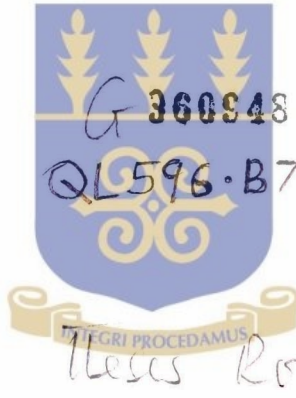

DECLARATION

The thesis is the result of a study I conducted between July 1997 and June 1998. The study is original, references are however cited to other people's work.

.....

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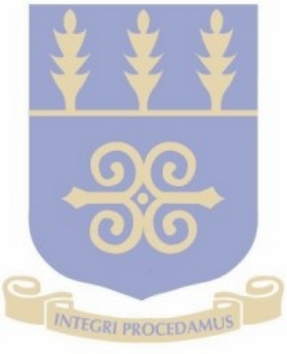

ABSTRACT

Bambara groundnuts are good nutrient sources of high protein and carbohydrate contents. The seeds suffer damage through infestation by *Callosobruchus maculatus* (Fab.) during storage. The efficacy of four protectants, namely actellic, steam, neem seed oil and neem kernel oil were tested on two varieties of Bambara groundnuts namely, Jabajaba and Piele Balgu.

Efficacy of the different treatments was demonstrated on Bambara groundnut through the number of adult *C. maculatus* that emerged. Actellic, steam and neem seed oil significantly ($P<0.05$) reduced emergence of *C. maculatus* on the seeds of the varieties used. Consequently these treatments also significantly ($P<0.005$) reduced the number of seeds that were damaged through *C. maculatus* infestation. Damage of seeds of the Piele Balgu variety ranged from 1.1(actellic treated) to 30.4 (neem kernel oil treated). In the Jabajaba variety, seed damage ranged from 1.1(actellic treated) to 28.6 (untreated). Each of the four treatments, however, significantly ($P<0.05$) reduced *C. maculatus infestation in the order of Actellic >Steam > Neem seed oil > Neem kernel oil.*

Persistence of treatment effects were tested for 90 days after treatment (due to time constraint). It was established that the seed testa of Jabajaba Bambara groundnut renders it less susceptible to *C. maculatus* infestation. Actellic did not lose its efficacy 90 days after treatment. Steam and neem seed oil significantly ($P<0.05$) reduced infestation of legumes by *Callosobruchus maculatus.*

Germination of treated seeds showed that steam treatment had a lethal effect on the seeds used. Neem seed oil promoted germination whilst Actellic significantly ($P<0.05$) reduced the activity. Neem seed oil promoted more vegetative growth in the Piele Balgu variety than in the jabajaba variety.

Protein content of treated Bambara groundnut seeds did not change significantly ($P>0.05$) from the untreated. Seeds of the jabajaba variety have more protein than the Piele Balgu seeds which were instead more susceptible to *C. maculatus* infestation. Neem seed oil offers a good protection to seeds of Bambara groundnut, does not affect the protein content and germination potential of seeds and can therefore be used as a substitute for actellic.

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CHAPTER ONE

1. INTRODUCTION

Bambara groundnut, *Vigna subterranea* (L.) Verdc. is cultivated throughout Africa but remains neglected by research scientists, in that it has not been studied extensively. Empirical evidence and fragmentary research results, however, suggest that it is a crop with much promise. It is drought-tolerant, thrives on poor soils and is generally free of diseases during growth. Despite the lack of research on the crop, its commercial use in Africa is increasing. The commercial canning of Bambara groundnut has been successful in Zambia and Ghana (Owusu-Domfeh,1972). It is one of the major grain legumes consumed in Africa ranking third to cowpea and groundnut (Sellschop,1962). Bambara groundnuts are cheap sources of carbohydrate and protein in diets of humans and livestock in Africa (Aregheore,1992). The seeds are highly nutritious (Rassel, 1960); they contain a higher lysine and methionine content than either cowpea or groundnut (Ezedinma and Maneke,1985). According to Watson (1971), Bambara groundnut has the following nutrient content; protein: 19.7%, carbohydrate : 54.6%, fat : 5.6 % , fibre: 5.3% , ash: 3.5%, calcium : 0.108%, magnesium: 0.195%, Iron : 0.00097% and 424 calories.

It forms pods and seeds on or just beneath the ground. It achieves this by its flower stalk elongating, and as its bulbous tip penetrates the soil, it creates a tunnel through which the fertilized flowers, attached a few centimeters behind the tip, are drawn into the soil. The pods are round and wrinkled and each contains one or two seeds. The

seeds are round and smooth and measure up to about 1.5 cm in diameter and when dried they are very hard. There are several cultivars of the Bambara groundnut. The seeds may be cream, brown, red, mottled or black-eyed. There is, however, a marked white hilium in most cultivars and every hundred seeds weigh approximately 50-75 g. It is a complete food and is eaten as either immature or fully ripe. The fresh semi-ripe seeds are eaten fresh or boiled, while the dry seeds are often pounded into flour and mixed with oil or butter to form a porridge or sometimes they are roasted in oil. The young whole pods are used in soups. The shelled nuts are very susceptible to insect infestation and so are sometimes fumigated with phosphine, carbon tetrachloride or carbon disulphide. Infestation of stored Bambara groundnut may commence from the field but serious insect damage occurs in store (Warui, 1984). Cornes (1971) named the cowpea beetle, *Callosobruchus maculatus* (Fab.) and *C. subinnotatus* (Pic.) as the major storage pests of Bambara groundnut while *C. ehaseoli* (Pic.) and *C. Pallidus* (Lin.) were considered to be of minor importance (Mbata, 1987). *C. maculatus* reared on Bambara groundnut have a comparatively shorter developmental period, more fecund females and long- living adults (Ofuya and Bambigbola,1991).

The nutrient content and diverse dietary usage of Bambara groundnut in Ghana suggest that it is naturally an inexpensive substitute for animal protein to the ordinary Ghanaian. Promotion of its cultivation in Ghana would be encouraged if there is an effective means to control the major pest which attacks it during storage. This will help increase the farmers income and ensure food security to the resource-poor growers in

Ghana. In November 1997, a survey was conducted in the Dangme East District of Ghana on the traditional methods used to protect stored Bambara groundnut against infestation by *C. maculatus*. The survey revealed that the average Bambara groundnut field in the area was two acres and farmers stored their produce in locally constructed silos for up to nine months. Some farmers use Actellic 25EC to protect stored Bambara groundnut. The sole dependence on synthetic insecticides for controlling stored product pests has given rise to several problems such as the development of resistant strains of insects, toxic residue in food and health hazards to the illiterate applicators. Furthermore, with the removal of subsidies on all agricultural inputs, pesticides have become too expensive to most small-scale farmers. These farmers need cheap and readily available materials to control pests during storage.

The protection of stored products using plant materials is a common practice among small-scale farmers in Africa (Delobel and Malonga,1987). Plant derived pesticides are effective against many storage pests as antifeedants, repellents or toxicants and are less hazardous (Prakash *et al.,* 1982). These materials are readily available and cheap. Steam treatment of seeds of Bambara groundnut is a physical method which affects the oviposition and development of *C. maculatus* since the beetle is selective in its site of oviposition. Several factors either stimulate or inhibit oviposition of the beetle. Amongst them are: (i) the roughness of the seed coat (Nwanze *et al.,*1975) (ii) surface odours of the seed which may either attract or repel the insect (Nwanze and Horber, 1976; Giga and Smith,1985) (iii) the shape or curvature of the seeds (Horber, 1978). Nwanze and Horber (1976) reported that differences in the seed coat of cowpea affect oviposition

and larval development of *C. maculatus*. The beetle prefers sound and healthy seeds to wrinkled and infested seeds (Pathak, 1986).

It is the objective of this study to explore phytochemical and physical methods to protect stored Bambara groundnuts against damage by insects. Among the specific objectives were;

(1)To compare the development of *C. maculatus* on Bambara groundnut treated with the following;

(a) Actellic

(b) Neem seed oil

(c) Neem kernel oil

(d) Steam

(e) Control (check)

(2) To assess the levels of damage caused by *C. maculatus* on Bambara groundnut with the above treatments.

(3) To investigate the effects of the above treatments on seed viability.

(4) To test the persistence of the various treatments.

(5) To determine the protein content of Bambara groundnut with the above treatments.

CHAPTER TWO

2. LITERATURE REVIEW

2.1 Recognition and identification of *Callosobruchus maculatus*

Adult *Callosobruchus maculatus* (Fab.) (Coleoptera: Bruchidae) has serrated antennae and is recognized by two dark patches situated halfway along each elytron and several small patches close to both the anterior and posterior ends and a paler brown cross shaped area covering the rest (Haines, 1991).

Southgate *et al.*, (1957) stated that the most distinguishing external feature of *C. maculatus* from other species of *Callosobruchus* is the presence of a long and prominent tooth-like structure of the inner carina of the hind femur. They also distinguished two forms of *C. maculatus on the basis of* morphlogical differences. These are the flight forms which are active fliers and the flightless which are sedentary. The flightless forms have strongly marked elytra with white pygidium. The pygidium in the flight male is longer than that found in the flightless male. The reverse is true for the female. Another distinguishing character of the flight form is the covering of the pygidium with a pale golden pubescence, thus making the dull black areas of bare cuticle stand out sharply. In the flight forms the pubescence is much reduced making the elytra pattern less definite.

2.2 Distribution and economic importance

C. maculatus originated in Africa where it is still the dominant species of

Callosobruchus found. It is now distributed throughout the tropics and subtropics, indicating that the species is very well adapted to hot climates (Anon.,1991). In tropical Asia, *C. chinensis* (Lin.) and *C. analis* (Fab.) are the dominant species. *C. phaseoli* (Gyl.) is found in East and West Africa, South Asia, Central and South America. *C. rhodesianus* (Pic.) is generally restricted to Southern Africa although it occurs occasionally in West Africa and commonly in coastal Kenya. *C. subinnotatus* (Pic.) is restricted entirely to West Africa (Anon 1991).

C. maculatus attacks a wide range of hosts, some of which are less frequently attacked than others or restricted to only under stress conditions. This phenomenon arises where the bruchid is not indigenous and the normal hosts are not encountered frequently, usually giving rise to an infestation of new hosts. Hence *C. maculatus* has been reported to attack several host plant in the family leguminosae. These include *Glycine max* (Lin.) Merr (Allotey and Oyewo,1993), *Vigna subterranea* (Verdc.) (Cornes, 1971), *Vigna unguiculata* (Lin.) Walp.(Ofuya, 1987) and *Vigna radiata* (Lin.) (Osman *et al.*, 1991).

2.3.Losses caused by *C. maculatus*

Infestation of cowpea by *C. maculatus* causes qualitative and quantitative losses. Complete development of *C. maculatus* larvae takes place in a single legume seed. Consumption of part of the cotyledon reduces the weight of the seed. The embryo may also be affected. The seed becomes contaminated with frass and debris during the development of *C. maculatus* from the larval stage to the adult stage (Allotey and

Oyewo, 1993). Adults of *C. maculatus* emerge out of the seed by creating windows through which they emerge. Infestation may cause a change in the seed and an unpleasant odour emanates from heavily infested cowpea seeds. Damage assessment of *C. maculatus* on *Vigna radiata* in an experiment conducted by Osman *et al.,* (1991) showed a 100% damage of the seeds Studies on 10 varieties of cowpea namely Udaipur 1, Udaipur 2, P1302, P1414, P454-457, IC 11352, CS152 , Co1, and Kanpur black showed that infestation by *C. maculatus* could cause 44.97% weight loss to seeds of the Udaipur 2 while the least affected variety, CO1, recorded 16.25% weight loss (Manoha and Yadava,1990).

2.4 Life history and behaviour of *C. maculatus*

The adult beetles do not feed and are short-lived. Usually the lifespan is not more than 12 days (Anon,1991; Allotey and Dankwah, 1994). Adult of an unmated flight form can live up to 33 days using its food reserves to prolong life (Caswell,1960). Newly emerged females soon assume a 'calling posture' and release a phenomone that attracts males (Ouedrago and Huignard,1981). A single mating of 3-8 minutes (Qi. and Burkholder, 1982) is usually sufficient to fertilize a female's eggs (Haines, 1991). Unmated females may delay egg laying (Caswell, 1960). In the flightless form copulation takes place soon after emergence and oviposition quickly follows in less than 24 hours (Wasserman, 1985). The ovary of the flight form is not developed when the adult emerges (Caswell, 1960). Eggs begin to develop some three or four days after emergence and then copulation takes place and oviposition begins (Utida, 1956). It has been reported that a female adult can lay up to 115 eggs in a life time (Anon, 1991), although the flight forms lay fewer numbers of eggs (Utida, 1956; Thanthianga and Mitchell, 1990). Several

factors determine the total number of eggs laid during the life time of the female. It depends on the following (i) the food on which the adults were bred (ii) humidity and temperature of the environment (El-Sawaf, 1956 ; Howe and Currie, 1964) (iii) the density of the adult beetles (Giga,1982) (iv) the plumpness or wrinkling of the seed-coat and perhaps by the size and hardness of seed as well as the odour (Nwanze *et al.*, 1975; Nwanze and Horber, 1976; Singh *et al.*, 1977). Presumably better food enable the larva to grow better into large adults. The weight of the adults affects egg laying (Credland *et al.*, 1986) and also affects the changes in the levels of total body lipid (Sharma *et al.*, 1983) or water content (Sharma and Sharma, 1984). It is also known that different strains of the bruchids differ in their fecundity and oviposition behaviour (Credland, 1986; Credland *et al.*, 1986). Fecundity may also be reduced if few beans are available (Thanthianga and Mitchell, 1990) or unattractive host is presented (Credland and Wright, 1990).

C. maculatus discriminates in oviposition due to several factors such as the roughness of the seed coat (Nwanze *et al.*, 1975), surface odour which attracts or repels (Nwanze and Horber, 1976; Singh *et al.*, 1977; Giga and Smith, 1985) and the shape or curvature of the seeds (Horber, 1978). These factors may either stimulate or inhibit oviposition. Nwanze and Horber (1976) reported that differences in the seed coats of cowpea affect oviposition and larval development of *C. maculatus*. The optimun temperature for oviposition is 30-35 °C (Anon, 1991). Eggs laid are about 650 μm long including a posterior funnel that emerges first and 300-400 μm wide at the wider point. In *Callosobruchus* species, eggs have a large air filled space between the developing embryo and the testa. The margins of the eggs are firmly glued to the testa. The shape

of the egg resembles an oval inverted saucer that is a doomed shaped structure with the embryo and yolk in a thickened central position. The funnel serves as a major and the only route for the exchange of respiratory gases and water uptake or loss (Credland, 1991). Newly laid eggs are translucent and grey. Hatched eggs are white and clearly visible to the unadided eyes due to the detritus or larval frass produced (Giga and Smith , 1986). Credland (1991), observed the that; (i.) the first instar breaks through the borrows into the seed, (ii) the larva opens a route into the core of the seed which is connected via the space beneath the remains of the egg with the atmosphere surrounding the seed, after larval eclosion, the funnel provides the primary route for gaseous exchange. The eggs take about five days to hatch and the developing larva feeds entirely within a single seed, excavating a chamber within the cotyledon as it grows. As many as 8 to 10 larvae can complete development within a single cowpea seed, but crowding causes delay in development, higher larva mortality and smaller size (Bellows, 1982). There are five larval instars and the minimum developmental period on cowpea is about 21 days at about 32 °C and 90% r.h. (Anon, 1991). Pupation takes 26 days after oviposition and the total development on cowpea is about 36 days at 32^{0}C and 90% r.h. (Adhikary, 1981). El -Sawaf (1956) found that *C. maculatus* fed with soybeans had poor survival and rate of development. Adults emerge through a 'window' which is created by the larva as a result of the latter chewing away the cotyledon to leave an area of the seed coat slightly opened, the seeds may be almost completely hollowed out by the feeding activities of the larvae and characteristics emergent holes are evident after the adults leave the seeds (Giga and Smith, 1983).

Laboratory strains lose their capacity for producing flight forms because flightless forms deposit their eggs before the flight forms begin to lay their eggs (Utida, 1972). In

established cultures more flight forms appear when the humidity of the food meduim rises as in the case of crowded culture (Utida, 1972). The complete life cycle, however, varies depending on the environmental conditions and the type of food available.

2.5 Control of infestation in storage

Infestation during storage may be controlled by five different methods. These are (a) Physical control (b) Plant resistance (c) Biological control (d) Chemical control (e) Use of phytochemicals. Physical control methods are the physical elimination of the pest or physical alteration of the environment to make it inimical or inaccessible to the pest. Exposure of cowpeas to temperature more than 57.3 °C in an oven for 1 hour killed all larvae and pupae of *C. maculatus* seeds and adults among seeds (Murdock and Shade,1991). Seeds of cowpea can be protected against *C. maculatus* with ash. This traditional method is extensively practised in Northern Cameroon. The amount of ash used and the details of the methodology varies among farmers (Wolfson *et al.*,1991). In Kenya it has been reported by Mueke and Apuuli (1986) that ash mixed with seeds of cowpea afforded satisfactory control of the bruchids. Various oils are used for protecting legume seeds against *Callosobruchus* infestation. Studies on the mode of action of vegetable oils on the eggs of *C. maculatus* revealed two possible mechanisms of action which may be complementary; that the oils exert lethal action slowly by drastically reducing respiratory activity and elimination of toxic metabolities as a result of the oil 'barrier effect' ; and direct toxic effects of oil or oil constituents that possibly penetrate the eggs (Don-Pedro,1989). Steam treatment of stored produce involves passing steam over grains to desinfest the grains and also to denature the surface of testa of the grains. This adversely affects oviposition of *Callosobruchus* whose eggs

are laid and glued against the seed surface. Certain varieties of legumes have developed a natural plant resistance to *C. maculatus* infestation.Thirty cowpea cultivars were evaluated as intact pods to determine if any possessed resistance to the bruchid, *C. maculatus*. Pod resistance was measured as pre-establishment larval mortality i.e. those larvae dying after egg hatch but before penetrating into the seeds, and as post-establishment within-seed mortality i.e. those larvae dying after penetrating into the seeds. Pre-establishment of larval mortality ranged from 57.9 to 99.4% .Ten varieties exhibited total intact pod mortality greater than 95% (Kitch *et al.*, 1991). Seeds of Bambara groundnut in storage also showed some degree of varietal resistance to *C. maculatus* infestation. Borchers *et al.*, (1947) reported that many leguminous seeds contain certain factors (mainly trypsin inhibitors) that affect the digestibility of protein by inhibiting proteolytic enzymes in bruchids. The trypsin inhibitors account for the differences in susceptibility of three varieties of Bambara groundnut to *C. maculatus* infestation in the order of Jabajaba > Piele kargu > Piele Balgu (Allotey and Dankwah, 1994).

Biological control of *C. maculatus* employs the use of a parasitic wasp *Bracon hebetor* Say. (Hymenoptera; Braconidae). *Bracon hebetor* is a cosmopolitan parasitic wasp which attacks many species of stored product moths (Press *et al.*, 1981). The wasp is also a parasitoid of *C. maculatus*. The wasp lays its eggs close to that of the beetle which is glued to the grain host. Under favourable conditions, *B. hebetor* develops from egg to adult in less than 2 weeks (Anon, 1979: Press *et al.*, 1981). The parasitoid's egg develops into a larva which feeds on the egg or developing larva of the cowpea beetle.

Considering the short developmental period of *B. hebetor* the parasitoid could be used effectively to suppress populations of *C. maculatus* on Bambara groundnut. *Uscana mukerjii* (Mani.) (Chalcidoidea : Trichogrammatidae) parasitizes eggs of *C. maculatus*. The male and female adult lives for 5.0 and 4.7 days, respectively . The maximum parasitization of host eggs takes place within the first 24 hours of egg laying and then declines thereafter. The parasite completes its development inside the host egg in 7.2 days at 27°C and 60-63 r.h.; it emerges after cutting a circular hole in the chorion of the host egg. Parasitized host eggs do not hatch. The degree of parasitization varied with the pulse on which the host eggs are laid. This egg parasite may be useful in controlling the populations of *C. maculatus* and thus help in reducing the damage caused (Kapila and Agarwal, 1995).

The use of synthetic chemicals in the control of insect pest of stored products is associated mainly with large scale storage. There are two types of synthetic insecticides, namely the contact insecticides and respiratory poisons or fumigants that are used to control insect pest in stored products. Contact insecticides like Dursban, Karate and lindane can confer long term protection, usually referred to as the residual effect, but often tend to be some what specific in their effect upon insect species and to produce resistance more than the respiratory poisons. Fumigants provide no residual effect but unlike contact insecticide, have the power to penetrate through stacks or bulks and to become absorbed into individual grains killing all stages of insect life within (Page and Lubatti, 1963). Methyl bromide and phosphine are two common fumigants, highly toxic to most stages of stored-product insects except the pupal stage (Applebaum

et al., 1968). Both methyl bromide and phosphine are highly poisonous to several species of Coleoptera (Fisk and Shepard, 1938). Synthetic insecticides are frequently used on stored products by local farmers even though the use of such formulation is being discouraged. In Togo, pirimiphos-methyl at 10 p,p,m. and deltamethrin at 1.p.p.m. were used to control the bruchids *Bruchidus atrolineatus* and *C. maculatus* in stored cowpeas. The two chemicals afforded effective control for six and four months, respectively (Adhikary, 1981).

The development of insecticide-base techniques for protecting grains in small traditional farm stores have only been partially successful because of problems such as high cost of synthetic insecticides and erratic supply due to foreign exchange constraints and poor storage structures. These problems have stimulated interest in the re-evaluation of traditional botanical pest control agents (Golob and Webley, 1980). Many small scale farmers in Africa commonly mix stored grain with different kinds of plant products for protection against pest damage in storage. The precise strategy used by different communities varies from place to place and appears to depend partly on the type and efficacy of suitable plant materials available in different localities (Hassanali *et al.*, 1990). An example is the use of dry ginger (*Zingiber officinale*) root powder to coat cowpeas for the control of *C. maculatus*. This reduced infestation of the cowpea seeds (Echendu,1988). There is a constant endeavour in bioprospecting to attain an advancement in the use of phytochemicals through the use of refined techniques in applying plant materials. The seeds of neem tree *Azadirachta indica (Aldous, 1992)* are rich in insect deterrent and insecticidal terpenoids which act as repellents and

antifeedants. Neem is a promising alternative to synthetic insecticides and is currently under investigation in several developing and advanced countries (Ivbijaro,1983; Jilani, 1983).Neem is the most widely investigated and successful plant material used to control insect pest of stored products (Dales, 1996). Aldous (1992) attributed the effect of *A. indica* to the presence of two azadirachtin components which have different effects. A decalin portion disrupts the insect's growth and development while the hydroxyfuran deters insects. In cowpea, early field experiments showed a 30 % yield increase after application of a methanolic neem kernel extract at 40 g/l while a methanolic leaf extract at 40 g/l gave 3 % yield increase (Adhikary, 1981). Insecticidal and fertilizing properties of neem cake applied to the soil around cowpea plants were demonstrated in field trials. The application significantly reduced the incidence of *Maruca vitrata* and increased the yield (Hongo and Karel,1986). Seeds of cowpea were protected from damage by *C. maculatus* for about 4 months by mixing with crushed Neem seeds at 1-3 g (Ivbijaro, 1983). Neem seed powder was recommended for short term protection of Bambara groundnut against *C. maculatus* at 1 g of crushed seeds on 40 g of Bambara groundnut (Allotey and Dankwah,1994). Neem oil at the rate of 30 mg/10 g of green grain seeds gave an anti-oviposition effect against *C. maculatus*. The oil block the funnel attached to the eggs of *Callosobruchus* thus preventing their development into larvae (Yadav ,1985). A treatment of cowpea seeds with Neem oil (5 ml/kg) resulted in a protection against *C. maculatus* for several months in West Africa (Tanzubil, 1986).

2.6 Steam treatment of seeds

Steam treatment of cowpea seeds have shown to be an effective measure to control *Callosobruchus* infestation of the legume (Cockfield, 1992). Steam treatment of cowpea reduces the availability of oviposition sites for *C. maculatus* and therefore reduces infestation by the beetle (Nwanze et al., 1975). This method is fairly cheap and offers no mamalian toxicity or poses no environmental problem as is the case of traditional pest control methods that are chemicals. Steam treatment also does not require any special training. Steaming of cowpea, however, does not offer any permanent protection although it is an effective sanitation technique (Cockfield,1992).

2.7 Seed viability

Viability is usually a measure of the ability of a seed lot to produce crop. The results of a germination test are used in two ways; to determine the suitability of a seed lot for sowing and to compare the value of different lots to provide a basis for trade in seeds. The results of a germination test indicate the proportion of pure seeds which have produced seedlings, capable of continued development into mature plants, when germinated under standardized conditions of substrate, moisture supply and temperature to ensure that the result is reproducible (Wellington, 1966). The principal environmental conditions necessary for seed germination are an adequate water supply and suitable temperature and composition of gases in the atmosphere. These requirements may vary according to the species, the conditions prevailing during seed formation and also by hereditary factors (Mayer and Poljakoff-Mayber, 1963).

Paper is used extensively in seed viability tests and is particularly suitable for small seeds and for seeds which may require light for germination. It should be porous and

may be sterilized to avoid cross-transmission of disease pathogens. The paper should be strong when wet and thick enough to supply adequate moisture. The principal function of the substrate is to supply moisture. The supply of oxygen in laboratory germination tests is largely determined when the water supply is inadequate (Chow and Draper, 1969) but excessive water physically impedes the uptake of oxygen (Chetram and Heydecker, 1967) which may also be competed for by fungi and bacteria in the medium (Roberts, 1960). The optimal temperature for germination is that at which the highest percentage of germination is attained in the shortest time. The range over which complete germination is attained may differ with the age of the seed, the range widens as the seed ages (Mayer and Poljakoff-Mayber, 1963). For seed testing purposes, germination as a laboratory test is defined as the emergence and development from the seed embryo of those essential structures which, for the kind of seed being tested, indicates the ability to develop into a normal plant under favourable conditions in the soil (Roberts,1960).

2.8 Biochemical changes during storage

Biochemical changes have been observed during storage. The dry weight of grain increases and this is explained by the fact that water is consumed during hydrolysis of starch. During storage alpha and beta-amylases attack the starches of grain converting them into dextrin and maltoses (Bottomley *et al.* ,1950). Starch hydrolysis increases the reducing sugar content of the grain but this is further consumed in respiration and converted into carbon dioxide and water. The total carbohydrate content of a stored grain therefore decreases slightly, but consistently higher in mould-damaged sample than in the corresponding sound flour (Daftary *et al.*, 1970). The relative increase on a

percentage basis can be explained by respiratory losses of carbohydrate. Protein values determined by a dye method is lower than the kjeldhal method because the dye cannot bind with damaged protein and lipid molecules. Proteolytic enzymes in grain hydrolyse protein into polypeptides and then into amino-acids (Crocker and Barton, 1953). Except for glutamic acid and the amides free amino acids in the endosperm end of a kernel generally increase as in the nitrogen molecules of stored seeds determined by the kjeldhal method is due to the breakdown of several substances in the stored food. Several nitrogen molecules may be present even though they may not necessarily come from protein molecules but instead from other compounds.

CHAPTER THREE

3. Materials and methods

3.1Market survey

In August 1997,a survey was conducted on five markets in and around Accra to assess the infestation of *Callosobruchus* on Bambara groundnut to determine the major pests of the legume. The markets visited were Malata, Makola, Madina, Kasoa, and Agbogbloshie. Samples of Bambara groundnut infested with *Callosobruchus* were randomly purchased from three spots in each of the five markets. In the laboratory, all foreign matter were removed from the seeds by sieving. The samples from each market were kept in different rearing jars till the emergence of adult *Callosobruchus*. Adult insects that emerged from these samples were removed daily with a mouth aspirator. *C. maculatus* were selected using the serrated antennae and the two dark patches situated halfway along each elytron (Haines, 1991).

3.2 Varieties of Bambara groundnut used

Two local varieties of Bambara groundnuts namely Jabajaba and Piele Balgu were purchased from the Accra Newtown Market and stored in a refrigerator at 0 ^{0}C till they were needed. The Jabajaba is the dominant variety grown in the Dangme East district of Ghana. The Piele Balgu variety is one of the common varieties grown in Northern Ghana. It has also the best mean growth ratio and is thus chosen for experiments to evaluate control methods (Allotey and Dankwah, 1994).

3.3 Rearing of insects

Adults of *C. maculatus* were reared on Bambara groundnut which had been sterilized at 60°C in a Gallen Kamp oven for one hour (Murdock and Shade, 1991). The parent stock of insects were obtained from infested cowpea bought from the Madina market in Accra. About 200 randomly selected unsexed adult *C. maculatus* were introduced into 600 g of sterilized Bambara groundnut in rearing jars (12 x 12 cm) and kept in the Physiology laboratory of the Zoology Department of the University of Ghana, Legon. The laboratory was maintained at 28 ± 2 °C and 60-65% r.h. .The seeds were sieved to remove all dead insects after 14 days. Progeny emergence began after 21 days and the emerging F1 adults were immediately used for the various assays. All equipment used for the experimental work were sterilized in a hot-box Gallenkamp oven at 100 °C for two hours. The set-ups were arranged in trays which were mounted over oil to prevent diseases or cross infestation (Allotey and Goswani, 1991).

3.4 Preparation of plant materials

Ripe neem fruits were harvested from trees on the University of Ghana, Legon Campus. The fruits were kept in the dark in the laboratory for two days to soften the mesocarp. The mesocarp were removed and the seeds dried in a shade for eight hours a day for nine sunny days. The endocarp of the seeds were cracked to remove the kernel which were milled with an electric mill to particle size of 5 μm (Allotey and Oyewo,1993) .An extract of the milled kernel was prepared using ethanol with the Soxhlet apparatus (Sanaa, 1988) at the Phsiology Biochemistry laboratory of the Cocoa Research Institute of Ghana Tafo. Five grams milled neem kernel was washed with 100 ml ethanol in the Soxhlet apparatus for five hours. The extract was concentrated using the kjedhal

apparatus at 100 ^{o}C. The oily extract was preserved in tightly corked 20 ml vials and stored in a refrigerator till they were needed (Islam,1983) .Neem kernel extract was applied at 1 % concentration (Sanaa, 1988).

3.5 Determination of effective concentration of neem seed oil

The neem seed oil used in this study was a standard formulation supplied by Cocoa Research Institute of Ghana, Tafo. Neem seed oil is applied at a concentration of 1-2 % to avoid phytotoxicity (Yadav, 1985). A preliminary test was conducted to determine the dosage at which the neem seed oil would allow for a low oviposition of the *C. maculatus. A stock solution of t*he neem seed oil was prepare by emulsifying with teepol at a ratio of 5:1 and then further diluting to one litre with distilled water. Dosages of 0.8, 1.0, 1.2, 1.5, and 1.8% were prepared. 1000 ml of each dosage was sprayed on 1000 seeds of Bambara groundnut with a hand sprayer. Three replicates were set for each treatment. The treated seeds were kept in breeding jars. The jars were covered with nylon cloth which were held in place with rubber bands. The number of surviving adult *C. maculatus* was recorded after three days. The surviving and dead insects were removed on the fourteenth day. The number of eggs laid on each batch of 100 seeds was recorded.

3.6 Actellic 25EC

Actellic is an organophosphorous insecticide with an active ingredient called pirimiphos-methyl. It is used to control both field and storage insects. In storage facilities, actellic controls flying and crawling insects including beetles, weevils, mites and borers of cereals and legumes. It is applied through admixture with the produce at a dosage of 16 ml of the commercial product in one litre of water. One thousand seeds of each variety of Bambara groundnut were treated with actellic. Treated seeds were air-dried for 24 hours.

3.7 Neem seed oil and neem kernel oil treatment

Ten millimeters each of the neem seed oil and neem kernel oil were first emulsified using 2 ml of teepol and then diluted to 1000 ml to form a 1% solution. The solutions were used to treat 1000 seeds of each variety of Bambara groundnut using a hand sprayer. The treated seeds were air dried for 24 hours.

3.8 Steam treatment

Steam produced from boiling water in a bowl was passed over batches of seeds in a Sieve for 15 minutes. Each batch had 1000 seeds. The seeds were oven dried at 60 °C for 30 minutes in a Gallen kamp oven.

The control seeds were not exposed to any treatment.

3.9 Assessment of damage cause by *C. maculatus*

Experiment one was set up to investigate the effect of the different treatments on the two varieties of Bambara groundnut on the biology of *C. maculatus* introduced onto the seeds a day after treatment was investigated. A pair of day-old adult *C. maculatus*

were used for the experiment because female *Callosobruchus* avoids laying on seeds already laden with eggs of another female *Callosobruchus* (Mbata, 1992). A pair of *Callosobruchus* was introduced onto the seeds which were contained in glass jars (5 × 12 cm) Each jar containing 100 treated seeds were covered with nylon mesh which were held in place with rubber bands. Ten replicates of each treatment were randomly arranged in wooden trays mounted over used engine oil. Jars containing different treatment were kept in separate trays. The progeny started emerging from the 22nd day after the experimental set-up which lasted 30 days. The experimental design was a Randomised Complete Block Design. The damage caused by infestation of the progeny of a pair of adult *C. maculatus* to 100 seeds was assessed. The *C. maculatus* laid their eggs on the seed coats. These eggs hatched and the larvae penetrated the seeds where they developed. The larvae fed on the cotyledon and embryo of the seeds. Their feeding created windows in the seed coat . The young adults emerged out of the seeds through the windows. Seeds with emergent holes were considered damaged. Both damaged and undamaged seeds were weighed separately. The experiment lasted 52 days. The percentage weight loss of the seeds was calculated using the method of FAO (1985);

$$\% \text{ weight loss} = \left[\frac{UaN - (U + D)}{UaN}\right] \times 100$$

where, U = weight of Undamaged fraction in the sample

Ua = average weight of undamaged grain.

D = weight of damaged fraction in the sample.

N = total number of seeds.

3.10 Progeny emergence

The seeds of Bambara groundnut were treated with 1% neem seed oil, 1% neem kernel oil and actellic at 20 µl/litre per 1kg of seeds. Some seeds were exposed to steam for 15 minutes and oven dried at 60 °C for 30 minutes. The control had no treatment. The seeds were stored in glass jars for 90 days due to time constraint after which they were exposed to infestation by adult *C. maculatus*. A pair of day-old *C. maculatus* were introduced into 100 seeds of Bambara groundnut. Each treatment was replicated ten times in breeding glass of size 5 cm x 12 cm. The jars were covered with nylon mesh and were arranged in wooden trays which were mounted over used engine oil. The pair of *C. maculatus* introduced were removed after 14 days. The *C. maculatus* adults which emerged each day were removed from the jars with a mouth aspirator and kept in Petri dishes. The insects in the Petri dishes were examined daily to assess the number which survived. Dead insects were removed with mouth aspirator. This constituted experiment two.

3.11 Germination test

Bambara groundnut seeds were treated as described in sections 3.6, 37 and 38. A viability test was carried out on the seeds. Ten seeds of each variety were separately sown in plastic Petri dishes in the laboratory. The Petri dishes were lined with two layers of Whatman No. 1 filter paper which were kept moistened throughout the experimental period. The seeds were arranged with their hilium touching the soaked filter paper to facilitate absorption of water. Each treatment was replicated four times. The Percentage germination of seeds after five days was recorded for the various treatments.

3.12 Determination of protein content of seeds

The amount of protein in the treated and untreated seeds was determined using the micro-kjedhal test. These seeds were stored for 90 days after which they were milled and digested. The product was used in a back-titration to estimate the amount of nitrogen in the sample and the protein content was then calculated. About 0.5 g of the oven-dried (100-105 °C) milled seeds was weighed. One milligram catalyst (a mixture of 0.2 g selenium, 5 g potassium sulphate and 1 g copper sulphate) was added. The sample was moistened with three drops of distilled water and 12 cm^3 of concentrated sulphuric acid was added. The mixture was allowed to stand for 10-15 minutes and then heated moderately on a hot heating mantle in a fume cupboard until a clear or colourless solution was obtained. The solution was allowed to cool and then diluted to 100 cm^3 The solution was kept overnight in the digestion flask. Twenty centimetres cubic 2% boric acid solution was pipetted into a 100cm^3 conical flask. Two drops of an indicator (0.1 methylene blue and .01 g methyl red in 95 % ethanol-made up to 50 ml) was added. Ten centimetre cubics of the digested sample was pipetted into the Markham distillation unit and to which 5cm^3 of 40 % sodium hydroxide was added. Ammonia was distilled into the boric acid solution and was made up to the 50cm^3 mark on the conical flask. There was a colour change from blue to light green. The boric acid-ammonia solution was titrated against 0.01m sulphuric acid from a burette where the colour changed from light green to blue.

Calculation, for a constant weight of 0.5 g sample used,

$$\% N = \frac{T \times 0.02}{\text{weight of sample}} \times \frac{14}{100} \times 10 \times 100$$

where $^{14}/_{100} \times 10 \times 100$ is a constant

$$\% N = 5.6T$$

where T = titre value

% crude protein is expressed as % N/gm dry matter

= 6.25 x % N

= 6.25 × 5.6 T

The percentage Nitrogen per grain dry weight of seeds of Bambara groundnut was determined by first weighing 100 gm of the seeds and repeatedly weighing the seeds after each hour of oven drying at 60 °C. The oven-drying was stopped when subsequent weights did not change. The dry weight of seeds was found to be 90.5 ± 0.00 gm. The percentage crude protein of seeds of Bambara groundnut was calculated as;

= 90.5 × 6.25 ×5.6 T

= 3167.5 T

3.13 Field evaluation

The field work was designed to test the performance of the various treatments in the field. It was carried out in a farmer's crib (Plate 1) at Addokoppe in the East Dangme District of Ghana. The crib was made of bamboo with a thatched roof. It was raised from the ground level to a height of 91.5cm to prevent crawling organisms from climbing the

crib. The main crib was constructed with split bamboo sticks which were nailed to a frame of whole tree trunks. The crib is also used to store maize and other cereals. The seeds of Bambara groundnut were treated as previously described in sections 3.6, 3.7 and 3.8. Five hundred grams of treated seeds were put into 12cm x 15 cm polypropylene sacks. The ends of the bags were carefully stitched. Three replicates were set for each treatment. They were arranged horizontally on each other in the crib. The sacks were inspected every 14 days for rodent and termite infestation. The sacks were removed after 120 days and the damage was assessed as previously described (FAO, 1985).

3.14 Analysis of data

The experimental design was a Randomised Complete Block Design. The data collected was transformed using $\log_{10}(n+1)$ and analysed with ANOVA for significant differences at 5% level. Means were seperated with LSD. Data on germination was transformed with the arcsine and analysed using ANOVA. LSD was used to separate the means.

Plate 1. A bamboo crib for storing legumes and cereals.

CHAPTER FOUR

Results

General survey

A survey of traditional methods of storage of Bambara groundnut in the Dangme East district in November 1997 showed that, infestation of stored Bambara groundnut by *C. maculatus* (Fab.) was the main problem facing the farmers (Table 1). Traditionally the farmers construct clay silos to store Bambara groundnut. Additionally a survey of five markets, namely, Malata, Makola, Madina, Kasoa and Agbogbloshie in November 1997 to identify the major pests of stored Bambara groundnut showed that two beetle species *C. maculatus* (Fab.) and *C. subinnotatus* (Pic.) were the major pests of the crop during storage (Table 1).

Progeny emergence and damage assessment

Tables 2 and 3 show the effect of the different treatments on adult emergence, damage and weight loss caused by *C. maculatus*. The number of adults that emerged on the untreated Piele Balgu variety was 50.4 (Table 2) and 38.6 on the untreated Jabajaba variety (Table 3). The trend of adult emergence, seed damage and weight loss in both the Jabajaba and Piele Balgu varieties were similar. Analysis of variance showed that adult emergence was significantly lowered when seeds were treated with actellic, steam and neem seed oil. A reduction in adult emergence consequently reduced damage. Actellic treated seeds had the lowest adult emergence and damage of seeds. Steam treatment of seeds also caused a significant ($P<0.05$) reduction in both adult emergence and damage. The neem seed oil significantly ($P<0.05$) suppressed emergence and

damage as well while the neem kernel oil failed to suppress the beetle's emergence and damage. The various treatments offered different degrees of protection to the Bambara groundnut. The Piele Balgu variety is more susceptible to *C. maculatus* infestation. The Jabajaba variety exhibits a natural resistance to *C. maculatus* infestation. Actellic and neem seed oil provided the greatest protection of the seeds against infestation by *C. maculatus*.

Developmental period and longevity of *C. maculatus* bred on treated and untreated seeds of Bambara groundnut.

A summary of the results of experiment two is shown in Table 4. The emergence lasted 30 days for the whole experiment. Analysis of variance on both varieties showed a significant reduction ($P<0.05$) in progeny production of the seeds treated with actellic steam and neem seed oil. Actellic caused the highest reduction in adult emergence. Neem seed oil and steam treatments were statistically equally effective in reducing the number of *C. maculatus* that emerged (Table 4). Longevity of adult *C. maculatus* was 13.9 days on the untreated seeds of both varieties. The developmental period of *C. maculatus* bred on untreated varieties of Bambara groundnut were 21.0 days (Jabajaba) and 21.2 days (Piele balgu). The developing larvae utilizes the seed content and the adult beetle survives on energy stored in the seeds during development (El-Sawaf, 1956). Developmental periods of insects bred on Actellic treated seeds (21.5 days) and steam treated seeds (22.2 days) were prolonged whilst the longevity of these same insects were drastically reduced (9.2 days and 9.6 days, respectively). Using the developmental periods and the longevity of insects bred on the untreated seeds of both

varieties of Bambara groundnut as standards, the steam and actellic treatments offered the developing larvae of *C. maculatus* poor conditions for development.

Germination Test

The percentage germination of seeds over a period of five days is summarized in Table 5. All the treatments except neem seed oil caused a significant ($P<0.05$) reduction in the germination of the Jabajaba and Piele Balgu varieties of Bambara groundnut compared to the untreated seeds. Steam treatment of seeds did not allow for any germination. Neem seed oil promoted germination of seeds of both legume varieties significantly ($P< 0.05$). Neem seed oil therefore activated germination of Bambara seeds whiles actellic hindered germination.

Protein content

Results of the analysis of the protein content of the seeds of both varieties are shown in Table 6. The untreated seeds of the jabajaba variety has a higher protein content than the untreated Piele Balgu seeds. Percentage protein content did not change significantly ($P> 0.05$) when the seeds were treated with actellic, neem seed oil, neem kernel oil and steam.

Field trial

Seeds treated and stored in a crib for a period of 120 days did not show signs of damage throughout the storage period. At the end of the 120 days the seeds had gained weight. The seeds were free of infestation mainly because they were first sterilized and also bagged to prevent re-infestation during the period of storage. Weight increase in the Jabajaba and Piele Balgu varieties were 12.3 g and 13.4g, respectively

in the untreated seeds which were significantly ($P<0.05$) different from the treated seed (Table 7). Steam treated seeds had significantly the lowest gain in weight of 5.0g and 5.2 g for the Jabajaba and Piele Balgu varieties, respectively. The general trend of weight gain in seeds is similar for both varieties of the Bambara groundnut and follows an increasing order of; steam, actellic, neem kernel oil, neem seed oil and the untreated.

Table 1: Mean number of insect species found in Bambara groundnut collected from five markets in and around Accra in November 1997.

MARKET	Mean No. of Species of *Callosobruchus* in sample	
	C. maculatus	*C. subinnotatus*
Malata	75.66	10.33
Makola	51.33	-
Madina	30.00	28.33
Kasoa	56.00	-
Agbogbloshie	21.66	59.33
Total	234.66	97.99
Percentage	70.54	29.28

Table 2: The efficacy of different treatments on the Piele Balgu variety of Bambara groundnut against infestation by *C. maculatus.*

Treatment	No.of adults that emerged (X ± S.E., N=10)	No.of seeds damaged (X ± S.E., N=10)	% Weight loss
Untreated	*50.4 ± 6.66^{b}*	*29.4 ± 2.78^{c}*	*23.2 ± 2.14^{c}*
Actellic	*1.1 ± 0.23^{a}*	*1.1 ± 0.23^{a}*	*0.8 ± 0.16^{a}*
Steam	*9.9 ± 2.81^{a}*	*9.7 ± 2.21^{b}*	*10.1 ± 1.73^{b}*
Neem seed oil	*18.4 ± 2.55^{a}*	*14.8 ± 1.97^{b}*	*11.5 ± 1.53^{b}*
Neem kernel oil	*49.0 ± 6.63^{b}*	*30.4 ± 2.17^{c}*	*23.7 ± 2.34^{c}*

Column means with the same alphabets are not significantly different (P>0.05).

Table 3: The efficacy of different treatments on the Jabajaba variety of Bambara groundnut against infestation by *C. maculatus.*

Treatment	No.of adults that emerged (X ± S.E., N=10)	No.of seeds damaged (X + S.E., N=10)	% Weight loss
Untreated	*38.6 ± 3.78* ^{d}	*28.6 ± 1.86* ^{d}	*20.3 ± 5.84* ^{c}
Actellic	*1.1 + 0.23* ^{a}	*1.1 + 0.23* ^{a}	*0.8 + 0.16* ^{a}
Steam	*10.0 + 0.52* ^{b}	*8.9 + 2.19* ^{b}	*6.9 + 1.67* ^{b}
Neem seed oil	*16.9 + 2.58* ^{c}	*13.4 + 2.19* ^{b}	*10.4 + 1.70* ^{c}
Neem kernel oil	*25.3 + 3.41*^{d}	*20.3 + 2.56* ^{c}	*15.0 + 2.00* ^{c}

Column means with the same alphabets are not significantly different (P>0.05).

Table 4 : Effect of treatments on developmental period and progeny emergence of *C. maculatus*.

eatment	Jabajaba Variety (X ± S.E) n=10			Piele Balgu variety (X±S.E) n=10		
	Emergence	Longevity (days)	Developtal Periods (days)	Emergence	Longevity (days)	Developtal Periods (days)
ntreated	40.3+4.10 ^{c}	13.9+2.07 ^{b}	21.0 + 0.25 ^{a}	50.6+3.16 ^{c}	13.9+0.93 ^{b}	21.2 ± 0.54 ^{a}
ctellic	0.9+0.27^{a}	9.2+0.60^{a}	21.5 + 3.34^{a}	0.9±0.34 ^{a}	8.3±0.76 ^{a}	22.9 ± 3.30^{a}
eam	18.9+1.59^{b}	9.6±1.35 ^{a}	22.2 + 0.70^{a}	23.1+1.60^{b}	11.3±0.70 ^{a}	22.8 ± 0.38^{a}
eem seed oil	19.2±0.64 ^{b}	13.6+1.47 ^{b}	21.1 ± 0.34 ^{a}	18.3+1.57^{b}	13.9+1.14^{b}	22.5 + 0.79^{a}
leem Kernel il	31.3+3.49^{c}	12.4±0.7 ^{b}	21.0 ± 0.37 ^{a}	50.0+2.36^{c}	13.3+1.38^{b}	21.4 + 0.43^{a}

Column means with the same alphabets are not significantly different (P>0.05).

Table 5: Percentage germination of Bambara groundnut exposed to the different treatments.

Treatment	(x + S.E) , n=4	
	Jabajaba Variety	Piele Balgu Variety
Untreated	82.0 ± 0.86 ^{d}	80. ± 0.57 ^{c}
Actellic	58.0 + 0.62 ^{b}	58.0 + 0.85 ^{b}
Steam	0.0 ± 0.00 ^{a}	0.0 ± 0.00 ^{a}
Neem Seed oil	85.0 ± 0.85 ^{d}	100.0 ± 0.00 ^{d}
Neem kernel oil	68.0 ± 1.49 ^{c}	74.0 ± 0.79 ^{c}

Column means with the same alphabets are not significantly different (P>0.05).

Table 6: Percentage protein content of seeds of Bambara groundnut.

Treatment	(x + S.E) , n=10	
	Jabajaba Variety	Piele Balgu Variety
Untreated	18.02[a]	16.53[a]
Actellic	18.29[a]	16.63[a]
Steam	18.2[a]	16.98[a]
Neem Seed oil	18.02[a]	16.67[a]
Neem kernel oil	17.92[a]	16.15[a]

Column means with the same alphabets are not significantly different (P>0.05).

Table 7 Weight gain observed in treated and untreated seeds of Bambara groundnut stored in a crib.

Treatment	Initial weight of sample (g)	Weight gained *(g)*	
		Jabajaba variety	Piele balgu variety
Untreated	*500.0*	*12.3 ± 0.43 c*	*13.4 ± 0.62 c*
Actellic	*500.0*	*9.0 ± 0.21 b*	*8.0 ± 0.26 b*
Steam	*500.0*	*5.0 ± 0.12 a*	*5.2 ± 0.62 a*
Neem seed oil	*500.0*	*11.9 ± 0.08 c*	*10.4 ± 0.43 c*
Neem kernel oil	*500.0*	*11.8 ± 0.08 c*	*10.2 ± 0.30 c*

Column means with the same alphabets are not significantly different (P>0.05).

CHAPTER FIVE

Discussion

Progeny emergence

Traditional clay silos in which farmers store harvested Bambara groundnut are usually sealed to prevent re-infestation. The internal environment of the silos are not conducive to the development of any hidden infestation and also avoid further contact with the pest. The survey of five major markets in the neighbourhood of Accra showed that two species of *Callosobruchus* namely *C. maculatus* and *C. subinnotatus* infest Bambara groundnut. Southgate *et al.*, (1957) reported that *C. maculatus* has both flight and flightless forms. The flight forms are active fliers and the flightless forms are sedentary. The flight forms of *C. maculatus* spread their eggs easily making it a more serious pest than *C. subinnotatus*. *C. subinnotatus* are less tolerant to adverse environmental conditions (Mbata,1987). A female *C. maculatus* can lay up to 115 eggs (Anon,1991) and each egg can develop into an adult in a minimum of 21 days on Bambara groundnut at about 30 °C and 70 % r.h. .The high oviposition numbers coupled with the short developmental period makes it possible for the beetle to multiply within a very short period of time.

An attempt to promote the cultivation of Bambara groundnut thereby increasing the farmer's income and also ensure food security to the resource-poor growers in Ghana should address the problems encountered in storage. Insecticides especially actellic 25EC are generally used to prevent infestation. The eggs of *C. maculatus* are laid glued to the host seeds. The larvae of *C. maculatus* develop inside host seeds. Development

of an individual beetle takes place in a single seed. The developmental period of an adult *C. maculatus* differs on different legumes. A long developmental period on a particular legume variety indicates that the food medium is not suitable for larval development or that the insect is unable to utilize the food material efficiently (Allotey and Oyewo, 1993). In the case of the Jabajaba variety, the untreated seeds did not differ significantly ($P>0.05$) from the treated seeds. None of the different treatments showed a significantly different developmental period from the other. The developmental period of *C. maculatus* from Steam treated seeds was the highest in both Bambara groundnut varieties. The shortest developmental period for *C. maculatus* reared on untreated seeds was because the process of metamorphosis was uninterrupted. Steam, however, delayed emergence by prolonging developmental period. This situation arises from the fact that the treatment hydrolyzes certain seed components which are vital for the development of the insect (Gatehouse *et al.,* 1979; Kuznetsov *et al.,* 1990). Developmental period was not significantly different ($P>0.05$) for the untreated seeds and those that were variously treated. Bruchids demonstrate a pattern of daily emergence on different varieties of legume (Allotey and Dankwah, 1994). The number of *C. maculatus* increases gradually from the first day of emergence to a peak and then declines until emergence of the beetles stops. The occurrence of a peak of emergence of *C. maculatus* earlier on a legume variety indicates that, that variety is more susceptible to infestation. The untreated seeds of the Piele Balgu variety yielded a peak of 92 insects on the eighth day while the untreated seeds of the Jabajaba variety had its peak of 80 insects on the ninth day. There are differences in the amount of amino-acids and saponins in seeds of pulses (Applebaum *et al.,* 1968).

Saponins may be regarded as specific metabolic defence mechanisms of pulses which have been evolved against insect attack. Protein digestion in bruchids is also affected by trypsin inhibitors in leguminous seeds (Borchers *et.al.*, 1947). Protein digestion in insects reared on different varieties of pulses would differ thereby affecting the rate of growth and development of these insects and their subsequent emergence from the seeds. The number of *C. maculatus* that emerged was significantly lower on treated seeds. The effect of the different treatments reduced the number of adult *C. maculatus* that emerged. Each of the treatments ($P<0.05$) significantly reduced emergence of *C. maculatus* for both the Jabajaba and Piele Balgu varieties. Actellic was the most potent product for the reduction of progeny of *C. maculatus*. Actellic is an insecticide which instantly kills adult *C. maculatus* on contact and also interrupts the development of eggs into adults. Steam on the other hand might have reduced the oviposition sites for *C. maculatus* since the beetle prefers laying on seeds with smooth testa. The neem seed oil is regarded as an ovicide which was lethal to the eggs of *C. maculatus*. The neem seed oil also served as an antifeedant which suppressed the feeding of *C. maculatus* larvae. Neem kernel oil also reduced the emergence of *C. maculatus* but at a lesser extent due to its lesser content of the active ingredient, azadirachtin, as compared to the neem seed oil (Wendt, 1991).

Damage assessment

Damage caused by *C. maculatus* was indicated by the presence of emergent holes on the host seeds. More than one insect can develop in a single seed. Fungal growth from spores on *C. maculatus* may also cause damage to the host seeds. This unidentified

fungus may cause a decolourization of the seeds. Moisture may be released from respiration of heavily infested seeds. Data on the damage assessment showed that seed damage of untreated seeds was significantly ($P<0.05$) higher than treated seeds for both the Jabajaba and Piele Balgu varieties. All the products evaluated reduced feeding by *C. maculatus* compared to the control. Weight loss results from feeding of *C. maculatus* larvae. The insects may make selective feeding on the host seeds resulting in nutritional loss. Actellic was the most effective product by reducing the percentage weight loss to the barest minimum amongst the treatments. The neem seeds oil also caused a significant ($P<0.05$) reduction in the feeding activity of the beetles.

The persistence of different treatments was assessed by determining their efficacy after 90 days following treatment. The sex ratio of *C. maculatus* reared on Bambara groundnut is 1:1 (Allotey and Dankwah,1994) but the F1 progeny cannot be predicted This is either due to the fecundity of the insects or the chemical content of the host seeds. Storage control methods also affect progeny emergence. Actellic reduced infestation of *C. maculatus* through impregnating the seeds and disrupting the development of the insects, whereas steam affects the oviposition sites for the beetles and thus reduces infestation. Toxicity of the Neem oils were demonstrated by their abilities to cause a depression of progeny emergence from seeds of the two varieties of Bambara groundnut. The Neem seed oil interfered with metamorphosis of *C. maculatus* and reduced the adult population (Ladd *et al.*, 1994). Azadirachtin, the active ingredient in neem is lost at a faster rate in neem kernel than in neem seed (Wendt,1991). The life

span of *C. maculatus* that emerges from seeds of legumes depends on the amount of food reserve in the insect. Edezinma and Maneke (1985) reported that higher adult longevities can be associated with better or more nutritious larval food medium. The female adult *C. maculatus* generally has a shorter life span (Haines,1991). Adult longevities of *C. maculatus* was 13.9 days for the untreated seeds of both Bambara groundnut varieties. For both legume varieties , the longevities of *C. maculatus* reared on Actellic and steam treated seeds were significantly ($P<0.05$) reduced. The pirimiphos-methyl present in the Actellic hastens the development of *C. maculatus* (Anon,1991) to yield adults which die earlier than normal. The steam treatment on the other hand causes hydrolysis of vital seed components notably carbohydrates which are essential for adult life span of *C. maculatus* (Gatehouse *et al.*,1979).

Seed viability and Protein content

Seed germination of the untreated legumes varieties after five days was about 80%. Steam treatment of seeds had a significant ($P<0.05$) effect on seed germination. All the seeds exposed to steam did not germinate. The steam treatment therefore affected germination. Actellic also retarded germination of seeds of the two varieties of Bambara groundnut. The rate of germination was promoted in the Neem treated seeds. Azadirachtin in neem promoted vegetative growth, enhanced seed germination and protected germinating seeds from pathogens which disturbed the process (Lehmann *et al.*, 1993). Different varieties of Bambara groundnut contain varying amount of protein but different treatments of seeds for storage can cause different degrees of the breakdown of non-protein and protein compound to release varying amounts of free Nitrogen detectable by the Micro kjedhal method. The breakdown of food substances to

yield free Nitrogen molecules reduces the nutritional value of seeds. The study showed that Jabajaba has a higher percentage protein content in the seeds than the Piele Balgu variety. The Jabajaba variety however is less susceptible to C. maculatus infestation due the nature of the seed testa.

Weight gain

The amount of weight gain over the 120 days is due to an accumulated moisture which reflects the amount of life activity in the seeds. The steam treated seeds had the lowest increase in weight while the neem seed oil recorded the highest increase amongst the treated seeds. Respiration in stored seeds uses reducing sugars and converts them into carbon dioxide and water, (Bottomley *et al.,* 1950). The carbon dioxide escapes whilst the moisture accumulates in the seeds. The polypropylene sacks kept the treated and untreated seeds free from re-infestation. The different treatments dis-infested the seed before they were concealed from further attack by *C. maculatus*. This procedure worked similarly as the local silos in the East Dangme district of Ghana.

Protectant Potential of the treatments.

Actellic persisted by impregnating the seeds and disrupting the development of *C. maculatus*. Steam treatment reduces the oviposition sites of *C. maculatus*. *Callosobruchus* normally prefers laying eggs on smooth seed testa. Neem oils caused a depression of progeny emergence because of its antifeedant property against the feeding larvae (Aldous, 1992). Actellic and Neem seed oil reduce re-infestation, a situation that is lacking in the application of steam. Neem seed oil is therefore a good alternative to the use of Actellic on stored Bambara groundnut.

The foregoing discussion of the efficacy and residual effects of alternatives to the utilization of synthetic chemicals should include an assessment of various methods through a social point of view to enable the ordinary farmer to appreciate and choose the best alternative to the use of synthetic chemical. Steam treatment of Bambara groundnut involves generating steam from water. The essential resources in the process, namely, fuel wood and water are both cheap and readily available in most farming communities. However, the burning of wood for heating contributes towards the depletion of the forest wood supply and also a source of polluting the atmosphere with smoke. Steam treatment is useful in reducing damage of Bambara groundnut by *C. maculatus* infestation. The viability of the seeds is, however, lost through the process. The method can therefore not be used for seed stock. Neem oil is obtained from neem seeds and can also be stored in a referigerator till it is needed for use. The process of preparing neem seed oil is diverse depending on the facilities available. Neem seed oil is, however, potent in reducing damage to Bambara groundnut by *C. maculatus*. The oil also promotes germination of seeds and vegetative growth as well. A comparison of steam and neem treatments as an alternative to the use of Actellic shows that both treatments are useful in preventing damage to Bambara groundnut seeds by *C. maculatus* infestation. Viability of seeds was hampered by the steam treatment but promoted by neem seed oil. Steam treatment is therefore appropriate for storage of seeds to be consumed while neem treatment can be applied to seeds destined for consumption or propagation.

Conclusion and Recommendations.

The main challenge facing the production of Bambara groundnut in Ghana is storage Seeds are damaged in store mainly by *C. maculatus*. Actellic 25EC is the most commonly used synthetic insecticide for protecting Bambara groundnut against infestation by the bruchid. Synthetic insecticides are expensive and detrimental to human health and the environment. The development of resistant strains of pests due to indiscriminate use of synthetic insecticides is of major concern to growers.

Conclusion

The study established that ;

1). *C. maculatus* is a pest of stored of Bambara groundnut. Infestation may start from the field.

2). The Piele Balgu variety of Bambara groundnut is more susceptible to *C. maculatus* infestation.

3). Infestation may be checked by reducing seed testa potential as an oviposition site of *C. maculatus*. Infestation was significantly ($P<0.05$) reduced by the four treatments namely actellic > steam > neem seed oil > neem kernel oil.

4). The efficacy of the treatments over a period of three months was actellic > neem seed oil > steam > neem kernel oil. Steaming does not offer a permanent protection to Bambara groundnut seeds.

5). Treatment of seeds did not significantly ($P>0.05$) affect protein content of seeds.

6). Neem seed oil promoted germination in Bambara groundnut. Steaming of Bambara groundnut reduces its germination potential. Actellic was lethal to Bambara groundnut.

7). Seeds of Bambara groundnut may be damaged through *C. maculatus* infestation when the insect feeds on the embryo and cotyledon of seeds or through treatment of seeds with certain synthetic insecticides like actellic.

RECOMMENDATIONS

Plant derivatives are effective in fighting infestation of crops. Neem seed oil proves to be effective against *Callosobruchus maculatus* on stored Bambara groundnut. Steaming of Bambara groundnut is also an effective sanitation technique but offers no permanent protection to stored Bambara groundnut. Infestation of Bambara groundnut may be tackled on the field. This will prevent infestation also during storage. Post harvest infestation can be prevented through steaming or spraying with neem seed oil and proper concealment to prevent *C. maculatus* from getting into contact with Bambara groundnut seeds.

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APPENDIX

Appendix 1. Bioassay of neem seed oil to determine the dosage at which *C. maculatus* would lay a minimum number of eggs.

Percentage Conc. Of neem seed oil	Mean No. of surviving female *C. maculatus*	Total Oviposition
0.8	8.66	227.66
1.0	7.00	202.33
1.2	6.66	200.66
1.5	6.30	196.33
1.8	6.66	206.66

Appendix 2. Total number of *Callosobruchus* collected from market survey

Markets	*C. maculatus*			*C. Subinnotatus*		
Malata	102	86	39	11	2	18
Makola	54	68	32	0	0	0
Madina	62	8	20	16	42	27
Kasoa	108	23	38	0	0	0
Agbogloshie	15	28	22	28	62	88

Appendix 3a. Adult emergence of *C. maculatus* from seeds of the JabaJaba Variety of Bambara groundnut.

Source of Variation	df	ss	ms	F
Treatment	4	8667.47	2166.86	23.43*
Error	55	5085.51	92.46	
Totals	59	13752.98		

LSD = t $\propto$ (error df) s_d

= 8.6

Appendix 3b. Adult emergence of *C. maculatus* from seeds of the Piele Balgu Variety of Bambara groundnut.

Source of Variation	df	ss	ms	F
Treatment	4	20765.79	5191.44	20.8382*
Error.	55	13702.61	249.13	
Totals	59	34468.40		

LSD = t $\propto$ (error df) s_d

= 14.18

Appendix 3c. Damaged seeds of the Jabajaba variety of Bambara groundnut through *C. maculatus* infestation.

Source of Variation	df	ss	ms	F
Treatment	4	4720.22	1180.05	26.9725*
Error.	55	2406.36	43.75	
Totals	59	7126.58		

LSD = t $\propto$ (error df) s_d

= 5.97

Appendix 3d. Damaged seeds of the Piele Balgu variety of Bambara groundnut through *C. maculatus* infestation.

Source of Variation	df	ss	ms	F
Treatment	4	6835.74	1708.93	28.6396*
Error.	55	3221.91	59.67	
Totals	59	10057.65		

LSD = t $\propto$ (error df) s_d

= 6.97

Appendix 3e. Weight loss observed in seeds of the Jabajaba variety of Bambara groundnut through *C. maculatus* infestation.

Source of Variation	df	ss	ms	F
Treatment	4	2528.06	632.01	21.0951*
Error.	55	1617.91	29.96	
Totals	59	4145.97		

LSD = t $\propto$ (error df) s_d

= 4.94

Appendix 3f. Weight loss observed in seeds of the Piele Balgu variety of Bambara groundnut through *C. maculatus* infestation.

Source of Variation	df	ss	ms	F
Treatment	4	1595.18	398.79	8.5983*
Error.	55	2550.79	46.38	
Totals	59	4145.97		

LSD = t $\propto$ (error df) s_d

= 6.15

Appendix 4a. Emergence of adult *C. maculatus* from seeds of the Jabajaba variety of Bambara groundnut stored for 90 days following treatment.

Source of Variation	df	ss	ms	F
Treatment	4	10777.52	2694.38	29.7065*
Error.	55	4988.41	90.70	
Totals	59	15765.93		

LSD = t ∝ (error df) s_d

= 8.60

Appendix 4b. Longevity of adult *C. maculatus* reared on seeds of the Jabajaba variety of Bambara groundnut stored for 90 days following treatment.

Source of Variation	df	ss	ms	F
Treatment	4	268.59	67.14	7.9174*
Error.	55	466.83	8.48	
Totals	59	735.42		

LSD = t ∝ (error df) s_d

= 2.63

Appendix 4c. Developmental period of *C. maculatus* emerging from the Jabajaba variety of Bambara groundnut stored for 90 days following treatment.

Source of Variation	df	ss	ms	F
Treatment	4	74.6	18.65	0.7486
Error.	55	370.05	24.91	
Totals	59	1444.60		

Appendix 4d. Emergence of adult *C. maculatus* from seeds of the Piele Balgu variety of Bambara groundnut store for 90 days following treatment.

Source of Variation	df	ss	ms	F
Treatment	4	21688.73	5422.18	81.4141*
Error.	55	3596.92	66.60	
Totals	59	25285.65		

LSD = t $\propto$ (error df) s_d

= 7.37

Appendix 4e. Longevity of adult *C. maculatus* reared on seeds of the Piele Balgu variety of Bambara groundnut stored for 90days following treatment.

Source of Variation	df	ss	Ms	F
Treatment	4	268.59	67.14	7.6208*
Error.	55	484.65	8.81	
Totals	59	753.24		

LSD = t $\propto$ (error df) s_d

= 2.68

Appendix 4f. Developmental period of *C. maculatus* emerging from the Piele Balgu variety of Bambara groundnut stored for 90 days following treatment.

Source of Variation	df	ss	ms	F
Treatment	4	28.21	7.05	0.4799
Error.	55	808.47	14.69	
Totals	59	1462.58		

Appendix 5a. Analysis of variance of the percentage germination of seeds of the Piele Balgu variety of Bambara groundnut.

Source of Variation	df	ss	ms	F
Treatment	4	5.82	1.45	3.6375*
Error.	15	6.00	0.40	
Totals	19	11.82		

LSD = t α (error df) s_d

= 0.12

Appendix 5b. Analysis of variance of the percentage germination of seeds of the Jabajaba variety of Bambara groundnut.

Source of Variation	df	ss	ms	F
Treatment	4	4.84	1.12	3.6011*
Error.	15	5.04	0.336	
Totals	19	9.88		

LSD = t α (error df) s_d

= 0.08

Appendix 6a. Analysis of variance of the percentage Nitrogen content of seeds of the Piele Balgu variety of Bambara groundnut.

Source of Variation	df	ss	ms	F
Treatment	4	0.01	0.00	∞
Error.	5	34.20	6.84	
Totals	9	34.21		

LSD = t ∝ (error df) s_d

= 3.00

Appendix 6b Analysis of variance of the percentage Nitrogen content of seeds of the Jabajaba variety of Bambara groundnut.

Source of Variation	df	ss	ms	F
Treatment	4	0.01	0.00	∞
Error.	5	32.96	6.592	
Totals	9	32.97		

LSD = t ∝ (error df) s_d

= 3.02.

Appendix 7a. Analysis of variance of weight gain observed in seeds of the Jabajaba variety of Bambara groundnut stored for 120 days in a crib.

Source of Variation	Df	ss	ms	F
Treatment	4	113.00	28.25	9.3234*
Error.	10	30.30	3.03	
Totals	14	143.30		

LSD = t $\propto$ (error df) s_d

= 2.45.

Appendix 7b. Analysis of variance of weight gain observed in seeds of the Piele Balgu variety of Bambara groundnut stored for 120 days in a crib.

Source of Variation	Df	ss	ms	F
Treatment	4	119.31	29.82	10.0768*
Error.	10	29.60	2.96	
Totals	14	148.91		

LSD = t $\propto$ (error df) s_d

= 2.42.