ml/kg) and untreated control compared favourably to each other (P>0.05) but were also not statistically different from the standards and the least effective treatment of *G. arborea* at an application rate of 8 ml/kg (Table 4). Three weeks after the application of plant material treatments, mortality readings of bruchid indicated that all the treatments including the untreated control were statistically comparable to the standards except treatments of *A. vera* (4 mg/kg) and *G. arborea* (8 ml/kg) which were statistically comparable to each other (P>0.05) but different from the other treatments (P<0.05) (Table 4).

4.5 Second Mortality Studies

When analysed using single factor (One-way) ANOVA and means separated using SNK at the 5% level of probability in 2009, the effect of treatments on the mortality of *C. subinnotatus,* from 24 hours after treatment showed that the standards, Actellic and neem caused significant mortality of the bruchid that was statistically not different from each other P>0.05) like in 2008. The oil treatment of *M. oleifera* at all the three levels under investigation (2, 4, and 8 ml/kg) and the drupe juice extract of *G. arborea* at application rate of 8 ml/kg bambara groundnut seed caused mortality of the bruchid that compared favourably to the standard treatments with neem and Actellic (P> 0.05) (Table 1). Unlike in the first trial, *G. arborea* at the application rate of 4 ml/kg did not compare to the standard treatments and its performance was significantly lower than the standards at 24 hours post application. It however, compared favourably to treatment with *G. arborea* at the application rate of 8 ml/ kg by not being statistically different from it (P>0.05). The other treatments showed variable efficacies compared to the standards (Table 1).

Results from 48 hours post application of plant materials indicated that treatments of *G. arborea* at the application rate of 4- and 8 ml/kg and *A. vera* at application rate of 8 mg/kg bambara groundnut seed showed comparable performance to the standards (Table 2) and *M. oleifera* at all three levels of application by not being significantly different from each other (P>0.05). Treatments with *M. indica* (8 ml/kg) and the untreated control showed comparable efficacy in causing the mortality of the bruchid that was significantly less than the standards (P<0.05). Mortality caused by the different treatments from 72 hours showed that *A. annua* at the application rate of 8 ml/kg and *M. indica* at an application rate of 2 ml/kg seed compared favourably to the standards. Treatments with *M. oleifera* at all three levels of application as well as *G. arborea* (4- and 8 ml/kg) caused higher mortality of the bruchid comparable to the two standards (P>0.05). However, the untreated control and treatment with *A. vera* at the application rate of 2 ml/kg and *M. indica* (8 ml/kg) did not compare to the standards and were statistically different from the standards (P<0.05) (Table 5). When tested at the 5% level of probability and means compared using SNK, results for the first week post treatment showed all the treatments at the different levels performing comparatively similar (P>0.05) except for the untreated control and *A. vera* at application rate of 2 mg/kg, which were not similar to the other treatments (P<

0.05) and were significantly different from each other ($P<0.05$) (Table 5). Results for the second and third weeks showed all the treatments including the untreated control not being statistically different from each other ($P>0.05$) and all being statistically higher than treatments of *A. vera* at an application rate of 2 mg/kg (Table 5).

Table 1: Mortality of *C. subinnotatus* 24 Hours after Infestation on Bambara Groundnuts Treated with Plant Products

Treatments	Concentration (v/w or w/w)	Mean No. of dead Insects/ 10 Adults	
		2008	2009
A. vera Gel	2.0	0.00^{a}	0.00^{a}
	4.0	2.00^{a}	2.00^{ab}
	8.0	0.67^{a}	4.00^{bc}
A. annua aqueous extract	2.0	0.33^{a}	2.00^{ab}
	4.0	2.00^{a}	3.00^{ab}
	8.0	1.67^{a}	1.33^{ab}
M. indica butter	2.0	2.33^{a}	2.67^{ab}
	4.0	2.33^{a}	1.67^{ab}
	8.0	1.33^{a}	0.00^{a}
G. arborea drupe juice	2.0	1.00^{a}	2.33^{ab}
	4.0	8.67^{b}	6.00^{cd}
	8.0	1.33^{a}	7.00^{de}
M. oleifera oil	2.0	10.0^{b}	10.00^{e}
	4.0	10.0^{b}	10.00^{e}
	8.0	10.0^{b}	10.00^{e}
Neem seed oil	2.0	8.67^{b}	9.00^{e}
Actellic dust	2.5	9.67^{b}	10.00^{e}
Untreated control	0.0	0.00^{a}	0.33^{a}
S.E ±		1.014 ±	1.07 ±

Means followed by the same letter within each column are not significantly different (P>0.05) Student Newman Keuls.

Table 2: Mortality of *C. subinnotatus* 48 hours after infestation on bambara groundnuts treated with plant products

		Mean No. of dead insects/10 Adults	
Treatments	**Concentration (v/w or w/w)**	**2008**	**2009**
A. vera gel	2.0	0.33[a]	1.67 [ab]
	4.0	3.00 [abc]	3.00 [ab]
	8.0	1.67[abc]	10.00 [c]
A. annua aqueous extract	2.0	2.00 [abc]	4.67 [ab]
	4.0	3.00 [abc]	5.00 [b]
	8.0	4.0 [bc]	3.00 [ab]
M. indica butter	2.0	5.00 [c]	4.00 [ab]
	4.0	5.00 [c]	3.33 [ab]
	8.0	3.00 [abc]	1.33 [a]
G. arborea drupe juice	2.0	3.00 [abc]	2.33 [ab]
	4.0	9.67 [d]	9.67 [c]
	8.0	1.33 [ab]	9.67 [c]
M. oleifera oil	2.0	10.00 [d]	10.00[c]
	4.0	10.00 [d]	10.00 [c]
	8.0	10.00 [d]	10.00 [c]
Neem seed oil	2.0	10.00 [d]	10.00 [c]
Actellic dust	2.5	10.00 [d]	10.00 [c]
Untreated control	0.0	0.67 [ab]	1.33 [a]
S.E ±		1.06 ±	1.06 ±

Means followed by the same letter within each column are not significantly different (P>0.05) Student Newman Keuls.

Table 3: Mortality of *C. subinnotatus* 72 hours after infestation on bambara groundnuts treated with plant products

		Mean No. of dead Insects/10	
Treatments	**Concentration (v/w or w/w)**	**2008**	**2009**
A. vera gel	2.0	2.67^{ab}	1.67^{a}
	4.0	3.67^{ab}	6.00^{b}
	8.0	3.00^{ab}	10.00^{c}
A. annua aqueous extract	2.0	4.00^{ab}	7.00^{cb}
	4.0	5.00^{abc}	7.00^{cb}
	8.0	6.00^{abc}	5.00^{b}
M. indica butter	2.0	7.00^{abc}	6.33^{cb}
	4.0	7.00^{abc}	6.00^{b}
	8.0	3.00^{ab}	1.67^{a}
G. arborea drupe	2.0	5.67^{abc}	6.00^{b}
juice	4.0	10.00^{c}	10.00^{c}
	8.0	1.67^{a}	10.00^{c}
M. oleifera oil	2.0	10.00^{c}	10.00^{c}
	4.0	10.00^{c}	10.00^{c}
	8.0	10.00^{c}	10.00^{c}
Neem seed oil	2.0	10.00^{c}	10.00^{c}
Actellic dust	2.5	10.00^{c}	10.00^{c}
Untreated control	0.0	2.67^{ab}	2.67^{a}
S. E ±		1.52 ±	0.075 ±

Means followed by the same letter within each column are not significantly different (P>0.05) Student Newman Keuls.

Table 4: Year One Weekly Analyses of Effect of Test Materials on Mortality of *C. subinnotatus*

		Mean Number of dead insects/10 Adults		
Treatments	**Concentration (v/w or w/w)**	**One Week**	**Two Weeks**	**Three Weeks**
A. vera gel	2.0	6.33^{ab}	7.00^{ab}	10.00^{b}
	4.0	5.33^{ab}	6.00^{ab}	7.33^{a}
	8.0	6.00^{ab}	9.33^{b}	10.00^{b}
A. annua aqueous extract	2.0	8.00^{ab}	9.33^{b}	10.00^{b}
	4.0	8.33^{ab}	10.00^{b}	10.00^{b}
	8.0	8.00^{ab}	10.00^{b}	10.00^{b}
M. indica	2.0	9.00^{b}	10.00^{b}	10.00^{b}
	4.0	9.00^{b}	10.00^{b}	10.00^{b}
	8.0	6.33^{ab}	7.67^{ab}	10.00^{b}
G. arborea	2.0	9.00^{b}	9.67^{b}	10.00^{b}
	4.0	10.00^{b}	10.00^{b}	10.00^{b}
	8.0	3.33^{a}	5.00^{a}	7.67^{a}
M. oleifera	2.0	10.00^{b}	10.0^{b}	10.00^{b}
	4.0	10.00^{b}	10.00^{b}	10.00^{b}
	8.0	10.00^{b}	10.00^{b}	10.00^{b}
Neem		10.00^{b}	10.00^{b}	10.00^{b}
	2.0			
Actellic	2.5	10.00^{b}	10.00^{b}	10.00^{b}
Control	0.0	5.67^{ab}	6.67^{ab}	10.00^{b}
S. E ±		0.45 ±	0.35 ±	0.18 ±

Means followed by the same letter within each column are not significantly different (P>0.05) Student Newman Keuls.

Table 5: Year Two Weekly Analyses of Effect of Test Materials on Mortality of *C. subinnotatus*

		Mean Number of dead insects/10 Adults		
Treatments	**Concentration V/w**	**One Week**	**Two Weeks**	**Three Weeks**
A. vera	2.0	1.67^{a}	1.67^{a}	1.67^{a}
	4.0	8.67^{c}	9.00^{c}	10.00^{b}
	8.0	10.00^{c}	10.00^{c}	10.00^{b}
A. annua	2.0	9.33^{c}	10.00^{c}	10.00^{b}
	4.0	8.67^{c}	10.00^{c}	10.00^{b}
	8.0	7.67^{cb}	10.00^{c}	10.00^{b}
M. indica	2.0	9.67^{c}	10.00^{c}	10.00^{b}
	4.0	9.00^{c}	10.00^{c}	10.00^{b}
	8.0	6.00^{cb}	10.00^{c}	10.00^{b}
G. arborea	2.0	8.00^{cb}	10.00^{c}	10.00^{b}
	4.0	10.00^{c}	10.00^{c}	10.00^{b}
	8.0	10.00^{c}	10.00^{c}	10.00^{b}
M. oleifera	2.0	10.00^{c}	10.00^{c}	10.00^{b}
	4.0	10.00^{c}	10.00^{c}	10.00^{b}
	8.0	10.00^{c}	10.00^{c}	10.00^{b}
Neem	2.0	10.00^{c}	10.00^{c}	10.00^{b}
Actellic	2.5	10.00^{c}	10.00^{c}	10.00^{b}
Control	0.0	5.00^{b}	7.33^{b}	9.67^{b}
SE ±		0.33 ±	0.16 ±	0.046 ±

Means followed by the same letter within each column are not significantly different (P>0.05) Student Newman Keuls.

4.6 Mode of Action Study

4.6.1 Contact Toxicity Study

Twenty four hours post application of the treatments by direct contact to the bruchid, those treated with the synthetic insecticide actellic and neem seed oil used as standards caused significant control of the bruchid by causing mortality. Treatment of *A. indica* showed control by causing adult bruchid mortality that was statistically superior to the other treatments and were statistically similar to the standards ($P > 0.05$) when compared using SNK. Bruchid treated with *M. oleifera* were not statistically different from treatments with *A. indica* ($P>0.05$) but were statistically not similar to those treated with actellic 2% dust ($P<0.05$) (Table 6). The other treatments were not statistically different from the untreated control at 5% level of significance (SNK). By 72 hours after application of treatments, bruchid treated with actellic, *M. oleifera, A. vera* and *A. indica* compared favourably to each other in causing mortality of the bruchid by contact toxicity that was significantly higher than all the other treatments and the untreated control ($P<0.05$) (Table 6). At seven days (168 hours) post treatment by contact toxicity, all the treatments showed significant control of the bruchid that compared favourably to each other (SNK) at the 5% level of significance and were statistically superior to the untreated control in causing mortality of the bruchid.

Table 6: Contact Toxicity of Treatments on *C. subinnotatus*

	Mean number of dead insects/ hours post application						
Treatment	24	48	72	96	120	144	168
M. indica	0.67^{c}	1.67^{b}	2.33^{b}	3.67^{c}	6.0^{b}	8.0^{ab}	9.33^{a}
A. vera	2.33^{c}	7.33^{a}	9.0^{a}	9.33^{ab}	9.33^{a}	9.67^{a}	10.0^{a}
A. annua	0.33^{c}	0.67^{b}	0.67^{b}	1.67^{cd}	3.0^{c}	6.0^{b}	8.33^{a}
G. arborea	1.67^{c}	3.0^{b}	4.67^{b}	7.0^{b}	9.0^{a}	9.67^{a}	9.67^{a}
M. oleifera	4.67^{b}	8.33^{a}	9.33^{a}	10.0^{a}	10.0^{a}	10.0^{a}	10.0^{a}
Neem	7.67^{ab}	10.0^{a}	10.0^{a}	10.0^{a}	10.0^{a}	10.0^{a}	10.0^{a}
Actellic	10.0^{a}	10.0^{a}	10.0^{a}	10.0^{a}	10.0^{a}	10.0^{a}	10.0^{a}
Untreated Control	0.0^{c}	0.0^{b}	0.0^{c}	0.33^{d}	0.33^{d}	0.67^{c}	0.67^{b}
SE ±	0.66 ±	0.66 ±	0.64 ±	0.66±	0.73±	0.81±	0.88±

Means followed by the same letter within each column are not significantly different (P>0.05) SNK.

4.6.2 Fumigant Toxicity of Plant Materials on *C. subinnotatus*

During the first and second trials on fumigant toxicity of test materials on *C. subinnotatus,* the standard treatments, *A. indica* and actellic dust showed significant fumigant mode of activity by causing mortality of the bruchid from 72 hours after application. The results indicated that the plant products, *M. indica*, *M. oleifera* and *A. vera* demonstrated the most fumigant toxicities against the insects by causing their mortality (Table 7) from 72 hours post treatment compared to the standards in the first trial. These treatments were not statistically different from each other in their performances ($P>0.05$) when compared using SNK. The untreated control compared favourably to treatments with *A. annua*, while *G. arborea* at 72 hours post treatment showed no effect in causing the mortality of the bruchid and was statistically lower than all the other treatments ($P<0.05$). By the 120th hour post treatment, all the treatments and untreated control were not statistically different from each other ($P= 0.05$). In the second year of the research work, treatments with Actellic, *A. indica* and *A. vera* caused the mortality of all the test insects by the third day (72 hours post application) and their effects were not statistically different from each other ($P>0.05$). Treatment with *G. arborea* caused mortality of the bruchid by fumigant toxicity that compared favourably to the standard treatments, *A. indica* and *A. vera* ($P>0.05$) as well as to treatments with *M. indica, A. annua* and *M. oleifera* by not being significantly different from these treatments ($P>0.05$) using SNK. All the treatments tested were significantly higher than the untreated control ($P<0.05$) which demonstrated poor fumigant toxicity in causing the mortality of the bruchid 72 hours post treatment. By 120 hours post treatment on the effect of treatments in causing mortality of the bruchid by fumigant toxicity, all the treatments compared favourably to each other ($P>0.05$) and were statistically higher than the untreated control, which showed significantly lower fumigant toxicity compared to the other treatments ($P<0.05$).

Table 7: Fumigant Toxicity of Different Treatments against *C. subinnotatus* in 2008 and 2009

Treatment	2008		2009	
	Hours after treatment		Hours after treatment	
	72	120	72	120
M. indica	10.0 [a]	10.0 [a]	5.0 [b]	10.0 [a]
A. vera	8.0 [a]	10.0 [a]	10.0 [a]	10.0 [a]
G. arborea	0.0 [c]	10.0 [a]	6.0 [ab]	8.0 [a]
A. annua	7.0 [ab]	7.0 [a]	4.0 [cb]	10.0 [a]
M. oleifera	10.0 [a]	10.0 [a]	5.0 [b]	10.0 [a]
Neem	10.0 [a]	10.0 [a]	9.0 [a]	10.0 [a]
Actellic	10.0 [a]	10.0 [a]	10 [a]	10.0 [a]
Untreated control	5.0 [b]	9.0 [a]	1.0 [d]	3.0 [b]
SE ±	0.88 ±	0.97 ±	0.96 ±	0.94 ±

Means followed by the same letter within each column are not significantly different (P>0.05) SNK.

4.6.3 Repellent Toxicity Study

The present study indicated that treatments with *A. annua* and *A. vera* both had negative values as percentage repellency or were non-repellent (Table 9). Treatments with the standards (neem and actellic 2% dust), *M. oleifera*, *G. arborea* and *M. indica* all showed positive repellency against the bruchid, thus these treatments induced avoidance in the bruchid as the insects settled more on the portion of the filter paper treated with distilled water than on the portion impregnated with the test materials. The plant products *A. vera* gel and *A. annua* extract both showed negative values indicating non- repellency (Table 9) with the test insects settling on the treated portions of the filter paper.

Table 8: Repellent Toxicity of Test Materials on *C. subinnotatus*

Treatment	Mean % Repellency	Mode of action
A. vera	-13.8	Non repellent
A. annua	-10	Non repellent
G. arborea	3.6	Repellent
M. indica	0.8	Repellent
M. oleifera	47.2	Repellent
Neem	43.6	Repellent
Actellic	55	Repellent

4.7 Effect of Treatment on Germination of Bambara Seeds

For the sake of this study, untreated refers to wholesome bambara groundnut not subjected to any form of experimental treatment other than the initial fumigation to remove any residual infestation. Control on the other hand, refers to the bambara groundnut subjected to the effects of *C. subinnotatus* with no plant product added to it. The batches for this study other than the untreated were drawn from that which was used for the main mortality study experiments. After seven days of test with distilled water to determine the germinability of seeds subjected to treatments with test materials and bruchid, the highest percentage germinated seeds was recorded from untreated seeds, which had 80% germination in the first year. The next highest was recorded from seeds treated with *M. indica* 8.0 ml/kg (76.67%) in the second trial, while the lowest was the control subjected to the bruchid and no test materials that recorded no germination in both years. The untreated bambara groundnut in the first trial also recorded the highest germination for that year (70%), followed by *A. vera* 8.0 ml/kg (66.7%) and 4.0 ml/kg (55%). Despite recording significant control of bruchid within 24 hours of treatment and suppression of progeny emergence, the standard treatments with neem and Actellic and the test plant oil of *M. oleifera* all recorded less than 50% germinability of seeds (Table 10).

Table 9: Mean Number of Germinated Seeds of Bambara Groundnuts at Five weeks post Treatment with Test Materials

Treatment		Germination (%)	
		2008	2009
	ml/kg		
Artemisia annua	2.0	53.3	36.67
	4.0	23.3	3.3
	8.0	16.7	26.7
Aloe vera	2.0	3.3	23.33
	4.0	16.67	60.0
	8.0	66.67	26.67
Mangifera indica	2.0	33.0	23.3
	4.0	36.0	36.67
	8.0	20.0	76.67
Gmelina arborea	2.0	33.0	26.67
	4.0	36.67	66.67
	8.0	20	23.33
Moringa oleifera	2.0	43.3	20.0
	4.0	10.0	10.0
	8.0	33.0	13.33
Actellic		43.3	63.33
Azadirachta indica		33.0	6.67
No bruchid and plant products		70.0	80.0
No plant products (control) with bruchid		0	0

4.8 Effect of Treatment on Progeny Emergence, Moisture Content and Weight of Treated Bambara Groundnut

By the end of mortality studies, record of adult emergence showed that bambara groundnuts treated with the standard treatments, neem seed oil (2 ml/kg) and Actellic 2% dust (10 g/kg) did not support any adult emergence (Table 10) in both 2008 and 2009. The bambara groundnut treated with *M. oleifera* oil (2, 4, and 8 ml/kg) also recorded no progeny emergence, while bambara groundnut treated with *A. annua* at an application rate of 2 ml/kg in the second year gave the overall highest mean progeny emergence that was statistically higher than all the other treatments (P>0.05) in that trial. In the first trial, treatments of *A. vera* at an application rate of 8 ml/kg recorded the highest progeny emergence of 151 adults (Table 10) which is statiscally higher than all other treatments (SNK = 0.05). All the other treatments are not significantly different from each other.

Results of the study indicated that none of the treatments was within the recommended moisture content at the termination of the experiment. Bambara groundnut treated with *A. vera* at the application rate of 0.5 ml/250 g had the highest percentage moisture content of 16.146. This was followed by *A. annua* (0.5) and *M. oleifera* (0.5) (Table 10). However, the moisture contents of the different treatments were statistically not significantly different from each other (SNK=0.05)

In the first year of the present mortality study, losses in weight were observed in the untreated control and treatments with *A. annua*, *M. indica* and *A. vera* at all the different levels (Table 10). On average, *M. oleifera* (0.5 ml/250g) and *G. arborea* maintained the same weight (2 ml/250 g), whereas treatments with treatment neem, *M. oleifera* (1.0 ml/250 g) and *A. annua* (2 ml/250 g) all showed slight increases in weight (Table 10). In the second year of the mortality studies, the untreated control, all levels of *A. annua* and *M. indica* all showed losses of weight, while *M. oleifera,* Actellic and *G. arborea* either had weight gain or retained the same weight as at the beginning of the experiment (Table 10). Though Actellic and neem had slight weight losses in one replicate each, on average, there was increase in weight in both treatments (Table 10). During the first trial, treatments with Treatments with *A. annua* (0.5 ml/250 g) in the second trial had the highest loss in weight with correspondingly higher progeny emergence that was statistically higher than the other treatments (SNK= 0.05). Generally, mean emergence was statistically higher on treatments with *A. annua* (2 ml/kg) in the second year than even on the untreated control. Whereas the highest level treatment with *A. annua* (2.0 ml/250 g bambara groundnut seed) in the first trial had no F1 progeny emergence, in the second year there was progeny emergence.

Table 10: Moisture Content, Weight and Progeny Emergence of Bambara Groundnut after Five Weeks of Mortality Studies

Treatment ml/250g bambara	Final weight/ g			**Mean Progeny emergence**	
	% m. c	**2008**	**2009**	**2008**	**2009**
M. indica 0.5	10.44	247.67	242.33	75.67 ^{a}	13.67^{a}
M. indica 1.0	9.891	245.33	246.67	0 ^{a}	37.67^{a}
M. indica 2.0	8.964	240.00	240.00	13.6 7 ^{a}	95 ^{a}
A. vera 0.5	16.146	241.67	235	133 ^{a}	0 ^{a}
A. vera 1.0	13.92	246.00	250.33	86.67 ^{a}	0 ^{a}
A. vera 2.0	9.149	247.00	238.33	185 ^{b}	33.67 ^{a}
A. annua 0.5	11.773	226.67	225	86 ^{a}	210.33 ^{b}
A. annua 1.0	6.316	233.33	235	151.6 7 ^{a}	104.33^{a}
A. annua 2.0	9.193	251.67	247.67	0 ^{a}	29.67 ^{a}
G. arborea 0.5	6.75	249.00	250.33	0 ^{a}	51.0 ^{a}
G. arborea 1.0	9.337	258.33	250	0 ^{a}	0 ^{a}
G. arborea 2.0	9.018	250.00	250	117.67 ^{a}	0 ^{a}
M. oleifera 0.5	11.0	250.0	262.67	0 ^{a}	0 ^{a}
M. oleifera 1.0	8.262	251.67	258.67	0 ^{a}	0 ^{a}
M. oleifera 2.0	8.833	256.67	261.33	0 ^{a}	0 ^{a}
Neem	8.037	253.33	252.33	0 ^{a}	0 ^{a}
Actellic	8.366	249.33	250.33	0 ^{a}	0 ^{a}
Control	9.695	228.33	226.67	81.33 ^{a}	77.67 ^{a}
SE±				60.4±	60.4±

Means separated by the same letter within the same column are not statistically different from each other SNK (P=0.05)

CHAPTER FIVE

DISCUSSION

The present study evaluated the effect of five plant products, *M. indica* seed butter, *A. vera* leaf gel, *G. arborea* drupe juice, *A. annua* whole plant crude aqueous extract and *M. oleifera* seed oil at three levels of application of 2, 4 and 8 ml/kg bambara groundnut seed at causing the mortality of adult *C. subinnotatus*. The study also investigated the mode of activity of the plant products on *C. subinnotatus* as well as the effect of the plant products and insect activity on the weight, moisture content of bambara groundnut seeds and progeny emergence of the bruchid. These were compared with the synthetic insecticide, Pirimiphos methyl 2% dust, neem seed oil as standards and an untreated control.

Adult *C. subinnotatus* started emerging from the sixth week after setting up the experiment. This agrees with earlier observations that states that the development period ranges from 34-42 days (Ofuya and Lale, 2001; Amatobi, 2007). The adults flew readily and both day olds (0-24 hours) as well as older (a few days to three weeks) adults flew easily. This is an indication that the strains used in the study are the active forms. They are also long-lived (up to three weeks and beyond in treatments) which is a characteristic of the active forms (Appleby and Credland, 2001) and showed increased dispersal tendencies. They also, emerged later (from the sixth week) which is a characteristic of active forms (Mbata *et al.,* 1999), this flight ability is a means of dispersal by the bruchid (Appleby and Credland, 2001).

The plant product, *A. indica* (neem), used as a standard in the present study, is of proven efficacy (Sowunmi and Akinnusi, 1983; Ivbijaro, 1983; Ali, 2002; Agrodok, 2004) against insect pests of agricultural, veterinary and medical importance. They have beenused successfully for the control of storage bruchid (Ofuya and Lale, 2001; Agrodok, 2004). The synthetic insecticide

Pirimiphos methyl (Actellic) has been used for years for the control of storage insect pests (Rahman, 1990; Singh and Singh, 1990; Daniel, 1991; Daglish *et al*., 1993; Agrodok, 2004). In the present study, they showed significant control of the bruchid by causing adult mortality from 24 hours post application. They also showed wide spectrum mode of activity, thus making them suitable standards with which to compare the effect of the plant materials under the present investigation.

The oil of *M. oleifera* also known as Ben oil compared favorably to the synthetic insecticide, Actellic and neem seed oil ($P \geq 0.05$) even at the lowest application rate of 2 ml/kg (0.5 ml/250g bambara groundnut seed). It was the most consistent of all the treatments under investigation in its efficacy. The low application dose of 2 ml/kg bambara groundnut therefore is assumed to be the least most effective dose of the levels tested for the control of the bruchid. Bambara groundnut treated with the oil had no eggs on the seeds or adult progeny emergence in the two years of the experiment, which may indicate an interruption of oviposition ability of the bruchid. The oil may also have killed the adults before eggs could be laid by the female. This contradicts the report by Ilesanmi and Gungula (2010) which stated that cowpeas treated with pure *M. oleifera* oil at levels of 2.5, 5.0 and 7.5ml/kg cowpea seeds had the highest ($p<0.05$) oviposition by *C. maculatus* compared to that treated with neem seed oil on cowpea infested with *C. maculatus* at the same levels. It could therefore be assumed to possess reproductive inhibition property against *C. subinnotatus* but may not be effective even at levels higher than 2 ml/kg for the control of *C. maculatus*. The plant, *M. oleifera* has been reported to be generally resistant to most pests and diseases in the field (Palada and Chang, 2003). This may be due to the possession of insecticidal principles in its system.

The drupe juice of *G. arborea* showed varying degrees of efficacy, which may be due to soil characteristics, water quality and collection site characteristics. The drupe juice did not effectively cause mortality of the bruchid at the lowest (2 ml/kg) and highest (8 ml/kg) levels of application in the first year of the trial. However, in the second year, both the highest (8 ml/kg) and middle (4 ml/kg) application rates showed control comparable to the standards (neem and Actellic dust) and *M. oleifera* oil from 24 hours post application. The treatments *A. vera*, *M. indica* and *A. annua* had poor effect at the levels tested up to 72 hours post treatment on their ability to cause mortality of the bruchid, which may indicate that they may not be used at these levels for reliable control of the bruchid especially given that they did not stop F1 progeny emergence. They however, caused mortality from seven days after application indicating that they may require longer exposure periods with the bruchid to cause mortality.

The plant, *A. vera* is reported to be used as an insecticide (Ali, 2002), and there are reports that the leaf and root ashes were used for the control of the secondary storage pest, *Tribolium casteneum* Herbst. on popcorn maize. The report showed mortality of adults and suppression of oviposition (Omotoso and Oso, 2005). In the present study, *A. vera* gel showed poor performance generally in the control of the bruchid in the first year, however, in the second year, the high application level of 8 ml/kg showed comparable efficacy as the standards and so may be used at this level for the control of the bruchid. This may be as a result of concentration of active principles, which may be higher in the specimens collected in the second than in the first year.

Contact insecticides, which are suitable for use on stored foods, are limited in number, owing to problems of taint or toxic residues, which may occur. A large number of those approved for use in storage environment were used to treat storage structures. Contact poisons are those with which the insect has to make some contact before they are effective. They kill on contact with the insect presumably by absorption through the cuticle. The gel from the leaves of *A. vera* and *M. oleifera* demonstrated positive contact toxicity comparable to the standards from 48 hours post application, which may be an indication that the plant products may be used to cause mortality of the bruchid by direct contact.

During the first year of the study, the oil of *M. oleifera* also showed positive fumigant toxicity, which was not observed in the second year probably as a result of loss of some of its potency due to storage and/or environmental conditions even though the oil is reported to be a long lasting oil with a shelf life of up to five years (miracletrees.org, 2011). Some plant products however, are reported to lose some of their potency sometimes from the first year (Nuhu, Pers. Comm.) depending on the plant and the stable nature of its active principles. Potency may also be lost as a result of storage conditions. The gel from *A. vera* showed positive fumigant toxicity in both years of the research 72 hours post treatment. This may indicate that the gel may require a modified environment such as a hermitic condition to manifest insecticidal property as a fumigant. The butter from *M. indica*, showed positive fumigant toxicity in the first trial and also demonstrated repellent toxicity. However, it had effect in causing mortality of the bruchid only from 48 hours post treatment and showed significant control of the bruchid from one week post treatment in the mortality study. This is an indication that the plant product may require prolonged contact with the bruchid as well as modified conditions such as airtight use as a fumigant in order to cause mortality or else it may be a slow acting biopesticide in respect to causing mortality to the bruchid.

Some plant constituents can be used to control storage insect pests' damage as repellents (Don Pedro, 1989; 1990). In the present study, the two standards and the plant products *M.*

oleifera oil, *M. indica* butter and *G. arborea* drupe juice all had positive repellency indicating that they can be used as surface treatment on containers of seeds to repel *C. subinnotatus* infestation of stored seeds. On the other hand, the gel from *A. vera* and extracts of *A. annua* did not have repellent toxicities and so may not be used as repellents against the test insects.

The plant products used in the present experiment resulted in the treated bambara groundnuts showing poor percentage germination rates except for those treated with *M. indica* butter and gels of *A. vera* both at 2.5ml/250g bambara groundnut seeds and the untreated bambara groundnuts. This may indicate that the other treatments may not be used for seed storage but for the storage of grains to be used as food since all the plant products used in this work can be used as food or medicine by humans and/or animals. They are therefore to be assumed to be safe at the levels used here for food grain storage.

Treatments with *A. annua* and untreated control had higher weight losses at the termination of the mortality studies; the treatments also showed higher progeny emergence with *A. annua* showing higher progeny emergence and correspondingly higher loss in weight than even the untreated control. However, treatment with *G. arborea* (8 ml/kg), which showed comparative control of the bruchid with the standards and *M. oleifera* in the second year's trial and recorded no loss in weight in both years, had progeny emergence in the first year trial higher than even the untreated control. This may indicate that the drupe juice may not suppress oviposition of the bruchid. The negative loss in weight recorded in the first year may be as a result of presence of immature stages in the kernel, which may add to the weight of the grain even though loss may be incurred as a result of insect activity however, this was not investigated. Treatments with *A. vera* (2 ml/kg) had high adult emergence, slight loss in weight and high moisture content. The high moisture content may be the reason the loss in weight was slight and this may be as a result of the moisture from the treatment gel, absorbed moisture from the atmosphere and moisture from the metabolic activities of the grains and/or the insects (Stoll, 1988).

The intermediate level of *G. arborea* also suppressed egg production and adult emergence of the bruchid in both years of the experiments comparable to the oviposition suppression demonstrated by the standards, neem and actellic dust and *M. oleifera*. It also demonstrated positive repellent toxicity to the adult bruchid. Its inability to suppress progeny emergence maybe due to its low oil content compared to the oils from *M. indica, M. oleifera,* and neem seed oil. This is because plant oils have been shown to confer protection to stored grains against damage by storage insect pests (van Huis, 1991; Lale, 1995). The mode of action of the oils has been described to include actions as physical barrier to respiration of insect eggs and young larvae (Credland, 1992; Daniel and Smith, 1994). Given that the juice had lower oil content than the other products, it may not have as much ability as the other products to act as an effective physical barrier. Treatments of *A. vera* gel at application rates of 2 and 4 ml/kg had no adult emergence in the second trial. Since the gel showed positive contact and fumigant toxicities, there may be some local contact or fumigant toxic effects exerted in the experimental unit that may account for the lack of progeny emergence in these treatments. Given that fresh materials were used at each set up of the experiment, it is probable that there may be more concentration of insecticidal principles at the time of application in the second year than in the first year. The plant, *A. annua*, a bitter aromatic plant is reported to contain the chemical absinthin, which is said to have anti-feedant property on insects (Shorey and Mckelvey, 1977). This may have been demonstrated in the present study on treatments with *A. annua* applied at rates of 8 ml/kg, which on average had the third lowest mean progeny emergence and no emergence in the first year. Treatments with anti-feedant property will deter the immature larvae from feeding on the germ in the process of boring into the seed and this may affect the eventual emergence of the adults by causing larval mortality from starvation. The adults that emerged may have fed on untreated portions of the seed, or bored through areas already eaten by other bruchid and thus contribute to the reason for the observed emergence however, this is subject to investigation. The earlier recorded significant mortality (48 hours post treatment) of treatments with *M. indica* seed butter in contact than in the mortality study (one week post treatment) may be because the materials were applied directly on the insect giving that the toxicity of a material is dependent on the extent to which the material has been in contact with the insect (Lale, 2002).

All the plant products under the present investigation demonstrated some potential as protectants against *C. subinnotatus.* However, not all caused direct and immediate mortality. The oil of *M. oleifera* at the three levels under investigation caused mortality of all test insects within 24 hours after application. This compared favourably to the standards, neem and actellic 2 % dust. The oily juice from the drupe of *G. arborea* was effective at application rates of 4 ml/kg in both years of the trial and at 8 ml/kg by the second year. The oily juice may be used as a grain protectant at the application rate of 4 ml/kg, which can be considered to be the least effective dose. Treatments with *M. indica* required longer exposure periods for its effect to manifest and also required either direct contact with the bruchid or an airless condition of application for immediate manifestation of its toxic effect as a fumigant on the bruchid to show. It caused low viability of treated seeds at low (2 ml/kg) and intermediate (4 ml/kg) levels of application. The butter however, had the highest percentage viability of treated seeds at the high application rate of 8 ml/kg (2.0 ml/250 g bambara seeds) which may indicate that it may be used as a fumigant to store planting seeds. Treatments with *A. vera* may require high doses of at least 8 ml/kg/seed or higher doses for it to cause mortality of the bruchid. It may protect the grains from oviposition although this effect was observed in lower doses of application. Being non-repellent, it may not be used as surface treatment to repel the bruchid. However, it could be used as a direct contact or fumigant poison against them.

The bruchid showed the highest tolerance to treatments with *A. annua* at all levels. The aqueous extract showed significant effect in causing mortality of the bruchid after exposure of about one week. It showed fumigant toxicity against the bruchid and so may be used under hermitic conditions. The extract had low progeny emergence of the bruchid in some treatments, which may indicate anti-feedant toxicity against the immature stages which may cause mortality as a result of starvation.

CHAPTER SIX

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary

The present study compared the efficacy of the oil of *Moringa oleifera* seed*, Mangifera indica* seed butter, the gel of *Aloe vera*, drupe juice of *Gmelina arborea and* aqueous extract of whole plant *Artemisia annua* at the application rates of 2, 4, and 8 ml/kg (v/w or w/w) and compared it to standard treatments of pirimiphos methyl 2 % dust at the rate of 10 g/kg (w/w), neem seed oil at the rate of 2 ml/kg/ bambara groundnut seeds and an untreated control. The study was carried out for a two year period in 2008 and 2009 and data taken included effect of treatments on mortality of the adult bruchid, *C. subinnotatus*, mode of action of test materials on the bruchid, the effect of the test materials on progeny emergence, weight, moisture content and seed viability of treated bambara groundnut. Results obtained showed that the oil from *M. oleifera* even at the lowest application rate of 2 ml/kg compared favourably to the standard insecticidal neem seed oil at the same application rate and 2% dust Pirimiphos methyl at the rate of 10 g/kg. It protected bambara groundnut seeds from oviposition by the bruchid as well as development of the bruchid on grains treated with the oil. The plant oil demonstrated contact, repellent as well as fumigant toxicities against the bruchid.

The other plant products, conferred variable degrees of protection to treated bambara groundnut against the test insects. They also showed different modes of activity against the bruchid. The results showed that the gel from *A. vera* and the butter from the seed of *M. indica* can both be used as fumigants under airtight conditions. The gel may also be used as a contact insecticide against the bruchid for seed treatment for short periods as it did not impair germination as much as the oils. If used as a grain protectant for long periods, it may not protect the grain from oviposition and progeny development of the bruchid. The drupe juice from *G. arborea* caused mortality of the bruchid at application rates of 4 ml/kg bambara groundnut seeds and above. The oily juice may be used as space and container treatment to repel the bruchid because of its positive repellent toxicity. The aqueous extract of *A. annua* had little effect on the bruchid compared to the other treatments.

6.2 Conclusion

From the results obtained in the present study, it can be concluded that the oil from the seed of *Moringa oleifera* can be used as a protectant against the bruchid, *C. subinnotatus* at application rates of 2 ml/kg bambara groundnut seeds. It has a wide range of mode of toxicities and as such makes it a good choice insecticide for resource poor farmers and grain commodity merchants who store grains for sometime before use or trading. The oil may however, act in impairing germination of treated seeds and as such should be used with caution in the protection of seeds to be used for planting. It can however, be used for the safe protection of grains to be used as human and animal food given that it is already being used as a source of food and medication by humans. The oily juice from the drupe of *G. arborea* may be used either as a grain protectant against the bruchid at application rates of 4 ml/kg seed or above. It can also be used under hermitic conditions as a fumigant or as space and container treatment to repel and stop grains from infestation by the bruchid as indicated in the present study. The gel from the fleshy leaf of *A. vera* may be used as a grain protectant against the bruchid at application rates of 8 ml/kg. It can also be used as a contact or fumigant biopesticides to control the bruchid. It can be used for protection of small quantities of seeds since it did not impair germination as much as the other treatments. However, caution should be exercised in its use as it can raise the moisture content of stored grains. The butter from seeds of *M. indica* was effective in causing the mortality of the bruchid compared to the standards only from one week post application in both years. The butter showed contact toxicity from the sixth day post application compared to the standards. This may indicate that the product required longer exposure period in contact with the bruchid for it to cause mortality. It can therefore be concluded that it can only be used successfully for the protection of bambara groundnuts against the bruchid, *C. subinnotatus* as a surface treatment of containers to repel the bruchid because of its positive repellent mode of activity. The extract from whole plant *A. annua* which caused mortality of the bruchid after three days contact with the bruchid, also had poor contact and repellent toxicities. Seeds treated with the aqueous extract showed higher loss in weight and high number of progeny emergence compared to the other treatments. It therefore can be concluded that aqueous extract of *A. annua* cannot be used as a plant insecticide against the bruchid, *C. subinnotatus* except in higher doses which may not be economically viable.

The biopesticides in the present study can be used taking advantage of their modes of action for effective utilization. These products can also be used as part of Integrated Pest

Management (IPM) along with other pest management strategies that will lead to the over all reduction in the quantity of environmentally unfriendly synthetic pesticides.

6.3 Recommendations

With the looming specter of the negative effect of climate change and the role of humans in perpetuating the effect through activities including the use of toxic substances like synthetic insecticides, there is the more urgent need for research to find more environmentally friendly biopesticides that are biodegradable and are often part of human and animal food and are often used as ethno-medicines. Their active principles should be isolated and large scale formulation be undertaken. Large-scale cultivation of plants with pesticidal potential should done and the plants used up should be re-planted periodically. Not only will these ensure sustainable availability of raw materials for pesticide formulations, but the plants will provide cleaner air, reduce desertification, a serious problem in sub Saharan Africa, and the by-products will provide food and medicine for humans and animals, as well as provide shelter for some animals and for the much needed biodiversity and gene pools for some threatened and disappearing useful plant species.

All research results beneficial to the small holder farmer, grain merchant or pesticide manufacturer should be made available to the potential beneficiaries instead of its remaining as an academic discovery to be recycled by researchers. No research work is ever exhaustive as there is room for improvement and new discoveries. This research is open to improvement especially in the area of isolation of active constituents of plant products used for insect pest control and standardization of the products to ensure uniformity of application. Use of biotechnology to formulate large scale biopesticides is another area for further research as well as use of local knowledge to find more plant products with insecticidal property that can be used at farm and household level grain storage.

More research work should be targeted at the mode of activity of the plant products with a view to their use in their most effective mode of activity. The parasitoid, *Eupelmus vuilleti* associated with the bruchid could play an important role in reducing the number of viable eggs and immature stages of the bruchid given that it has been associated with related bruchid. The potential of the parasitoid as a biological control agent for the control of *C. subinnotatus* should be studied as well its incorporation into IPM schemes is recommended.

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APPENDICES

Appendix I: Proximate Composition of Raw and Processed Bambara Groundnut Seeds (% Dry Matter).

Bambara variety	Dry matter	Crude protein [N x 6.25]	Crude fibre	Ether extract	Total ash	N-free extract
Raw KAB-3	91.1	20.6	3.2	6.7	3.5	66.0
Raw Oturkpo local	92.4	21.4	3.4	6.4	3.8	65.0
Cooked KAB-3	88.7	18.8	2.9	6.3	3.0	69.0
Cooked Oturkpo local	90.1	19.8	3.2	5.9	3.5	67.6
Autoclaved KAB-3	92.9	20.2	3.0	6.6	3.4	66.8
Autoclaved Oturkpo local	91.3	20.2	3.3	6.5	3.8	68.8

Appendix II: Calorific Value of Bambara Groundnuts Compared to Other Common Legumes

	Bambara groundnut without shell	Shelled groundnut	Vigna cowpea
Calories/100g	365	546	342
Protein	17.7	24.6	23.5
Fat	6.3	43.3	1.8
Calcium mg	73	52	76
Iron mg	7.6	1.9	5.7
Vitamin A u	30.1	30	40
Vitamin B1[Thiamine]mg	0.28	0.84	0.92
Riboflavin mg	0.12	0.12	0.18
Niacin mg	2.1	16.0	1.9
Vitamin C[ascorbic acid] mg	1.0	0	2.0

Appendix III: Year One Data Analyses for Effect of Treatments on Mortality of *C. subinnotatus*

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
MagCoc1	8	66	8.25	7.642857
MagCoc1	8	61	7.625	10.26786
MagCoc1	8	63	7.875	8.125
AloeCoc1	8	55	6.875	19.83929
AloeCoc1	8	46	5.75	18.5
AloeCoc1	8	38	4.75	20.78571
artmCoc1	8	57	7.125	15.55357
artmCoc1	8	56	7	16
artmCoc1	8	48	6	18.28571
GmelCoc1	8	55	6.875	11.55357
GmelCoc1	8	62	7.75	12.21429
GmelCoc1	8	58	7.25	17.07143
MoriCoc1	8	80	10	0
MoriCoc1	8	80	10	0
MoriCoc1	8	80	10	0
NeemCoc 1	8	79	9.875	0.125
NeemCoc 1	8	78	9.75	0.5
NeemCoc 1	8	79	9.875	0.125
ActelCoc1	8	80	10	0
ActelCoc1	8	79	9.875	0.125
ActelCoc1	8	80	10	0
Blank	8	46	5.75	15.64286
Blank	8	39	4.875	19.83929
Blank	8	52	6.5	21.71429
MagCoc2	8	66	8.25	7.642857
MagCoc2	8	61	7.625	10.26786
MagCoc2	8	63	7.875	8.125
AloeCoc2	8	70	8.75	3.357143
AloeCoc2	8	39	4.875	10.41071
AloeCoc2	8	29	3.625	14.83929
artmCoc2	8	55	6.875	19.26786
artmCoc2	8	49	6.125	18.69643
artmCoc2	8	71	8.875	4.410714
GmelCoc2	8	80	10	0
GmelCoc2	8	77	9.625	0.553571

GmelCoc2	8	78	9.75	0.5
MoriCoc2	8	80	10	0
MoriCoc2	8	80	10	0
MoriCoc2	8	80	10	0
MagCoc3	8	60	7.5	12.28571
MagCoc3	8	53	6.625	9.982143
MagCoc3	8	41	5.125	19.55357
AloeCoc3	8	52	6.5	15.71429
AloeCoc3	8	54	6.75	14.21429
AloeCoc3	8	46	5.75	20.78571
artmCoc3	8	58	7.25	11.64286
artmCoc3	8	55	6.875	13.83929
artmCoc3	8	66	8.25	7.642857
GmelCoc3	8	44	5.5	21.14286
GmelCoc3	8	35	4.375	10.26786
GmelCoc3	8	34	4.25	13.35714
MoriCoc3	8	80	10	0
MoriCoc3	8	80	10	0
MoriCoc3	8	80	10	0

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1557.104	53	29.37932	3.157742	7.6E-11	1.374475
Within Groups	3516.875	378	9.303902			
Total	5073.979	431				

LS= Very highly significant ***

Appendix IV: Year Two Data Analyses for Effect of Treatments on Mortality of *C. subinnotatus*

Anova: Single Factor

SUMMARY

Groups	Count	Sum	Average	Variance
MagCoc1	8	71	8.875	3.267857
MagCoc1	8	61	7.625	10.26786
MagCoc1	8	56	7	18.28571
AloeCoc1	6	26	4.333333	19.86667
AloeCoc1	6	26	4.333333	19.86667
AloeCoc1	6	23	3.833333	22.96667
TremCoc1	8	57	7.125	13.26786
TremCoc1	8	71	8.875	4.410714
TremCoc1	8	61	7.625	13.41071
GmelCoc1	8	60	7.5	9.714286
GmelCoc1	8	57	7.125	12.41071
GmelCoc1	8	59	7.375	12.83929
MoriCoc1	8	80	10	0
MoriCoc1	8	80	10	0
MoriCoc1	8	80	10	0
NeemCoc 1	8	80	10	0
NeemCoc 1	8	79	9.875	0.125
NeemCoc 1	8	78	9.75	0.5
ActelCoc1	8	80	10	0
ActelCoc1	8	80	10	0
ActelCoc1	8	80	10	0
Blank	8	53	6.625	14.55357
Blank	8	34	4.25	23.07143
Blank	8	52	6.5	16.57143
MagCoc2	8	62	7.75	9.928571
MagCoc2	8	58	7.25	13.35714
MagCoc2	8	60	7.5	12
AloeCoc2	8	67	8.375	3.125
AloeCoc2	8	51	6.375	18.26786
AloeCoc2	8	58	7.25	15.07143
TremCoc2	8	65	8.125	6.125
TremCoc2	8	67	8.375	8.553571

TremCoc2	8	59	7.375	9.125
GmelCoc2	8	76	9.5	2
GmelCoc2	8	77	9.625	1.125
GmelCoc2	8	74	9.25	3.071429
MoriCoc2	8	80	10	0
MoriCoc2	8	80	10	0
MoriCoc2	8	80	10	0
MagCoc3	8	46	5.75	21.92857
MagCoc3	8	45	5.625	24.55357
MagCoc3	8	56	7	16.28571
AloeCoc3	8	75	9.375	3.125
AloeCoc3	8	72	9	8
AloeCoc3	8	75	9.375	3.125
TremCoc3	8	57	7.125	14.125
TremCoc3	8	58	7.25	11.07143
TremCoc3	8	52	6.5	11.71429
GmelCoc3	8	79	9.875	0.125
GmelCoc3	8	77	9.625	1.125
GmelCoc3	8	74	9.25	3.071429
MoriCoc3	8	80	10	0
MoriCoc3	8	80	10	0
MoriCoc3	8	80	10	0

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	1209.991	53	22.83002	2.906119	2.05E-09	1.375033
Within Groups	2922.375	372	7.855847			
Total	4132.366	425				

LS= very highly significant***

Appendix V: Year One Analysis of Variance of effect of plant product treatments on *C. subinnotatus* 24hrs after application

Source	Sum of Squares	Df	Mean Square	F	Sig.
					.000
					.000
			.	.	.
Repetition	4.000	2	2.000	1.291	.288
Trt	847.333	17	49.843	32.177	.000***
Error	52.667	34	1.549		
Total	1768.000	54			
Corrected Total	904.000	53			

L.S. Very highly significant***
SNK 5%

Appendix VI: Year one SNK ranking of means of effect of plant product treatments on *C. subinnotatus* 24hrs after application

Student-Newman-Keuls

Trt	N	
AloeCoc1	3	.00[a]
Blank	3	.00 [a]
ArtmCoc1	3	.33 [a]
AloeCoc3	3	.67 [a]
GmelCoc1	3	1.00 [a]
GmelCoc3	3	1.33 [a]
MagCoc3	3	1.33 [a]
ArtmCoc3	3	1.67 [a]
AloeCoc2	3	2.00 [a]
ArtmCoc2	3	2.00 [a]
MagCoc1	3	2.33 [a]
MagCoc2	3	2.33 [a]
GmelCoc2	3	8.67 [b]
NeemCoc 1	3	8.67 [b]
ActelCoc1	3	9.67 [b]
MoriCoc1	3	10.00 [b]
MoriCoc2	3	10.00 [b]
MoriCoc3	3	10.00 [b]
Sig.		.498

Means separated by the same letter are not statistically different from each other at 5% level of probability using Student Newman Keuls test

Appendix VII: Year One Analysis of Variance of effect of plant product treatments on *C. subinnotatus* 48hrs after application

Tests of Between-Subjects Effects

Dependent Variable: 48hr

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	715.574(a)	19	37.662	22.480	.000
Intercept	1400.463	1	1400.463	835.907	.000
Year	.000	0	.	.	.
Repetition	2.370	2	1.185	.707	.500
Trt	713.204	17	41.953	25.041	.000***
Error	56.963	34	1.675		
Total	2173.000	54			
Corrected Total	772.537	53			

L.S. Very significant***
SNK 5%

Appendix VIII: Year One SNK ranking of means of effect of plant product treatments on *C. subinnotatus* 48hrs after application

Student-Newman-Keuls

Trt	N	
AloeCoc1	3	.33[a]
Blank	3	.67[ba]
GmelCoc3	3	1.33 [ab]
AloeCoc3	3	1.67 [abc]
ArtmCoc1	3	2.00 [bac]
AloeCoc2	3	3.00 [bac]
ArtmCoc2	3	3.00 [bac]
GmelCoc1	3	3.00 [bac]
MagCoc3	3	3.00 [bac]
ArtmCoc3	3	4.00 [bc]
MagCoc1	3	5.00 [c]
MagCoc2	3	5.00 [c]
GmelCoc2	3	9.6[d]
ActelCoc1	3	10.00 [d]
MoriCoc1	3	10.00 [d]
MoriCoc2	3	10.00 [d]
MoriCoc3	3	10.00 [d]
NeemCoc 1	3	10.00 [d]
		.

Means separated by the same letter are not statistically different from each other at 5% level of probability using Student Newman Keuls test

Appendix IX: Year One ANOVA of effect of plant product treatments on *C. subinnotatus* 72hrs after application

Tests of Between-Subjects Effects

Dependent Variable: 72hr

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	502.741(a)	19	26.460	7.663	.000
Intercept	2065.852	1	2065.852	598.250	.000
Year	.000	0	.	.	.
Repetition	1.926	2	.963	.279	.758
Trt	500.815	17	29.460	8.531	.000***
Error	117.407	34	3.453		
Total	2686.000	54			
Corrected Total	620.148	53			

L.S. Very highly significant***
SNK 5%

Appendix X: Year One SNK ranking of means of effect of plant product treatments on *C. subinnotatus* 72hrs after application

Student-Newman-Keuls

Trt	N	Subset
GmelCoc3	3	1.67[a]
AloeCoc1	3	2.67 [ab]
Blank	3	2.67 [ab]
AloeCoc3	3	3.00 [ab]
MagCoc3	3	3.00 [ab]
AloeCoc2	3	3.67 [ab]
ArtmCoc1	3	4.00 [ab]
ArtmCoc2	3	5.00 [abc]
GmelCoc1	3	5.67 [abc]
ArtmCoc3	3	6.00 [abc]
MagCoc1	3	7.00 [abc]
MagCoc2	3	7.00 [abc]
ActelCoc1	3	10.00[c]
GmelCoc2	3	10.00 [c]
MoriCoc1	3	10.00 [c]
MoriCoc2	3	10.00 [c]
MoriCoc3	3	10.00 [c]
NeemCoc 1	3	10.00 [c]
Sig.		.158

Means separated by the same letter are not statistically different from each other at 5% level of probability using Student Newman Keuls test

Appendix XI: Year One ANOVA of effect of plant product treatments on *C. subinnotatus* one week after application

Tests of Between-Subjects Effects

Dependent Variable: 1wk

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	231.019(a)	19	12.159	3.282	.001
Intercept	3472.019	1	3472.019	937.169	.000
Year	.000	0	.	.	.
Repetition	18.037	2	9.019	2.434	.103
Trt	212.981	17	12.528	3.382	.001**
Error	125.963	34	3.705		
Total	3829.000	54			
Corrected Total	356.981	53			

L. S.=Highly significant **

Appendix XII: Year One SNK Ranking of means of effect of plant product treatments on *C. subinnotatus* one week after application

Student-Newman-Keuls

Trt	N	Subset
		1
GmelCoc3	3	3.33[a]
AloeCoc2	3	5.33 [ab]
Blank	3	5.67 [ab]
AloeCoc3	3	6.00 [ab]
AloeCoc1	3	6.33 [ab]
MagCoc3	3	6.33 [ab]
ArtmCoc1	3	8.00 [ab]
ArtmCoc3	3	8.00 [ab]
ArtmCoc2	3	8.33 [ab]
GmelCoc1	3	9.00[b]
MagCoc1	3	9.00[b]
MagCoc2	3	9.00[b]
ActelCoc1	3	10.00[b]
GmelCoc2	3	10.00[b]
MoriCoc1	3	10.00[b]
MoriCoc2	3	10.00[b]
MoriCoc3	3	10.00[b]
NeemCoc 1	3	10.00[b]
Sig.		.067

Means separated by the same letter are not statistically different from each other at 5% level of probability using Student Newman Keuls test

Appendix XIII: Year One ANOVA of effect of plant product treatments on *C. subinnotatus* two weeks after application

Tests of Between-Subjects Effects

Dependent Variable: 2wks

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	153.074(a)	19	8.057	3.670	.000
Intercept	4302.296	1	4302.296	1960.054	.000
Year	.000	0	.	.	.
Repetition	12.704	2	6.352	2.894	.069
Trt	140.370	17	8.257	3.762	.000
Error	74.630	34	2.195		
Total	4530.000	54			
Corrected Total	227.704	53			

L.S. Very highly significant***
SNK 5%

Appendix XIV: Year One SNK ranking of means of effect of plant product treatments on *C. subinnotatus* two weeks after application

Student-Newman-Keuls

Trt	N	Subset
		1
GmelCoc3	3	5.00[a]
AloeCoc2	3	6.00 [ab]
Blank	3	6.67 [ab]
AloeCoc1	3	7.00 [ab]
MagCoc3	3	7.67 [ab]
AloeCoc3	3	9.33[b]
ArtmCoc1	3	9.33[b]
GmelCoc1	3	9.67[b]
ActelCoc1	3	10.00[b]
ArtmCoc2	3	10.00[b]
ArtmCoc3	3	10.00[b]
GmelCoc2	3	10.00[b]
MagCoc1	3	10.00[b]
MagCoc2	3	10.00[b]
MoriCoc1	3	10.00[b]
MoriCoc2	3	10.00[b]
MoriCoc3	3	10.00[b]
NeemCoc 1	3	10.00[b]
Sig.		.203

Means separated by the same letter are not statistically different from each other at 5% level of probability using Student Newman Keuls test

Appendix XV: Year One ANOVA of effect of plant product treatments on *C. subinnotatus* three weeks after application

Tests of Between-Subjects Effects

Dependent Variable: 3wks

Source	Type III Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	35.611(a)	19	1.874	3.315	.001
Intercept	5104.167	1	5104.167	9028.179	.000
Year	.000	0	.	.	.
Repetition	2.111	2	1.056	1.867	.170
Trt	33.500	17	1.971	3.486	.001***
Error	19.222	34	.565		
Total	5159.000	54			
Corrected Total	54.833	53			

L. S.=Very highly significant ***

Appendix XVI: Year One SNK ranking of effect of plant product treatments on *C. subinnotatus* three weeks after application

Student-Newman-Keuls

Trt	N	Subset
		1
AloeCoc2	3	7.33[a]
GmelCoc3	3	7.67 [a]
ActelCoc1	3	10.00[b]
AloeCoc1	3	10.00 [b]
AloeCoc3	3	10.00 [b]
ArtmCoc1	3	10.00 [b]
ArtmCoc2	3	10.00 [b]
ArtmCoc3	3	10.00 [b]
Blank	3	10.00 [b]
GmelCoc1	3	10.00 [b]
GmelCoc2	3	10.00 [b]
MagCoc1	3	10.00 [b]
MagCoc2	3	10.00 [b]
MagCoc3	3	10.00 [b]
MoriCoc1	3	10.00 [b]
MoriCoc2	3	10.00 [b]
MoriCoc3	3	10.00 [b]
NeemCoc 1	3	10.00 [b]
Sig.		.591

Means separated by the same letter are not statistically different from each other at 5% level of probability using Student Newman Keuls test

Appendix XVII: Year Two ANOVA of effect of plant product treatments on *C. subinnotatus* 24hrs after application

Tests of Between-Subjects Effects

Dependent Variable: 24hr

Source	Type II Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	758.852(a)	19	39.940	23.161	.000
Intercept	1102.519	1	1102.519	639.363	.000
Year	.000	0	.	.	.
Repetition	12.704	2	6.352	3.684	.036
Trt	746.148	17	43.891	25.453	.000***
Error	58.630	34	1.724		
Total	1920.000	54			
Corrected Total	817.481	53			

L.S.= Very highly significant***

Appendix XVIII: Year Two SNK Ranking of Means of effect of plant product treatments on *C. subinnotatus* 24hrs after application

Student-Newman-Keuls

trt	N	Subset
		1
AloeCoc1	3	.00[a]
MagCoc3	3	.00 [a]
Blank	3	.33 [a]
ArtmCoc3	3	1.33 [ab]
MagCoc2	3	1.67 [ab]
AloeCoc2	3	2.00 [ab]
ArtmCoc1	3	2.00 [ab]
GmelCoc1	3	2.33 [ab]
MagCoc1	3	2.67 [ab]
ArtmCoc2	3	3.00 [ab]
AloeCoc3	3	4.00 [bc]
GmelCoc2	3	6.00[cd]
GmelCoc3	3	7.00[de]
NeemCoc 1	3	9.00[e]
ActelCoc1	3	10.00 [e]
MoriCoc1	3	10.00 [e]
MoriCoc2	3	10.00 [e]
MoriCoc3	3	10.00 [e]
Sig.		.177

Means separated by the same letter are not statistically different from each other at 5% level of probability using Student Newman Keuls test

Appendix XIX: Year Two ANOVA of effect of plant product treatments on *C. subinnotatus* 48hrs after application

Tests of Between-Subjects Effects

Dependent Variable: 48hr

Source	Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	693.833(a)	19	36.518	21.782	.000
Intercept	1980.167	1	1980.167	1181.152	.000
Year	.000	0	.	.	.
Repetition	2.333	2	1.167	.696	.506
Trt	691.500	17	40.676	24.263	.000***
Error	57.000	34	1.676		
Total	2731.000	54			
Corrected Total	750.833	53			

L.S. Very highly significant***
SNK 5%

Appendix XX: Year Two SNK Ranking of Means of effect of plant product treatments on *C. subinnotatus* 48hrs after application

Student-Newman-Keuls

Trt	N	Subset
		1
Blank	3	1.33[a]
MagCoc3	3	1.33 [a]
AloeCoc1	3	1.67 [ab]
GmelCoc1	3	2.33 [ab]
AloeCoc2	3	3.00 [ab]
ArtmCoc3	3	3.00 [ab]
MagCoc2	3	3.33 [ab]
MagCoc1	3	4.00 [ab]
ArtmCoc1	3	4.67 [ab]
ArtmCoc2	3	5.00[c]
GmelCoc2	3	9.67[d]
GmelCoc3	3	9.67 [d]
ActelCoc1	3	10.00 [d]
AloeCoc3	3	10.00 [d]
MoriCoc1	3	10.00 [d]
MoriCoc2	3	10.00 [d]
MoriCoc3	3	10.00 [d]
NeemCoc 1	3	10.00 [d]
Sig.		.072

Means separated by the same letter are not statistically different from each other at 5% level of probability using Student Newman Keuls test

Appendix XXI: Year Two ANOVA of effect of plant product treatments on *C. subinnotatus* 72hrs after application

Tests of Between-Subjects Effects

Dependent Variable: 72hr

Source	Type II Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	467.185(a)	19	24.589	14.179	.000
Intercept	2787.852	1	2787.852	1607.568	.000
Year	.000	0	.	.	.
Repetition	3.704	2	1.852	1.068	.355
Trt	463.481	17	27.264	15.721	.000
Error	58.963	34	1.734		
Total	3314.000	54			
Corrected Total	526.148	53			

L.S. Very highly significant***
SNK 5%

Appendix XXII: Year Two SNK Ranking of Means of effect of plant product treatments on *C. subinnotatus* 72hrs after application

Student-Newman-Keuls

Trt	N	Subset
		1
MagCoc3	3	1.67[a]
AloeCoc1	3	1.67 [a]
Blank	3	2.67 [a]
ArtmCoc3	3	5.00[b]
AloeCoc2	3	6.00[b]
GmelCoc1	3	6.00 [b]
MagCoc2	3	6.00 [b]
MagCoc1	3	6.33 [bc]
ArtmCoc1	3	7.00 [bc]
ArtmCoc2	3	7.00 [bc]
ActelCoc1	3	10.00[c]
AloeCoc3	3	10.00 [c]
GmelCoc2	3	10.00 [c]
GmelCoc3	3	10.00 [c]
MoriCoc1	3	10.00 [c]
MoriCoc2	3	10.00 [c]
MoriCoc3	3	10.00 [c]
NeemCoc 1	3	10.00 [c]
Sig.		.625

Means separated by the same letter are not statistically different from each other at 5% level of probability using Student Newman Keuls test

Appendix XXIII: Year Two ANOVA of effect of plant product treatments on *C. subinnotatus* one week after application

Tests of Between-Subjects Effects

Dependent Variable: 1wk

Source	Type II Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	261.685(a)	19	13.773	7.123	.000
Intercept	3935.574	1	3935.574	2035.412	.000
Year	.000	0	.	.	.
Repetition	2.259	2	1.130	.584	.563
Trt	259.426	17	15.260	7.892	.000
Error	65.741	34	1.934		
Total	4263.000	54			
Corrected Total	327.426	53			

L.S. Very highly significant***
SNK 5%

Appendix XXIV: Year Two SNK Ranking of Means of effect of plant product treatments on *C. subinnotatus* one week after application

Student-Newman-Keuls

Trt	N	Subset
		1
AloeCoc1	3	1.67[a]
Blank	3	5.00[b]
MagCoc3	3	6.00 [bc]
ArtmCoc3	3	7.67 [bc]
GmelCoc1	3	8.00 [bc]
AloeCoc2	3	8.67[c]
ArtmCoc2	3	8.67 [c]
MagCoc2	3	9.00 [c]
ArtmCoc1	3	9.33 [c]
MagCoc1	3	9.67 [c]
ActelCoc1	3	10.00 [c]
AloeCoc3	3	10.00 [c]
GmelCoc2	3	10.00 [c]
GmelCoc3	3	10.00 [c]
MoriCoc1	3	10.00 [c]
MoriCoc2	3	10.00 [c]
MoriCoc3	3	10.00 [c]
NeemCoc 1	3	10.00 [c]
Sig.		1.000

Means separated by the same letter are not statistically different from each other at 5% level of probability using Student Newman Keuls test

Appendix XXV: Year Two ANOVA of effect of plant product treatments on *C. subinnotatus* two weeks after application

Tests of Between-Subjects Effects

Dependent Variable: 2wks

Source	Type II Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	209.074(a)	19	11.004	24.459	.000
Intercept	4629.630	1	4629.630	10290.557	.000
Year	.000	0	.	.	.
Repetition	.704	2	.352	.782	.466
Trt	208.370	17	12.257	27.245	.000***
Error	15.296	34	.450		
Total	4854.000	54			
Corrected Total	224.370	53			

L.S. Very highly significant***
SNK 5%

Appendix XXVI: Year Two SNK Ranking of Means of effect of plant product treatments on *C. subinnotatus* two weeks after application

Student-Newman-Keuls

Trt	N	Subset
		1
AloeCoc1	3	1.67[a]
Blank	3	7.33[b]
ArtmCoc3	3	8.67[c]
AloeCoc2	3	9.00[c]
ActelCoc1	3	10.00[c]
AloeCoc3	3	10.00[c]
ArtmCoc1	3	10.00[c]
ArtmCoc2	3	10.00[c]
GmelCoc1	3	10.00[c]
GmelCoc2	3	10.00[c]
GmelCoc3	3	10.00[c]
MagCoc1	3	10.00[c]
MagCoc2	3	10.00[c]
MagCoc3	3	10.00[c]
MoriCoc1	3	10.00[c]
MoriCoc2	3	10.00[c]
MoriCoc3	3	10.00[c]
NeemCoc 1	3	10.00[c]
Sig.		1.000

Means separated by the same letter are not statistically different from each other at 5% level of probability using Student Newman Keuls test

Appendix XXVII: Year Two ANOVA of effect of plant product treatments on *C. subinnotatus* three weeks after application

Tests of Between-Subjects Effects

Dependent Variable: 3wks

Source	Type II Sum of Squares	Df	Mean Square	F	Sig.
Corrected Model	196.185(a)	19	10.326	270.824	.000
Intercept	4892.519	1	4892.519	128323.771	.000
Year	.000	0	.	.	.
Repetition	.037	2	.019	.486	.619
Trt	196.148	17	11.538	302.629	.000***
Error	1.296	34	.038		
Total	5090.000	54			
Corrected Total	197.481	53			

L.S. Very highly significant***
SNK 5%

Appendix XXVIII: Year Two Ranking of means of effect of plant product treatments on *C. subinnotatus* three weeks after application

Student-Newman-Keuls

Trt	N	Subset
		1
AloeCoc1	3	1.67[a]
Blank	3	9.67[b]
ActelCoc1	3	10.00 [b]
AloeCoc2	3	10.00 [b]
AloeCoc3	3	10.00 [b]
ArtmCoc1	3	10.00 [b]
ArtmCoc2	3	10.00 [b]
ArtmCoc3	3	10.00 [b]
GmelCoc1	3	10.00 [b]
GmelCoc2	3	10.00 [b]
GmelCoc3	3	10.00 [b]
MagCoc1	3	10.00 [b]
MagCoc2	3	10.00 [b]
MagCoc3	3	10.00 [b]
MoriCoc1	3	10.00 [b]
MoriCoc2	3	10.00 [b]
MoriCoc3	3	10.00 [b]
NeemCoc 1	3	10.00 [b]
Sig.		1.000

Means separated by the same letter are not statistically different from each other at 5% level of probability using Student Newman Keuls test

Appendix XXIX: Single Factor Analysis of Variance of Contact Mode of Action of Treatments on *C. subinnotatus*

Anova: Single Factor

SUMMARY

Groups	*Count*	*Sum*	*Average*	*Variance*
Mang	7	65	9.285714	2.238095
Aloe	7	57	8.142857	7.47619
Artm	7	53	7.571429	9.619048
Gmel	7	31	4.428571	19.61905
Mor	7	61	8.714286	6.904762
Neem	7	60	8.571429	8.952381
Blank	7	42	6	6

ANOVA

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	125.3469	6	20.89116	2.404855	0.043508	2.323994
Within Groups	364.8571	42	8.687075			
Total	490.2041	48				

Appendix XXX: Analysis of Variance for Repellency Toxicity of Test Materials against *C. subinnotatus*

Anova: Single Factor

SUMMARY

Groups	Count	Sum	Average	Variance
aloe1	5	205.53	41.106	369.0315
gmel1	5	143.57	28.714	97.87273
mor1	5	209.78	41.956	537.4701
mang1	5	133.27	26.654	111.1043
artm1	5	107.91	21.582	137.9469

ANOVA

Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups	1649.767	4	412.4417	1.645258	0.202135	2.866081
Within Groups	5013.702	20	250.6851			
Total	6663.469	24				

Appendix XXXI: Year one analysis of variance of test materials on mortality of *C. subinnotatus*

Treatment	24hrs	48hrs	72hrs	1week	2weeks	3weeks
AloeCoc1	$.00^{a}$	$.33^{a}$	2.67^{ab}	6.33^{ab}	7.00^{ab}	10.00^{b}
Blank	$.00^{a}$	$.67^{ba}$	2.67^{ab}	5.67^{ab}	6.67^{ab}	10.00^{b}
ArtmCoc1	$.33^{a}$	2.00^{bac}	4.00^{ab}	8.00^{ab}	9.33^{b}	10.00^{b}
AloeCoc3	$.67^{a}$	1.67^{bc}	3.00^{ab}	6.00^{ab}	9.33^{b}	10.00^{b}
GmelCoc1	1.00^{a}	3.00^{bac}	5.67^{abc}	9.00^{b}	9.67^{b}	10.00^{b}
GmelCoc3	1.33^{a}	1.33^{ab}	1.67^{a}	3.33^{a}	5.00^{a}	7.67^{a}
MagCoc3	1.33^{a}	3.00^{bac}	3.00^{ab}	6.33^{ab}	7.67^{ab}	10.00^{b}
ArtmCoc3	1.67^{a}	4.00^{b}	6.00^{abc}	8.00^{ab}	10.00^{b}	10.00^{b}
AloeCoc2	2.00^{a}	3.00^{bac}	3.67^{ab}	5.33^{ab}	6.00^{ab}	7.33^{a}
ArtmCoc2	2.00^{a}	3.00^{bac}	5.00^{abc}	8.33^{ab}	10.00^{b}	10.00^{b}
MagCoc1	2.33^{a}	5.00^{c}	7.00^{abc}	9.00^{b}	10.00^{b}	10.00^{b}
MagCoc2	2.33^{a}	5.00^{c}	7.00^{abc}	9.00^{b}	10.00^{b}	10.00^{b}
GmelCoc2	8.67^{b}	9.6^{d}	10.00^{d}	10.00^{b}	10.00^{b}	10.00^{b}
NeemCoc 1	8.67^{b}	10.00^{d}	10.00^{d}	10.00^{b}	10.00^{b}	10.00^{b}
ActelCoc1	9.67^{b}	10.00^{d}	10.00^{d}	10.00^{b}	10.00^{b}	10.00^{b}
MoriCoc1	10.00^{b}	10.00^{d}	10.00^{d}	10.00^{b}	10.00^{b}	10.00^{b}
MoriCoc2	10.00^{b}	10.00^{d}	10.00^{d}	10.00^{b}	10.00^{b}	10.00^{b}
MoriCoc3	10.00^{b}	10.00^{d}	10.00^{d}	10.00^{b}	10.00^{b}	10.00^{b}

Means followed by the same letter within each column are not significantly different ($P>0.05$). Student Newman Keuls.

Appendix XXXII: Year two analysis of variance of test materials on mortality of *C. subinnotatus*

Treatment	24hrs	48hrs	72hrs	1week	2weeks	3weeks
AloeCoc1	0.00^{a}	1.67^{ab}	1.67^{a}	i.67^{a}	1.67^{a}	1.67^{a}
Blank	0.33^{a}	1.33^{a}	2.67^{a}	5.00^{b}	7.33^{b}	9.67^{b}
ArtmCoc1	2.00^{ab}	4.67^{ac}	7.00^{bc}	9.33^{c}	10.00^{c}	10.00^{b}
AloeCoc3	4.00^{bc}	10.00^{e}	10.00^{c}	10.00^{c}	10.00^{c}	10.00^{b}
GmelCoc1	2.00^{ab}	2.33^{ba}	6.00^{b}	8.00^{bc}	10.00^{c}	10.00^{b}
GmelCoc3	7.00^{de}	9.67^{d}	10.00^{c}	10.00^{c}	10.00^{c}	10.00^{b}
MagCoc3	0.00^{a}	1.33^{a}	1.67^{a}	6.00^{bc}	10.00^{c}	10.00^{b}
ArtmCoc3	1.33^{ab}	3.00^{ab}	5.00b	7.67^{bb}	8.67^{c}	10.00^{b}
AloeCoc2	2.00^{ab}	3.00^{ab}	6.00^{b}	8.67^{c}	9.00^{c}	10.00^{b}
ArtmCoc2	3.00^{ab}	5.00^{c}	7.00^{bc}	8.67^{c}	10.00^{c}	10.00^{b}
MagCoc1	2.67^{ab}	4.00^{ab}	6.33^{bc}	9.67^{b}	10.00^{c}	10.00^{b}
MagCoc2	1.67^{ab}	3.33^{ab}	6.00^{b}	9.00^{b}	10.00^{c}	10.00^{b}
GmelCoc2	6.00^{bd}	9.67^{d}	10.00^{d}	10.00^{b}	10.00^{c}	10.00^{b}
NeemCoc 1	9.00^{e}	10.00^{d}	10.00^{d}	10.00^{b}	10.00^{c}	10.00^{b}
ActelCoc1	10.00^{e}	10.00^{d}	10.00^{d}	10.00^{b}	10.00^{c}	10.00^{b}
MoriCoc1	10.00^{e}	10.00^{d}	10.00^{d}	10.00^{b}	10.00^{c}	10.00^{b}
MoriCoc2	10.00^{e}	10.00^{d}	10.00^{d}	10.00^{b}	10.00^{c}	10.00^{b}
MoriCoc3	10.00^{e}	10.00^{d}	10.00^{d}	10.00^{b}	10.00^{c}	10.00^{b}

Means followed by the same letter within each column are not significantly different (P>0.05). Student Newman Keuls.

APPENDIX XXXIII: Duncan Grouping of Repellency mode of Action of Treatments on *C. subinnotatus.*

Duncan Grouping	Mean	N	trt.
A	42.14	5	T3
A	41.11	5	T1
A	26.65	5	T4
A	21.58	5	T5
A	21.31	5	T2
A	33.9	5	T6
A	47.9	5	T7

Key: T1 = *Aloe vera* gel
T2 = *gmelina arborea* drupe juice
T3 = *Moringa oleifera* oil
T4 = *Mangifera indica* butter
T5 = *Artemisia annua* extract
T6= *Azadirachta indica* oil
T7= Actellic dust

Means with the same letter are not significantly different (DNMRT) at 5% level of probability.